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Transanal Specimen Extraction After Laparoscopic Sigmoidectomy for Sigmoid Volvulus

Sigmoid Volvulus Hastalarında Laparoskopik Sigmoidektomi Sonrası Transanal Spesmen Çıkarımı

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ABSTRACT

Objective: Sigmoid resection can be performed using conventional and laparoscopic methods. Specimen removal from the natural orifice after laparoscopic surgery is increasingly preferred. This approach can reduce wound complications and the length of hospitalization. In this study, we present the results from cases of sigmoid volvulus treated with laparoscopic surgery and transanal specimen removal.

Methods: A retrospective analysis was performed on eight cases in which patients diagnosed with sigmoid volvulus underwent elective laparoscopic sigmoid colon resection and transanal specimen extraction. The patients were evaluated in terms of age, gender, comorbidities, operation time, surgical difficulties, length of hospital stay, and complications.

Results: Laparoscopic sigmoid resection and transanal specimen extraction were performed on eight patients. All patients were male, and the median age was 68 years (28-86 years). Five of the patients had comorbidities. The median operative time was 195 minutes (180-360). Transanal specimen extraction was successful in all patients. Anastomotic leakage occurred in one patient and subileus occurred in two patients. The median hospital stay was 5.5 days (3-21).

Conclusion: Transanal specimen extraction after laparoscopic resection is an easy, feasible, and safe method. Sigmoid volvulus is the ideal disease for the application of this procedure because it does not involve mass-like lesions such as tumors and diverticula.

Keywords: Colon, surgery, laparoscopy, sigmoid volvulus, natural orifice

ÖZ

Amaç: Sigmoid rezeksiyon konvansiyonel ve laparoskopik yöntemlerle yapılabilmektedir. Literatürde sigmoid volvulus tanılı hastalarda doğal delikten spesmen çıkarımı bildirilen az sayıda yayın mevcuttur. Biz burada sigmoid volvuluslu hastalarda yapılacak laparoskopik cerrahi sonrasında transanal spesmen çıkarımının teknik olarak uygulanablir bir yöntem olduğunu literatüre sunmayı amaçladık.

Yöntemler: 2018 ile 2019 tarihleri arasında sigmoid volvulus nedeniyle opere edilen hastaların dosyaları geriye dönük olarak tarandı. Elektif laparoskopik sigmoid kolon rezeksiyonu uygulanarak spesmen çıkarımı için transanal kullanılan olgular çalışmaya alındı. Hastalarda cinsiyet, yaş, komorbidite, operasyon süresi, operatif zorluklar, komplikasyonlar, yatış süresi ve mortalite bulguları analiz edildi.

Bulgular: Toplam sekiz hastaya laparoskopik sigmoid rezeksiyon ve transanal spesmen çıkarımı uygulandı. Olguların hepsi erkek olup, median yaş 68 (28-86) idi. Hastaların %62,5 da komorbidite mevcuttu. Operasyon süresi 195 dk. (180-360) idi. Hastaların hiçbirinde yara yeri enfeksiyonu görülmedi. Anastomoz kaçağı bir hastada görüldü. Hastanede yatış süresi median 5,5 gün (3-21) idi.

Sonuç: Doğal delikten spesmen çıkarımı cerrahisi giderek artan sıklıkla tercih edilmektedir. sigmoid volvulusta kolon çapı geniş olduğundan dolayı doğal delik cerrahisinin daha kolay ve güvenilir bir şekilde yapılabileceğini düşünmekteyiz.

Anahtar Sözcükler: Kolon, ameliyat, laparoskopi, sigmoid volvulus, doğal delik

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INTRODUCTION

Sigmoid volvulus is an important problem, especially in the elderly population. Unfortunately, surgical resection of the redundant sigmoid colon is currently the only radical treatment option. Emergency and elective colon surgery is always high risk in this elderly population, and the less invasive the treatment, the better. Endoscopic derotation is the first treatment approach in patients presenting with volvulus. Afterward, the sigmoid colon is removed with minimally invasive surgery under elective conditions.

The advantages of laparoscopic surgery are also seen in patients with sigmoid volvulus, with shorter hospital stays and lower morbidity compared with open surgery (1). However, in laparoscopic approaches, specimen extraction necessitates either widening of the existing incision or the creation of an extra incision. These alterations may cause complications, such as increased wound infection and incisional hernia (2,3). The advantage of natural orifice specimen extraction (NOSE) after laparoscopic surgery is that no extra incision is required for specimen removal. Additionally, patients' pain and length of hospital stay are reduced with NOSE (4,5). However, natural orifice surgery is still rarely performed in patients with sigmoid volvulus. This study evaluates the potential advantages of NOSE in patients undergoing elective surgery for sigmoid volvulus.

MATERIALS AND METHODS

The retrospective study was conducted at Inonu University's Faculty of Medicine and at the Gastroenterology Surgery Clinic at Gaziantep Dr. Ersin Arslan Training and Research Hospital. The research was conducted in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments, or comparable ethical standards. The study was approved by the Gaziantep University Ethics Committee (decision number: 2020/178, date: 02.07.2020) and registered in an international database (ClinicalTrials.gov NCT04740619). The files of surgical patients with sigmoid volvulus at İnönü University Faculty of Medicine and Gaziantep Dr. Ersin Arslan Training and Research Hospital from November 2018 to September 2019 were retrospectively reviewed. Patients with missing data and those who underwent emergency or conventional, open and laparoscopic sigmoid resections were excluded from the study. The patients' gender, age, comorbidities, operation times, operative challenges, complications, duration of hospital stay, morbidity, and mortality data were analyzed.

Statistical Analysis

Statistical analyses were performed using SPSS v.22.0 (IBM, Armonk, N.Y., USA). Quantitative variables were expressed as means ± standard deviation (SD), a median, and a range. Qualitative variables were reported as numbers and percentages (%). The means and SD were recorded for homogeneous distributions, while medians and ranges were recorded for heterogeneous distributions. Qualitative variables were compared using Fisher's exact test. Heterogeneous distributions were analyzed using the Mann-Whitney U test, and homogeneous distributions with the Student's t-test. A p-value of less than 0.05 was considered statistically significant.

Surgical Technique

Informed consent was obtained from patients, and elective operations were scheduled after successful derotation. Because the patients were operated on after endoscopic derotation, only a rectal enema was performed before the surgery. Subcutaneous low molecular weight heparin (0.1 mg/kg) was administered preoperatively. A single dose of third-generation cephalosporin (1 g, IV) and metronidazole (500 mg, IV) was administered one hour before the operation for antibiotic prophylaxis. The patient was taken to the operating table in the lithotomy position, and fixed to prevent falling. General anesthesia was then administered. An incision was made under the umbilicus to place a 12 mm trocar. A pneumoperitoneum was created using a Veress needle. A total of four trocars were used. The 30-degree camera trocar was inserted when intra-abdominal pressure reached 12-15 mmHg. Then, two main 5 mm, and 12 mm trocars were placed in the right paracolic area. An assistant trocar was placed in the left paracolic area. The sigmoid colon was resected, preserving the mesentery, toward the distal end of the colon, descending to 5 cm above the peritoneal reflection. The staple line on the distal rectal stump was opened, and the stump walls were held open with a grasper (Figure 1). Resection of the sigmoid colon was aided by a clamp inserted through the anal canal and into the lumen. The specimen was retrieved through a transanal approach. (Figure 2). A 31-33 mm circular stapler was placed via the anus, and then the anvil was taken into the abdomen, and the shaft removed. The open rectal stump was closed again, with a stapler. The anvil was inserted at the end of the proximal colon through the stapler line, and the open area was closed with a stapler. The resected stapler lines were removed through the trocar using an endo bag. In seven of the eight patients, a side-to-end anastomosis was performed with a circular stapler, while in only one patient, the anastomosis was performed side-by-side with a linear stapler. The stapler opening was closed with 3-0 polypropylene. In most of the patients a 32F rectal tube was placed at the proximal end of the anastomosis. Following an air leakage test, the operation was completed by placing a drain. The rectal tube was removed on the third postoperative day.

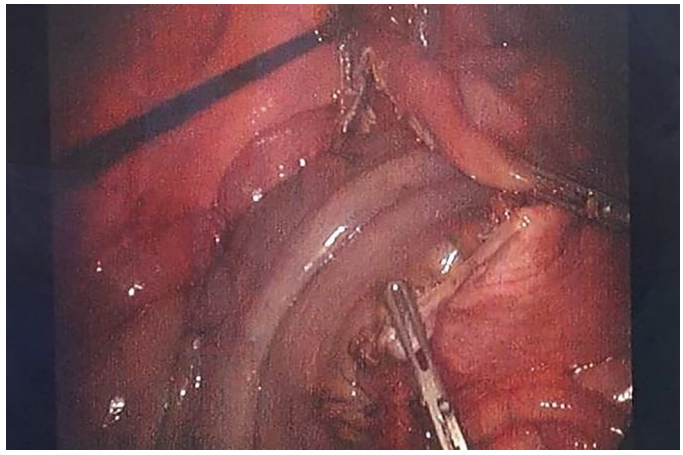

Figure 1. Extraction of the specimen from the rectal lumen.

RESULTS

Laparoscopic sigmoid resection and transanal specimen extraction were performed on a total of eight patients. All patients were male, with a median age of 68 (range =28-86) years. Comorbidity was present in 62.5% of the patients (Table 1). The median operation time was 195 min. (range =180-360). Operative challenges were encountered in two (25%) patients. In one case, there was an exploration problem due to colon dilatation, which resulted in the longest operation time (360 min.). In the second case, difficulties were encountered during surgery when attempting to insert an anvil into the proximal colon. This led to fecal contamination, which necessitated a colotomy. Postoperative complications occurred in three (37.5%) patients. In one patient, this complication was an anastomotic leak, which manifested as a fistula from the trocar site. The problem was resolved with conservative treatment. The complication in the other two patients was the development of subileus. One of these patients required further surgery seven days after the original operation, during which a procedure known as milking was performed. The other patient with subileus recovered with conservative follow-up. Wound infection was not observed in any of the patients.

The median length of hospitalization was 5.5 (range =3-21) days. The length of hospitalization for the subileus patient treated conservatively, the subileus patient treated by reoperation, and the patient who developed anastomotic leakage was 10 days, 12 days, and 21 days, respectively. None of the remaining five patients stayed in the hospital for more than six days. No mortality occurred during postoperative hospitalization. However, one (12.5%) of the patients died in the fourth month after surgery due to myocardial infarction.

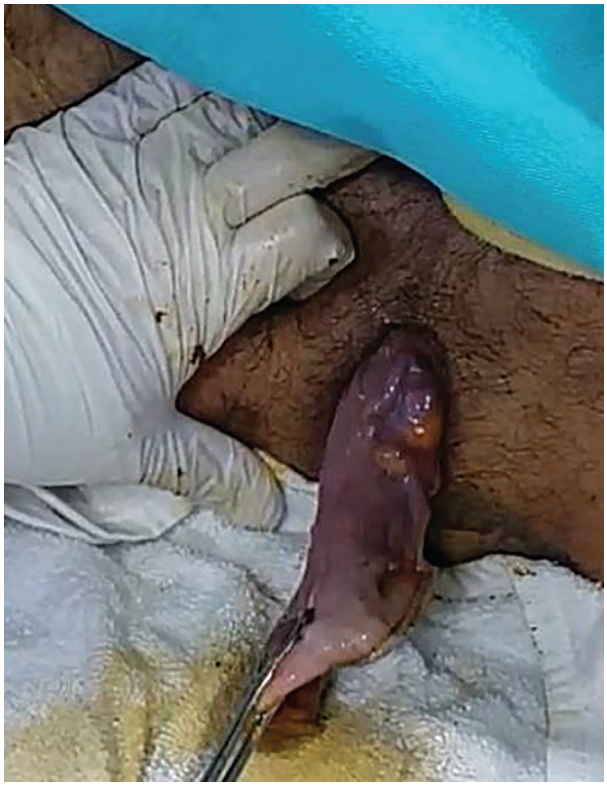

Figure 2. Extraction of the specimen from the anal canal.

DISCUSSION

The results indicate that laparoscopic sigmoid colon resection and transanal specimen extraction are straightforward and feasible in elective sigmoid volvulus surgery. Clinical studies and case reports have supported the application of NOSE surgery in colorectal cases, including in patients with sigmoid volvulus (6-10). A study of sigmoid resection with transvaginal Natural Orifice Transluminal Endoscopic Surgery was also reported. (11). In addition, a clinical trial of NOSE surgery performed in six patients with sigmoid volvulus was reported in the most recent publication (12). Open surgery, conventional laparoscopic surgery, and single-port laparoscopic resection can all be used to treat sigmoid volvulus (2). However, in laparoscopic approaches, either the incision needs to be enlarged or an extra incision needs to be created for specimen extraction. These incisional alterations may lead to complications such as increased wound infection and incisional hernia (3,13). The greatest advantage of NOSE after laparoscopic surgery is that no extra incision is needed for specimen extraction. This surgical method is being increasingly accepted by surgeons because the comfort and aesthetic outcome for the patient is more favorable. Because the specimen extraction is performed through a transanal approach, the patient requires only the trocar site incisions (Figure 3). Trocar site herniation after elective laparoscopic colon surgery has been reported at rates of 1.5-2.9% (14,15). The incidence of incisional hernia was reported as 5.2% at 1-year follow-up and 8.5% at 4-year follow-up after laparoscopic colorectal surgery (16). In our study, incisional hernias were not observed because the specimens were extracted using the transanal approach and incisions were not needed.

NOSE has been used to treat both benign and malignant disease, and similar oncological results have been reported as with other methods, and therefore, the popularity of NOSE is increasing (17).

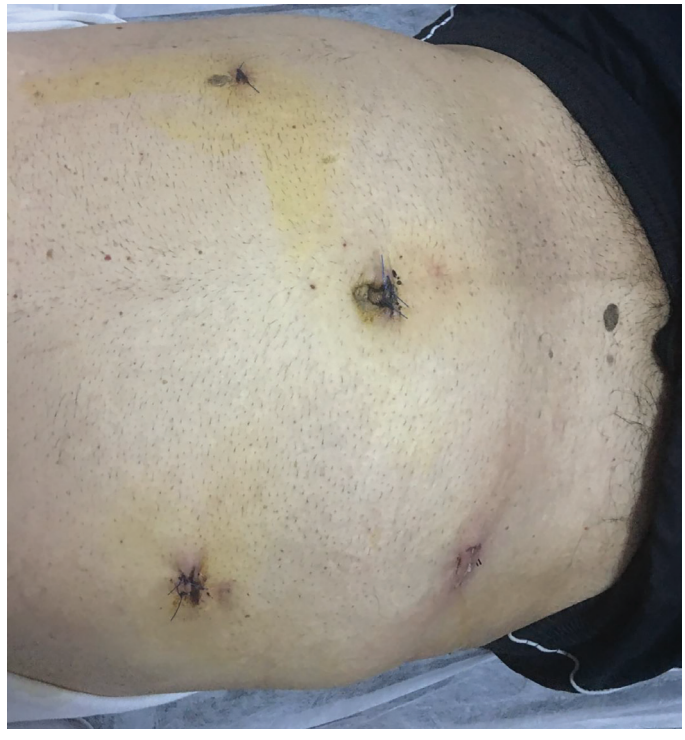

Figure 3. Postoperative trocar incision sites.

Table 1. Demographic, clinical, and surgical patient data

Patients features	1	2	3	4	5	6	7	8
Age	79	86	79	67	28	43	67	68
Gender	Male	Male	Male	Male	Male	Male	Male	Male
Comorbidity	HT, Alzheimer's disease	None	None	None	Epilepsy	Epilepsy, MR	None	HT, CAHD
Anastomosis	SECS	SECS	SSCS	SECS	SECS	SECS	SECS	SECS
Operative challenges	None	None	Exploration trouble	None	Fecal contamination	None	None	None
Rectal tube	No	Yes	Yes	No	Yes	Yes	Yes	No
Operation time (minutes)	180	240	360	180	240	210	180	180
Postoperative complication	None	Anastomotic leakage	Paralytic ileus	None	None	Ileus	None	None
Duration of hospitalization (days)	3	12	10	6	5	21	4	4
Mortality	None	None	None	None	None	None	None	None

SECS: Side to end circular stapler, SSCS: Side to side circular stapler, MR: Mental retardation, HT: Hypertension, CAHD: Coronary artery disease

In female patients, both transvaginal and transanal extraction methods are equally preferred by clinicians. While transvaginal extraction is recommended for larger masses, transanal extraction is recommended for smaller masses due to its suitability for the smaller diameter of the anal lumen (18). Because both ends of the colon are closed in transvaginal extraction, the risk of fecal contamination is very low; however, since the colon must be temporarily opened for specimen extraction, fecal contamination may still occur. Patience and caution are required in this regard.

In a sigmoid volvulus specimen extraction reported by Sia et al. (9) a nylon band was applied to prevent contamination while transecting the colon, and specimen extraction was performed with a modified Alexis retractor. To create end-to-end anastomosis, a purse-string suture was used for anvil placement. When difficulties were encountered in extracting the specimen, the author suggested splitting the colon. In our procedure, we transected the colon with a linear stapler. After pushing the anvil into the colon, we placed it so that the anvil protruded from the sidewall, not from the colon end. Our colon anastomosis was therefore side-to-end. We used the mesenteric division technique to efficiently remove the specimen (19). In addition, we used a camera sleeve, similar to the one used in laparoscopy, instead of an incision protector to remove specimens (20).

In laparoscopic colorectal surgery, specimen extraction from the natural orifice is a more complex procedure than the conventional laparoscopic method of specimen extraction. With increased experience of the surgeon, the procedure can be easily performed in most patients. In our experience, the opening of the distal rectal stump and the placement of the anvil were the most challenging and time-consuming parts of this method. However, serious operational difficulties were encountered in only two of our patients. These conditions were the difficulty of exploration due to dilatation in the proximal intestines, and the difficulty experienced during anvil placement. Despite this, we completed these two operations without the need for conversion to other methods.

In the Anastomotic Leakage After Colon Cancer Surgery study, 8% of patients who underwent colectomy had anastomotic leakage, and 80% of these patients required reoperation (21). It has been reported that no complications, mortality, or wound infection were observed in three patients who underwent natural hole surgery to treat sigmoid volvulus (10-12). In our study, anastomotic leakage developed in one patient, who did not require reoperation Perioperative mortality was not observed, although one patient died due to myocardial infarction in the fourth month after surgery.

Surgical site infection rates are as high as 14% to 26% after colorectal surgery (22). In a cohort study, surgical site infection was 8.2% and 4.1% in open and laparoscopic sigmoidectomies, respectively (23). However, in a study comparing conventional and natural specimen extraction during laparoscopic surgery for colorectal cancer, no significant difference was found between the two methods regarding frequency of wound infection (24). Perioperative wound infection was not observed in the patients in our study. The mean length of hospital stay for patients who underwent laparoscopic sigmoid resection for sigmoid volvulus was reported as 7±1 days (25). There are insufficient data on the effect of NOSE surgery on the length of hospital stay in colorectal cancer (24,26). In our study, the median hospital stay was 5.5 (3-21) days. It was determined that the management of the three patients who developed complications prolonged the average length of stay. Except for these three patients, the maximum hospital stay was six days.

In our previous study, we reported a success rate of 2/3 in transanal specimen extraction after laparoscopic colon resection (27). However, because there is no mass present in the rectosigmoid colon in patients with sigmoid volvulus, specimen extraction by the transanal approach is straightforward. All patients in our study were male, and, while a narrow pelvis could be a challenge for transanal specimen extraction in NOSE, we experienced no difficulty removing the colon segments.

Study Limitations

Our study has limitations as it is retrospective and includes a small number of patients. However, we think that the results of this study, which was conducted with a homogeneous patient group, will contribute to the literature.

CONCLUSION

The laparoscopic approach is frequently preferred in elective sigmoid volvulus surgery. Colon diameter is larger in sigmoid volvulus than in other diseases, which provides an advantage for NOSE procedures. In the treatment of sigmoid volvulus with laparoscopic surgery, transanal specimen extraction is an easy and safe method.

Ethics

Ethics Committee Approval: The study was approved by the Gaziantep University Ethics Committee (decision number: 2020/178, date: 02.07.2020).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: U.U., F.S., C.K., Concept: U.U., Design: U.U., F.S., Supervision: F.S., C.K., Resources: U.U., R.G., Material: U.U., C.K., F.S., Data Collection or Processing: U.U., R.G., Y.M.B., A.A., Analysis or Interpretation: U.U., A.A., C.K., Literature Search: U.U., F.S., Y.M.B., A.A., Writing: U.U., R.G., Y.M.B., A.A., Critical Review: C.K., F.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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Autoimmune Hemolytic Anemia in the Course of Pediatric Acute Leukemia and After Hematopoietic Stem Cell Transplantation

Pediatrik Akut Lösemi Tedavisinde ve Hematopoetik Kök Hücre Nakli Sonrasında Gelişen Otoimmün Hemolitik Anemi

Sırma Karamercan, Serap Kırkız Kayal, Büşra Topuz Türkcan, Zühre Kaya, Ülker Koçak

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ABSTRACT

Objective: Autoimmune hemolytic anemia (AIHA), thrombocytopenia, neutropenia and some other autoimmune diseases can be observed in the context of lymphoproliferative diseases and after hematopoietic stem cell transplantation (HSCT). Among these, AIHA is a rare red blood cell disorder; however, the cause cannot be determined in almost half of the cases.

Methods: This retrospective study analyzed nine pediatric leukemia patients who developed hemolytic anemia during chemotherapy or following HSCT. Patients were diagnosed with hemolysis based on laboratory markers, including hemoglobin (Hb), haptoglobin, reticulocyte count, lactate dehydrogenase (LDH), and direct antiglobulin test (DAT), and peripheral blood smear findings. Infectious and genetic causes of hemolytic anemia were also investigated.

Results: The mean age of the patients was 9.5±4.5 years (2.2-16.7 years). Six patients were being followed with acute lymphoblastic leukemia (ALL) whereas three had biphenotypic leukemia. Three of the ALL patients underwent allogeneic HSCT. During AIHA attacks, reticulocyte and LDH were high, whereas Hb and haptoglobin levels were low. While indirect bilirubin elevation was not detected in one case, the direct antiglobin test was positive at different rates in all, except three cases. Significant hemolysis findings were observed in all peripheral smears. The hemolytic attacks were thought to be related to chemotherapy, infection, and drugs. Steroids, intravenous immunoglobulin, rituximab were used for treatment.

Conclusion: In the clinical follow-up of leukemia patients, the significant increase in reticulocytes with low levels of Hb and haptoglobin and the increase in LDH and/or indirect bilirubin levels along with DAT positivity should suggest AIHA. Diagnosis and management planning must be undertaken accordingly.

Keywords: Leukemia, hemolytic anemia, autoimmune

ÖZ

Amaç: Otoimmün hemolitik anemi (OİHA), trombositopeni, nötropebi ve diğer otoimmün hastalıklar lenfoproliferatif hastalıklar sürecinde ve hematopoetik kök hücre nakli (HKHN) sonrası gelişebilir. Bunlardan OİHA olgularının yaklaşık yarısının etiyolojisi net değildir.

Yöntemler: Bu çalışmada lösemi tedavi sırasında ve HKHN sonrasında OİHA gelişen dokuz hasta değerlendirildi. Hastalarda hemoliz parametreleri olan hemoglobin (Hb), retikülosit, haptoglobulin, laktat dehidrogenaz (LDH), direkt antiglobulin test değerlendirildi. Ayrıca hastalarda enfeksiyöz ve genetik nedenler de tetkik edildi.

Bulgular: Hastaların ortalama yaşı 9,5±4,5 (2,2-16,7) idi. Altı hasta akut lenfoblastik lösemi, 3 hasta bifenotipik lösemi tanılıydı. Üç hastaya allojenik HKHN yapıldı. Otoimmün hemolitik anemi atakları sırasında retikülosit ve LDH yüksek, Hb ve haptoglobin düzeyleri düşük idi. Bir olguda indirekt bilirubin yükselmesi saptanmadı ve üç hasta dışında tüm olgularda direkt antiglobin testi farklı oranlarda pozitifti. Periferik yayma incelemesinde hemoliz bulguları gözlendi. Hemolitik atakların kemoterapi, enfeksiyon ve ilaçlarla ilişkili olduğu düşünüldü. Tedavi için steroidler, intravenöz immünoglobulin ve rituksimab kullanıldı.

Sonuç: Lösemi takibi sırasında gelişen retikülositteki anlamlı artış, hemoglobin düşüşü ve diğer hemoliz parametlerindeki değişimlerin tespiti ile otoimmün hemolitik anemi düşünülerek tedavi hızla yapılmalıdır.

Anahtar Sözcükler: Lösemi, hemolitik anemi, otoimmün

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INTRODUCTION

Autoimmune hemolytic anemia (AIHA) is a condition characterized by acute hemolysis caused by autoantibodies that develop against red blood cell (RBC) surface antigens. Clinically, fatigue, pallor and jaundice are frequent symptoms. While hemoglobin (Hb) and haptoglobin levels decrease, reticulocyte, bilirubin, and lactate dehydrogenase (LDH) levels are increased. The direct antiglobulin test (DAT) is usually positive. However, it may not be positive in all cases (1,2). The incidence of AIHA has been reported as 0.8/100,000 for those under 18 years of age (1). AIHA can be due to warm, cold, or mixed autoantibody types. Warm autoantibodies, which are the most common cause of AIHA, are typically a member of the IgG class. The DAT identifies IgG antibodies and/or C3, attached to the patient's RBCs after the introduction of the antihuman globulin reagent. Cold autoantibodies are usually of the IgM class and activate complement (usually C3), which is found on the RBC membrane. Despite being a routine and effective diagnostic tool for AIHA, the DAT is falsely negative in 3-11% of patients (3,4). Immunodeficiency, infections, medications, and malignancies can cause AIHA. Lymphoproliferative diseases are known to be accompanied by hemolytic anemia. AIHA has been reported to develop in acute lymphoblastic leukemia (ALL), acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, some lymphomas, and after hematopoietic stem cell transplantation (HSCT) (2,4,5). The association of AIHA with chronic lymphocytic leukemia has been clearly described, in which apoptosis of B lymphocytes, lack of adequate immunoglobulin release, deterioration of T lymphocytes involved in antigen presentation, and loss of immune tolerance have been involved in the pathogenesis (2). AIHA is rarely observed in solid tumors. Passenger lymphocyte syndrome is implicated in the etiology of AIHA after solid organ transplantation. It is thought to be caused by lymphocyte dysfunction (6). Anemia is an expected side effect during leukemia treatment and follow-up. However, attributing it solely to myelosuppression is not sufficient. Therefore, in this study, etiological factors (immune, non-immune) of anemia and the development of AIHA, which developed during the course and treatment of acute leukemia and after HSCT in children, were investigated and discussed.

MATERIALS AND METHODS

This study included leukemia patients who were diagnosed, treated, and continued to be followed up in our pediatric hematology unit between May 1 and November 30, 2021. All the records of these patients were reviewed retrospectively. Of these records, the patients who developed anemia (<-2 standard deviation for age) were selected and their peripheral blood smears were evaluated for the hemolysis indicators (anisocytosis, poikilocytosis, polychromasia, etc.). Complete blood count, bilirubin, LDH, haptoglobin, and DAT of the patients were also recorded.

The DAT was analyzed using the Grifols diagnostic DG gel system with the Erytra® Automated System Devices. Additionally, infectious agents [Epstein-barr virus (EBV), cytomegalovirus (CMV), hepatitis, Brucella, mycoplasma] and genetic causes of hemolytic anemia [thalassemia, deficiencies of glucose 6 phosphate dehydrogenase, pyruvate kinase (PK), etc.] that could assist in the etiology of anemia were also investigated. Patients without hemolysis indicators in peripheral blood smears were excluded.

The study was conducted through retrospective file review and was approved by the Gazi University Ethics Committee (approval number: 2025-1105, date: 17.06.2025).

RESULTS

Peripheral blood smears of 54 patients with anemia (<-2 standard deviation for age) who had acute leukemia were evaluated. Records of nine patients (5 females, 4 males) with hemolysis indicators: (anisocytosis, poikilocytosis, polychromasia, etc.) were included in the study.

The mean age of the patients was 9.5±4.5 years (2.2-16.7 years). Six patients were followed with ALL; three had biphenotypic leukemia (BL). Three of the ALL patients underwent HSCT. Laboratory values showed a mean Hb level of 9±0.5 g/dl (8.6-10). Reticulocyte count increased in all cases with a mean value of 8.6±2.6% (6.4-14.5%); LDH was also increased in the range of 269-889 IU/L. Indirect hyperbilirubinemia was detected in all cases except in one patient. All patients had anisocytosis, poikilocytosis (mostly spherocytes and schistocytes) and polychromasia in the peripheral blood smear. The direct antiglobin test was positive in six patients. In the three DAT negative patients, although they have typical laboratory and clinical findings for AIHA, cold agglutinins were also negative. Haptoglobin levels were low in seven patients, and high levels in the remaining two cases were thought to result from systemic infections. Serological tests showed positivity for EBV in two patients and CMV in one patient. One patient had a DAT positive anemia concomitant with PK deficiency. PK deficiency was incidentally identified when tests were conducted due to a sudden drop in Hb levels in this particular patient. In terms of treatment modalities, antiviral therapy was administered for CMV in patients with infection, while rituximab was used to treat EBV. Four patients who developed autoimmune anemia received steroid treatment (2 mg/kg/day for 3 days and 1 mg/kg/day for four days). In three of these patients who did not respond, an increase in hemoglobin was observed following intravenous immunoglobulin (IVIG) treatment (1g/kg/day for 2 days). For a patient with AIHA who was febrile and neutropenic, IVIG was preferred over steroid treatment. Table 1 shows the demographic and clinical findings of the patients.

Discussion

Leukemia patients suffer from anemia due to the disease itself, bleeding, chemotherapy, loss of appetite, and vomiting. Autoimmune hemolysis might also contribute to the severity of anemia, for several reasons, such as malignant disease, chemotherapy, immune deficiency, and infections (1-3). Reticulocyte, indirect bilirubin, and LDH levels are expected to be high in hemolytic anemia, while hemoglobin and haptoglobin levels are decreased, along polychromasia in the blood smear (2-5). However, none of these tests is essential for diagnosing hemolysis. Liver disease can lead to increased LDH and decreased haptoglobin; milder hemolysis can cause normal bilirubin levels. In 20% of patients, reticulocytopenia might be seen despite erythroid hyperplasia (7). Moreover, DAT is usually positive except in 3-11% of the cases (4). Direct antiglobin

Table 1. Patient characteristics

Patient	Age (years)	Gender	Diagnosis	Time	Co-morbidity	Hb (10.8-13.3 gr/dL)	Ret (%)	LDH (120-250 ıu/L)	Hapto (30-200 mg/dL)	IDB (<0.8 mg/dL)	DAT	Cold agglutinin	Peripheral blood smear	Treatment
1	13	F	ALL	After HSCT (3th month)	EBV	8.2	9.5	654	4	0.45	+	N/A	Acanthocytes, schistocytes, spherocytes	IVIG
2	13.6	F	ALL	After HSCT (1stmonth)	CMV	9	6.8	352	2	0.46	+	N/A	Anisocytosis, acanthocytes, spherocytes	Antiviral treatment
3	16.7	M	ALL	After REZ-BFM-ALL F2 block	Down syndrome	9.6	6.9	889	3	1.08	++	N/A	Spherocytse, polychromasia	IVIG
4	11.3	M	ALL	Maintenance CT (17th.wks)	PK defiency	8.8	14.5	563	7	2.5	+	-	Schistocytes	Stop CT
5	2.2	F	ALL	After CT (Bortezomib-VCR-Doxo-dexamethazone)	Infection (neutropenia)	9	6.4	269	187	0.38	+	N/A	Acanthocytes, polychromasia	IVIG-rituximab
6	8	F	BL	After REZ-BFM-ALL F2 block	-	8.6	7.9	373	28	0.32	+++	N/A	Polychromasia, schistocytes	Steroid
7	7.5	M	BL	Interfant protocol (Octatad-day 22	Infection (neutropenia)	10	7.5	389	246	1.17	-	-	Anisocytosis, polychromasia	IVIG
8	5	M	BL	Interfant protocol (MARMA- day 10	-	9	11.2	270	2	2.68	-	N/A	Polychromasia, schistocytes	IVIG
9	8.5	F	ALL	After HSCT (Day 47)	EBV	9.5	6.8	330	25	0.4	-	-	Acanthocytes, schistocytes, spherocytes	Rituximab

ALL: Acute lymphoblastic leukemia, BL: Biphenotypic leukemia, CT: Chemoterapy, EBV: Ebstein-Barr virus, CMV: Cytomegalovirus, PK: Pyruvate kinase, Hb: Hemoglobin, Ret: Reticulocyte, LDH: Lactate dehydrogenase, Hapto: Haptoglobin, IDB: Indirect bilirubin, DAT: Direct antiglobin test, IVIG; Intravenous immunglobulin, HSCT: Hematopoetic stem cell trasnplantation

IgG was found to be positive in only six of our cases. The negative DAT results in our three cases may be due to antibody types, such as IgA, that are not detected by DAT or the low-affinity antibody titer of IgG (3). In these situations, specific tests need to be performed for confirmation, which unfortunately are not available at most centers. Although the positivity of cold agglutinin can also cause DAT negativity, this was not the case in any of our DAT negative patients. AIAHs and their subtypes are defined by typical laboratory and clinical findings in the absence of standard diagnostic criteria. Clinical studies indicate that the use of different criteria for diagnosis can lead to varying treatment responses (8).

Children and adults with leukemia have been reported to develop AIHA similar to our ALL and BL patients (9,10). Three of our patients had developed AIHA secondary to infections while on immunosuppressive therapy after HSCT. The etiology of AIHA in these patients consists of both immunosuppression and infections due to chemotherapy and/or HSCT. Posttransplant EBV-associated AIHA has been reported to develop years after kidney and heart transplantation (11,12). AIHA after HSCT has been defined in transplants from mismatched unrelated donors, chronic graft versus host disease (GVHD) and non-malignant primary disease (13). Two of our three patients who developed AIHA after HSCT had been transplanted from an unrelated donor. In all three, GVHD of different severities had also been developed. One of these cases who was DAT negative received intensive immunosuppressive therapy for grade IV acute gastrointestinal GVHD with an additional EBV positivity. Moreover, CMV positivity accompanied AIHA in another transplant patient. Wang et al. (14) examined 553 adult HSCT patients. AIHA was detected in 19 of them. Although CMV positivity was detected in 10 of these cases, the difference was not statistically significant. However, CMV-associated AIHA has been reported in the past in immunosuppressed patients (15). Two of our three patients with hemolytic anemia after chemotherapy had concomitant neutropenic fever. IVIG treatment was given because the patient had both culture-negative neutropenic fever and AIHA. Chemotherapy-associated anemia develops through immune, microangiopathic and/or oxidative mechanisms (16). Three of our cases were DAT negative, but there were no other findings supporting microangiopathy or oxidative hemolysis. An increase in hemoglobin value following IVIG supported our diagnosis of immune hemolytic anemia. In AIHA, low haptoglobin, high LDH, and indirect hyperbilirubinemia are expected (4). Haptoglobin was elevated in two of our patients with febrile neutropenia and hemolysis. Since it is an acute phase indicator, this elevation was not considered significant.

The main treatment modalities of AIHA are, removal of the etiological factors, corticosteroids, IVIG and rituximab. If there is no response with these treatments, other immunosuppressive agents are used as line therapy (17). Our patients improved with the above treatments accompanied by removal of etiological factors. In conclusion, effective management of AIHA was achieved through a comprehensive approach that involved IVIG, corticosteroids, and addressing underlying factors, highlighting the importance of tailored therapeutic strategies in these cases.

Study Limitations

The study's retrospective design, relying on the review of past patient records, introduces inherent limitations. Moreover, the findings and conclusions are dependent on the availability and completeness of patient records. Incomplete or missing data could impact the accuracy and reliability of the results. As this is a pilot study, it will inform multicentric prospective studies. The study included a limited number of patients from a single center. These findings may not be generalized to a larger population or other healthcare settings. Another important limitation of this study is that it was focused on leukemia patients who developed AIHA during the course of their disease. This selection may introduce bias, as patients with specific characteristics or conditions might be overrepresented or underrepresented. Determining the exact etiological factors contributing to AIHA can be complex. The study aimed to investigate both immune and non-immune causes, but some factors, such as low-titer antibodies and IgA DAT, were not fully explored. External factors such as changes in treatment protocols, new developments in medical practices, or variations in patient demographics over time were also not considered in the study.

CONCLUSION

Although the diagnosis of AIHA typically involves a stepwise approach focused on identifying both laboratory and clinical evidence of hemolysis, it is important to note that the interpretation of laboratory parameters can occasionally be misleading. Besides, differential diagnosis could be challenging in leukemia and stem cell transplanted patients. Pediatric hemato/oncologists should be aware of hemolysis in leukemia patients, as detailed examination, prompt diagnosis, and immediate treatment have paramount importance to decrease morbidity and mortality. This would also contribute to the survival of the children with leukemia.

Ethics

Ethics Committee Approval: The study was conducted through retrospective file review and was approved by the Gazi University Ethics Committee (approval number: 2025-1105, date: 17.06.2025).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.K., Ü.K., Concept: S.K., Ü.K., Design: S.K., S.K.K., Supervision: Z.K., Ü.K., Resources: S.K., Material: S.K., Data Collection or Processing: S.K., B.T.T., Analysis or Interpretation: S.K., S.K.K., B.T.T., Literature Search: S.K., S.K.K., Writing: S.K., Critical Review: S.K., Ü.K.

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Original Investigation | Özgün Araştırma

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Antibiotic Sensitivity of Microbial Isolates Causing Asymptomatic Bacteriuria During Pregnancy, in General Heet Hospital, Western Iraq

Gebelik Sırasında Asimptomatik Bakteriüriye Neden Olan Mikropların Antibiyotik Duyarlılığı, Genel Heet Hastanesi, Batı Irak

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ABSTRACT

The goal of this work is to determine the prevalence of asymptomatic bacteriuria (ASB) during pregnancy, identify the causative organisms, and analyze the antibiotic susceptibility pattern of the isolates. This study was conducted on 139 pregnant women in Iraq. Clinically, all the women had no symptoms of a urinary tract infection. Clean catch midstream urine samples were collected from all patients. The microscopic and cultural methods were used to check all urine samples. Identification of isolates was performed using the VITEK 2 system and antibiotic sensitivity was assessed using the same technique. The results showed that 70 (50.36%) of the 139 pregnant women tested positive for ASB. The age group of 15-20 years had the highest prevalence (60.86%) of ASB. The most common etiological agent causing infections among pregnant women was *Staphylococcus* species (66.65%), followed by *Escherichia coli (E. coli)* (10.52%), and *Klebsiella pneumoniae (K. pneumoniae)* (8.77%). Also, the susceptibility pattern of *E. coli* and *K. pneumoniae* showed that most of the isolates were highly sensitive (100%) to piperacillin/tazobactam, ertapenem, imipenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, and tigecyclin. *E. coli* isolates were highly sensitive to cefoxitin (100%). Coliforms *(E. coli and K. pneumoniae)* were highly resistant to β-lactams, including : ampicillin (100%), ceftazidime (100%), cefazolin (100%), ceftriaxone (100%), and ciprofloxacin (100%). Based on the resistance profiles, all isolates of *K. pneumoniae* and *E. coli* (100%) were extended-spectrum beta-actamase producers.

Keywords: Antibiotic susceptibility test, asymptomatic bacteriuria, antibacterial agent, urinary tract infection, pregnancy

ÖZ

Bu çalışmanın amacı, gebelik sırasında asemptomatik bakteriüri (ASB) prevalansını belirlemek, neden olan organizmaları tanımlamak ve izolatların antibiyotik duyarlılık desenini analiz etmektir. Bu çalışma, Irak'ta 139 gebe kadın üzerinde yapılmıştır. Klinik olarak, tüm kadınlarda idrar yolu enfeksiyonu belirtileri bulunmamaktadır. Tüm hastalardan temiz orta akış idrar örnekleri alınmıştır. Mikroskobik ve kültürel yöntemler kullanılarak tüm idrar örnekleri incelenmiştir. İzolatların tanımlanması VITEK 2 sistemi ile yapılmış ve antibiyotik duyarlılığı aynı teknikle değerlendirilmiştir. Sonuçlar, 139 gebe kadından 70'inin (%50,36) ASB için pozitif sonuç verdiğini göstermektedir. On beş ila yirmi yaş grubundaki kadınlar, en yüksek prevalansa (%60,86) sahipti. Gebelerde enfeksiyonlara neden olan en yaygın etyolojik ajan Staphylococcus türleri (%66,65) olup, bunu *Escherichia coli (E. coli)* (%10,52) ve *Klebsiella pneumoniae (K. pneumoniae)* (%8,77) izlemektedir. Ayrıca, *E. coli* ve *K. pneumonin*'nin duyarlılık deseni, izolatların çoğunun piperacillin/tazobactam, ertapenem, imipenem, amikasin, gentamisin, siprofloksasin, levofloksasin ve tigeciklin'e yüksek derecede duyarlı olduğunu (%100) göstermektedir. *E. coli* izolatları, sefoxitine karşı da yüksek derecede duyarlıdır (%100). Koliformlar (*E. coli ve K. pneumoniae*) β-laktamlara karşı yüksek derecede dirençlidir; bunlar arasında ampisilin (%100), seftazidim (%100), sefazolin (%100), seftriakson (%100) ve siprofloksasin (%100) yer almaktadır. Direnç profillerine dayanarak, tüm *K. pneumoniae ve E. coli* izolatlarının (%100) geniş spektrumlu beta-laktamaz üreten organizmalar olduğu bulunmuştur.

Anahtar Sözcükler: Antibiyotik duyarlılık testi, asemptomatik bakteriüri, antibakteriyel ajan, idrar yolu enfeksiyonu, gebelik

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INTRODUCTION

Urinary tract infections (UTIs): "the colonization and multiplication of bacteria in the urinary tract". The presence of bacteria in the collected urine samples is called bacteriuria (1). Moreover, the genitourinary tract is sterile in normal circumstances. Bacteriuria appears when bacteria from a faecal reservoir enter the bladder via urethral ascent (2). In pregnant women, as in women who aren't pregnant, UTIs are classified either as symptomatic infections (acute pyelonephritis and acute cystitis) when bacteria invade urinary tract tissues involving an inflammatory response, or asymptomatic bacteriuria (ASB) when the infection is limited to the growth of bacteria in urine (in general, defined as true bacteriuria in the absence of specific symptoms of acute UTI) (3).

ASB is one of the common infectious conditions in pregnancy that requires antibiotic treatment. Growth above 10^5 colony forming units (CFU) in a midstream urine culture taken from an individual who does not exhibit symptoms or indications of bacteriuria is referred to as ASB (4).

Among active, premenopausal women, the prevalence of ASB is 2-10% (3,5). However, pregnancy-related physiological and anatomical changes to the urinary tract, like displaced bladders, along with immune system modifications, raise the prevalence of ASB and can occasionally result in symptomatic UTIs and other pregnancy complications (such as preterm delivery and pyelonephritis). This matter puts the fetus, newborn, and mother at grave risk (5,6). Pregnant women may be more susceptible to UTIs due to parity, aging, diabetes, urinary tract diseases, sickle cell anemia, and a history of UTIs (6,7).

Preterm labor, pyelonephritis, anemia, low birth weight, amnionitis, toxic septicemia, bacteremia, and pre-eclampsia are some of the issues that can arise from bacteriuria during pregnancy if antibiotics are not administered (6,7). However, therapy for bacteriuria during pregnancy decreases the complications risk. Thus, during pregnancy, the treatment of bacteriuria and screening for early diagnosis in women is essential for preventing the complications associated with bacteriuria (8).

The bacteriuria spread in Iraqi pregnant women was observed to be 48-64.6% (9,10). These percentages are close to the results of our current study. Determining the frequency of UTI, ABS, and the most prevalent pathogenic bacteria is, therefore, an important diagnostic tool in a variety of countries.

This study aims to evaluate the spread of UTI, ASB, and infections associated with bacteriuria in pregnant women in Heet, Iraq. It was also done to determine patterns of bacteria isolated from UTIs and to test antibiotic susceptibility to see which antibiotics might prevent the bacterial isolates that cause ASB in pregnant women from growing.

MATERIALS AND METHODS

Urine Specimen Collection

An appropriate number of 139 pregnant women participated in this investigation who visited the basic care facilities, and admitted to General Heet Hospital's general wards, Anbar, Iraq, who consented to provide us with your data for the current study between October 1, 2021, and March 30, 2021, a period of six months.

Each woman was interviewed for about 15 minutes. The prepared questionnaire was used to interview the pregnant women and obtain data. Nevertheless, the current questionnaire included the following variables: general urine examination, patient complaint, medical and obstetrical history, maternal socio-demographic factors, and acute UTI symptoms. Those taking antibiotics or having taken them for at least 14 days before the presentation were not included in this study. Moreover, the information for each case was recorded in a form prepared for this purpose.

In the current work, the samples of midstream urine were collected from (139) pregnant women a mean age 25.97 ranges (15-40 years),(apparently healthy without any symptoms or signs of UTI), attended the General Heet Hospital were looked at for possible ASB. Moreover, all urine samples were gathered and submitted for regular urinalysis, Gram stain, dipstick, bacterial culture, and urine microscopy.

Asymptomatic Bacteriuria Diagnosis and Culture Test

Un-centrifuged urine samples were incubated at 37°C for 24 hours. For ASB, bacterial growth exceeding 105 CFU/mL was deemed significant. However, the bacterial growth less than 105 CFU/mL was considered contaminated. However, the sample was confirmed negative for ASB if no growth occurred. (ASB is growth of more than 100000 CFU/mL in the bacterial culture of the sample of midstream urine).

Diagnosis Isolation and Antibiotic Susceptibility Test

All isolates were bacteriologically identified, and antimicrobial susceptibility test was investigated using the VITEK 2 system, as mentioned by bioMérieux (11).

Ethical Aspects

The University of Anbar's College of Medicine, the Ethical Committee, examined and approved the study's current protocol. This study was conducted in accordance with the ethical standards of the University of Anbar (approval number: 21, date: 18.03.2025) by the Ethics Committee. Informed consent was obtained from all participants, ensuring they were fully aware of the study procedures, risks, and benefits before voluntarily agreeing to participate.

Statistical Analysis

A p-value of less than 5% was used as the threshold for statistical significance. The goodness of fit test, utilizing the chi-square test (cross tabulation), within non-parametric statistics, was used to determine if there were any significant differences. The chi-square test was utilized to examine the differences between the two test types (the antibiotic types used by the VITEK 2 system).

Results

Severe maternal illness and perinatal morbidity are caused by ASB during pregnancy. This morbidity can be decreased by giving appropriate treatment, and the screening of pregnant mothers. In the current work, the incidence of ASB was 50.36% (Figure 1).

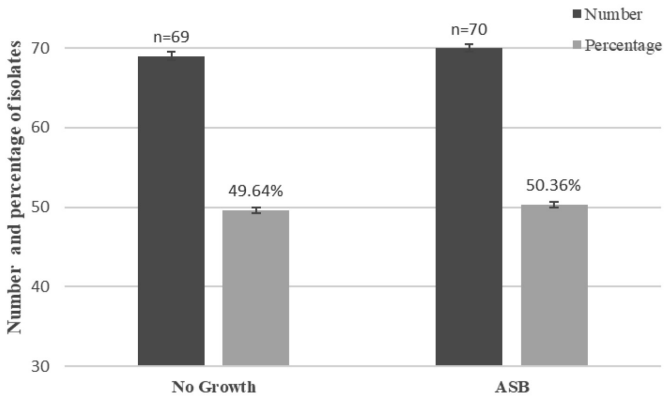

Figure 1. Asymptomatic bacteriuria (ASB) in pregnancy. Microbial growth >105 CFU/mL was considered significant for ASB (total n=139).

As per this work, there was a greater incidence of ASB in the first trimester of pregnancy, 66.66% (8/12 isolates), then 62.96% (17/27 isolates) in the second trimester, and while 42.66% (32/75 isolates) were in the third trimester (Table 1). The 15 to 20 years age group (60.86%) had the greatest rate of ASB (Table 2). This may be because women in this age range engage in sexual activity.

The results of the current research exhibited that species of *Staphylococcus (S.)* (66.65%) were the most frequently occurring causative agents of infection among pregnant women, followed by *Escherichia coli (E. coli*) (10.52%) (Table 3).

In this work, *E. coli* was the second most common (10.52%) bacterium after *Staphylococcus* ssp. (Table 3). Numerous research studies were published. *E. coli* was the most common bacterium found in pregnant women's urine cultures (27,28,29). Moreover, the majority of bacteria in the vaginal and rectal areas are *E. coli*.

The other isolated strains were *Klebsiella pneumoniae* (*K. pneumoniae*) (8.77%), *Kocuria* spp. (3.5%), *Aeromonas hydrophila* (1.75%), *Enterococcus faecium* (1.75%), *Sphingomonas pancimobilis* (1.75%), *Dermacoccus nishinomiyaensis* (1.75%), Mixed Growth [*Staphylococcus hominis* (*S. hominis.)* + *Aerococcus viridans*] (1.75%). Only *Candida albicans* (1.75%) was the fungus that was isolated in this study (Table 3).

Antimicrobial Susceptibility Pattern of Staphylococcal Species Isolates

Our study revealed that linezolid (100%), vancomycin (100%), nitrofurantoin (100%), tigecycline (96.96%), clindamycin (93.93%), teicoplanin (93.93%), rifampicin (93.93%), tobramycin (87.87%), gentamicin (84.84%), levofloxacin (75.75%), moxifloxacin (75.75%) and trimethoprim/sulfamethoxazole (72.72%) were relatively effective antibiotics against *Staphylococcus* uropathogen species (Table 4).

However, isolates of *Staphylococcus* spp. were highly resistant to the β-lactam class [benzylpenicillin (100%), oxacillin (93.93%)], and fusidic acid (78.78%). low resistance to both erythromycin (66.66%) and tetracycline (42.42%) was identified (Table 4). Out of all *Staphylococcus* spp. isolates (n=33), multi-drug resistance (MDR) was recorded for 25 (75.75%) isolates (Table 5).

Pattern of Antimicrobial Susceptibility of Klebsiella pneumoniae and E. coli isolates

In this study, the susceptibility pattern of *K. pneumoniae* and *E. coli* exhibited that most of the isolates were highly sensitive to piperacillin/tazobactam (100%), ertapenem (100%), imipenem (100%), amikacin (100%), gentamicin (100%), ciprofloxacin (100%), levofloxacin (100%), and tigecyclin (100%) (Table 5). Also, *E. coli* isolates were highly sensitive to cefoxitin (100%), (Table 5). In addition, the sensitivity level of *E. coli* and *K. pneumoniae* bacteria to gentamicin, nitrofurantoin, and trimethoprim/sulfamethoxazole was (83.33%, 60%), (66.66%, 60%), and (66.66%, 60%), respectively (Table 5). Likewise, *K. pneumonia* isolates were moderately resistant to cefoxitin (60%) (Table 5).

As a result, this research is required due to the bacteria isolates identified in this study, which have shown high resistance to the majority of routinely used antimicrobial drugs. This follow-up study could explain the molecular foundation for bacteria developing resistance to highly effective antibacterial agents.

Discussion

Severe maternal illness and perinatal morbidity are caused by ASB during pregnancy. In the current work, the incidence of ASB was 50.36% (Figure 1). This result agreed with similar research done in Iraq, Saudi Arabia, and Nigeria where the incidence of ASB was reported from 48-64.6% (7-10,12).

The slight variation in the ratio between the studies above could be the result of variations in the patients' socioeconomic position, social habits, education, and access to community health services (13).

Table 1. Relationship between ASB and the duration of pregnancy

Gestation period	Total no. screened	ASB	
		No.	%
First trimester	12	8	66.66
Second trimester	27	17	62.96
Third trimester	75	32	42.66
Total	114	57	50

ASB: Asymptomatic bacteriuria

Table 2. Relationship between age distribution and ASB during pregnancy

Age in years	Total no. screened	ASB	
		No.	%
15-20	23	14	60.86
21-25	37	16	43.24
26-30	35	17	48.57
31-35	15	8	53.33
35-40	4	2	50
Total	**114**	**57**	**50**

ASB: Asymptomatic bacteriuria

Table 3. The isolated pathogenic bacteria from pregnant women with ASB

No.	Isolates		No.	%
1	***Staphylococcus* species**		**38**	**66.65**
	Staphylococcus haemolyticus	**Coagulase-negative *staphylococci* (CoNS)**	12	
	Staphylococcus epidermidis		10	
	Staphylococcus hominis		9	
	Staphylococcus warneri		1	
	Staphylococcus capitis		1	
	Staphylococcus lentus		1	
	Staphylococcus aureus		4	
2	***Escherichia coli***		**6**	**10.52**
3	***Klebsiella pneumonia***		**5**	**8.77**
4	***Kocuriae* spp.**		**2**	**3.5**
	Kocuriae varians		1	
	Kocuriae kristinae		1	
5	Aeromonas hydrophila		**1**	**1.75**
6	***Enterococcus faecium***		**1**	**1.75**
7	***Sphingomonas pancimobilis***		1	**1.75**
8	***Dermacoccus nishinomiyaensis***		1	1.75
9	**Mix growth (*Staphylococcus hominis* + *Aeroccocus viridans*)**		**1**	**1.75**
10	Candida albicans		1	1.75
Total			**57**	**100**

ASB: Asymptomatic bacteriuria

Moreover, one of the major risk factors for symptomatic bacteriuria is ASB. Also, ASB constitutes around 40% of pyelonephritis and 30% of cystitis in unscreened pregnant women. Moreover, ASB has been indirectly linked to anemia and preeclampsia (9,14).

As per this work, there was a greater incidence of ASB in the first trimester of pregnancy, 66.66% (8/12 isolates), then 62.96% (17/27 isolates) in the second trimester, and while 42.66% (32/75 isolates) were in the third trimester (Table 1). This corresponds with prior studies by Chandel et al. (15) and is attributed hormonal changes occurring prior to anatomical changes. In this respect, Azami et al. (6) confirmed in their study that the highest and lowest prevalence of ASB were noticed in the 1st trimester (11.7%) and 3rd trimester (6.1%), respectively (6). In another study (12,16,17), the 3rd trimester was observed to have a greater prevalence of the condition being studied. As pregnancy progresses, urinary stasis increases, leading to a higher incidence of bacteriuria in the final trimester (17). This, combined with poor cleaning techniques and the heavily distended belly of pregnant women in their third trimester, may illustrate the high spread of bacteriuria noticed among pregnant women in their 3rd trimester (12). According to research results and other studies, monitoring for ASB is recommended to be conducted in each of the three trimesters of pregnancy in order to avoid the major consequences that might result from ASB during pregnancy (13). The 15 to 20 years age group (60.86%) had the greatest rate of ASB (Table 2), and this almost matches previous research findings (9,13). This may be because women in this age range engage in sexual activity.

The results of the current research exhibited that species of *Staphylococcus (S.)* (66.65%) were the most frequently occurring causative agents of infection among pregnant women, followed by *E. coli* (10.52%) (Table 3). In a similar study by Almukhtar (18) in Iraq, and Kalgo et al. (19) in Nigeria, the most common isolates were *Staphylococcus* species, with incidences of 20.6% and 51.6% respectively.

The most common *Staphylococcus* species uropathogens were *Staphylococcus* This result is consistent with separate scientific works achieved in northern Iraq by Assafi et al. (20) and Al-Naqshbandi et al. (21). Gram-positive cocci known as coagulase-negative staphylococci (CoNS), are grouped due to their shared absence of the virulence factor coagulase (22). When it comes to CoNS, the species most commonly found together are *S. epidermidis* and *S. haemolyticus*, which are clinically defined species. Other species that have historically been included in this group include *S. hominis, S. capitis, S. warneri,* and *S. lentus*. (23), can be caused cystitis in young sexually active women, they have been found to make up a noteworthy percentage in the current study as well (24). The findings indicate that this organism may be becoming more well-known as a potential cause of UTIs during pregnancy, probably related to its occurrence as part of normal vaginal flora (25).

Prior researchers regarded the coagulase-negative *Staphylococcus* that were identified from the urine sample, as contaminants and did not assign them any importance. However, a considerable number of coagulase negative *Staphylococcus* have been identified as the agents of UTIs in recent years (26). Additionally, because the urine of pregnant women contains high levels of albumin and amino acids, it serves as a suitable substrate supporting the growth of most infections. Additionally, pregnant women's defenses are weakened during pregnancy, leaving them more vulnerable to infections, particularly *Staphylococcus* species (18).

In this work, *E. coli* was the second most common (10.52%) bacterium after Staphylococcus ssp. (Table 3). Numerous research studies were published. *E. coli* was the most common bacterium found in pregnant women's urine cultures (27-29). Moreover, the majority of bacteria in the vaginal and rectal areas are *E. coli*. Pregnancy may increase the risk of contracting a UTI from *E. coli* due to changes in function and anatomy as well as challenges with maintaining personal hygiene (30). Because it promotes the colonization of certain bacterial strains, Urine stasis during pregnancy is the main cause of increased isolates of *E. coli* (31).

The other isolated strains were *Klebsiella pneumoniae* (*K. pneumoniae*) (8.77%), *Kocuria* spp. (3.5%), *Aeromonas hydrophila* (1.75%), *Enterococcus faecium* (1.75%), *Sphingomonas pancimobilis* (1.75%), *Dermacoccus nishinomiyaensis* (1.75%), Mixed Growth [*Staphylococcus hominis* (*S. hominis.)* + *Aerococcus viridans*] (1.75%).

Table 4. Result of minimal inhibitory concentration (MIC) of antimicrobial susceptibility test for isolates of *Staphylococcus ssp.* by VITEK2 system

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																	MDR
			FOX	BEN (a)	OXA (a)	GEN (b)	TOB (b)	LVX (c)	MOX (c)	ERY (d)	CLI (e)	LZD (f)	TEC (g)	VAN (g)	TET (h)	TGC (i)	NIT (j)	FA (k)	RIF (l)	SXT (m)	
1	P44	*S. haemolyticus*	+	R	R	S	S	R	I	R	S	S	S	S	R	S	S	R	S	S	5* (+)
2	P47	*S. haemolyticus*	*No results																	
3	P49	*S. haemolyticus*	*No results																	
4	P54	*S. haemolyticus*	+	R	R	I	S	R	I	R	S	S	S	S	R	S	S	R	S	S	5* (+)
5	P59	*S. haemolyticus*	+	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	3* (+)
6	P63	*S. haemolyticus*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S	3* (+)
7	P64	*S. haemolyticus*	+	R	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	R	6* (+)
8	P79	*S. haemolyticus*	+	R	R	S	S	R	R	R	S	S	S	S	R	S	S	R	R	R	7* (+)
9	P89	*S. haemolyticus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
10	P91	*S. haemolyticus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
11	P95	*S. haemolyticus*	+	R	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	5* (+)
12	P104	*S. haemolyticus*	+	R	R	S	S	R	I	R	S	S	S	S	R	S	S	R	S	S	5* (+)
13	P5	*S. epidermidis*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	2* (-)
14	P29	*S. epidermidis*	+	R	R	S	I	R	I	R	R	S	S	S	R	S	S	R	S	R	7* (+)
15	P31	*S. epidermidis*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	2* (-)
16	P46	*S. epidermidis*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1* (-)
17	P51	*S. epidermidis*	*No results																	
18	P67	*S. epidermidis*	+	R	R	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	2* (-)
19	P68	*S. epidermidis*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	3* (+)
20	P72	*S. epidermidis*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S	3* (+)
21	P80	*S. epidermidis*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	R	5* (+)
22	P98	*S. epidermidis*	+	R	R	R	I	S	S	S	S	S	S	S	S	S	S	R	S	S	3* (+)
23	P25	*S. hominis*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	R	S	R	4* (+)
24	P36	*S. hominis*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S	3* (+)
25	P62	*S. hominis*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	2* (-)
26	P66	*S. hominis*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
27	P71	*S. hominis*	+	R	R	S	S	S	S	R	S	S	R	S	S	S	S	R	S	R	5* (+)
28	P77	*S. hominis*	+	R	R	S	S	S	S	R	S	S	I	S	S	S	S	R	S	S	3* (+)
29	P104	*S. hominis*	+	R	R	S	S	R	I	R	S	S	S	S	R	S	S	R	S	S	5* (+)
30	P106	*S. hominis*	*No results																	
31	P112	*S. hominis*	*No results																	
32	P27	*S. aureus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
33	P35	*S. aureus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	R	5* (+)
34	P58	*S. aureus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
35	P110	*S. aureus*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1* (-)
36	P11	*S. capitis*	-	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1* (-)

Table 4. Continued

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																		MDR
			FOX	BEN (a)	OXA (a)	GEN (b)	TOB (b)	LVX (c)	MOX (c)	ERY (d)	CLI (e)	LZD (f)	TEC (g)	VAN (g)	TET (h)	TGC (i)	NIT (j)	FA (k)	RIF (l)	SXT (m)	
37	P34	*S. warneri*	-	R	S	S	S	S	S	S	R	S	S	S	S	R	S	S	R	S	4* (+)
38	P102	*S. lentus*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1* (-)
No.&% R			31 (93.93)	33 (100)	31 (93.93)	3 (9.09)	2 (6.06)	8 (24.24)	3 (9.09)	22 (66.66)	2 (6.06)		1 (3.03)		14 (42.42)	1 (3.03)		26 (78.78)	2 (6.06)	9 (27.27)	25 (75.75)
No.&% S			2 (6.06)		2 (6.06)	28 (84.84)	29 (87.87)	25 (75.75)	25 (75.75)	11 (33.33)	31 (93.93)	33 (100)	31 (93.93)	33 (100)	19 (57.57)	32 (96.96)	33 (100)	7 (21.21)	31 (93.93)	24 (72.72)	8 (24.24)
No.&% I						2 (6.06)	2 (6.06)		5 (15.15)				1 (3.03)								

*No Results: The results of Antimicrobial Susceptibility Test have not been read by the VITEK 2 system due to a technical error in system. MDR: Multidrug resistant, (a)- β-Lactams class, (b)- Aminoglycosides class, (c)- Quinolones class, (d)- Macrolides class, (e)- Lincosamides class, (f)- Oxazolidinones class, (g)- Glycopeptides class, (h)- Tetracyclines class, (i)- Glycylcyclines class, (j)- Nitrofurans class, (k)- Fusidane class, (l)- Ansamycins class, (m)- Folate pathway inhibitors class, (*)- Classes of antibiotics which resistance by *Staphylococcus ssp.* Isolate.
FOX: Cefoxitin Screen, BEN: Benzylpenicillin, OXA: Oxacillin, GEN: Gentamicin, TOB: Tobramycin, LVX: Levofloxacin, MOX: Moxifloxacin, ERY: Erythromycin, CLI: Clindamycin, LZD: Linezolid, TEC: Teicoplanin, VAN: Vancomycin, TET: Tetracycline, TGC: Tigecycline, NIT: Nitrofurantoin, FA: Fusidic Acid, RIF: Rifampicin, SXT: Trimethoprim/Sulfamethoxazole. R: Resistant, I: Intermediate and S: Sensitive; by Vitek 2 system, (+): Positive, (-): Negative.

Only *Candida albicans* (1.75%) was the fungus that was isolated in this study (Table 3). From setting to setting, differences and similarities in etiological agents of ASB exist. It might be because each location has different environmental conditions, socioeconomic and educational attainment levels, and personal cleanliness practices (32).

Because pregnancy increases the risk of significant consequences, treatment for ASB is advised. When a pregnant woman with ASB goes untreated, up to 40% of these women develop acute pyelonephritis, which can have major consequences for the fetus as well as the mother (29).

Antibiotics should thus be given just when necessary, with proper patient education on the need for compliance, because failing to take antibiotics for the full period indicated can also lead to resistance (4). This fragment requires additional context to form a complete sentence. For example: "in order to treat and eliminate infections in pregnant women, healthcare providers often prescribe antibiotics." When treating ASB, the practitioner must consider the antimicrobial susceptibility pattern of UTI bacteria to choose and employ the most potent antimicrobial drug (32).

Antimicrobial Susceptibility Pattern of Staphylococcal Species Isolates

The bulk of *Staphylococcus* spp. in their investigation showed resistance to different antibiotics such as oxacillin, benzylpenicillin, erythromycin, and tetracycline, ranging from 60 to 100% (35).

Therefore, these medications would not be appropriate for the empirical treatment of ASB in expectant mothers. This was confirmed by the present research, where the vast majority of isolates (93.93%) were positive for cefoxitin screen. These tests are suitable for assessing the degree of oxacillin resistance in all CoNS species that is mediated by the *mecA* gene (11,36).

On the other hand, cefoxitin screen (oxacillin resistance) is a marker for *MRS*, involving *methicillin-resistant coagulase-negative staphylococci (MR-CoNS)* and *MRS aureus (MRSA*) (37) (Table 4). Antibiotic misuse and self-medication may be the cause of the rise in antibiotic resistance. Additionally, it is said that the rates of antimicrobial resistance among frequently isolated uropathogens are increasing to several routinely used drugs; their susceptibility varies depending on the time and location (38).

Out of all *Staphylococcus* spp. isolates (n=33), MDR was recorded for 25 (75.75%) isolates (Table 5). An MDR isolate is one that is resistant to agents in three or more antimicrobial categories (39). A similar result was reported in Northeast Ethiopia (72.4%) (40), Eastern Uganda (72.4%) (41), Western Ethiopia (74.4%) (33), and Somaliland (75%) (42).

Widespread antibiotic usage (inappropriate medication use, taking antibiotics for the wrong length of time, or using antibiotics when they are not needed, including for viral illnesses) and lax antibiotic monitoring are linked to the rise in MDR. This causes bacteria to choose antibiotic resistance mechanisms (41). MDR strains can also emerge from a biological mechanism that confers resistance to multiple drugs, from the genetic linkage of genes that confer resistance to various antibiotics on a chromosome or plasmid, or from the evolution of multiple mutations that confer resistance to multiple antibiotics in a host (43).

Table 5. Result of minimal inhibitory concentration (MIC) of antimicrobial susceptibility test for isolates of *Escherichia coli* and *Klebsiella pneumonia* by VITEK 2 system

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																
			ESBLs	AM (a)	P/T (a)	CZ (a)	FOX (a)	CEF (a)	CRX (a)	CP (a)	ERP (a)	IMI (a)	AMI (b)	GEN (b)	CIP (c)	LVX (c)	TGC (d)	NIT (e)	SXT (f)
1	**P21**	***Escherichia coli***	**+**	**R**	**S**	**R**	**S**	R	R	R	S	S	S	R	S	S	S	I	R
2	P26	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	**S**	**S**	**I**	**R**
3	P45	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
4	P57	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
5	P60	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
6	P78	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
No.&% R			100	100		100		100	100	100				16.66					33.33
No.&% S					100		100				100	100	100	83.33	100	100	100	66.66	66.66
No.&% I																		33.33	

No.	No. of isolate	Type of Isolate	Result of (MIC) of antimicrobial susceptibility test																
			ESBLs	AM (a)	P/T (a)	CZ (a)	FOX (a)	CEF (a)	CRX (a)	CP (a)	ERP (a)	IMI (a)	AMI (b)	GEN (b)	CIP (c)	LVX (c)	TGC (d)	NIT (e)	SXT (f)
1	**P3**	***K. pneumoniae***	**+**	**R**	**S**	**R**	R	R	R	R	S	S	S	S	S	S	S	S	R
2	**P37**	***K. pneumoniae***	**+**	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
3	**P42**	***K. pneumoniae***	**+**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	S	S	S	S	S	S	S	S
4	**P76**	***K. pneumoniae***	**+**	**R**	**S**	**R**	**R**	**R**	R	R	S	S	S	I	S	S	S	I	R
5	**P83**	***K. pneumoniae***	**+**	**R**	S	R	S	R	R	R	S	S	S	R	S	S	S	R	S
No.&% R			100	100		100	60	100	100	100				20				20	40
No.&% S					100		40				100	100	100	60	100	100	100	60	60
No.&% I														20				20	

R: Resistant, I: Intermediate and S: Sensitive; by VITEK 2 system, (+): Positive, (-): Negative. (a)- β-Lactams class, (b)- Aminoglycosides class, (c)- Quinolones class, (d)- Glycylcyclines class, (e)- Nitrofurans class, (f)- Folate pathway inhibitors class.

ESBLs: Extended-spectrum β-lactamases, AM: Ampicillin, P/T: Piperacillin/Tazobactam, CZ: Cefazolin, FOX: Cefoxitin, CEF: Ceftazidime, CRX: Ceftriaxone, CP:Cefepime, ERP: Ertapenem, IMI: Imipenem, AMI: Amikacin, GEN: Gentamicin , CIP: Ciprofloxacin, LVX: Levofloxacin, , TGC: Tigecyclin, NIT: Nitrofurantoin, SXT: Trimethoprim/Sulfamethoxazole

Pattern of Antimicrobial Susceptibility of *Klebsiella pneumoniae* and *E. coli* isolates

Similar results from earlier research conducted in Iraq (35), Uganda (41) Indonesia (44), and India (45) have been published coliforms (*E. coli and K. pneumoniae*) were highly resistant to β-lactams, involving ampicillin (100%), cefazolin (100%), ceftazidime (100%), ceftriaxone (100%), and ciprofloxacin (100%). Likewise, *K. pneumonia* isolates were moderately resistant to cefoxitin (60%) (Table 5).

Similarly, in a multicenter study by Seni et al. (46) from Tanzania reported *E. coli* and *K. pneumoniae* These isolates showed strong resistance to Ampicillin, with resistance rates of 94.5% and 98%, respectively. Our finding was relatively higher than a meta-analysis study by Chelkeba et al. (29). Ampicillin resistance was found in around 80% and 75% of *E. coli and K. pneumoniae* species, respectively.

Also, in the published work which was done in Iraq by Naqid et al. (35), there were significant findings. *K. pneumoniae* showed strong resistance to ampicillin and ceftriaxone, and exhibited resistance patterns resembling those of *E. coli.*

β-lactam antibiotics, such as cephalosporins and penicillins, are generally used to treat UTIs throughout the gestational period and are thought to be safe during pregnancy (47). In recent years, these drugs have not been suitable for empirical treatment of ASB in these pregnant women. The confirmation of this is provided by the results of the current study; according to the resistance profiles, 100% of the isolates of *K. pneumoniae* and *E. coli* were extended-spectrum beta-lactamase (ESBL) producers (Table 6).

ESBLs have been detected predominantly in *Klebsiella* spp. and *E. coli* among the enterobacteriaceae. ESBLs clinically diminish the effectiveness of β-lactam, especially cephalosporins, and are linked with prolonged hospital stays and increase significant morbidity and mortality (47,48). The gram-negative isolates' synthesis of extended spectrum β-lactamase, their acquisition of resistance genes, the downregulation of receptors, and drug efflux, are the causes of their resistance to β-lactam antibiotics.

This is primarily, because using third-generation cephalosporins puts selective pressure on the body (41). In the present study, multidrug resistance was not seen among *E. coli* and *K. pneumoniae* isolates.

The authors are currently conducting research to identify and describe virulence and resistance genes responsible for MDR *in Staphylococcus spp.* and ESBLs in gram-negative bacteria associated with ASB in pregnant women in Iraq. MRS, including MRSA and MRCoNS strains, has become an emerging threat to humans, considerably contributing to morbidity, mortality, and socioeconomic expenses (49). The *mec*A gene is acquired by MRSA and MRCoNS,

Table 6. Result of minimal inhibitory concentration (MIC) of antimicrobial susceptibility test for isolates of *Escherichia coli* and *Klebsiella pneumonia* by VITEK2 system

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																
			ESBLs	AM (a)	P/T (a)	CZ (a)	FOX (a)	CEF (a)	CRX (a)	CP (a)	ERP (a)	IMI (a)	AMI (b)	GEN (b)	CIP (c)	LVX (c)	TGC (d)	NIT (e)	SXT (f)
1	P21	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	R	S	S	S	I	R
2	P26	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	I	R
3	P45	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
4	P57	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
5	P60	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
6	P78	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
No.&% R			**100**	**100**		**100**		**100**	**100**	**100**				**16.66**					**33.33**
No.&% S					**100**		**100**				**100**	**100**	**100**	**83.33**	**100**	**100**	**100**	**66.66**	**66.66**
No.&% I																		**33.33**	

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																
			ESBLs	AM (a)	P/T (a)	CZ (a)	FOX (a)	CEF (a)	CRX (a)	CP (a)	ERP (a)	IMI (a)	AMI (b)	GEN (b)	CIP (c)	LVX (c)	TGC (d)	NIT (e)	SXT (f)
1	P3	*K. pneumoniae*	+	R	S	R	R	R	R	R	S	S	S	S	S	S	S	S	R
2	P37	*K. pneumoniae*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
3	P42	*K. pneumoniae*	+	R	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S
4	P76	*K. pneumoniae*	+	R	S	R	R	R	R	R	S	S	S	I	S	S	S	I	R
5	P83	*K. pneumoniae*	+	R	S	S	S	R	R	R	S	S	S	R	S	S	S	R	S
No.&% R			**100**	**100**		**100**	**60**	**100**	**100**	**100**				**20**				**20**	**40**
No.&% S					**100**		**40**				**100**	**100**	**100**	**60**	**100**	**100**	**100**	**60**	**60**
No.&% I														**20**				**20**	

R: Resistant, I: Intermediate and S: Sensitive; by VITEK 2 system, (+): Positive, (-): Negative.
(a) β-Lactams class
(b) Aminoglycosides class
(c) Quinolones class
(d) Glycylcyclines class
(e) Nitrofurans class
(f) Folate pathway inhibitors class.
ESBLs: Extended-spectrum β-lactamases, AM: Ampicillin, P/T: Piperacillin/Tazobactam , CZ: Cefazolin, FOX: Cefoxitin, CEF: Ceftazidime, CRX: Ceftriaxone, CP:Cefepime, ERP: Ertapenem, IMI: Imipenem, AMI: Amikacin, GEN:Gentamicin , CIP: Ciprofloxacin, LVX: Levofloxacin, TGC: Tigecyclin, NIT: Nitrofurantoin, SXT: Trimethoprim/ Sulfamethoxazole ,

leading to resistance. The *Staphylococcus* cassette chromosome mec (SCCmec) carries the mec operon, which contains the *mec*A gene. SCCmec I through XIII have been identified to date (37). Enzyme groups, known as ESBLs and mediated by plasmids, hydrolyze aztreonam, extended-spectrum cephalosporins, and penicillins (48).

As a result, this research is required due to the bacteria isolates identified in this study, which have shown high resistance to the majority of routinely used antimicrobial drugs. This follow-up study could explain the molecular foundation for bacteria developing resistance to highly effective antibacterial agents.

CONCLUSION

The incidence of ASB among pregnant women attending prenatal general wards at General Heet Hospital was relatively high in the study presented here. As a result, it is critical to screen every pregnant woman who visits an antenatal hospital in Iraq and other developing countries for ASB. The majority of bacterial isolates found in pregnant women's urine samples at General Heet Hospital were *Staphylococcus* species, *E. coli*, and *K. pneumoniae*. *Staphylococcus* spp. have been identified as a typical causative agent of ASB in pregnant women. As a result, routine screening for gram-positive bacteria as the causative agent of ASB should be considered. The vast majority of *Staphylococcus* species isolates were sensitive to linezolid, vancomycin, nitrofurantoin, tigecycline, clindamycin, teicoplanin, and rifampicin. The majority of the isolates were resistant to commonly used antimicrobials, particularly to the β-lactams class (benzylpenicillin and oxacillin). The high level of resistance among isolates of *Staphylococcus* spp. that cause ASB limits the use of antimicrobial drugs for therapy. Moreover, the dissemination of MDR isolates poses a risk to medical practice.

The susceptibility patterns of *E. coli* and *K. pneumoniae* showed that most of the isolates showed greater sensitivity to piperacillin/tazobactam, ertapenem, imipenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, and tigecycline. Also, the susceptibility of *E. coli* isolates to cefoxitin was greater compared to other antibiotics tested. Coliforms (*E. coli* and *K. pneumoniae*) were highly resistant to β-lactams, including ampicillin, cefazolin, ceftazidime, ceftriaxone, and ciprofloxacin. As a result, clinicians should base their empirical antibiotic selection on knowledge about the local prevalence of bacterial profiles and antibiotic susceptibility testing in Iraq, rather than adhering to universal guidelines.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the ethical standards of the University of Anbar (approval number: 21, date: 18.03.2025) by the Ethics Committee.

Informed Consent: Informed consent was obtained from all participants, ensuring they were fully aware of the study procedures, risks, and benefits before voluntarily agreeing to participate.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: A.S.J., N.A.B., Concept: A.S.J., N.A.B., Design: A.S.J., N.A.B., Supervision: A.S.J., Resources: M.N.O., Material: A.S.J., N.A.B., Data Collection or Processing: A.S.J., N.A.B., Analysis or Interpretation: A.S.J., M.N.O., Literature Search: A.S.J., M.N.O., Writing: A.S.J., M.N.O., Critical Review: M.N.O.

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The Clinical and Radiologic Features of Patients with Myelin Oligodendrocyte Glycoprotein (MOG) Antibody-Associated Disease (MOGAD) in the City of Sakarya, Türkiye

Türkiye, Sakarya İlinde Myelin Oligodendrosit Glikoprotein (MOG) Antikor İlişkili Hastalığı (MOGAD) Olan Hastaların Klinik ve Radyolojik Özellikleri

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ABSTRACT

Objective: The study aims to share our knowledge on myelin oligodendrocyte glycoprotein antibody (anti-MOG) seropositivity in patients with demyelinating diseases, focusing on their clinical, serologic, and radiologic characteristics, as well as treatment options for MOG associated disease (MOGAD) cases.

Methods: This retrospective study included 332 of 450 demyelinating disease cases, aged 18 to 65 years, who were referred to our clinic from 2017 to 2023 with clinical and/or radiological signs of demyelination, followed by testing for the anti-MOG antibody. We applied the revised 2017 McDonald criteria and the 2023 MOGAD diagnostic criteria to those who tested positive for anti-MOG. Cases of anti-MOG seronegative multiple sclerosis (MS) and non-MOGAD were excluded. We detailed the clinical, serologic, and radiologic characteristics and treatment protocols of anti-MOG-positive/low-positive cases.

Results: Among the cases, 16 were clear/low anti-MOG seropositive; of these, 10 were diagnosed with MOGAD, three were MS associated with anti-MOG seropositivity, and three were considered possible MOGAD and followed up. Four MOGAD cases (40%) were double positive for anti-MOG and oligoclonal bands. Three MOGAD cases also had autoimmune diseases. Rare clinical presentations included sixth cranial nerve palsy, tetraparesis secondary to acute disseminated

ÖZ

Amaç: Çalışmamız demyelinizan hastalığa sahip olgularımızda miyelin oligodendrosit glikoprotein antikoru (anti-MOG) seropozitifliğini ve MOG ilişkili hastalıklar (MOGAD) tanısı alan olgularımızda klinik, serolojik, radyolojik özellikleri, tedavi seçenekleri hakkındaki deneyimlerimizi paylaşmayı amaçlamaktadır.

Yöntemler: Bu retrospektif çalışmaya, 2017-2023 yılları arasında kliniğimize klinik ve/veya radyolojik demiyelinizasyon bulgularıyla sevk edilen, 18-65 yaş aralığındaki 450 demiyelinizan hastalık olgusundan, anti-MOG antikor testi yapılan 332'si dahil edildi. Anti-MOG pozitif olgulara revize edilmiş 2017 McDonald kriterlerini ve 2023 MOGAD tanı kriterlerini uyguladık. Anti-MOG pozitif/düşük pozitif vakaların klinik, serolojik ve radyolojik özelliklerini ve tedavi protokollerini ayrıntılı olarak açıkladık. Anti-MOG seronegatif multipl skleroz (MS) ve non-MOGAD olguları çalışma dışı bırakıldı. Anti-MOG pozitif/düşük pozitif olguların klinik, radyolojik özellikleri ve tedavi protokolleri incelendi.

Bulgular: Üç yüz otuz iki olgudan anti-MOG pozitif 16 olgu tespit edildi. Üçü anti-MOG sero-pozitifliğinin eşlik ettiği MS, 10'u MOGAD tanısı aldı. Üç olgu ise olası MOGAD olarak değerlendirildi ve yakın takibe alındı. MOGAD'da eşlikçi oto-immun hastalıklar 3 olgumuzda mevcuttu. Dört olgumuz anti-MOG ve oligoklonal bant dual pozitifliğine sahipti (%40). MOGAD olgularımızdaki ender klinik prezentasyonları sırasıyla;

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ABSTRACT

encephalomyelitis, wall-eyed bilateral internuclear ophthalmoplegia and progressive transverse myelitis in adulthood. A total of 300 cases were diagnosed with MS, and 1% of these cases were anti-MOG with low levels of seropositivity.

Conclusion: The pathogenesis, treatment, and prognosis of MOGAD differ from those of other demyelinating diseases. We aim to highlight the importance of recognizing MOGAD due to its potential association with autoimmune diseases, progressive nature, and dual seropositivity. Thus, it should be considered for its unique clinical and radiologic features.

Keywords: Myelin oligodendrocyte glycoprotein, MOG, myelin oligodendrocyte glycoprotein antibody associated disease, atypical demyelinating diseasesi MOGAD.

ÖZ

altıncı kraniyal sinir tutulumu, erişkinlik döneminde akut dissemine ensefelomiyelit, Wall-eyed bilateral internükleer oftalmopleji kliniği ve progresif seyirli transvers myelit kliniğine sekonder tetraparezi idi. Üç yüz otuz olgu MS tanısı aldı. MS olgularında anti-MOG düşük-seropozitifliği %1 idi.

Sonuç: MOGAD'ın patogenezi, tedavisi ve prognozu diğer demiyelinizan hastalıklardan farklıdır. Çalışmamızda nadiren otoimmün hastalıklarin eşlikçi olduğu, progresif seyrin ender olduğu, dual sero-pozitiflik gösterebilen MOGAD'a dikkat çekmek, klinik ve radyolojik özellikleriyle akılda tutulması gereken bir hastalık olduğunu vurgulamak istedik.

Anahtar Sözcükler: Miyelin oligodendrosit glikoprotein, MOG, miyelin oligodendrosit glikoprotein ilişkili hastalıklar, atipik demiyelinizan hastalıklar, MOGAD

INTRODUCTION

Myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) has been identified as a distinct immune-mediated demyelinating disease of the central nervous system, and recently published diagnostic criteria for MOGAD have facilitated its identification (1,2). Although the global prevalence of the disease is still uncertain, studies suggest an incidence of 1.6-3.4 cases per million people per year in Europe, with a prevalence of 20 per million (3,4). The median age of onset is between 20 and 30 years, with similar frequencies observed in both genders (2,5).

Clinically, MOGAD is characterized by either monophasic or relapsing attacks that may include unilateral or bilateral optic neuritis (ON), acute disseminated encephalomyelitis (ADEM), cerebral monofocal or polyfocal deficits, brainstem or cerebellar deficits, and cerebral cortical encephalitis, often associated with epilepsy (2). The phenotype varies with age of onset, typically presenting as ON in adults and ADEM in children (6). The histopathologic mechanisms of MOGAD are distinct from other demyelinating diseases, such as multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD), and this distinction extends to imaging features, treatment options, and responses. Standard immunomodulatory treatments for demyelinating diseases are often ineffective in MOGAD and may even worsen the disease (7,8).

In this study, we investigated the presence and frequency of MOG antibodies in a cohort of patients with demyelinating diseases, along with the clinical, radiologic, and serologic features and treatment options for these cases based on the 2023 MOGAD diagnostic criteria in Sakarya, Türkiye.

MATERIALS AND METHODS

Patient Selection

This study has a single-center, retrospective, observational research design. Following our inclusion criteria, we included patients aged 18 to 65 years with one or more demyelinating diseases, such as ON, myelitis; cerebral monofocal or polyfocal deficits; brainstem or cerebellar deficits; or cerebral cortical encephalitis often associated with epilepsy, along with typical or atypical demyelinating lesions on cranial and spinal magnetic resonance imaging (MRI). A total of 450 cases were referred to our outpatient demyelinating disease clinic between 2017 and 2023. Patients who were not tested for MOG antibodies were excluded (n=96). We collected demographic, clinical, radiologic, and serologic data for the included cases. We systematically applied the revised 2017 McDonald criteria to 332 cases evaluated for anti-MOG antibodies, and the 2023 MOGAD diagnostic criteria to those that were anti-MOG seropositive (2,9). Cases that were anti-MOG seronegative and diagnosed with MS according to the 2017 revised McDonald criteria, as well as those with anti-MOG seronegative demyelinating features that did not meet MS diagnostic criteria, were excluded from the study. Anti-MOG seropositive cases were evaluated in subgroups as part of our research (Figure 1 and Figure 2).

Radiologic Methods

MRI scans were performed using a 1.5 Tesla scanner (Voyager, GE Medical Systems, USA). Routine imaging included axial pre- and post-contrast T1-weighted, axial and sagittal T2-weighted, and axial fluid-attenuated inversion recovery images of the brain. In addition, cervical spine imaging included sagittal T1-weighted, sagittal T2-weighted, and post-contrast sagittal T1-weighted fat-saturated images. A radiologist with a decade of experience retrospectively reviewed these images.

Laboratory Methods

Serologic tests related to demyelinating diseases, including anti-MOG, anti-neuromyelitis optica aquaporin-4 (AQP4), and oligoclonal band (OCB), were performed before intravenous steroid administration (10). Isoelectric focusing followed by immunoblotting was used to detect OCBs in serum and cerebrospinal fluid (CSF) in the neuroimmunology laboratory. A cell-based indirect immunofluorescence assay was used to detect AQP4 antibodies in serum (11). Detection of MOG antibodies in serum was performed using a live cell-based assay method at Koç University Research Center for Translational Medicine (12).

Statistical Analysis

Data analysis was performed using SPSS 23.0 (IBM) software. Normality and homogeneity of the data were assessed by the Kolmogorov-Smirnov test and the Levene's test, respectively. Data

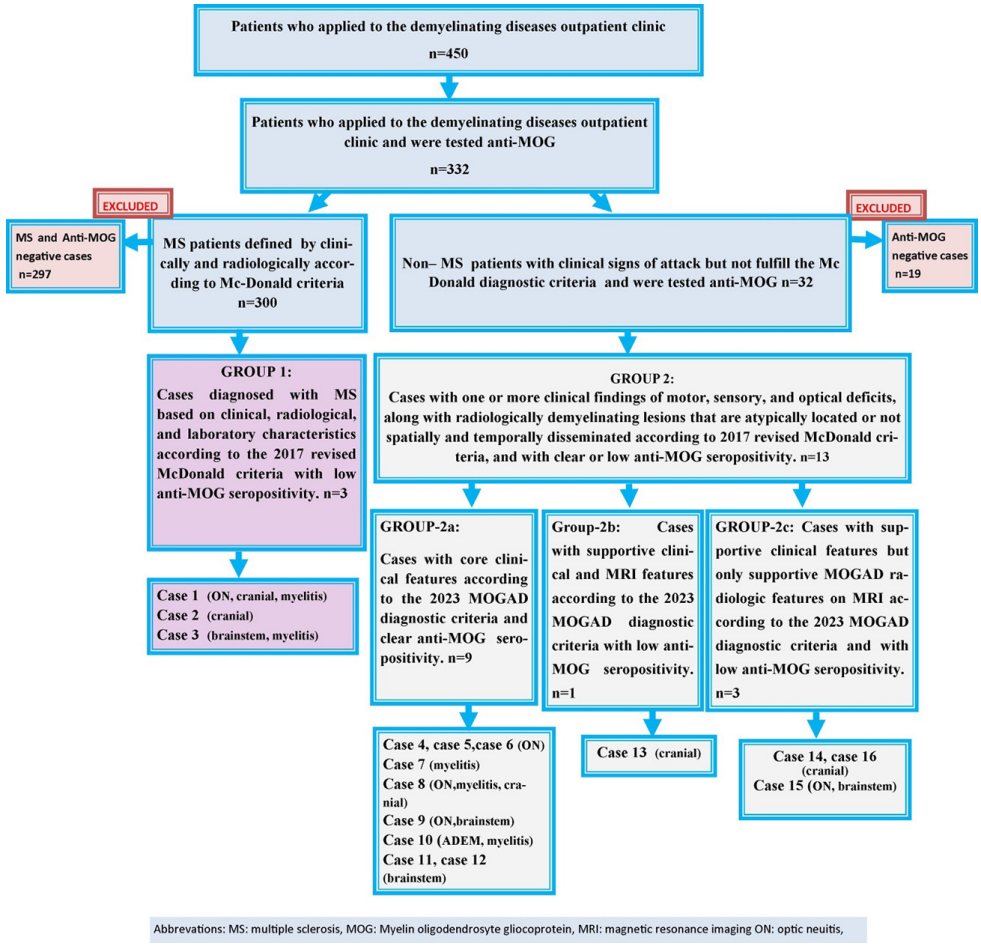


Figure 1. The schema of patient selection and grouping of the cases within the scope of the study.

distributions were presented as mean ± standard deviation or median (minimum-maximum) based on normality and homogeneity. All tests were two-tailed, and $p<0.05$ was considered statistically significant.

Ethical approval was obtained from the Ethics Committee of Sakarya University Faculty of Medicine on June 30, 2022 (approval number: 146336, date: 30.06.2022).

RESULTS

We evaluated 332 cases with one or more clinical core demyelinating events according to the MOGAD diagnostic criteria 2023, and known serologic status for anti-AQP4, anti-MOG, and OCB. Among these, 16 cases with clear or low-positive anti-MOG serology were investigated for MOGAD (Figure 2) and categorized based on clinical, radiologic, and serologic features. Detailed data are shown in Tables 1-5.

Group 1: Cases diagnosed with MS based on clinical, radiological, and laboratory characteristics according to the 2017 revised McDonald criteria with low anti-MOG seropositivity.

There were three cases in this group: one male and two females. The median disease duration was 3 years (range 3-33), and the median age at onset was 52 years (range 42-54). Detailed clinical, radiologic, and serologic features, as well as treatment options, are summarized in Table 1 and Table 2.

These cases exhibited core clinical features (Figure 2) but lacked additional supportive clinical or MRI features according to the 2023 MOGAD criteria. Despite being diagnosed with MS according to the 2017 McDonald criteria, they also had anti-MOG seropositivity. In our study, the rate of low anti-MOG seropositivity in MS cases was 1%.

Group 2: Cases with one or more clinical findings of motor, sensory, and optical deficits, along with radiologically demyelinating lesions that are atypically located or not spatially and temporally disseminated according to 2017 revised McDonald criteria, and with clear or low anti-MOG seropositivity.

This group included 13 cases evaluated according to the 2023 MOGAD diagnostic criteria (Figure 1). All cases met the core clinical features of MOGAD and were further divided into three subgroups according to their clinical, radiologic, and serologic characteristics.

Group 2a: Cases with core clinical features according to the 2023 MOGAD diagnostic criteria and clear anti-MOG seropositivity.

This group included nine cases, three males, and six females. The median disease duration was 3.5 years (1-22 years) and the median age at onset was 39.5 years (21-58 years). One case (case 4) had a late onset (≥50 years), while the others developed the disease in adulthood (18-49 years). Detailed clinical, radiologic, and serologic features, as well as treatment options, are presented in Tables 1-5. Three cases had accompanying autoimmune diseases.

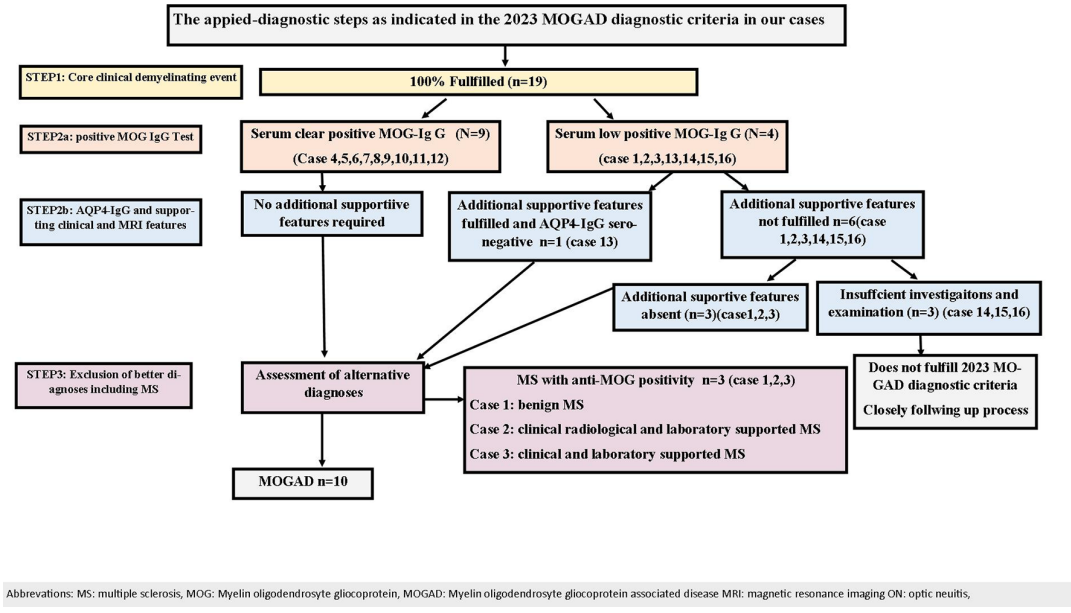


Figure 2. The schema of patient selection and grouping of the cases according to the 2023 MOGAD diagnostic criteria.
MOGAD: Myelin oligodendrocyte glycoprotein antibody-associated disease

Rare clinical presentations included sixth cranial nerve involvement in case 9; adult-onset ADEM and recurrent myelitis in case 10; wall-eyed bilateral internuclear ophthalmoplegia (WEBINO), in case 12; and tetraparesis secondary to transverse myelitis (TM) with a relapsing-progressive course in case 7. Indeed, case 7 was refractory to intravenous methylprednisolone (IVMP) and plasmapheresis, requiring ongoing treatment with rituximab and intravenous immunoglobulin (IVIG). The clinical courses of the cases were as follows: five were relapsing, three were monophasic, and one had a relapsing-progressive course. OCB seropositivity was detected in four cases. The dual positivity rate, defined as the OCB seropositivity alongside clear anti-MOG seropositivity, was 40% among the MOGAD cases in our study. Treatment options varied, with four cases receiving azathioprine, three receiving rituximab, and one receiving a combination of IVIG and rituximab. Demographic, clinical, radiologic, and serologic characteristics and treatment options are summarized in Tables 1-5.

Group 2b: Cases with supportive clinical and MRI features according to the 2023 MOGAD diagnostic criteria with low anti-MOG seropositivity.

Case 13, a female, had a disease duration of 3 years. Her clinical, radiologic, and serologic features and treatment options are detailed in Table 1 and Table 5.

Group 2c: Cases with supportive clinical features but only supportive MOGAD radiologic features on MRI according to the 2023 MOGAD diagnostic criteria and with low anti-MOG seropositivity.

There were three cases in this group: one male and two females. The median disease duration was 3 years (2-9 years) and the median age was 22 years (21-29 years). Clinical presentations included isolated ON and brainstem attacks in case 15 and cerebral cortical deficit findings in cases 14 and 16. OCB was negative in all cases (Table 1). Despite low anti-MOG seropositivity, these cases were considered suspicious for MOGAD, and were followed closely, as their clinical and radiologic features did not meet the revised 2017 McDonald criteria or the additional supporting clinical and MRI criteria for 2023 MOGAD. Detailed demographic, clinical, radiologic, and serologic data are presented in Tables 1-5.

DISCUSSION

MOGAD differs from other central neuroinflammatory diseases because of its unique clinical, radiologic, and immunologic features (13) and specific diagnostic criteria (2). In our study, 10 cases were diagnosed with MOGAD. It is noteworthy that MOG serology in the same patient can fluctuate from seropositivity to low seropositivity or even seronegativity within six months of symptom onset (14). In our study, three cases with low anti-MOG seropositivity were closely followed clinically, radiologically, and serologically because they did not fully meet the diagnostic criteria. We believe that the monophasic nature of the disease and the timing of anti-MOG serologic testing (>6 months after symptom onset) may contribute to the diagnostic challenges.

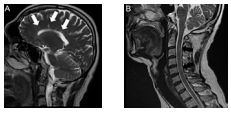
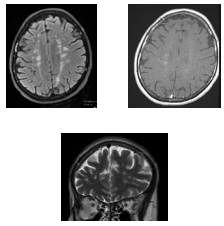
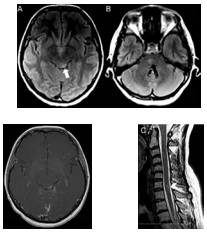
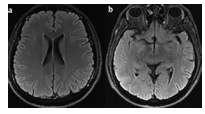
Anti-MOG seropositivity is found in 0-2.5% of MS cases (15). The coexistence of anti-MOG and OCB seropositivity may complicate the diagnosis of demyelinating diseases. In our study, three out of 300 MS cases had low anti-MOG seropositivity, but none had additional data supporting a core clinical event of MOGAD. Our results indicate a 1% rate of anti-MOG seropositivity in MS, which is consistent with the existing literature. Dual seropositivity for anti-MOG and OCB occurs in 15-50% of MOGAD cases (16,17). Although the clinical significance of this dual serology is not fully understood, it is associated with a higher incidence of polyfocal clinical presentations, greater lesion burden on MRI, more brain lesions, lesser optic nerve enhancement, and a higher relapse rate compared with anti-MOG monoseropositivity (17,18). In our study, 40% of the 10 MOGAD cases were dual seropositive. These cases, in line with the literature, showed a greater lesion burden on MRI, multifocal clinical presentation, and minimal contrast enhancement of the optic nerve.

Table 1. The clinical and laboratory features of the cases

Patients	Clinics	Finally diagnosis	Anti-MOG	OCB	CSF cells (/ µL)	CSF protein (mg/dL)	Ig G Index	Anti-AQP4
Case-1 (group 1)	Cranial, spinal attacks, ON	MS with anti MOG positivity	Low positive	Unknown	Unknown	Unknown	Unknown	Unknown
Case-2 (group 1)	Trunkal ataxia, hypoesthesia, ms	Clinical, radiological and laboratory supported MS- MS with anti MOG positivity	Low positive	Patern 2 positive (CSF)	21	33.9	1.25	Negative (serum)
Case 3 group 1)	Paraparesis, transvers myelitis Ms	Clinical and laboratory supported MS- MS with anti MOG positivity	Low positive	Patern 2 positive (8 bant)	20 eritrocyte	80.3	0.843	Negative (serum)
Case-4 (group 2a)	ON	MOGAD	Positive	Tip 4 positive (CSF)	Non	31.1	0.51	Negative (serum)
Case-5 (group 2a)	Recurrent ON	MOGAD	Positive	Negative (CSF)	Unknown	Unknown		Negative (serum)
Case-6 (group 2a)	ON	MOGAD	Positive	Negative (CSF)	10 eritrocyte	31.4	0.826	Negative (serum)
Case-7 (group 2a)	Paraparesis, transvers myelitis	MOGAD	Positive	Negative (CSF)	Non	30.2	0.5	Negative (serum)
Case-8 (group 2a)	ON, Thoracal spinal attack	MOGAD	Positive	Tip-2 positive (11 bant)	10 eritrocyte	8.54	0.85	Negative (serum)
Case 9 (group 2a)	6.cn palcy.	MOGAD	Positive	Patern 3 positive	Neither erytrocye or leucocyte	30.40	0,51	Negative (serum)
Case-10 (group 2a)	ADEM, myelitis	MOGAD	Positive	Negative (CSF)	20 leucocyte	31	0.40	Negative (serum)
Case-11 (group 2a)	Ataxia, brainstem attack	MOGAD	Positive	Negative (CSF)	No cell	35.4	0.36	Negative (serum)
Case-12 (group 2a)	Bilateral INO, cranial attack	MOGAD	Positive	Tip 3 positive	No cell	17.4	0.72	Negative (serum)
Case-13 (group 2b)	Hemiparesis, cranial attack	MOGAD	Low positive	Not tested	Not tested	Not tested		
Case-14 (group 2c)	Vertigo, ON. cranial lesion	Following up	Low positive	Patern 1	No cell	43.7	0.34	Negative (serum)
Case-15 (group 2c)	ON	Following up	Low positive	negative (CSF)	No cell -	30.1	0.71	Negative (serum)
Case-16 (group 2c)	Vertigo, dizziness, cranial attack	Following up	Low positive	negative (CSF)	No cell -	32.3	0.62	Negative (serum)

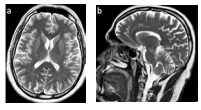
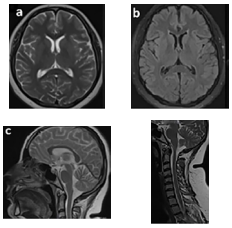
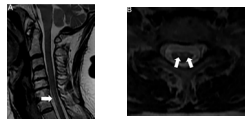
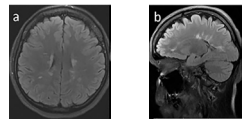
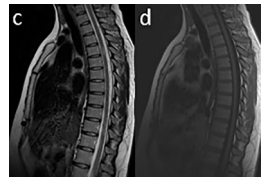
ADEM: Acute disseminated encephalomyelitis, MS: Multiple sclerosis, MOG: Myelin oligodendrocyte glicopretein, MOGAD: Myelin ologodendrocyte glicoprotein associated disease MRI: Magnetic resonance imaging, ON: Optic neuritis, OCB: Oligoclonal band, Anti-AQP4: Anti-aquoporin 4, CSF: Cerebrospinal fluid

Table 2. The clinical and radiological features of anti-MOG positive cases/1

Patients	Case 1	Case 2	Case 3	Case 4
Current age	52 years	41 years	50 years	49 years
Age of onset /gender	21 years/male	39 years female	49 years female	47 years male
Clinical presentation	Optic attacks, cranial, myelitis	Numbness and hypoesthesia on arm and legs, and truncal ataxia	paraplegia	Optic attack
Clinical course	Three (the last attack was ten years ago)	Two	Two	Relapsing
Attack count	Relapsing	Relapsing	Relapsing	Two
Radiologic Imaging / electrophysiologic findings	Periventricular hyperintense lesions perpendicular to the corpus, cervical sagittal T2-weighted hyperintense lesions not exceeding one vertebra length are observed in the spinal cord at the C2-3 and C5 levels	Cerebral white matter hyperintense lesions some of which extend perpendicular to the callosal septal interface in the periventricular area on FLAIR and T2 series on MRI, contrast enhancement on the left centrum semiovale.	Hyperintense lesions in mesencephalon medulla oblongata and a hyperintense lesion not exceeding one vertebra length in the spinal cord C7-T1, contrast enhancement on the left lateral tectum	No lesion
				
Acute treatment option	Methyl prednisolone IV	Pulse methyl prednisolone 1000 mg/day IV for 5 days	Pulse methyl prednisolone 1000mg/day IV for 5 days	First: 7 days IV 1000mg/ day pulse steroid second: no response to 3 days IV 1000mg/day pulse steroid therapy then plasmaphereses.
Finally diagnosis	MS with anti MOG positivity	Clinical, radiological and laboratory supported MS MS with anti MOG positivity	Clinical, radiological and laboratory supported MS MS with anti MOG positivity	MOGAD
Maintanence Treatment option	No treatment	Ocrelizumab	Ocrelizumab	Rituximab
Disease duration	30 years	3 years	1 years	2 years
Recovery clinical	Partial	Partial	Partial	
Resolution of lesion radiologic	Chronic lesions	Chronic lesions	Chronic lesions	No lesion
Additional disease	Non	Non	N	Ankylosing spondylitis, left eye was blind as a sequele.

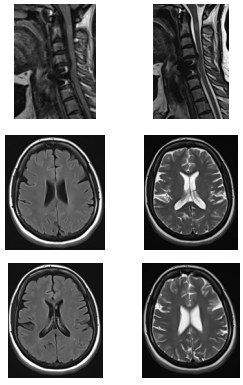
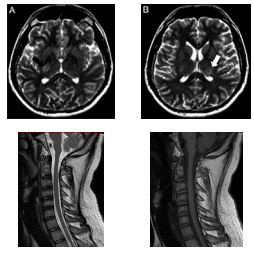
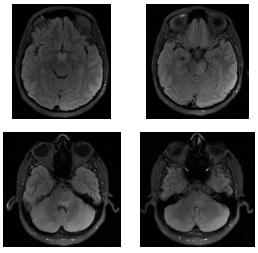
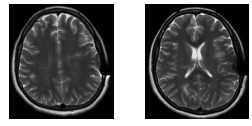
MS: Multiple sclerosis, MOG: Myelin oligodendrocyte glicopretein, MOGAD: Myelin ologodendrocyte glicoprotein associated disease MRI: Magnetic resonance imaging, FLAIR: Fluid-attenuated inversion recovery

Table 3. The Clinical and radiological features of anti-MOG positive cases/2

Patients	**Case 5**	**Case 6**	**Case 7**	**Case 8**
Current age	42 years	19 years	55 years	27 years
Age of onset /gender	23 years male	18 years female	54 years female	26 years/female
Clinical presentation	Optic attacks with different sides	Left Optic attack	Two Spinal attack-paraplegia	Optic attack, myelitis, and cranial hemi-hypoesthesia on the right
Clinical course	Relapsing	Monophasic	Relapsing-progressive	Two
Attack count	Four	One	Two	Relapsing
Radiologic Imaging / electrophysiologic findings	No lesion	No lesion visual field tests at pre/post treatment of third case at below.	A lesion in the size of two vertebrae, involving the anterior segment of the cord, is observed at the level of the C6-7 intervertebral disc.	Multiple demyelinating lesions in the periventricular area one of them was contrast enhanced and a two-segment-long lesion in the thoracal
				 
Acute treatment option	IV 1000 mg/day pulse steroid	Methyl prednisolone IV 1000mg/day for 7 days and followed by oral 1mg/kg/day with a gradually reduced dose was performed during follow-up	Methyl prednisolone IV 1000mg/day for 5 days and followed by 0.75mg/kg/day oral long-term steroid therapy which was gradually reduced monthly	First: no therapy. second: methyl prednisolone IV 1000mg/day for 5 days and long-term steroid therapy
Finally diagnosis	MOGAD	MOGAD	MOGAD	MOGAD
Maintanence Treatment option	Azathioprine	Azathioprine	Rituximab/IVIG (because of progression)	Rituximab
Disease duration	20 years	1 year	1 year	1 year
Recovery clinical	Partial	Complete	Progressively	Complete
Resolution of lesion radiologic	No lesion	No lesion	Partial	Persistant
Additional disease	Non	Non	Non	Non

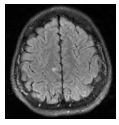
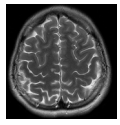
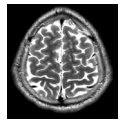
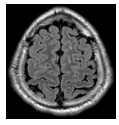
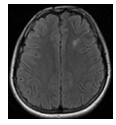
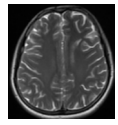
MS: Multiple sclerosis, MOG: Myelin oligodendrocyte glicopretein, MOGAD: Myelin ologodendrocyte glicoprotein associated disease MRI: Magnetic resonance imaging, ON: Optic neuritis

Table 4. The clinical and radiological features of anti-MOG positive cases/3

patients	**Case 9**	**Case 10**	**Case 11**	**Case 12**
Current age	46 years	31years	23 years	29 years
Age of onset /gender	42 years/female	27 years/male	23 years/female	29 years/female
Clinical presentation	ON, 6. CN palcy	ADEM, myelitis	Brainstem	Bilateral INO, dizziness
Attacks count	Two	Two	One	One
Clinical course	Relapsing	Relapsing	Monophasic	Monophasic
Radiologic Imaging / electrophysiologic findings	T2 hyperintense lesion at the level of C2 vertebra, Millimetric hyperintense lesion on T2 and Flair sequences located in deep white matter in both parietal lobes and right temporal lobe	Hyperintense lesions right globus pallidus to the posterior leg of the internal capsule posterior to the left thalamus	Hyperintensity in flair and T2 at the level of the mesencephalon, in the left half of the pons, in the left middle cerebellar peduncle and adjacent to the 3rd ventricle, without significant enhancement appearance on cranial MRI	The subcortical white matter lesions in fronto-temporal lobe on left side, and in the occipital lobe on right side that had signal changes of hypointense in T1A series, hyperintense in T2A series and had contrast enhancement appearance
				
Acute treatment option	Pulse methyl prednisolone 1000mg/day IV for 7 days	Methyl prednisolone 1000mg/day IV for 5 days	Methyl prednisolone 1000mg/day intravenously for 5 days	Methyl prednisolone IV 1000mg/day for 5 days and Plasmapheresis
Finally diagnosis	MOGAD	MOGAD	MOGAD	MOGAD
Maintanence Treatment option	Azathioprine 150 mg/day	Azatioprine	Rituximab	Rituximab
Disease duration	4 years	4 years	1 year	1 year
Recovery clinical	Complete	Complete	Complete	Complete
Resolution of lesion radiologic	Chronic lesions	No lesion	Complete	No
Additional disease	Diabetes mellitus, hashimato, asthma	Non	Non	Familial mediterranean fever

ADEM: Acute disseminated encephalomyelitis, MOG: Myelin oligodendrocyte glicopretein, MOGAD: Myelin ologodendrocyte glicoprotein associated disease MRI: Magnetic resonance imaging, ON: Optic neuritis, INO: Internuclear ophalmoplegia, FLAIR: Fluid-attenuated inversion recovery

Table 5. The clinical and radiological features of anti-MOG positive cases/4

Patients	**Case 13**	**Case 14**	**Case 15**	**Case 16**
Current age	27 years	21 years	42 years	29 years
Age of onset /gender	24 years Female	19 years Male	39 years Female	26 years Female
Clinical presentation	Numbness, hypoesthesia, hemiparesis, allodinia	Intermittent dizziness, unsteady gait, blurred vision and numbness on face	Left optic attack and sixth nerve palsy.	Vertigo, dizziness
Attacks count	Two		One	One
Clinical course	Relapsing	Recurrent attack	Monophasic	Monophasic
Radiologic Imaging / electrophysiologic findings	Millimetric flair t2 hyperintense lesion in the subcortical white matter in the right parietal at the vertex level	Periventricular a few and subcortical 3-4 hyperintense in T2A series on cranial MRI	The hyperintense lesion in T2A and Flair series had irregular borders extending towards the mesencephalon at the level of the pons	Nonspecific hyperintense lesion in the t2a series with a diameter of about 5 mm adjacent to the frontal horn of the left lateral ventricle on ventricular cross-sections.
	   		Not available	 
Acute Treatment option	Pulse methyl prednisolone 1000mg/ day IV for 5 days	No treatment	Oral prenisolone	No treatment
Finally diagnosis	MOGAD	Following up	Following up	Following up
Maintanence Treatment option	Rituximab	No treatment	No treatment	No treatment
Disease duration	3 years	3 years	3 years	3 year
Recovery clinical	Partial	Complete	Complete	
Resolution of lesion radiologic	Chronic lesions	Chronic lesions	No lesion	Chronic lesions
Additional disease	Non	Non	Non	Non

MS: Multiple sclerosis, MOG: Myelin oligodendrocyte glicopretein, MOGAD: Myelin ologodendrocyte glicoprotein associated disease MRI: Magnetic resonance imaging, ON: Optic neuritis

The incidence of MOGAD is approximately 1.6-2.39 per million people per year, with a similar gender distribution (2,19). However, our study found a female predominance (70%), which differs from the literature. We attribute this discrepancy to the small size of our disease cohort.

In adults, ON is the most common clinical manifestation of MOGAD (20). Consistent with the literature, ON was the most common phenotype in our study (n=5), while there was only one case that presented with ADEM, which is rare in adults. MOGAD-ON typically presents bilaterally, either synchronously or sequentially (20), and often follows a relapsing course (6). In our study, the clinical courses of the three MOGAD-ON cases were different: one had bilateral ON occurring sequentially, one had monophasic ON, and one had relapsing unilateral ON. We believe that the sequela of optic atrophy in the contralateral eye prevented bilateral ON presentation, in case 4. According to the literature, the radiologic phenotype of MOGAD-ON includes an edematous, swollen, and tortuous optic nerve with T2 hyperintensity along the prechiasmatic pathwayff and typical peripheral enhancement of the optic nerve and orbital fat on orbital MRI (21). In our study, radiologic imaging appeared normal for MOGAD-ON. We believe that the delay between clinical onset and imaging, along with the subsequent resolution of symptoms, may have influenced our radiologic findings. Although MOGAD-ON may improve without treatment, corticosteroids are highly effective in the acute phase (21,22). All of our ON cases showed clinical recovery, supporting these findings.

TM is the second most common clinical manifestation of MOGAD, occurring in 26% of cases (20). TM may occur as an isolated disease, in association with ON, or as part of ADEM (23). Longitudinal involvement of three or more vertebral segments is a common radiologic feature, although shorter, fragmented, or multifocal spinal cord lesions are also seen. The clinical course of our three MOGAD-TM cases varied: one presented with isolated monophasic myelitis, another with myelitis associated with a history of ON, and the third with ADEM and relapsing myelitis. Radiologic features included a two-segment long T2 hyperintense lesion in the anterior segment

of the spinal cord at the C6-7 level in case 7, a two-segment long contrast-enhanced lesion on thoracic MRI in case 8, and a vertebra-long focal T2 hyperintense lesion in the left anterior horn of the spinal cord in case 10. Although MOGAD-TM generally has a good prognosis (2), case 7 had a progressive course and was refractory to treatment, contrary to typical expectations.

MOGAD brain lesions are predominantly located in the supratentorial region and typically present as a few (≤3) bilateral, hyperenhancing, ill-defined T2 hyperintense lesions (24). These lesions often appear in the deep gray matter (5) and middle cerebellar peduncles (25), with diffuse involvement of the pons and areas adjacent to the fourth ventricle more common than in seropositive NMOSD (25). Most MOGAD brain lesions resolve on follow-up MRI (60-79%), although some persist (2,8). In our study, radiological findings were resolved completely in three cases, partially in one case, and persisted in two cases.

Two cases presented with rare neuro-ophthalmologic manifestations: one with WEBINO and the other with sixth nerve palsy. Only one case of WEBINO and two cases of sixth nerve palsy have been reported in MOGAD (26,27). Our understanding of MOGAD-related optic nerve involvement beyond ON is expanding, with new phenotypic findings such as WEBINO and sixth nerve palsy being documented.

Recent studies suggest that the late onset of inflammatory demyelinating disease may be associated with more severe clinical findings and higher levels of disability (28). Our seventh MOGAD case is consistent with this hypothesis, as it involved an age of onset over 50 years, a progressive course of paraplegia, and an inadequate response to treatment. In the literature, MOGAD has been associated with comorbid rheumatologic diseases such as SLE and Sjögren's syndrome (29,30). In our study, three cases had concomitant autoimmune diseases: ankylosing spondylitis (AS), Familial Mediterranean Fever (FMF), and Hashimoto's thyroiditis. Only one AS+MOGAD case has been documented, suggesting that TNF-alpha inhibitors may exacerbate MOGAD symptoms (31). Our AS + MOGAD case did not receive any immunosuppressive or TNF-alpha inhibitor treatment at diagnosis. Therefore, we believe that our case may be the first in the literature. While MS is often associated with FMF, the association between FMF and MOGAD is poorly documented in the literature (32). Thus, our case of FMF + MOGAD is considered rare. Hashimoto's thyroiditis is known to coexist with both MS and MOGAD (33,34). Our case of MOGAD + Hashimoto's thyroiditis supports these findings.

The acute treatment regimen for MOGAD typically involves high-dose IV corticosteroids for 3-5 days, similar to other demyelinating diseases, although there is no consensus on tapering steroids after acute treatment (35,36). IVIG or plasma exchange is often used when standard treatment fails to produce adequate improvement (38,39). In our study, two cases underwent plasma exchange after 5 days of IVMP, one case received IVIG after IVMP, and another was treated with IVMP for 5 days followed by gradual tapering of long-term oral steroids, which were reduced gradually over the following months. Long-term treatment includes various immunosuppressive agents based on the experience of individual centers, mycophenolate mofetil, azathioprine, rituximab, and IVIG, administered either intravenously or subcutaneously (37). In our study, azathioprine, rituximab, and repeated IVIG were used for maintenance therapy.

Study Limitations

The limitations of our study can be divided into three main categories. First, the small number of cases diagnosed with MOGAD. Secondly, a lack of access to CSF and complete radiological data for all cases. Finally, a delay in performing serologic tests was observed in three cases. Our study emphasizes the need for more extensive research to better understand the diagnosis, clinical presentation, effective treatment, and course of MOGAD.

CONCLUSION

We aimed to highlight the importance of recognizing MOGAD due to its potential association with autoimmune diseases, progressive nature, and dual seropositivity. Thus, it should be considered for its unique clinical and radiologic features. Despite the limited size of our series, our findings contribute to the literature supporting the generally mild clinical course of MOGAD.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ethics Committee of Sakarya University Faculty of Medicine on June 30, 2022 (approval number: 146336, date: 30.06.2022).

Informed Consent: Retrospective study.

Acknowledgment

The authors declare no competing interests. The anti-MOG assays in this study were performed at Koç University Research Center for Translational Medicine (KUTTAM). This project was supported by TUBITAK (project number: 118S397). We thank Dr. Atay Vural for supervising the MOG-IgG assays.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.S., O.T., M.Y., T.D., D.K., Concept: S.S., E.Ç., O.T., D.K., Design: S.S., D.K., Data Collection or Processing: S.S., D.K., Analysis or Interpretation: S.S., D.K., Literature Search: S.S., D.K., Writing: S.S., E.Ç., O.T., M.Y., D.K.

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Development of Machine Learning Prediction Models to Predict ICU Admission and the Length of Stay in ICU for COVID-19 Patients Using a Clinical Dataset Including Chest Computed Tomography Severity Score Data

COVID-19 Hastalarının Yoğun Bakım Ünitesine Yatışlarını ve Hastanede Kalış Sürelerini Tahmin Etmek için Makine Öğrenimi Modellerinin Geliştirilmesi

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ABSTRACT

Objective: The chest computed tomography severity score (CT-SS) is significantly associated with the severity of the disease and subsequently intensive care unit (ICU) admission in coronavirus disease-19 (COVID-19) patients. However, there was a lack of information about the prognostic role of radiological manifestations in combination with demographics, clinical manifestations, and laboratory predictors to predict ICU admission and the length of stay (LOS) in the ICU (ICU LOS) of COVID-19 patients. The machine learning (ML) approach is a new and, non-invasive digital technology that can present an efficient risk prediction model for clinical problems. The purpose of the present study was to develop an effective ML model for predicting ICU admission and ICU LOS for COVID-19 patients using a more comprehensive dataset including imaging findings.

Methods: A COVID-19 hospital-based registry database that contained medical records of 6,854 patients was retrospectively reviewed. The incomplete records with missing values of more than 70% were excluded, and the remaining missing values were imputed using the mean and mode values for the continuous and discrete variables,

ÖZ

Amaç: Göğüs bilgisayarlı tomografi şiddet skoru (BT-SS), koronavirüs hastalığı-19 (COVID-19) hastalarında hastalığın şiddeti ve sonrasında yoğun bakım ünitesine (YBÜ) yatışla önemli ölçüde ilişkilidir. Ancak, COVID-19 hastalarının YBÜ yatışını ve YBÜ'de kalış süresini (LOS) (YBÜ LOS) tahmin etmek için demografik özellikler, klinik belirtiler ve laboratuvar öngörücüleriyle birlikte radyolojik bulguların prognostik rolü hakkında bilgi eksikliği vardı. Makine öğrenimi (ML) yaklaşımı, klinik sorunlar için etkili bir risk tahmin modeli sunabilen yeni ve invaziv olmayan bir dijital teknolojidir. Mevcut çalışmanın amacı, görüntüleme bulgularını içeren daha kapsamlı bir veri seti kullanarak COVID-19 hastaları için YBÜ yatışını ve YBÜ'de kalış süresini tahmin etmek için etkili bir ML modeli geliştirmekti.

Yöntemler: Altı bin sekiz yüz elli dört hastanın tıbbi kayıtlarını içeren bir COVID-19 hastane tabanlı kayıt veritabanı retrospektif olarak incelendi. %70'ten fazla eksik değere sahip eksik kayıtlar hariç tutuldu ve kalan eksik değerler, sürekli ve ayrık değişkenler için sırasıyla ortalama ve mod değerleri kullanılarak hesaplandı. Grupların veri sayılarındaki dengesizlik, sentetik azınlık örnekleme tekniği algoritması

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ABSTRACT

respectively. The imbalance in the data numbers of groups was resolved using the synthetic minority over-sampling technique algorithm. Two sets of prediction models were separately developed to predict ICU admission and ICU LOSs of COVID-19 patients. The most important and related predictors selected by the Boruta feature selection method were used to develop ML prediction models. The parameters obtained from the confusion matrix were used to evaluate the performance of the prediction models. The performance evaluation of the developed ML models for predicting ICU LOS of the patients utilized correlation coefficient, mean absolute error, and root mean squared error metrics.

Results: The records of 815 positive reverse transcription polymerase chain reaction (RT-PCR) patients were included in the study after applying the inclusion/exclusion criteria. Of the 815 positive RT-PCR patients, only 185 patients were admitted to the ICU to receive intensive care. The number of records in the ICU admission group was raised to 630 to deal with the data imbalance problem. For predicting the ICU admission of COVID-19 patients, k-nearest neighbors (k-NN) yielded better performance than J48, support vector machine, multi-layer perceptron, Naïve Bayes, logistic regression, random forest (RF), and XGBoostbased ML models. The sensitivity, specificity, accuracy, precision, F-measure, and area under the curve of the k-NN algorithm were 97.0%, 89.7%, 93.3%, 90.4%, 93.6%, and 99.1%, respectively. Results showed that with a correlation coefficient of 0.42, a mean absolute error of 2.01, and a root mean squared error of 4.11, the RF algorithm with a correlation coefficient of 0.42, mean absolute error of 2.01, and root mean squared error of 4.11demonstrated the best performance in predicting the ICU LOS of COVID-19 patients.

Conclusion: The ML approach, utilizing a more comprehensive dataset that includes CT-SS, could efficiently predict ICU admission and ICU LOS of COVID-19 patients. Timely prediction of ICU admission and ICU LOS of COVID-19 patients would improve patient outcomes and lead to the optimal use of limited hospital resources.

Keywords: Computed tomography, COVID-19, machine learning, intensive care unit, length of stay

ÖZ

kullanılarak çözüldü. COVID-19 hastalarının YBÜ yatışını ve YBÜ'de kalış sürelerini tahmin etmek için ayrı ayrı iki tahmin modeli seti geliştirildi. Boruta özellik seçimi yöntemi ile seçilen en önemli ve ilgili tahmin ediciler, ML tahmin modellerini geliştirmek için kullanıldı. Karışıklık matrisinden elde edilen parametreler, tahmin modellerinin performansını değerlendirmek için kullanıldı. Hastaların YBÜ kalış sürelerini tahmin etmek için geliştirilen ML modellerinin performans değerlendirmesinde korelasyon katsayısı, ortalama mutlak hata ve kök ortalama kare hata ölçümleri kullanıldı.

Bulgular: Dahil etme/dışlama kriterleri uygulandıktan sonra 815 pozitif ters transkripsiyon polimeraz zincir reaksiyonu (RT-PCR) hastasının kayıtları çalışmaya dahil edildi. Sekiz yüz on beş pozitif RT-PCR hastasından yalnızca 185 hasta yoğun bakıma alınmak üzere YBÜ'ye yatırıldı. YBÜ kabul grubundaki kayıt sayısı, veri dengesizliği sorunuyla başa çıkmak için 630'a çıkarıldı. COVID-19 hastalarının YBÜ kabulünü tahmin etmek için k-NN, J48, destek vektör makinesi, çok katmanlı algılayıcı, Naïve Bayes, lojistik regresyon, rastgele orman (RF) ve XGBoost tabanlı ML modellerinden daha iyi performans gösterdi. k-NN algoritmasının duyarlılığı, özgüllüğü, doğruluğu, kesinliği, F-ölçüsü ve eğri altında kalan alanı sırasıyla %97,0, %89,7, %93,3, %90,4, %93,6 ve %99,1 idi. Sonuçlar, 0,42 korelasyon katsayısı, 2,01 ortalama mutlak hata ve 4,11 kök ortalama kare hatası ile 0,42 korelasyon katsayısı, 2,01 ortalama mutlak hata ve 4,11 kök ortalama kare hatası olan RF algoritmasının COVID-19 hastalarının YBÜ kalış süresini tahmin etmede en iyi performansı gösterdiğini gösterdi.

Sonuç: BT-SS'yi içeren daha kapsamlı bir veri setini kullanan ML yaklaşımı, COVID-19 hastalarının YBÜ yatışını ve YBÜ kalış süresini etkili bir şekilde tahmin edebilir. COVID-19 hastalarının YBÜ yatışının ve YBÜ kalış süresinin zamanında tahmin edilmesi hasta sonuçlarını iyileştirecek ve sınırlı hastane kaynaklarının optimum şekilde kullanılmasını sağlayacaktır.

Anahtar Sözcükler: Bilgisayarlı tomografi, COVID-19, makine öğrenimi, yoğun bakım ünitesi, kalış süresi

INTRODUCTION

In December 2019, the novel coronavirus disease-19 (COVID-19), also known as severe acute respiratory syndrome coronavirus 2, was detected in Wuhan City, China. Since the first reports, over 690 million cases of infection with COVID-19 and 9.6 million deaths among these individuals have been reported (19 Jun 2023) (1). The COVID-19 virus has heterogeneous and mutable clinical manifestations which result in poor outcomes (2). Its clinical presentations range from asymptomatic to severe complications and death in some cases (2,3). For many patients with mild symptoms, clinical manifestations of the disease can rapidly change to severe complications including acute respiratory distress syndrome and multi-organ failure, which lead to intensive care unit (ICU) hospitalization (2,4).

Approximately 20% of COVID-19 patients over 80 years old needed hospital care, and ICU care requirements of COVID-19 in-hospital patients ranged from 5% to 32%, depending on the location of the study and characteristics of the studied community (5). The mortality rate of patients with severe disease manifestations is as high as 50% (6).

The complex clinical features and mutable clinical manifestation patterns of the disease have increased the demand for hospitalization and healthcare services. Under normal conditions, more than 50% of ICU resources are occupied (5,7), and during the outbreak of diseases such as COVID-19, there will be a significant limitation in medical resources and hospital beds. Therefore, early risk stratification has a critical role in patient management and medical resource allocation.

Artificial intelligence (AI) is a new and non-invasive digital technology that can present an efficient risk prediction model for clinical problems. In pioneer studies, an AI approach had also been implemented to predict the ICU admission and lengths of stay of COVID-19 patients. The machine learning (ML) approach, as a subfield of AI, is used to generate risk prediction models in retrospective datasets (2,7,8). In several studies, ML prediction models have been developed to predict the ICU admission and ICU LOS of COVID-19 patients (2,7, 9-15). In these studies, prognostic factors related to the severity of the disease, the ICU admission, and ICU LOSs of the patients, including age, comorbidities, and laboratory results were reported. There was a lack of information about the prognostic role of radiological manifestations in predicting ICU admission and ICU LOSs of COVID-19 patients.

Chest imaging is an indispensable diagnostic method for the diagnosis, monitoring, and management of COVID-19 patients.

The severity of pulmonary involvement on computed tomography (CT) scans, or the chest CT severity score (CT-SS), is significantly associated with the severity of the disease, ICU admission, and mortality of patients (16-19).

Therefore, CT-SS could improve the prognostic performances of the ML algorithms to predict the ICU admission and ICU LOSs of COVID-19 patients. To the best of our knowledge, the prognostic role of imaging manifestations in combination with demographics, clinical manifestations, and laboratory predictors to predict ICU admission and ICU LOS of COVID-19 patients has not yet been evaluated.

The purpose of the present study is to develop an effective ML prediction model the ICU admission and ICU lengths of stay of COVID-19 patients using a more comprehensive dataset including demographics, clinical manifestations, laboratory results, and imaging findings. Therefore, the most relevant predictors related to ICU admission and ICU LOSs of the patients were first determined. In the next step, using these predictors, we developed and evaluated ML prediction models to predict ICU admission and ICU LOSs of the COVID-19 patients.

MATERIALS AND METHODS

Ethics Approval and Consent to Participate

This article is extracted from a research project supported by Abadan University of Medical Sciences and all experimental protocols were approved by the ethical committee of Abadan University of Medical Sciences (approval number: IR.ABADANUMS.REC.1402.108, date: 14.11.2023). All methods of the study were performed in accordance with the relevant guidelines and regulations of the Ethics Committee of Abadan University of Medical Sciences. Participation was voluntary and informed consent was obtained from all subjects and/or their legal guardians. Participants had the right to withdraw from the study at any time.

Dataset Description

In this study, a COVID-19 hospital-based registry database that contained 6,854 patients was retrospectively reviewed. Of the 6,854 patients, 1,853 cases were COVID-19-positive, 2,472 cases were COVID-19-negative, and 2,529 cases were unspecified. In this COVID-19 registry database, demographic information (eight features), clinical pictures (21 features), comorbidities (13 features), laboratory results (28 features), and radiological findings (CT-SS) were collected at the time of patient admission. The primary outcome was defined as admission to the ICU, and the output feature was registered as either ICU-admission or non-ICU-admission.

CT-SS quantifies the severity of pulmonary involvements in CT images. For radiological evaluation of the patients, each lung lobe was visually scored as 0 (no involvement), 1 (less than 5% involvement), 2 (5%-25% involvement), 3 (25%-50% involvement), 4 (50%-75% involvement), and 5 (more than 75% involvement). The sum of these lobar scores determines CT-SS, which ranges from 0 to 25. For each patient, chest CT images were separately evaluated by two radiologists in a double-blind fashion. Any disagreements between them were resolved through further discussions and consulting with a third attending radiologist with 23 years of experience.

Data Pre-processing

Data pre-processing is used to address irrelevant, redundant, and unreliable instances in ML studies. A substantial number of inconsistencies could be resolved using data pre-processing. Data pre-processing will be performed before the training of the ML models. First, the presence of missing values is one of the common issues for the independent features of a medical dataset (20). To deal with this issue, incomplete records with many missing values (more than 70%) were excluded. For the continuous and discrete variables, the remaining missing values were imputed using the mean and mode values, respectively. Noisy, abnormal, and meaningless data were checked, and if necessary, we contacted the corresponding physicians.

The patients with negative reverse transcription polymerase chain reaction (RT-PCR) COVID-19 test, unknown dispositions, discharge or death from the emergency department, missing data >70%, and ages younger than 18 years old were excluded from the study. Figure 1 illustrates the schematic representation of the study inclusion and exclusion criteria. The final sample size of the included cases was 815 patients who tested positive with RT-PCR.

This dataset contains 630 and 185 cases in the non-ICU-admitted and ICU-admitted groups, respectively. The imbalance in the sample sizes of these groups delivers biased results toward the dominant class. To deal with this issue, the synthetic minority over-sampling technique (SMOTE) was used (https://imbalanced-learn.org/stable/). SMOTE generates synthetic data until the minority cases are balanced with the majority.

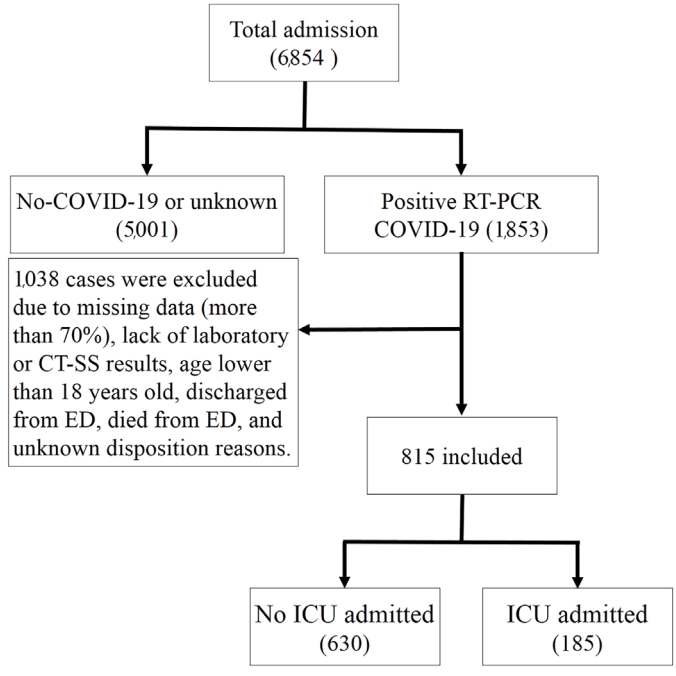

Figure 1. Flow chart describing patient selection.

COVID-19: Coronavirus disease-19, ICU: Intensive care unit, CT-SS: Computed tomography severity score, RT-PCR: Reverse transcription polymerase chain reaction, ED: Emergency department

Feature Selection

In data mining studies, the most important and related predictors are commonly determined using the feature selection process (21). Overfitting, as one of the critical problems in developing ML models, could be considerably avoided using the feature selection procedure (22).

In this study, the Boruta feature selection package implemented in the R programming environment (version 4.0.3; https://www.r-project.org/) was used. This method is a wrapper algorithm built upon the random forest algorithm. The Boruta algorithm determines the importance magnitudes of all relevant features in the prediction of the target output. The Boruta algorithm assesses the significance of each feature using the importance values derived from shadow attributes. These shadow attributes are generated by shuffling the values of the original attribute across the subjects. This approach involves extending the information system by adding copies of all variables, which are then shuffled to eliminate any correlations between the features and the output. The random permutation of features among the studied populations results in a decrease in classification accuracy. The importance of a given attribute is quantified using a Z score, which is calculated by dividing the average loss of classification accuracy by the attribute's standard deviation. The maximum Z score (MZSA) for the shadow attributes is identified, and any attribute with an importance value higher than this score is classified as a hit. Conversely, attributes with importance values lower than MZSA are considered unimportant and are subsequently removed from the information system. This process continues with the elimination of shadow attributes until the significance of each feature is established. For all feature selections, the maximal number of importance source runs and the verbosity level (doTrace) were set to 500 and 2, respectively.

Model Development

In this study, two sets of prediction models were separately developed to predict ICU admission and the ICU in COVID-19 patients. For ICU admission prediction, eight ML models including the J48 decision tree (J48), support vector machine (SVM), multi-layer perceptron (MLP), k-nearest neighbors (k-NN), Naïve Bayes (NB), logistic regression (LR), random forest (RF), and eXtreme gradient boosting (XGBoost) algorithms were developed. Gaussian processes (GP), linear regression (LinR), MLP, SVM, k-NN, locally weighted learning (LWL), M5-Prime (M5P), M5-based decision list for regression problems using separate-and-conquer (M5Rules), and RF algorithms were used to predict the ICU LOS in COVID-19 patients. All ML prediction models were implemented using Waikato Environment for Knowledge Analysis (Weka) software (version 3.9.2; University of Waikato, New Zealand). The 10-fold cross-validation method was used for evaluating the performance of the developed prediction models. In the ten-fold cross-validation method, the samples are randomly divided into ten subgroups. In this method, 10 training and validation iterations are performed with different data folds. For each iteration, nine subgroups of data are used to train the model, and the remaining subgroup is the validation dataset. The parameters obtained from the confusion matrix, including accuracy, precision, sensitivity, specificity, F-measure, and area under the curve (AUC) receiver operating characteristic (ROC) metrics, were used to evaluate the performance of prediction models for predicting ICU admission of the patients. The average magnitudes of the accuracy, sensitivity, specificity, among other indices, for these ten iterations are considered to evaluate the model performance.

For the performance evaluation of the developed ML models to predict ICU LOS of the patients, correlation coefficient, mean absolute error, and root mean squared error metrics were used.

RESULTS

The COVID-19 hospital-based registry database that contained 6,854 suspected cases was retrospectively reviewed. Records of 815 positive RT-PCR patients were included in the study after applying the inclusion/exclusion criteria. The mean age of COVID-19 patients was 57.22±16.76 years, and 54.85% of the study population was male.

Of the 815 positive RT-PCR patients, only 185 cases were admitted to ICU to receive intensive care. Therefore, there was a considerable data imbalance between the ICU-admission and non-ICU-admission groups (185 vs. 630 cases). To deal with this data imbalance, the SMOTE technique was used and the number of records in the ICU admission group was raised to 630.

Feature Selection

The magnitudes of importance of the features to predict ICU admission and ICU LOS of COVID-19 patients are shown in Figure 2. In this figure, the X and Y axes represent the features and their corresponding importance values. The irrelevant, tentative, and relevant features were represented by red, yellow, and green box plots, respectively. The blue box plots show the minimum, average, and maximum shadow variables.

Evaluation of The Developed Models

In this study, ML models for predicting the ICU admission of COVID-19 patients were developed using J48, SVM, MLP, k-NN, NB, LR, RF, and XGBoost algorithms. The ICU LOS of these patients was also predicted using nine ML models, including GP, LinR, MLP, SVM, k-NN, LWL, M5Rules, M5P, and RF algorithms. The ML prediction models were trained and tested using the selected features in the previous step. The sensitivity, specificity, accuracy, precision, F-measure, and AUC metrics were used to evaluate the prognostic performances of the ML models for predicting the ICU admission of COVID-19 patients. The performance metrics of the ML prediction models for the ICU admission of COVID-19 patients were listed in Table 1. Results of the performance evaluation for the developed ML models that are to predict the ICU LOS of COVID-19 patients are listed in Table 2.

For predicting the ICU admission of COVID-19 patients, k-NN yielded the best performance compared to other ML models. The sensitivity, specificity, accuracy, precision, F-measure, and AUC of the k-NN algorithm were 97.0%, 89.7%, 93.3%, 90.4%, 93.6%, and 99.1%, respectively. The comparison of the area under the ROC curves of the ML prediction algorithms for predicting the ICU admission of COVID-19 patients is presented in Figure 3.

For predicting the ICU length of stay of COVID-19 patients, results showed that the RF algorithm yielded better performance than other ML algorithms. The correlation coefficient, mean absolute error, and root mean squared error of the RF algorithm were 0.42, 2.01, and 4.11, respectively.

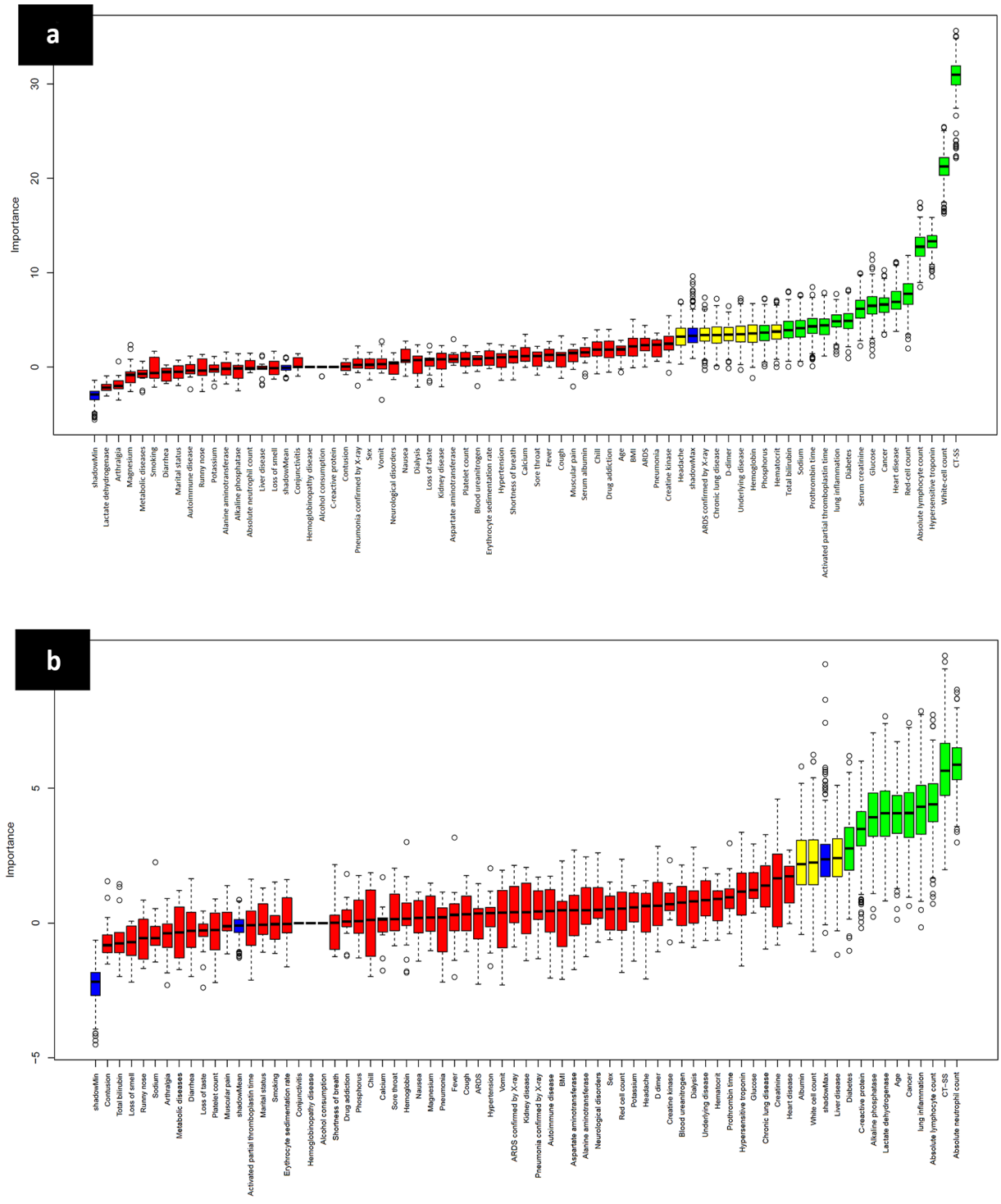

Figure 2. The plots show feature selections used to predict a) ICU admission and b) ICU LOS of COVID-19 patients using the Boruta algorithm. Green, yellow, and red boxes represent the relevant, tentative, and irrelevant features. Blue boxes denote the minimum, mean, and maximum of shadow variables.

COVID-19: Coronavirus disease-19, ICU: Intensive care unit, LOS: Length of stay

Table 1. Performances of ML algorithms for predicting ICU admission in COVID-19 patients

ML algorithm	Sensitivity	Specificity	Accuracy	Precision	F-measure	AUC
Decision tree	86.3	81.7	84.0	82.5	84.4	88.1
SVM	76.2	80.5	78.3	79.6	77.9	78.3
MLP	96.7	87.0	91.8	88.1	92.2	95.0
k-NN	97.0	89.7	93.3	90.4	93.6	99.1
Naïve Bayes	73.7	77.8	75.7	76.8	75.2	84.1
Logistic regression	76.2	81.3	78.7	80.3	78.2	85.4
Random forest	97.6	87.9	92.8	89.0	93.1	98.6
XGBoost	97.0	85.1	91.0	86.8	91.6	91.1

ML: Machine learning, AUC: Area under the curve, SVM: Support vector machine, MLP: Multi-layer perceptron, k-NN: k-nearest neighbors, XGBoost: eXtreme gradient boosting, COVID-19: Coronavirus disease-19

Table 2. Performances of ML algorithms to predict the ICU LOS of COVID-19 patients

	Gaussian processes	Linear regression	MLP	SVM	kNN	LWL	M5 rules	M5P	RF
Correlation coefficient	0.3541	0.3475	0.1821	0.1318	0.3696	0.2122	0.38	0.3714	0.4225
Mean absolute error	2.3453	2.3504	3.5832	1.632	1.9131	2.2123	2.1704	2.1937	2.0106
Root mean squared error	4.2317	4.2516	6.9122	4.7523	4.2789	4.4817	4.2022	4.2279	4.1096

LWL: Locally weighted learning, SVM: Support vector machine, RF: Random Forest, MLP: Multi-layer perceptron, ICU: Intensive care unit, SVM: support vector machine, k-NN: k-nearest neighbors, COVID-19: Coronavirus disease-2019

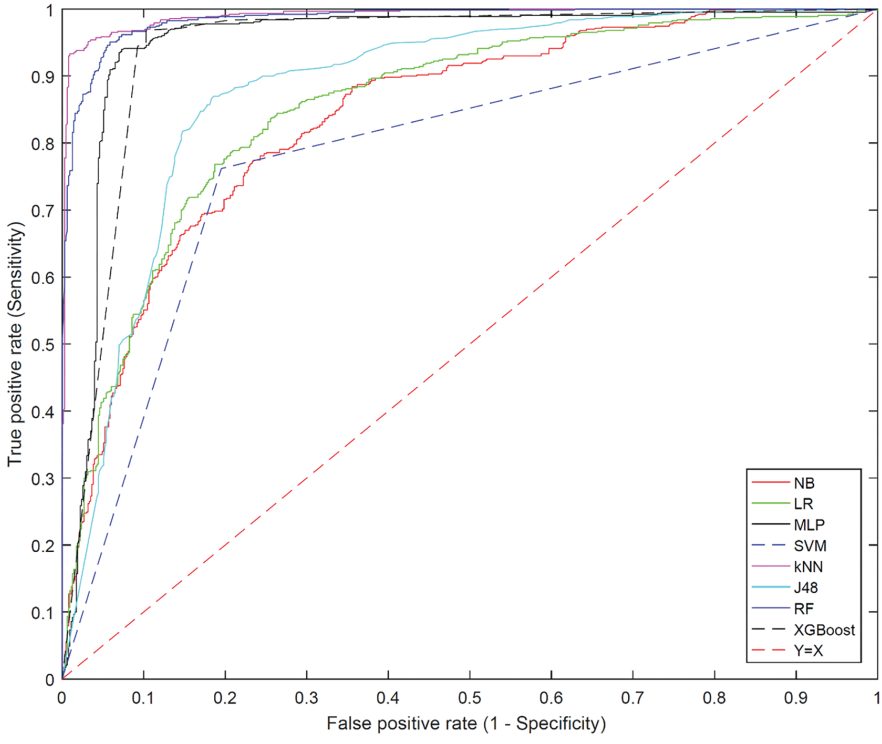

Figure 3. ROC curves for ML algorithms in the prediction of ICU admission in COVID-19 patients.

COVID-19: Coronavirus disease-19, ROC: Receiver operating characteristic, ICU: Intensive care unit, ML: Machine learning, NB: Naive Bayes, LR: Logistic regression, MLP: Multi-layer perceptron, SVM: Support vector machine, kNN: k-nearest neighbors, RF: Random forest

DISCUSSION

During the outbreak of a disease such as the COVID-19 pandemic, predicting a patient's disease course is essential for accurate triage and efficiently allocating resources (11,23). A high rate of mortality was reported for ICU-admitted COVID-19 patients, which varies between countries from 16% to 67% (5,24). Thus, early risk stratification could significantly improve patient outcomes and limit morbidity and mortality as much as possible.

The prediction models as a clinical decision-support tool would enable healthcare providers to identify the patients at significant risks associated with COVID-19 disease before the deterioration of their health condition (23). Determining the predictors related to patient ICU admission and identifying early the patients who will most likely benefit from increased care can assist in the effective allocation of limited hospital resources and optimization of patient management (4,5,7).

Chest CT is a common imaging method for diagnosing and screening patients with COVID-19, due to its high sensitivity to depict interstitial pneumonia (17,18). The severity of pulmonary involvements is significantly correlated with the severity of COVID-19, ICU admission, and patient mortality (16-19).

In the pioneering studies, ML-based prediction models were developed using demographics, clinical manifestations, and laboratory predictors (2,7,9-15).

There was a lack of imaging manifestations in ML prediction models to predict the ICU admission, and ICU LOS of COVID-19 patients. In our study, CT-SS was the most important predictor for the prediction of the likelihood of transferring COVID-19 patients to the ICU. ICU-admitted patients have higher CT-SSs; in other words, patients with higher CT-SS need more intensive care. There was a significant difference in the CT-SS index between the ICU-admitted and non-ICU-admitted patients ($p<0.001$). CT-SS was also the second most important feature to predict ICU LOS in COVID-19 patients. There is a statistically significant linear relationship between the CT-SS and ICU LOS of the patients ($r=0.257$, $p<0.001$).

These results showed that CT-SS is a strong predictor of ICU admission and ICU LOS of COVID-19 patients. Therefore, we evaluated the prognostic role of CT-SS in combination with demographics, clinical manifestations, and laboratory predictors to predict ICU admission and LOS of COVID-19 patients. In this study, we determined the most important features related to ICU admission and ICU LOS of COVID-19 patients. ML models for predicting ICU admission and ICU LOS of the patients were developed using these predictors. The results showed that k-NN yielded the best performance for predicting the ICU admission of COVID-19 patients. The sensitivity, specificity, accuracy, precision, F-measure, and AUC of the k-NN algorithm for predicting the ICU admission of COVID-19 patients were 98.1%, 83.5%, 90.8%, 85.6%, 91.4%, and 98.8%, respectively. For predicting the ICU LOS of COVID-19 patients, the RF algorithm, with a correlation coefficient of 0.42, mean absolute error of 2.01, and root mean squared error of 4.11, yielded better performance than other ML algorithms.

In previous studies, ML prediction models were also evaluated to predict ICU admission and ICU LOS of COVID-19 patients. Islam et al. (25) evaluated eight ML classifiers, including RF, SVM, k-NN, XGBoost, MLP, LR, extra trees, and gradient boosting models, for predicting ICU admission in patients with COVID-19 infection eight ML classifiers including RF, SVM, k-NN, XGBoost, MLP, LR, extra trees, and gradient boosting models were evaluated. In this study, a clinical dataset containing 156 positive COVID-19 patients was retrospectively reviewed. Results showed that RF, ET, k-NN, and LR were the four top ML models for predicting ICU admission for patients. The stacking model developed by integrating RF, ET, and k-NN algorithms with a sensitivity of 84.48%, specificity of 84.47%, overall accuracy of 84.48%, weighted precision of 84.45%, and F1-score of 83.64% yielded the best performance. The main limitation of this study was the number of parameters studied. In this study, only 9 parameters from clinical, radiological, and laboratory indices were examined, of which 5 were selected as the most important parameters in the feature selection step. These prediction models were developed using 5 features, including C-reactive protein, chest CT lung tissue affected (%), age, time between disease onset and hospital admission (days), and fibrinogen indices. In our study, 73 clinical features were examined, and the most relevant features from this dataset were used to develop predictive models. Utilizing this comprehensive dataset, we developed predictive models with improved performance.

In Shanbehzadeh et al. (7) study, ICU admission of COVID-19 patients was predicted using decision tree models including decision stump, Hoeffding tree, LMT, J48, RF, random tree, and REP-Tree algorithms. Twelve features, which included demographics, clinical manifestations, and laboratory predictors from the records of 512 COVID-19 patients, were used to develop ML models. The J48 algorithm, with a sensitivity of 92.4%, specificity of 65.9%, accuracy of 81.9%, F-score of 81.4%, and AUC of 84.5, had the best performance for estimating ICU admission of COVID-19 patients. Our results showed that the severity of pulmonary involvement in radiological images is one of the most relevant features for predicting the ICU admission and ICU LOS of COVID-19 patients. In our study, in addition to a more comprehensive dataset from demographics, comorbidities, clinical manifestations, and laboratory predictors, to develop ML models. Although the results of the Shanbehzadeh et al. (10) study were in close agreement with our findings, ML models with better classification performance were obtained in our study.

Saadatmand et al. (10) also evaluated ML approaches for predicting ICU admission, ICU LOS, and mortality at the ICU in COVID-19 patients (12). Demographic data, comorbidities, clinical features, and laboratory results from 956 patients admitted to the ICU was a registered dataset. RF with a sensitivity of 91%, specificity of 96%, accuracy of 93%, and AUC of 97.6% achieved the best result for predicting patient admission to the ICU. For predicting ICU LOS, the XGB model with a sensitivity of 88%, specificity of 40%, accuracy of 78%, and AUC score of 79.5% had the best performance. In this study, the mean ICU LOS was considered 7 days, and the performances of the developed models were checked to determine how effectively they could predict whether the patient will be hospitalized for more than 7 days or not. In our study, the prognostic performances of the ML models in predicting ICU LOS of the patients were evaluated.

These observations showed that ML prediction models can help in the early and accurate identification of critical patients who need intensive care. Timely prediction of ICU admission in COVID-19

patients would improve patient outcomes and lead to the optimal use of limited hospital resources. Our results showed that the ML approach utilizing a more comprehensive dataset including CT-SS could efficiently predict ICU admission and ICU LOS of COVID-19 patients.

Study Limitations

This study had some limitations that must be acknowledged. First, this study was performed in a retrospective manner, and consequently, ML prediction models for ICU admission and ICU LOS of COVID-19 patients were not evaluated prospectively. Second, this is a single-center study, and external validation of the ML prediction models requires future multi-center studies with a larger sample size.

CONCLUSION

In this study, the predictive performance of ML models for ICU admission and ICU LOS of COVID-19 patients were evaluated using a dataset including CTSS data. Our results showed that the ML approach fed by a more comprehensive dataset including CT-SS could efficiently predict ICU admission and ICU LOS of COVID-19 patients. Timely and accurate prediction of ICU admission and ICU LOS of COVID-19 patients at the time of admission improves patient outcomes and leads to the optimal use of limited hospital resources.

Ethics

Ethics Committee Approval: This article is extracted from a research project supported by Abadan University of Medical Sciences and all experimental protocols were approved by the Ethical Committee of Abadan University of Medical Sciences (approval number: IR.ABADANUMS.REC.1402.108, date:14.11.2023).

Informed Consent: Participation was voluntary and informed consent was obtained from all subjects and/or their legal guardians. Participants had the right to withdraw from the study at any time.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.S.Z., N.N., H.K., Concept: S.S.Z., H.K., Design: S.S.Z., H.K., Supervision: S.S.Z., H.K., Resources: H.K., Material: S.S.Z., H.K., Data Collection or Processing: S.S.Z., N.N., H.K., Analysis or Interpretation: S.S.Z., H.K., Literature Search: S.S.Z., N.N., H.K., Writing: S.S.Z., N.N., H.K., Critical Review: S.S.Z., N.N., H.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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Anxiety, Depression, and Post-Traumatic Stress Disorders in Pediatric Oncology Patients and Their Mothers

Çocuk Onkoloji Hastalarının ve Annelerinin Anksiyete, Depresyon ve Travma Sonrası Stres Bozukluğunun Değerlendirilmesi

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ABSTRACT

Objective: This prospective study aims to investigate the prevalence of anxiety, depression, and post-traumatic stress disorder (PTSD) in pediatric oncology patients and their mothers.

Methods: All patients (n=61) aged 8-18 years who were actively treated patients (ATP) and patients in remission (ReP) and their mothers (n=61) were recruited as the study group. The first control group for anxiety and depression consisted of healthy mothers and children (n=60). In contrast, the second control group for PTSD consisted of mothers and children who had experienced non-disease trauma, such as divorce, death of a parent, and loss of income (n=30). The questionnaires were administered to the children and mothers.

Results: There was no significant difference between ATP and ReP in depression, anxiety, or PTSD (p=0.35, p=0.56, p=0.20). The children of the patient group were significantly more depressed and anxious than the healthy controls (HCs) (p=0.001, p=0.005). ATP mothers were more anxious than ReP mothers (p=0.004), but there was no difference in depression and burnout between the two groups (p=0.09, p=0.526). Mothers in the patient group were more anxious and depressed than mothers in the HC (p<0.001). The patient group and their mothers showed more PTSD symptoms compared to the HCs with trauma (p<0.05).

Conclusion: Pediatric cancer is a significant stressor for both children and mothers. A combination of medical treatment with psychosocial support is imperative.

Keywords: Children, cancer, post-traumatic stress disorder, anxiety, mothers

ÖZ

Amaç: Bu kesitsel çalışmanın amacı, pediatrik onkoloji hastaları ve annelerinde anksiyete, depresyon ve travma sonrası stres bozukluğu (TSSB) yaygınlığını sağlıklı kontrollerle (SK) karşılaştırmalı olarak araştırmaktır.

Yöntemler: Aktif tedavi gören ve remisyondaki 8-18 yaş arası tüm hastalar (n=61) ve anneleri (n=61) çalışma grubu olarak alınmıştır. İki farklı karşılaştırma grubu oluşturulmuştur. İlk karşılaştırma grubu, sağlıklı kontrol grubu ve anneleridir. TSSB karşılaştırması için ikinci kontrol grubu ise, hastalık dışında travması olan çocuklar ve annelerinden (n=30) oluşturulmuştur. Anketler çocuklara ve annelere klinisyen eşliğinde ayrı ayrı uygulanmıştır.

Bulgular: Depresyon, anksiyete ve TSSB açısından aktif tedavi gören ve remisyonda izlenen hastalar arasında anlamlı bir fark saptanmamıştır. (p=0,35, p=0,56, p=0,20). Hasta grubunun çocukları, sağlıklı kontrol grubu çocuklarına göre anlamlı derecede daha depresif ve kaygılı bulunmuştur (p=0,001, p=0,005). Aktif tedavi gören hastaların anneleri sağlıklı kontrol grubu annelerine göre daha kaygılıdır (p=0,004) ancak depresyon açısından fark saptanmamıştır (p=0,09). Hasta grubunun anneleri sağlıklı kontrol grubunun annelerine göre daha kaygılı ve depresif bulunmuştur (p≤0,001). Aktif tedavi gören ve remisyonda izlenen çocuklar ve anneleri travma geçiren ikinci kontrol grubuna göre daha fazla TSSB belirtisi göstermiştir (p<0.05).

Sonuç: Bu bulgular, pediatrik kanserin hem çocuklarda hem de annelerde önemli bir stres faktörü olduğuna işaret etmektedir. Psikososyal destek tıbbi tedavi ile eş zamanlı uygulanmalıdır.

Anahtar Sözcükler: Çocuklar, kanser, travma sonrası stres bozukluğu, anksiyete, anneler

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INTRODUCTION

Although childhood cancer is rare, its incidence is increasing worldwide. Advances in early diagnosis and treatment have significantly improved survival rates, transforming childhood cancers from fatal diseases into chronic conditions. However, the psychological burden of cancer extends beyond the physical illness, affecting both pediatric patients and their caregivers. Studies indicate that children with cancer are at higher risk of developing anxiety, depression, and post-traumatic stress disorder (PTSD) due to the stress of diagnosis, intensive treatment protocols, and long-term hospitalizations (1,2). Parents (especially mothers) who are primary caregivers experience significant emotional distress. The constant fear of disease progression, the burden of caregiving, and disruptions in daily life contribute to heightened levels of anxiety and depression among parents (3). Previous research has shown that mothers of children undergoing cancer treatment report higher levels of psychological distress compared to those of healthy children, and these symptoms may persist even after the completion of treatment (4). Understanding the psychological impact of childhood cancer on both patients and their families is crucial for ensuring better treatment adherence and overall well-being. Mental health disorders that emerge during this period may affect the child's response to treatment and long-term recovery. Given the increasing survival rates, addressing the psychosocial needs of both children and caregivers has become a key aspect of pediatric oncology care (5). This study aims to evaluate and compare the levels of anxiety, depression, and PTSD symptoms in pediatric oncology patients and their mothers with those in a healthy control (HC) group. By distinguishing between patients undergoing active treatment and those in remission, the study seeks to explore the psychological burden associated with different phases of the illness trajectory, particularly in mothers. During the long-term follow-up of patients, different psychiatric symptoms in the cognitive and emotional domains may emerge. With the increase in cancer survival rates, improving the quality of life of patients and their families has become increasingly important. Early recognition and treatment of psychiatric disorders that may occur during this period are crucial for ensuring treatment compliance and long-term success (6). In this context, it is expected that children diagnosed with cancer will display higher levels of anxiety, depression, and PTSD symptoms than their healthy peers. Similarly, psychological distress in mothers is anticipated to vary depending on the treatment stage, with greater emotional burden observed during the active treatment phase. These findings aim to contribute to the growing body of literature emphasizing the importance of holistic care in pediatric oncology.

MATERIALS AND METHODS

The sample group of our study was randomly selected from patients aged 8-18 years, who were followed up in the pediatric oncology department with a diagnosis of malignancy. The distribution of disease types in the sample group is shown in Table 1. The actively treated patients (ATP) for at least 1 month, patients in remission (ReP) between 3 months and 60 months, as well as their mothers (n=122), were taken as the sample group. The sample group distribution is shown Table 2. During the follow-up visit at our institute or by phone, we contacted the participants and invited them to participate in the study with their family caregivers. As a control group, healthy children and their mothers (n=60) who applied to the general pediatric outpatient clinic, were compared in terms of anxiety and depression. For the comparison of PTSD, a group of healthy children and their mothers (n=30) who had experienced trauma from various causes were selected as the second control group. The ATP for at least 1 month, ReP between 3 months and 60 months, as well as their mothers (n=122), were taken as the sample group. We were contacted and were invited to participate in the study with their family caregivers. As a control group, healthy children and their mothers (n=60) who applied to the general pediatric outpatient clinic, were compared in terms of anxiety and depression. For the comparison of PTSD, a group of healthy children and their mothers (n=30) who had experienced trauma from various causes were selected as the second control group.

The ATP for at least 1 month, ReP between 3 months and 60 months, and their mothers (n=122) were taken as the sample group. We were contacted during the follow-up visit at our institute or by phone and were invited to participate in the study with their family caregivers.

As a control group, healthy children and their mothers (n=60) who applied to the general pediatric outpatient clinic were compared in terms of anxiety and depression. For the comparison of PTSD, a group of healthy children and their mothers (n=30) who have experienced trauma from various causes were selected as the second control group. The ATP for at least 1 month, ReP between 3 months and 60 months, and their mothers (n=122) were taken as the sample group. We were contacted during the follow-up visit at our institute or by phone and were invited to participate in the study with their family caregivers.

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A clinician performed a one-on-one interview with each member of the second control group to evaluate PTSD, while a questionnaire was used to measure anxiety and depression in the first control group. The demographic information was obtained via a retrospective file review, the hospital information management system database, and personal information forms from their mothers. The mothers participating in the study were given questionnaires including the Beck Depression Scale (7), Maslach Burnout Inventory MBI (8), Social Support Scale (9), Problem-Solving Inventory (10), and State-Trait Anxiety Inventory (STAI-I/STAI-II) (11). Children and adolescents were administered the Child Depression Inventory (12) and STAI-I/STAI-II for children (13) questionnaires, by their age groups. To assess PTSD symptoms, two scales were administered: the Clinician-Administered PTSD Scale (CAPS) (14) and the Clinician-Administered PTSD Scale for Children and Adolescents, CAPS-CA (15), were administered to the children and their mothers in separate rooms during one-to-one interviews, with a clinician. The exclusion criteria were unwillingness to participate in the study, voluntary withdrawal, duration of diagnosis or treatment less than 1 month, and a patient child or mother with mental retardation. Because there were only five (16.67%) relapsed patients in the

Table 1. Disease characteristics of the patient group

Diseases	Actively treated patients		Patients in remission	
	n	%	n	%
Hodgkin lymphoma	0	0	4	12.9
Non-Hodgkin's lymphoma	6	20.3	7	22.6
Diffuse pons glioma	1	3.3	0	0
Ewing Sarcoma	2	6.7	1	3.2
Germ cell testicular tumor	2	6.7	0	0
Glioblastoma multiforme	1	3.3	0	0
Alveolar soft part sarcoma	1	3.3	1	3.2
Intracranial germ cell tumor	0	0	1	3.2
Langerhans cell histiocytosis	2	6.7	1	3.2
Malignant mesenchymal tumor	1	3.3	0	0
Medulloblastoma	4	13.3	2	6.5
Nasopharyngeal carcinoma	1	3.3	2	6.5
Neuroblastoma	4	13.3	1	3.2
Osteosarcoma	1	3.3	1	3.2
Ovarian germ cell tumor	1	3.3	1	3.2
Rhabdomyosarcoma	1	3.3	3	9.7
Renal cell carcinoma	0	0	1	3.2
Sertoli Leydig cell tumor	1	3.3	0	0
Synovial sarcoma	0	0	2	6.5
Thyroid papillary carcinoma	0	0	1	3.2
Wilms tumor	1	3.3	2	6.5

Table 2. Sample group distribution

Study Center	Children	Mothers	Total number
ATP	30	30	60
ReP	31	31	62
HC	30	30	60
HC-trauma	15	15	30

ATP: Actively treated patients, ReP: Patients in remission, HC: Healthy control

study sample group, relapsed patients were excluded from further analyses as a statistically significant comparison could not be made. The study was conducted with the approval of the Ethics Committee of Gazi University Faculty of Medicine (number of documents: E.41646, research code number: 2017-108, number: 77082166-302.08.01 2017-108, date: 07.03.2017), Gazi University, Ankara, and in compliance with the Declaration of Helsinki (1951) by the World Medical Association. The verbal and informed consent of the parents was obtained before the questionnaire and after a detailed clarification of the study's objectives and conduct.

Statistical Analysis

Statistical evaluations were performed using the SPSS 21.0 IBM software package. Non-parametric tests were used in all evaluations. Descriptive analysis methods were used to evaluate socio-demographic data; the t-test was used for comparisons between groups; Mann Whitney U test and chi-square test were used if the groups constituting the sample were less than 30 people. The Pearson correlation method was used to examine the relationship between dependent variables. The significance level is set at below 0.005.

RESULTS

In this section, the results of the anxiety, depression, and PTSD scales administered to children with and without oncologic disease and their mothers will be presented. While there was no significant difference between the patient groups in terms of gender distribution (X^2=3.655, p=0.301), there was a significant difference in terms of age distribution (X^2=15.243, p<0.01). According to the post-hoc analysis, the mean age of the ReP (13.90±3.44) was significantly higher than the mean age of both ATP (11.67±3.49) and HC (10.77±2.75). There was no significant difference between ATP and ReP in terms of depression (p = 0.35), anxiety (p=0.56), and PTSD symptoms (p=0.20)(p=0.35, p=0.56, p=0.20). The patient group was found to be significantly more depressed and anxious than the HC (p=0.001, p=0.005). the patient group showed more trauma-related avoidance behavior and hyperarousal symptoms than the HCs with a history of trauma, and they experienced more current and lifelong trauma.

Mothers of ATP had higher anxiety than ReP (p=0.004), whereas there was no discernible difference between the two groups in terms of burnout (p=0.09) and depression (p=0.526). Mothers of the patient group were found to be more anxious, depressed, and exhausted than the mothers of HC (p=0.001, p<0.001). The patient group and their mothers had more present and lifetime PTSD symptoms than HC with a history of trauma (p<0.05). Sample group comparison of PTSD Scale Scores is shown in Table 3.

None of the control group mothers, who had a history of trauma while having HC, were diagnosed with PTSD. This suggests that illness trauma causes more post-traumatic stress symptoms and PTSD diagnoses than other traumatic events. It was found that as the social support level of the mothers in the patient group decreased, their depression, anxiety, emotional exhaustion, and total burnout levels increased; their current PTSD levels increased; and their problem-solving skills and personal achievement decreased. The patient group's depression scores did not significantly differ from those of their mothers (r=0.233, p=0.070); however, there was a positive correlation between the anxiety scores and the lifetime PTSD total scores (r=0.655, p<0.01; STAI 2-continuous anxiety, p<0.01; PTSD-Present Total, r=0.578, p<0.01). It was found that as the PTSD symptoms and anxiety of mothers increased, similar symptoms increased in their children.

DISCUSSION

The process of diagnosing, treating, and following up on cancer is exhausting and stressful for both the child and the family. Recent studies in the literature suggest that emotional, behavioral, and psychiatric disorders are common in cancer patients and that they should receive psychological support, at the same time as their diagnosis and treatment (16,17). In addition to the effects of medical care and medication, the relationship between family members plays a role in the success of the fight against childhood cancer. Studies in the literature suggest that in our study, it would be most useful to evaluate the patients and their mothers together (18). There are many studies in the literature comparing depression, anxiety, and PTSD in cancer patients at different time points with a HC (19-22). However, to our knowledge, no other studies have compared patients and their mothers during active treatment and remission. In our study, no significant difference was found between ATP and ReP in terms of depression, anxiety, and PTSD symptoms. In contrast, the patient group was found to be more depressed and anxious than HC, as reported in the literature (19-22). In our study, similar to the literature, we found that ATP and ReP had higher PTSD symptoms, compared to HC with a history of trauma (23). In the literature, unlike our study, some studies found no significant PTSD difference between cancer patients and HC (19,20). They defended this result by emphasizing that the adaptation mechanisms of cancer patients were more effective (19,20,24). As demonstrated in our study, the patient group has a higher risk of anxiety, depression, and PTSD compared to HC. Therefore, it has been suggested in the literature that cancer patients should receive psychological support at the same time as their diagnosis and treatment (22,25). A recent study confirmed that this support improves patient compliance, quality of life, and treatment success (26). Studies in the literature on caregivers of children with cancer have found severe emotional disturbance, anxiety, depression, health problems, and alcoholism in these families (23). In our study, patient group mothers were found to be more anxious, depressed, and exhausted than control group mothers, similar to what is reported in the literature (27). While no significant difference in depression was found between the mothers of ATP and ReP, higher levels of anxiety and PTSD were found in the mothers of ATP. These findings support our hypothesis that the depressive mood of mothers persists after the diagnosis of the disease and even during the remission period. In our study, similar to the literature, mothers with children diagnosed with cancer were found to be more depressed and anxious than mothers of HC (27). The results suggest that the course of the disease, the demanding treatment process, the duration of hospitalization, and the uncertainty about the future increase mothers' anxiety. However, in our results, persistent anxiety was also found in about half of the mothers of ReP. These findings suggest that mothers of cancer patients continue to be concerned about the possibility of a recurrence of the disease in their children. In our study, ATP mothers had more PTSD symptoms than ReP mothers, but there was no significant difference in burnout and depression. To our knowledge, there are no studies in the literature comparing anxiety, depression, and PTSD symptoms in mothers of ATP and mothers of ReP. In our study, when mothers of ill children and mothers of healthy children with a history of trauma were compared in terms of PTSD, they showed significantly more trauma-related avoidance behaviors, hyperarousal, and re-experiencing symptoms, similar to the literature (28,29). None of the control group mothers who experienced trauma was found to have PTSD symptoms. This suggests that illness trauma causes more PTSD symptoms than other traumatic events. Similar to the literature, a positive correlation was found between trait anxiety and lifetime post-traumatic stress symptoms in the patient group and their mothers. In contrast, there was no significant relationship in terms of depressive symptoms (19,28). It was thought that environmental factors besides mothers' emotional states might be effective in the development of depression in children. Our study, in line with previous research, found no correlation between children in the HC group and characteristics of their mothers. Still, it did find a positive correlation between lifetime PTSD among the mothers of the patient group and the patients themselves.

Table 3. Sample group comparison of PTSD Scale scores

Scales	Group	n	Rank mean	Average total	U	p
CAPS-CA child present total	ATP	25	22.96	551	181	0.373
	ReP	18	19.56	352		
CAPS-CA child lifetime total	ATP	25	20.36	509	184	0.312
	ReP	18	24.28	437		
CAPS-CA child present total	ReP	18	20.86	375.5	29.5**	**0.000**
	HC-trauma	13	9.27	120.5		
CAPS-CA child lifetime total	ReP	18	21.25	382.5	22.5**	**0.000**
	HC-trauma	13	8.73	113.5		
CAPS-CA adolescents present total	ATP	5	5	25	0	0.051
	HC-trauma	2	1.5	3		
CAPS-CA adolescents lifetime total	ATP	5	4.6	23	2	0.241
	HC-trauma	2	2.5	5		
CAPS-CA adolescents present total	ReP	13	9	117	0*	**0.027**
	HC-trauma	2	1.5	3		
CAPS-CA adolescents lifetime total	ReP	13	8.77	114	3	0.088
	HC-trauma	2	3	6		
CAPS-CA adolescents present total	ReP	13	9	117	0*	0.027
	HC-trauma	2	1.5	3		
CAPS-CA adolescents lifetime total	ReP	13	8.77	114	3	0.088
	HC-trauma	2	3	6		
CAPS-CA child present total	ATP	25	24.1	578.5	33.5***	**0.000**
	ReP	13	9.58	124.5		
CAPS-CA child lifetime total	ATP	25	24.32	608	42***	**0.000**
	ReP	13	10.23	133		
CAPS present total	ATP mothers	30	29.78	863.5	6.5**	**0.000**
	HC-trauma mothers	15	8.43	126.5		
CAPS lifetime total	ATP mothers	30	25.78	773.5	141.5*	**0.044**
	HC-trauma mothers	15	17.43	261.5		
CAPS present total	ReP mothers	31	30.06	932	29*	**0.000**
	HC-trauma mothers	15	9.93	149		
CAPS lifetime total	ReP mothers	31	28.31	877.5	83.5*	**0.000**
	HC-trauma mothers	15	13.57	203.5		

*p<.05, **p<.01, ***p<.001.
CAPS: Clinician-Administered Scale, CAPS-CA: Clinician-Administered Post-Traumatic Stress Disorder Scale for Children and Adolescents, ATP: Actively treated patients, ReP: Patients in remission, HC: Healthy control

(19,29). Studies support that psychological problems occurring in cancer patients and their caregivers negatively affect their compliance with treatment and quality of life. Furthermore, early recognition of psychological problems and simultaneous support increases compliance with treatment (22,30). When the results of our study are evaluated in relation to the literature, high levels of cancer-related anxiety and PTSD findings are observed in both survivors and the mothers of caregivers even years after the end of treatment (26). Therefore, we believe that child psychiatrists should intermittently evaluate patients with cancer to detect possible adjustment or mental problems related to the disease and its treatment. At the same time, the results of our study confirmed that the caregiver's mental problems triggered the child's cognitive problems and reduced the patient's adherence to treatment. This made us think caregivers should be referred to the psychiatry department at regular intervals, and evaluated by psychiatrists. Our study found an inverse and statistically significant relationship between social support and burnout. In our study, a

negative correlation was found between the social support levels of the mothers of the patient group and anxiety, depression, and PTSD and between the problem-solving skills of the mothers of the patient group and anxiety, depression, burnout levels, and post-traumatic stress symptoms, similar to the literature (31). The literature emphasizes the importance of problem-solving skills training for parents of children with chronic diseases (32).

Study Limitations

In this study, depression, anxiety, and PTSD, which are mental problems that may be caused by the disease and treatment of children with cancer, were examined at any time during the treatment period and remission period. We believe that it would be meaningful to recognize the changes in the symptoms of the patients by performing child psychiatry consultation at intermittent periods, such as the first, third, and sixth months of treatment from the time of diagnosis, and to recognize them early in terms of early psychiatric diagnosis and treatment.

Our study was limited to the pediatric oncology department service and the outpatient clinic of our hospital. It is thought that coping methods for psychosocial problems and psychiatric illnesses are significantly affected by upbringing, culture, and socioeconomic level. For this reason, collecting data from cities with different cultural characteristics may be more useful to reach a more general conclusion and develop guidelines on the subject.

CONCLUSION

Pediatric cancer treatment and follow-up are major stressors for both children and their mothers. Clinicians should be aware of the psychiatric symptoms of children with cancer and their caregivers during the challenging treatment process. Psychosocial support should be provided simultaneously with medical treatment.

Ethics

Ethics Committee Approval: The study was conducted with the approval of the Ethics Committee of Gazi University Faculty of Medicine (number of documents: E.41646, research code number: 2017-108, number: 77082166-302.08.01 2017-108, date: 07.03.2017).

Informed Consent: The verbal and informed consent of the parents was obtained before the questionnaire and after a detailed clarification of the study's objectives and conduct.

Footnotes

Authorship Contributions

Concept: E.Ö.Ö., A.O., E.G., E.G.S., F.G.P., Design: E.Ö.Ö., A.O., E.G., E.G.S., F.G.P., Data Collection or Processing: E.Ö.Ö., Analysis or Interpretation: E.Ö.Ö., E.G., E.G.S., Literature Search: E.Ö.Ö., A.O., E.G., Writing: E.Ö.Ö., A.O., E.G.,

Conflict of Interest: No conflict of interest was declared by the authors.

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Left Dominant Coronary Circulation is Associated with Poorer Left Ventricular Function but Not Long-Term Mortality After ST Elevation Myocardial Infarction

Sol Dominant Koroner Dolaşım, ST Elevasyonlu Miyokard Enfarktüsü Sonrası Azalmış Sol Ventrikül Fonksiyonu ile İlişkilidir, Ancak Uzun Dönem Mortalitede Farklılık Göstermemektedir

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ABSTRACT

Objective: To evaluate the effect of coronary dominance (CD) on the left ventricular systolic function in patients with first ST-elevational myocardial infarction (STEMI), and to evaluate the relationship between CD and long-term mortality.

Methods: We included 471 patients with first STEMI. The patients were categorized as right-dominant and left-dominant according to their CD pattern. The left ventricular Wall Motion Score Index (WMSI) and left ventricular ejection fraction (LVEF) were used to evaluate the extent of left ventricular systolic dysfunction. The COX regression regression analysis was used to assess the relationship between CD and long-term mortality.

Results: Left CD was present in 41 (8.7%) of the 471 patients. WMSI was significantly higher in the left dominant group than in the right dominant group (1.74±0.38 vs. 1.56±0.35, p=0.002). The frequency of LVEF <40% was significantly higher in patients with the left CD group than in the right CD group (39% vs. 15.8%, p<0.001). The patients with left CD had higher peak creatine kinase (CK) and CK-myocardial band levels (3269±2988 U/L vs. 2355±1511 U/L, p=0.007; 390±303 U/L vs. 241±172 U/L, p<0.001, respectively). Nevertheless, mortality was similar between the left and right dominance groups [13 (40.1%) vs. 85 (30.7%), p=0.201]. In COX regression analysis, CD was not related to long-term mortality.

Conclusion: Patients with left dominance had significantly lower left ventricular systolic function early after STEMI. However, long-term mortality was similar in patients with left and right dominant circulation.

Keywords: Coronary, angiography, circulation, myocardial infarction

ÖZ

Amaç: Bu çalışmada, ilk kez ST elevasyonlu miyokard enfarktüsü (STEMI) geçiren hastalarda koroner dominans (KD) tipinin sol ventrikül sistolik fonksiyonları üzerine etkisi ve uzun dönem mortalite ile ilişkisi değerlendirildi.

Gereç ve Yöntem: Çalışmaya ilk STEMI geçiren toplam 471 hasta dahil edildi. Hastalar koroner dominans tipine göre sağ dominant ve sol dominant olarak iki gruba ayrıldı. Sol ventrikül sistolik fonksiyonlarının değerlendirilmesinde sol ventrikül duvar hareket skor indeksi (WMSI) ve sol ventrikül ejeksiyon fraksiyonu (LVEF) kullanıldı. Uzun dönem mortalite ile koroner dominans arasındaki ilişki COX regresyon analizi ile araştırıldı.

Bulgular: Bulgular: Hastaların 41'inde (%8,7) sol dominant sirkülasyon mevcuttu. Sol dominant grupta WMSI değeri sağ dominant gruba göre anlamlı şekilde daha yüksekti (1,74±0,38 vs. 1,56±0,35, p=0,002). LVEF <%40 olan hasta oranı da sol dominant grupta belirgin şekilde daha fazlaydı (%39 vs. %15.8, p<0,001). Ayrıca, bu grupta pik kreatin kinaz (CK) ve CK-miyokardiyal bant seviyeleri anlamlı olarak daha yüksekti. Ancak, uzun dönem mortalite oranları iki grup arasında anlamlı fark göstermedi (%40,1 vs. %30,7, p=0,201). COX regresyon analizinde, koroner dominansın uzun dönem mortalite ile ilişkili olmadığı gösterildi.

Sonuç: Sol dominant koroner sirkülasyona sahip hastalarda STEMI sonrası sol ventrikül sistolik fonksiyonları daha belirgin bozulmuştur. Ancak, uzun dönem mortalite açısından sağ ve sol dominant gruplar arasında anlamlı fark bulunmamıştır.

Anahtar Sözcükler: Koroner, anjiyografi, dolaşım, miyokard enfarktüsü

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INTRODUCTION

The morbidity and mortality associated with acute myocardial infarction (MI) are strongly related to the amount of tissue necrosed during infarction (1,2). Global left ventricular function is related to the size of infarcted myocardium, and left ventricular ejection fraction (LVEF) is the most important predictor of mortality after acute MI (3,4). The extent of the myocardial necrosis was influenced by several angiographic features such as the presence of stenoses in other coronary arteries, the presence of collateral vessels, ischemic preconditioning, and the location of the culprit lesion in the involved artery (5,6). In addition, the quantity of myocardium supplied by the obstructed vessel is one of the principal determinants of infarct size (6,7).

There is considerable variation between patients with ischemic heart disease with respect to the pattern of coronary arterial distribution [so-called "coronary dominance" (CD)]. Previous coronary angiographic data have shown that approximately seventy-five percent of subjects undergoing coronary angiography had a right dominance pattern, 10% had left CD, and 15% of patients had a co-dominant (balanced) circulation (8). Although the left coronary artery supplies the majority of the left ventricular myocardium in patients with a right CD, all of the left ventricular myocardium was supplied by the left coronary artery in patients with left dominant circulation. Therefore, patients with a dominant left coronary arterial system may be at greater risk after a MI. However, previous studies demonstrated conflicting results about the effect of CD on mortality after MI. While some studies demonstrated a poor prognosis after MI in left dominant circulation, others revealed no effect of CD on prognosis after MI (9,10). Therefore, we aimed to evaluate the effect of CD on the left ventricular systolic function in patients with first ST-elevational myocardial infarction (STEMI), and the relationship between CD and long-term mortality.

MATERIALS AND METHODS

Patients with STEMI were prospectively included in the study. STEMI was defined by a typical chest pain at least 30 min, the presence of ST-segment elevation of ≥2 mm (1 mm =0.1 mV) in at least two contiguous leads, and by increased cardiac biomarkers concentrations above twice the upper normal values. Patients were excluded if they had non-ST elevation AMI, heart disease other than coronary artery disease, a history of revascularization procedures, or a poor acoustic window for performing transthoracic echocardiography. Patients who died at the index hospitalization were also excluded from the study. The study was approved by the Gazi University Faculty of Medicine Local Ethics Committee (approval number: 275, date: 21.11.2005) of our institution, and all patients gave written informed consent. Cardiovascular risk factors, time from pain onset to hospital admission, time from pain onset to revascularization, baseline complete blood count, creatinine levels, blood lipids, peak creatine kinase (CK), and CK-myocardial band (MB) levels were recorded. Revascularization methods (primary percutaneous coronary intervention (PCI), fibrinolytic treatment) were also recorded.

Echocardiography

Patients included in the study underwent complete echocardiographic examinations at a median of 2 days (25^{th} and 75^{th} percentiles 1-3 days) after admission. Echocardiographic studies were performed using a Vingmed CFM System Five (GE Medical, Horten, Norway) with a 2.5-MHz transducer and were recorded on digital media. Standard parasternal long- and short-axis, apical 4- and 2-chamber views were recorded in the left lateral position at rest. The left ventricle was analyzed according to the 16-segment model as proposed by the American Society of Echocardiography (11). Regional wall motion in each segment was graded visually, using a four-point scoring system: 1= normal, normal wall motion; 2= hypokinesia, marked decrease in endocardial motion; 3= akinesia, absence of inward wall motion; 4= dyskinesia, paradoxical wall motion away from the left ventricular lumen in systole. If more than two segments in the infarct zone or 4 or more of all 16 segments were not visualized, the study was considered inadequate, and these patients were excluded. The left ventricular Wall Motion Score Index (WMSI) and LVEF were used to evaluate the extent of left ventricular systolic dysfunction. WMSI was calculated by dividing the sum of the segmental scores by the number of segments visualized. The modified biplane Simpson method was used to measure LVEF. Severe left ventricular dysfunction was defined as LVEF <40%. All echocardiograms were analyzed 2 experienced observers who were blinded to the clinical and angiographic data.

Coronary Angiography

Coronary angiography was performed at a median of 1 day (25^{th} and 75^{th} percentiles 0-3 days) after admission. Coronary artery stenoses were estimated visually by two independent observers while blinded to the identity and clinical information of the patients. Single vessel disease was defined as greater than 50% diameter stenosis in only one coronary artery. Two- and three-vessel disease is defined according to the same criteria. Left main disease is regarded as two-vessel disease.

Patients were classified into 2 categories according to the type of coronary circulation: right dominance and left dominance. Right dominance was defined if the right coronary artery (RCA) gave off both a posterior descending artery and a branch that continued beyond the crux along the distal portion of the posterior atrioventricular groove, providing at least one posterolateral branch that supply the diaphragmatic surface of the left ventricle (12). Left dominance was defined as the RCA being very small, terminating before reaching the crux cordis, and off any branches to the left ventricle. In these cases, the posterior descending artery and all the posterolateral branches are provided by the distal circumflex (Cx) artery. Co-dominance or balanced circulation was defined as when the RCA gives rise to the posterior descending artery, with the Cx artery providing all the posterolateral branches. Patients with balanced circulation were included in the right dominant group. The location of the culprit lesion was determined from the coronary angiography.

Mortality Information

Survival data were extracted by searching the governmental electronic death notification system and hospital electronic health records. Survival data was extracted in May 2020. The follow-up time was calculated by subtracting the admission date from May 2020 or the time of death. The follow-up duration was expressed in months.

Statistical Analysis

SPSS 22.0 software for Windows was used to analyze the data. For continuous variables, the normality of distribution was tested using

the Kolmogorov-Smirnov test. The results were presented as mean ± standard deviation for variables with normal distribution and as median (interquartile range 25-75) for variables with abnormal distribution. For the comparison of the continuous variables between left CD and non-left CD patients, independent samples t-test or Mann-Whitney U test were used where appropriate. Categorical variables were analyzed using the chi-square test, or Fisher's exact test. The log rank test was used to detect univariate effects of the particular study variables on mortality. The Kaplan–Meier survival estimates were calculated. The cyclooxygenase (COX) regression analysis was used to assess the relationship between CD and long-term mortality. A p-value <0.05 was considered statistically significant.

RESULTS

Between November 2004 and February 2006, 471 patients with first STEMI were included in the study. The location of acute MI was anterior in 248 (52.7%) patients; inferior or lateral in 223 (47.3%) patients. Primary PCI was performed in 175 of the patients, and thrombolytic therapy was administered to 193 of them. Elective angiography was performed on 103 patients who either presented 12 hours after the onset of symptoms and had no pain. The infarct-related artery was the non-dominant RCA in 2 patients who presented with ST elevation in inferior leads. The clinical characteristics of patients with right and left dominance were summarized in Table 1.

Significant differences between groups were not found in age, gender, prevalence of cardiovascular risk factors, the use of reperfusion therapy, or time to reperfusion therapy. There were no significant differences in time to coronary angiography and time to echocardiography between the two groups.

Angiographic Findings

The angiographic findings are presented in Table 2. The left dominant circulation was present in 41 (8.7%) patients. The dominance of the RCA was present in 430 (91.3%) patients, and 70 (16.3%) of these patients had co-dominant (balanced) circulation. As expected, the patients with left dominance had a higher prevalence of left anterior descending (LAD) artery culprit location than the patients with right dominant circulation. The number of diseased vessels and culprit lesion location in the infarct artery was similar between patients with left and right dominant circulation. The prevalence of collaterals to the infarct artery was also similar among groups.

Laboratory and Echocardiographic Findings

The peak CK, CK-MB, LVEF, and WMSI are presented in Table 3. The WMSI was significantly higher in the left dominant group than in the right dominant group. The LVEF was significantly lower in the left dominant group than in the right dominant group. In addition, the frequency of LVEF <40% was significantly higher in patients with left dominant circulation than in right dominant circulation. Patients with left dominant circulation had significantly higher values of peak serum CK and CK-MB than patients with right dominant circulation. When the patients with right dominant circulation were sub-grouped as co-dominant (70 patients) and true right dominant circulation (360 patients), the LVEF and WMSI in patients with co-dominant circulation were similar to those of true right dominant circulation (LVEF, 48.4±8.2 vs. 49.1±9.5 p=0.586; WMSI, 1.56±0.36 vs. 1.56±0.35, p=0.986) and higher than those with left dominant circulation (LVEF, 48.4±8.2 vs. 45.1±11.1, p=0.080; WMSI, 1.56±0.36 vs. 1.74±0.38, p=0.017).

Table 1. Demographic and clinical characteristics of the study population

		Right dominant group	Left dominant group	p
Patients	n (%)	430 (91.3)	41 (8.7)	
Age (years)	n(%)	56.2±11.0	59.2±11	**0.095**
Gender (male)	n (%)	357 (83)	36 (87.8)	**0.431**
Family History	n (%)	103 (24.0)	7 (17.1)	**0.302**
Smoker	n(%)	278 (64.7)	23 (56.1)	**0.276**
Hypertension	n(%)	131 (30.5)	16 (39)	**0.258**
Diabetes	n (%)	78 (18.1)	6 (14.6)	**0.575**
Total cholesterol (mg/dL)		192±42	182±43	**0.142**
Creatinine (mg/dL)		1.07±0.50	1.04±0.22	**0.723**
Primary PCI or trombolytic therapy n (%)		339 (78.8)	29 (70.7)	**0.230**
Time from symptom onset (minutes)				
To hospital admission		149 (75-257)	195 (90-330)	**0.108**
To reperfusion therapy		165 (110-270)	210 (130-250)	**0.382**
Time to angiography (days) (median, 25^{th}-75^{th} percentile)		1 (0-3)	0 (0-4)	**0.449**
Time to echocardiography (days) (median, 25^{th}-75^{th} percentile)		2 (1-3)	2 (1-3)	**0.876**

Values are expressed as mean ± SD or number of patients (the percent value).
IHD: Ischemic heart disease, PCI: Percutaneous coronary intervention, TT: Thrombolytic therapy, CK: Creatine kinase, SD: Standard deviation

Table 2. Angiographic findings.

	Right dominant group n (%)	Left dominant group n (%)
	430 (91.3)	41 (8.7)
Vessel disease		
1	257 (59.8)	23 (56.1)
2	117 (27.2)	15 (36.6)
3	56 (13)	3 (7.3)
IRA		
LAD	219 (50.9)	31 (75.6)* p<0.001
RCA	175 (40.7)	
Cx	36 (8.4)	2 (4.9)
		8 (19.5)
TIMI 2/3 flow	361 (84)	34 (82.9)
Location of culprit lesion		
Proximal	112 (26)	10 (24.4)
Distal	318 (74)	31 (75.6)
Presence of collaterals to IRA	58 (13.5)	3 (7.3)

*p<0.001. Values are expressed as numbers of patients (percent value).
CAD: Coronary artery disease IRA: Infarct related artery, LAD: Left anterior descending, RCA: Right coronary artery, Cx: Circumflex

Table 3. Echocardiographic left ventricular systolic indexes and peak CK/CKMB values

	Right dominant group	Left dominant group	p
WMSI	1.56±0.35	1.74±0.38*	<0.05
LVEF, %	49±9.3	45.1±11.1*	<0.05
LVEF <40%	68 (15.8)	16 (39)**	<0.001
Peak CK (U/L)	2355±1511	3269±2988*	<0.05
Pik CK-MB (U/L)	241±172	390±303**	<0.001

*p<0.05, **p<0.001. Values are expressed as mean ± SD or numbers of patients (percent).
WMSI: Wall motion score index, LVEF: Left ventricular ejection fraction, CK: Creatine kinase, CK-MB: Creatine kinase-myocardial band, SD: Standard deviation

All-cause Mortality Findings

Survival information was available for 308 patients, but the mortality information for the remaining 163 patients was not available. Baseline demographic features, LVEF, WMSI, number of patients with LVEF <40%, peak CK, CK-MB levels, initial reperfusion strategy, and angiographic features of the followed-up patients were similar to patients lost to follow-up (Supplementary Table 1). Eighty-five deaths (30.7%) occurred in the right dominant group and 13 deaths (40.1%) occurred in the left dominant group. The COX regression model, including age, gender, hypertension, diabetes, receiving emergent reperfusion therapy, infarct-related artery, Gensini score, presence of depressed LV systolic functions, and CD variables, demonstrated that age and presence of depressed LV systolic functions were independently related to all-cause mortality. CD was not related to long-term all-cause mortality (Table 4).

Table 4. COX regression analysis for the very long-term mortality

	Exp(B)	95.0% CI for Exp. (B)		
		Lower	Upper	p
Age	1.055	1.034	1.076	<0.001
Gender (male)	0.655	0.386	1.113	0.118
Diabetes mellitus	1.051	0.590	1.874	0.866
Hypertension	0.959	0.612	1.501	0.854
Coronary dominance (left)	1.536	0.795	1.342	0.201
Receiving emergent reperfusion	0.848	0.535	1.118	0.481
IRA				0.425
RCA vs LAD	1.183	0.754	1.857	0.465
CX vs. LAD	0.676	0.295	1.546	0.353
EF <40%	1.800	1.069	3.031	0.027

CX: Circumflex artery, EF: Ejection fraction, IRA: Infarct related artery, LAD: Left anterior descending artery, RCA: Right coronary artery, CI: Confidence interval

DISCUSSION

The present study demonstrates that the pattern of coronary circulation is related to the left ventricular dysfunction after STEMI. Patients with left dominant coronary circulation had significantly decreased systolic function after STEMI, as evidenced by higher WMSI and lower LVEF. In addition, peak CK and CK-MB levels were increased in patients with left dominant circulation consistent with larger infarct sizes. Nevertheless, there was no impact of CD on long-term mortality. To the best of our knowledge, this study evaluates the effect of CD on mortality in patients with ST elevation MI.

The variability of the coronary artery distribution can be accepted as the most frequent variation of coronary circulation, and the determinants of the CD are thought to be multifactorial (13,14). The blood supply of the left ventricle myocardium is provided only by the left coronary artery in the left dominant circulation pattern. Therefore, the effect of ischemic insult on left ventricular functions and mortality after MI is thought to be different depending on coronary circulation patterns. In 741 patients with acute MI who presented with acute MI and underwent revascularization with primary PCI, Veltman et al. (15) found that LVEF was significantly lower in the early period (48 hours) in the group with left CD. At the 12-month follow-up, no difference was found between the coronary dominant circulation groups in terms of systolic functions. Unlike our results, early period WMSI was found to be similar between groups in this study. In the study of Hanboly et al. (16) which included 300 patients who underwent primary PCI after acute MI, significantly higher WMSI and lower LVEF values were found in the left dominant group in the early period. At the end of 3 months, there was no difference between the groups in terms of systolic functions (16). In our study, LVEF value was significantly lower in the left dominant group, and the number of patients with LVEF <40% was significantly higher. The infarct related artery was non-dominant RCA in 2 patients who were presented with ST elevation in inferior leads. These patients had right ventricular infarction and their left ventricular function was normal. We thought that these patients ought to be included in the study. If we excluded these patients from

the left dominant group, the difference between the groups' left ventricular systolic functions were more prominent.

Why do patients with left dominant circulation have more extensive necrosis after an AMI? The most reasonable explanation is that the entire left ventricular myocardium is supplied by two vessels instead of three vessels in patients with left dominant circulation. Therefore, the LAD or Cx coronary arteries supply blood to a larger mass of myocardium, and occlusion in the same site of the LAD or Cx coronary artery, results in a greater size of the myocardial necrosis in patients with left dominant circulation. In addition, coronary collaterals may also play a role. Coronary collaterals are protective in the presence of coronary artery stenosis and can reduce the extent of myocardial necrosis and contractile dysfunction following acute coronary occlusion. A wide variety of collateral pathways exist in patients with coronary artery disease. One of the major pathways of coronary collaterals is between the LAD and the RCA via septal branches; and another is between the Cx and the RCA via posterolateral branches. In the presence of the LAD or Cx occlusion, collateralization to the LAD or Cx artery from the RCA could be reduced in patients with left dominance than those patients with right dominance (17). This may, in part, contribute to more extensive necrosis in patients with left dominance circulation. In our study, the number of cases is very low for the assessment of the collateral vessel to an infarct-related artery. However, although not statistically significant, the patients with left dominant circulation had a lower prevalence of collaterals to the infarct-related artery.

Previous studies and our study consistently showed the association between CD and left ventricle systolic functions after MI, but the same conclusion cannot be made for mortality. The results of the short- and long-term cardiovascular outcomes demonstrate some discrepancies. Abu Assi et al. (18) examined the relationship between CD and prognosis in 767 patients who underwent primary PCI for STEMI. In the mean follow-up of 40 months, more deaths and reinfarctions occurred in the group with left dominant circulation (18). In the study conducted by Hanboly et al. (16) higher cardiac mortality, heart failure, non-fatal MI, revascularization, and stroke were observed in the group with left dominant coronary circulation in the hospital and in the 3-month period. Veltman et al. (10) published the longest follow-up study in 1131 patients presenting with acute MI. There was no difference between the two groups in terms of long-term mortality in a 5-year follow-up. The same study revealed an increased risk of all-cause death, cardiac death, and reinfarction in the left dominant group in the first month of the infarction. In our study, median follow-up duration was nearly 15 years, and we found that very long-term mortality was not associated with CD. In this study, we did not include STEMI patients who were dead at the index hospitalization, therefore we cannot draw conclusions regarding the effect of CD on early mortality. The aforementioned studies (15,16) demonstrated that although left ventricular systolic function was more depressed in the left dominant groups during the early phase of the infarction, this depression disappeared at the long-term follow-up. Both of the studies demonstrated that the significant difference in the left ventricle systolic functions between the right and left dominant groups disappeared during the follow-up. This can be an explanation for early increased mortality, but similar mortality at later stages, in the left dominant group.

Study Limitations

Compared to studies in the literature, our study had a relatively small sample size. The number of patients with available mortality data decreased further due to the lack of follow-up information for some patients. However, our follow-up period was extensive. In our study, only general mortality could be evaluated. Cardiac adverse events such as cardiovascular death and reinfarction were not known to occur. Our study sample was collected from cases of acute MI that underwent coronary angiography, and a selection bias might therefore exist because the CD pattern may be different in patients who did not undergo coronary angiography. We included only the patients with STEMI because the culprit lesion and infarct-related artery often are easily identified with coronary angiography after infarction. Therefore, our findings may not be generalizable to other presentations of coronary artery disease.

Conclusion

In conclusion, the pattern of coronary circulation is related to left ventricular dysfunction after STEMI. As a result, patients with left dominance had significantly lower left ventricular systolic function. However, long-term mortality was similar in patients left and right dominant circulation.

Ethics

Ethics Committee Approval: The study was approved by the Gazi University Faculty of Medicine Local Ethics Committee (approval number: 275, date: 21.11.2005) of our institution.

Informed Consent: All patients gave written informed consent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Concept: A.A., Design: A.A., Supervision: E.K., Material: Y.B.Ş., Data Collection or Processing: Y.B.Ş., Analysis or Interpretation: E.K., Literature Search: S.T., Writing: Y.B.Ş., Critical Review: E.K.

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Supplementary Table 1. Comparison of angiographic and clinical parameters between followed-up and lost to follow-up patients

	Total n (%) 471 (100)	**Lost follow up n (%) 163 (34,6)**	**Follow up n (%) 308(65,4)**	**p**
Vessel disease				
1	280 (59.4)	95 (58.3)	185 (60.1)	0.573
2	132 (28)	44 (27.0)	88 (28.6)	
3	59 (12.5)	24 (14.7)	35 (11.4)	
IRA				
LAD	250 (53.1)	75 (46.0)	175 (56.8)	0.077
RCA	177 (37.6)	72 (44.2)	105 (34.1)	
Cx	44 (9.3)	16 (9.8)	28 (9.1)	
TIMI 2/3 flow in IRA	395 (83.9)	133 (81.6)	262 (94.8)	0.429
Location of culprit lesion on IRA				
Proximal	122 (25.9)	42 (25.8)	80 (26.0)	0.961
Distal	349 (74.1)	121 (74.2)	228 (74.0)	
Presence of collaterals to IRA	61 (12.9)	25 (15.3)	36 (11.7)	0.227
Right dominancy	430 (91.3)	153 (93.3)	277 (89.9)	0.150
WMSI	1.58±0.36	1.58±0.38	1.57±0.34	0.918
LVEF, %	48.6±9.5	47.4±9.5	49.2±9.5	0.052
LVEF <40%	84 (17.8)	34 (20.9)	50 (16.2)	0.212
Peak CK (U/L)	2083 (1325-3235)	1975 (1201-3281)	2161 (1329-3269)	0.901
Peak CKMB (U/L)	222 (130-318)	232(120-306)	209 (131-307)	0.813

IRA: Infarct related artery, LAD: Left anterior descending, RCA: Right coronary artery, Cx: Circumflex, TIMI: Thrombolysis In Myocardial Infarction, WMSI: Wall Motion Score Index, LVEF: Left ventricular ejection fraction CK: Creatine kinase

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Do We Cause Dysphagia When Treating Spasmodic Dysphonia with Botox?

Spasmodik Disfoni'yi Botoksla Tedavi Ederken Disfajiye Neden Oluyor muyuz?

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ABSTRACT

Objective: Spasmodic dysphonia (SD) is a neurological movement disorder involving the laryngeal muscles. There are three main types: adductor, abductor, and mixed type. Adductor type is the most common and mixed type is the rarest. Botox, the gold standard in treatment, is applied to the affected muscle group according to the type of SD. Dysphagia often occurs as a side effect of botulinum toxin injection treatment in spasmodic dystonia. Dysphagia may sometimes be seen secondary to SD.

Methods: This study included 8 patients with adductor SD without dysphagia and 8 healthy subjects. The total number is 16. Swallowing evaluation of both groups was performed by fiberoptic endosopic evaluation of swallowing (FEES), electromyography (EMG) and ultrasound (US).

Results: Swallowing functions of patients with adductor SD were reevaluated after botox injection into the thyroarytenoid muscle. No significant difference was observed in both groups .

Conclusion: In our study, our patient group consisted of patients with SD without dysphagia, and dysphagia was not observed in patients evaluated with FEES, EMG and US after Botox.

Keywords: Swallowing/dysphagia, voice/dysphonia, laryngeal dystonia/tremor

ÖZ

Amaç: Spazmodik disfoni (SD) laringeal kasları içeren nörolojik bir hareket bozukluğudur. Üç ana tipi vardır: addüktör, abdüktör ve mikst tip. Addüktör tip en sık görülen, mikst tip ise en nadir görülen tiptir. Tedavide altın standart olan botoks, SD tipine göre etkilenen kas grubuna uygulanır. Spazmodik distonide yutma güçlüğü sıklıkla botulinum toksin enjeksiyon tedavisinin bir yan etkisi olarak ortaya çıkar. Disfaji bazen SD sekonder olarak da görülebilir.

Yöntemler: Bu çalışmaya disfajisi olmayan adduktor SD olan 8 hasta ve 8 sağlıklı birey dahil edildi. Toplam sayı 16'dır. Her iki grubun yutma değerlendirmesi fiberoptik endosopik yutma değerlendirmesi (FEES), elektromiyografi (EMG) ve ultrasonografi (USG) ile yapıldı.

Bulgular: Addüktör SD'li hastaların yutma fonksiyonları tiroaritenoid kasa botoks enjeksiyonundan sonra tekrar değerlendirildi. Her iki grupta da anlamlı bir fark gözlenmedi

Sonuç: Çalışmamızda hasta grubumuz disfajisi olmayan SD'li hastalardan oluşmakta olup, Botoks sonrası FEES, EMG ve USG ile değerlendirilen hastalarda disfaji gözlenmemiştir

Anahtar Sözcükler: Yutma/disfaji, ses/disfoni, laringeal distoni/tremor

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INTRODUCTION

Spasmodic dysphonia (SD) or laryngeal dystonia is a neurologic disorder characterized by involuntary intermittent contraction of intrinsic laryngeal muscles (1,2). According to the muscles involved, there are three forms of SD: the adductor, the abductor, and the mixed form. Adductor SD (ADSD) is characterized by an effortful and strained voice as if choking by contracting the muscles that provide adduction and blocking the airflow In ADSD, the cords do not close during phonation due to abductor paralysis, which is associated with dyspnea and aphonia (3,4). ADSD is the most common type in the clinic; mixed type is less common and it is difficult to diagnose (5).

The treatment of SD involves botulinum toxin (BT) injection into the thyroarytenoid muscle for the adductor type and the posterior cricoarytenoid muscle for the abductor type. This procedure is sometimes performed under electromyography (EMG) guidance. In some cases, surgical procedures and voice therapy may also be considered. Botox injection is the gold standard. Botox prevents muscle contractions by binding to acetylcholine receptors at the neuromuscular junction and inhibiting the release of acetylcholine. Surgical techniques include type 2 thyroplasty (6,7), myectomy (thyroarytenoid muscle) (8) and selective laryngeal denervation-reinnervation (9). These surgical procedures are used to treat for adductor type of SDVoice therapy is a part of the treatment, but its effectiveness is limited. It can be used in combination with other treatments.

Swallowing has some phases; it is a complex behavior that takes place with the work of oral, pharyngeal, and esophageal muscles (10,11). Dysphagia can be defined as the difficulty in the process that starts with chewing in the mouth and continues as food moves to the stomach. Oral dysphagia can be defined as any difficulty in the preparation of a bolus. Pharyngeal dysphagia may be caused by absence or delay of the swallowing reflex, and esophageal dysphagia may be caused by esophageal or sphincter disorder (10,12).

Dysphagia may also occur as a result of Botox injections administered for the treatment of spasmodic dystonia. One of the most common side effects after botox injection in cervical dystonias and spasmodic dysphonias is defined with an incidence rate ranging from 10% to 90% (13-15). The use of ultrasound (US) and EMG during injections can help reduce these side effects (14). Very few studies have evaluated swallowing before and after treatment (16-18). However, many studies have not focused extensively on dysphagia secondary to botox (19-21). The involuntary pharyngeal phase of swallowing is particularly affected, and premature leaking is observed with fluids. This condition, which can be treated with diet modifications, is often overlooked because of its transient nature.

No study has objectively and multimodally evaluated the effect of BT in on swallowing function in these patients. Therefore, we aimed to evaluate the swallowing functions in patients with spasmodic dystonia without dysphagia symptoms and to investigate the effect of BT treatment on the swallowing function.

MATERIAL AND METHODS

The University of Health Sciences Türkiye, Ankara Etlik City Hospital No. 1 Clinical Research Ethics Committee (decision number: AEŞH-EK1-2023-597, date: 04.10.2023) approved before the research began. We obtained informed consent from all participants.

Assessment Tools

First, demographic and disease characteristics were obtained from all participants. Then, fiberoptic endoscopic evaluation swallowing (FEES) electrophysiology, and ultrasonographic (USG) evaluation were performed to evaluate swallowing functions.

Demographic and disease characteristics: patients and volunteers, including age, gender and educational status, as well as dystonia duration of patients were recorded.

Swallowing evaluation: the following three assessment methods were used to evaluate the swallowing functions of the participants.

a) Fiberoptic Endoscopic Evaluation

b) Swallowing Electrophysiology

c) Ultrasonographic Evaluation

Flexible Fiberoptic Endoscopic Evaluation of Swallowing (FEES)

The most important and gold standard diagnostic methods for the diagnosis of dysphagia (OD) are FEES and videofluoroscopic swallowing study (VFSS) (22). The advantages of FEES are that it can be performed in any environment, including the patient's bedside; it has no radiation effect. Its disadvantages are that the passage cannot be seen clearly when the pharynx is closed during swallowing (22). Endoscopic evaluation of the patients was performed by the same specialist, with the patient in a sitting position, using a 3.4 mm diameter channelless fiberoptic nasopharyngoscope, light source, camera, monitor, and DVD recorder (Karl Storz GmbH & Co KG, Tuttlingen, Germany). Local anesthetics were not used during this test. Aspiration or penetration of water up to 100 milliliters was used to determine the residue. Yogurt was used as a semi-solid and biscuits were used as a solid. The findings were recorded and examined according to the Dzeiwas endoscopic evaluation protocol, to score the dysphagia levels of our patients between 1 and 6. Score 1 was considered “normal swallowing function”, while scores between 2 and 6 were considered “dysphagia” and were graded from minimal to severe (23).

Swallowing Electrophysiology

The physical medicine and rehabilitation specialist performed the electrophysiological evaluation with a 10-channel EMG device by Medelec Synergy (Oxford, England) (24,25). The motor components and muscles involved in swallowing are evaluated with sEMG. In particular, sEMG provides the amplitude, peak, and latency of muscle contraction, and magnitude and temporal parameters such as duration and frequency (26,27). During swallowing, the shMs between the mandible and the hyoid provide hyoid elevation, and these muscles have important roles in the pharyngeal phase of swallowing (26,28). The patients were asked to sit with their heads in a neutral position. An active disk electrode was placed on the submental muscles, a reference disk electrode was placed on the chin, and a laryngeal (piezoelectric) sensor was placed in the coniotomy area and fixed in place. The signals were recorded with a channel, filtered with a band-pass of 0.01-20 Hz. The first of the two deviations obtained with the piezoelectric sensor indicated the elevation of the larynx, and the second indicated the end of the pharyngeal reflex phase. The beginning of the first deviation is called “0”, while the beginning of the second deviation that indicates

the end of the pharyngeal reflex is called "2". The 0-2 interval is the time elapsed during the elevation and floating of the larynx. In other words, the 0-2 interval is the time that triggers the swallowing reflex. The time between the point (A) that is the beginning of the SM-EMG and the first point (0) that the swallowing reflex begins. The "A-0" time interval is the time between the voluntary contraction of the submental muscle complex and the triggering of the swallowing reflex, which provides the duration of the oral phase. The A-C time interval is recorded as the total oropharyngeal swallowing time , representing the period during which SM muscle activity is present.

Ultrasonographic (USG) Evaluation

USG is also used in the evaluation of swallowing (29). The evaluation includes grading the cross-sectional area (CSAs), thickness, contractility, and echogenicity of the muscle (30). The oral and shMs can be easily identified using USG, and these measurements can also be made during muscle movement, thus they can be used to evaluate muscle function (31).

All measurements were performed in the supine position. Real-time imaging and CSAs (geniohyoid and bilateral anterior digastric muscles) were acquired with an USG device (GE Logiq P5, General Electric, Korea) and a 7-12 MHz linear array transducer. The geniohyoid and anterior digastric muscles were measured while the patients were in a relaxed position with their tongue in the mouth. The distance between the mandible bone and the hyoid bone was measured, and the skin was marked one-third of the way behind the inferior border of the mandible. The transducer was placed in the coronal plane to measure the CSAs of the muscles.

Study Protocol

All subjects were assessed for swallowing. The pre-treatment results in the patient group and the healthy group were compared. Evaluation of parameters for patients was performed at 1 month after botulinum toxin administration. The impairment levels for the patient group were compared pre- and post-treatment.

Participants

A total of 16 subjects were included in the study, consisting of 8 patients with SDbut without dysphagia symptoms who were planned to undergo botulinum toxin injection into their thyroaritenoid muscles, and 8 healthy volunteers who were age- and gendermatched to these patients.

Subjects who had any metabolic/endocrine and progressive central or peripheral nervous system diseases, had a past surgical history, or were using drugs that can cause swallowing dysfunction were excluded from the study. In addition, patients with multifocal or segmental dystonia who described difficulty swallowing were also excluded from the study.

Botulinum Toxin Injection

In SD, involuntary closure of the vocal cords causes speech difficulties. The gold standard treatment for these patients is periodic injections of botulinum toxin A (BTX-A) into these muscles to prevent them from contracting involuntarily. In the adductor type, the improvement in voice after Botox is 8.0 to 15.1 weeks (32,34). Commonly reported side effects of BTX-A injections include a slightly breathy voice (25-35% of patients) and cough or dysphagia (especially with liquids), affecting 10% of patients. The dose and timing of the patient's next injection are determined by the patient's side effects (35).

In our study patients with adductor type SD were injected with 2.5 units of onabotulinum toxin Type A (Botox®) botulinum toxin into both thyroarytenoid muscles with EMG guidance. After treatment, patients' satisfaction was assessed using a Likert scale with options: "I am satisfied", "I am not satisfied", and "I am neither satisfied nor dissatisfied".

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences, version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Normality of the continuous variables was assessed by the Kolmogorov-Smirnov test. Descriptive statistics were shown as mean (SD: standard deviation) for continuous variables and frequencies (%) for nominal variables. Statistically significant differences in repeated measurements within the groups were evaluated with the Wilcoxon-Friedman tests. The Mann-Whitney U test was used for the significant differences between groups. The results were considered significant for $p<0.05$.

RESULTS

While 5 of the 16 (31.3%) patients were female, 11 (68.7 %)were male and the mean age was 45.12±7.28 years of study subjects (n=16). Distribution and comparison of demographic characteristics of subjects, according to the groups, are presented in Table 1.

The disease duration of the patients was 2.74 (SD=1.26) years. None of the subjects had dysphagia according to fiberoptic endoscopic evaluation. Comparison of electrophysiological and USG pre- and post-treatment evaluation results of groups is shown in Table 2 and Table 3.

Electrophysiologically, while the swallowing triggering reflex times (0-2) and total swallow durations (A-C) of patients were longer than those of healthy individuals ($p<0.05$), the oral phase time was similar ($p>0.05$). Moreover, the geniohyoid muscle area was larger than that of the healthy group ($p=0.023$).

It was seen that the swallowing triggering reflex time and total swallow durations of patients were similar to that of healthy individuals during the first month of follow-up after Botulinum toxin

Table 1. Demographic characteristics of subjects according to groups

	Patient group	Healthy group	p
Age (years) mean (SD)	47.26 (7.19)	42.60±8.94	0.187
Gender n (%)			
Male	6 (75)	5 (63.5)	0.271
Female	2 (25)	3 (37.5)	
Educational status n (%)			
5 years	1 (12.5)	0	
8 years	1 (12.5)	3 (37.5)	
11 years	2 (25)	1 (12.5)	0.094
More than 11 years	4 (50)	4 (50)	

SD: Standard deviation

Table 2. Electrophysiological and ultrasonographic pre-treatment evaluation results of subjects

	Patient group mean (SD)	Healthy group mean (SD)	p
Swallowing intervals (msn)			
0-2 interval	525.20 (96.23)	317.28 (86.22)	**0.011**
A-0 interval	175.01 (76.31)	168.14 (44.71)	0.537
A-C interval	681.52 (104.14)	487.43 (113.65)	**0.007**
Anterior digastric (cm^2)			
Right	0.99 (0.34)	0.97 (0.25)	0.762
Left	0.97 (0.41)	0.91 (0.89)	0.613
Geniohyoid (cm^2)	1.97 (0.85)	1.42 (0.21)	**0.023**

SD: Standard deviation

Table 3. Electrophysiological and ultrasonographic post-treatment evaluation results of subjects

	Patient group (post-treament) mean (SD)	Healthy group mean (SD)	p
Swallowing intervals (msn)			
0-2 interval	401.14 (87.62)	317.28 (86.22)	0.078
A-0 interval	173.15 (64.98)	168.14 (44.71)	0.619
A-C interval	513.87 (79.33)	487.43 (113.65)	0.092
Anterior digastric (cm^2)			
Right	0.98 (0.47)	0.97 (0.25)	0.915
Left	0.96 (0.72)	0.91 (0.89)	0.261
Geniohyoid (cm^2)	1.95 (0.23)	1.42 (0.21)	**0.028**

SD: Standard deviation

application. After treatment, all patients were satisfied (n=8, 100%). Moreover, 5 patients (62.5%) said that it was easier to eat solid foods than before. Also, none of the patients had aspiration findings.

DISCUSSION

Botox injection in SD was first performed by Blitzer et al. (36) in 1984. Dysphagia may be observed after BTX injection, and its frequency is between 10-90% (15,37,38), and this side effect can be reduced by performing BTX injection with EMG and USG guidance (38). In some studies, no signs of dysphagia were detected when the swallowing evaluation was compared before and after treatment (16,17,18). Alterations in swallowing, such as the presence of food in the epiglottic vallecula due to delayed swallowing reflex, have been described as changes in the pharyngeal phase of swallowing in these patients before treatment (39-41). In our study, this was not detected in the swallowing evaluation, and patients without dysphagia were included to show whether Botox has side effects.

In the literature, dysphagia can be seen in patients with cervical dystonia before and after Botox treatment or surgery. This dysphagia has been explained by two different mechanisms. One of these mechanisms is the abnormal position of the neck, which causes anatomical asymmetry in swallowing, in cervical dystonia (42-45). However, this interpretation does not explain dysphagia in spasmodic dystonias without abnormal neck movements and in some oromandibular dystonias. The second possibility is neurogenic dysfunction, which causes delayed swallowing and other oropharyngeal findings (42-45). Since our patient group consists of patients with adductor type spasmodic dysphonia, there is no anatomical asymmetry.

FEES described by Langmore et al. (46) is the gold standard for swallowing evaluation. FEES and videofluoroscopic swallowing evaluation are crucial methods used in the diagnosis of oropharyngeal dysphagia (OD) (22). FEES has many advantages, including no radiation exposure, portability, applicability to neurological patients with limited mobility at the bedside, and visual monitoring of swallowing, salivation, and residual food transit. Although FEES was initially developed by a speech and language pathologist, it is very commonly performed by healthcare professionals (22,23,46). In the present study, all patients were evaluated with FEES before and after Botox injection, and no signs of dysphagia were detected in any evaluation' or 'In the present study, all patients were evaluated with FEES before and after Botox injection. No signs of dysphagia were detected in any evaluation. However, a multidisciplinary approach is essential for the evaluation of dysphagia, including not only FEES but, also videofluoroscopic swallowing evaluation, EMG, and USG.

Some studies have examined the utility of surface EMG (sEMG) of the submental muscles in swallowing rehabilitation. In particular, sEMG has been used to assess swallowing and to examine hyolaryngeal elevation, pre- and post-swallow muscle contraction, and its duration (47-50). sEMG is a valid and reliable method for assessing normal swallowing (51). sEMG is a non-invasive tool for assessing specific aspects of the complex muscle activity involved in swallowing. sEMG is simple and reliable to perform (52,53). There are SEMG studies in the literature on neck muscle activity during squeezing or chewing in patients with normal swallowing and temporomandibular disorders. However, fewer studies have examined the SEMG behavior of neck muscles during swallowing (54-58). It records electrical activity from the anterior digastric muscle and suprahyoid area muscles (i.e., geniohyoid and mylohyoid) (54). The most important things for swallowing are sEMG findings showing hyoid elevation in the anterior compartment and contraction of the submental muscles (49). In the literature, swallowing time varies between 0.80 and 1.60 seconds (55,56). This time does not change from age 12 to age 70. After the age of 70, swallowing time increases significantly (51,52,57,58). In the study by C. Ertekin et al. (17) prolonged SM muscle complex activity during swallowing (68%) was observed. Prolonged laryngeal displacement was observed in 42% of patients with cervical dystonia, while decreased SM muscle activity was observed in 31%. These two findings are also seen in Parkinson's disease (17).

In the present study, before Botox, the patients' swallowing reflex time (0-2) and total swallowing time (A-C) were significantly longer than those of healthy individuals ($p<0.05$), while oral phase times were similar ($p>0.05$).' or 'In the present study, the swallowing reflex duration (0-2) and total swallowing time (A-C) of patients before Botox were significantly longer than those of healthy individuals ($p<0.05$), and the oral phase durations were similar ($p>0.05$).

In the first month after botulinum toxin injection, it was seen that, patients' swallowing reflex time and total swallowing time were similar to healthy individuals. All patients were satisfied after treatment (n=8, 100%). In addition, 5 patients (62.5%) said that eating solid foods was easier than before. Moreover, none of the patients had any signs of aspiration. In this study, the selection of patients with SD affects the results.

Coordinated contraction of the suprahyoid muscle (shM) complex, which includes the digastric, mylohyoid, and geniohyoid muscles, causes displacement of the hyoid bone and promotes bolus propulsion into the esophagus. Most studies have evaluated the thickness, CSAs, and echo density of the tongue or other swallowing muscles (digastric, geniohyoid, and mylohyoid) when evaluating dysphagia with USG (59-62). During swallowing, the shM contracts and a change in thickness and upward movement occur. The severity of dysphagia depends on the difference in the displacement of these muscles which play an important role in the pharyngeal phase of swallowing (63-65). In some studies, the shM complex and displacement of stroke, ALS, MG (64-70), and inflammatory myopathy, whose dysphagia was assessed with VFSS, were further evaluated with USG. It was observed that the findings from the USG indicating the severity of dysphagia were correlated (71). In our study, no significant difference was found between patients with SD and the healthy group in terms of USG evaluation. Although there was no statistically significant difference, it was observed that the geniohyoid muscle was larger in patients with spasmodic dysphonia, than in the healthy group'. If a sentence or statement is unclear, consider rephrasing it for clarity.It seems that there is a statistically significant difference in the geniohyoid muscle, in the results section.

Dysphagia may occur in patients with SD due to muscle involvement. Some studies have shown that this symptom can also develop secondary to Botox injection, which is the gold standard treatment. Although our patient group consisted of individuals, no complaints were observed after botox administration.

Study Limitations

The number of patients in our study is quite limited and in a larger group of patients this study would give more acceptable results. In addition, the study would be more comprehensive if videofluroscopic swallowing evaluation was performed in patients evaluated with FEES.

CONCLUSION

Even if they do not describe symptoms of swallowing dysfunction, their swallowing function may still be affected when compared to healthy individuals. Our patient group did not complain of dysphagia. FEES, EMG, and USG evaluation revealed no findings related to swallowing dysfunction. In addition, no change was observed in swallowing functions after botulinum toxin injection.

Ethics

Ethics Committee Approval: The University of Health Sciences Türkiye, Ankara Etlik City Hospital No. 1 Clinical Research Ethics Committee (decision number: AEŞH-EK1-2023-597, date: 04.10.2023) approved before the research began.

Informed Consent: We obtained informed consent from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.A., E.M.T., Concept: E.A., E.K.U., E.M.T., Design: E.A., E.K.U., Data Collection or Processing: E.A., E.B., E.K.U., E.M.T., Analysis or Interpretation: E.A., E.B., E.K.U., E.M.T., Literature Search: E.A., E.B., E.K.U., E.M.T., Writing: E.A., E.B., E.K.U., E.M.T.

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Theory of Planned Behavior (TPB) Explaining Late Presentation of Breast Cancer in the West Coast of Sabah: A Structural Equation Modelling Approach

Planlı Davranış Teorisi (TPB) Meme Kanserinin Geç Sunumunu Açıklıyor: Yapısal Eşitlik Modelleme Yaklaşımı

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ABSTRACT

Objective: Early detection potentially reduces mortality rates, yet instances of delayed treatment-seeking after symptom onset have been observed, posing significant risks, as late presentation escalates mortality rates among patients. This study aims to delineate the sociodemographic profile of late-presenting breast cancer patients and investigate the Theory of Planned Behavior's (TPB's) (attitude, subjective norm and perceived behavioural control) on intention to seek medical consultation after breast cancer symptoms, that contributing to delayed presentation.

Methods: This cross-sectional study spanned from January 2022 to December 2022, and 111 eligible participants were selected for inclusion through simple random sampling. Researchers obtained written consent and offered assurances of confidentiality. Ethical approval was granted by the Medical Research and Ethics Committee (MREC) under the Ministry of Health Malaysia (MOH). The questionnaire encompassed socio-demographic data, clinical information, and the TPB constructs. SPSS AMOS version 22 facilitated Structural Equation Modeling for data analysis. Demographic variables and the TPB constructs were integrated into the model.

Results: Participants predominantly fell into the 40-49 and 50-59 age groups (36.9% and 35.1% respectively), were mostly married (78.4%), and had secondary school education (46.8%). The majority belonged to the B40 economic group (78.4%) and resided within 25 km of the hospital (41.4%). Stage II cancer was found to be prevalent during diagnosis (43.2%) and mostly presented 3-6 months after experiencing

ÖZ

Amaç: Erken teşhis, potansiyel olarak ölüm oranlarını azaltabilir; ancak semptomların başlamasından sonra tedavi arayışında gecikmeler gözlemlenmiştir. Bu gecikmeler önemli riskler teşkil eder, çünkü geç başvuru hasta ölümlerini artırmaktadır. Bu çalışma, geç başvuran meme kanseri hastalarının sosyodemografik profilini tanımlamayı ve planlanmış davranış teorisi'nin (PDT) bu gecikmeli başvurular üzerindeki etkisini araştırmayı amaçlamaktadır.

Yöntemler: Kesitsel bu çalışma Ocak 2022 ile Aralık 2022 arasında yürütülmüştür ve basit rastgele örnekleme yöntemiyle 111 uygun katılımcı seçilmiştir. Araştırmacılar yazılı onam almış ve gizlilik güvencesi sağlamıştır. Etik onay, Malezya Sağlık Bakanlığı'na (MOH) bağlı Tıbbi Araştırma ve Etik Komitesi (MREC) tarafından verilmiştir. Anket, sosyodemografik veriler, klinik bilgiler ve PDT yapılarını içermektedir. Veri analizi için SPSS AMOS sürüm 22 kullanılarak Yapısal Eşitlik Modellemesi uygulanmıştır. Demografik değişkenler ve TPB yapıları modele entegre edilmiştir.

Bulgular: Katılımcıların çoğu 40-49 ve 50-59 yaş gruplarındaydı (sırasıyla %36.9 ve %35.1), büyük kısmı evliydi (%78.4) ve lise eğitimi almıştı (%46.8). Katılımcıların çoğu B40 ekonomik grubuna aitti (%78.4) ve hastaneye 25 km mesafede ikamet etmekteydi (%41.4). Tanı konulduğunda genellikle Evre II kanser mevcuttu (%43.2) ve semptomların görülmesinden 3-6 ay sonra başvuru yapılmıştı (%81.1). Yapısal model makul bir uyum gösterdi. Tutum, niyeti anlamlı şekilde etkiledi (β1=0.844, p<0.001); ardından algılanan davranış kontrolü

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the symptoms (81.1%). The structural model indicated a reasonable fit. Attitude significantly influenced intention (β1=0.844, p<0.001), followed by perceived behavioural control (β2=0.178, p=0.004). Subjective norm did not significantly affect intention (β2=0.088, p=0.199), suggesting a negative influence on patients' intention to seek medical consultation.

Conclusion: Interventions enhancing subjective norm efficacy are recommended, emphasising partner, family members, peers, physician, and media involvement.

Keywords: Breast cancer, late presentation, theory of planned behaviour

geldi (β2=0.178, p=0.004). Öznel normun niyet üzerinde anlamlı bir etkisi yoktu (β2=0.088, p=0.199), bu da hastaların tıbbi danışmanlık arama niyeti üzerinde olumsuz bir etkisi olduğunu göstermektedir.

Sonuç: Partner, aile üyeleri, arkadaşlar, hekimler ve medyanın katılımını vurgulayan, öznel normun etkinliğini artıran müdahalelerin önerilmesi gerekmektedir.

Keywords: Meme kanseri, geç başvuru, planlanmış davranış teorisi

INTRODUCTION

Breast cancer stands as the most prevalent malignancy among women globally, with over two million new cases diagnosed worldwide in 2018 (1). Efforts to control breast cancer incidence encompass prevention, early detection, diagnosis, and treatment. Although early detection potentially reduces mortality, many patients delay seeking treatment after symptom onset, posing significant risks. Late presentation of breast cancer significantly escalates mortality rates among patients (2). Patient delay in seeking medical consultation refers to the interval between symptom awareness and initial medical intervention. Most studies adopt a 3-month threshold to distinguish early from delayed presentation, as delays exceeding 3 months significantly worsen survival rates (3-5). The causes of delay is a result of the interplay with the patient's socio-cultural context, individual characteristics that influence symptom interpretation and decision-making, interaction with the social network and types of support obtained, and aspects of the local health services (6).

Understanding factors influencing delays is essential for developing effective strategies. Therefore, patient behaviour should be considered in assessing late presentation. An explicit model of human emotions, cognitions, and behaviour is necessary to evaluate psychosocial risk factors (5). Proposed by Ajzen, the Theory of Planned Behaviour (TPB) has been utilised in numerous health studies, particularly in breast cancer research. According to this theory, an attitude, subjective norm, and perceived behavioural control (PBC) shape behavioural intention, ultimately guiding subsequent actions (7-9). Attitude reflects beliefs toward a specific behaviour, subjective norm involves beliefs regarding approval or disapproval of key referents, and PBC pertains to the extent to which an individual perceives a behaviour as easy or difficult to perform. These variables are crucial in determining patient intention to perform certain behaviours. This study employs the structural equation modelling (SEM) based on the TPB to examine patients' behavioural intention to seek medical consultation after experiencing breast cancer symptoms.

The study seeks to identify sociodemographic data on of late-presenting breast cancer patients and to investigate the impact the of TPB components on the late presentation in of breast cancer patients.

MATERIAL AND METHODS

Participant and Procedure

This cross-sectional study was executed from January 2022 to December 2022 in three phases. Initially, patient data regarding late presentation were sourced from hospital breast cancer records. Subsequently, 111 patients who fulfilled the inclusion criteria were selected as participants via random sampling. In the final stage, the researcher or representative conversed with the patients during follow-up visits to the clinic or via phone calls after finalising the patient list. Written consent was obtained from all those listed, and assurances were given regarding patient confidentiality. Ethical approval was secured from the Medical Research and Ethics Committee (MREC) (approval number: NMRR-20-32-52564 (IIR), date: 27.02.2020), Ministry of Health Malaysia (MOH). Approval and data collection has been extended until 2022 due to the pandemic of Covid-19.

Questionnaire Development

The questionnaire comprised three sections: socio-demographic information, clinical data, and the TPB construct questionnaire. The TPB construct questionnaire was adapted from prior studies (10,11). Validation and reliability of the questionnaire was done in accordance with the TPB founders' methodology (12). Content clarity and appropriateness were reviewed by two expert committees experienced in the TPB questionnaire development. Translation and backward translation were conducted by Universiti Malaysia Sabah Translation and Editing Unit and an independent translator. A pilot survey confirmed the instrument's overall reliability, with by Cronbach's alpha >0.8, indicating adequate internal consistency. Additionally, reliability coefficients for attitude, subjective norm, PBC, and intention were 0.977, 0.826, 0.870, and 0.922, respectively. The participants' responses were systematically recorded using a five-point Likert scale to ensure consistency in data collection.

Statistical Analysis

The SEM was conducted using the Analysis of Moment Structure (AMOS) software, version 22, for data analysis. Demographic variables and the TPB constructs were entered into the model and analysed. SEM is widely regarded as the optimal method for simultaneous assessment of overall model fit, individual parameter

estimates, regression coefficients comparisons, and the examination of variances within and across multiple groups (13).

RESULTS

Sample Characteristics

Table 1 presents the sample characteristics. The majority of participants fell into the age groups of 40-49 and 50-59 years, accounting for 36.9% and 35.1%, respectively. Most patients were married (78.4%), and the highest proportion had a secondary school educational level (46.8%), with a significant portion being housewives (48.6%). The majority of patients belonged to the B40 economic status (78.4%), followed by M40 (19.8%) and T20 (1.8%). Approximately 41.4% of patients resided within a distance of less than 25 km from the hospital. The distribution of patients by cancer stage was as follows: stage II (43%), stage III (33%), stage I (16%), and stage IV (8%). About 97% of the patients presented with self-discovered symptoms of breast cancer, and the most frequent initial symptom was a breast lump (83%), while few had other symptoms. 20.9% of the patients had a family history of breast cancer. Despite having listed one or more reasons to delay seeking medical consultation, most patients (81%) reported presenting within 3-6 months of discovering their symptoms, 5% reported presenting between 6 months to 1 year, and 14% delayed their presentation for one year or more.

Structural Equation Modelling (SEM)

This study employed the SEM approach to examine the proposed model's relationships, following two-stage model-building process: (i) measurement model, and (ii) structural model. In the proposed model, three constructs (attitude, subjective norm, and PBC were specified as exogenous variables, while the endogenous variable was the patients' intention to seek medical consultation after experiencing breast cancer symptoms.

Evaluation of the Measurement Model

The measurement model was assessed for reliability, convergent validity, and discriminant validity of the construct measures. According to Hair et al. (13), a good model fit is indicated by a comparative fit index (CFI) above 0.9, a chi-square normalised by degrees of freedom (χ^2/df) below 3, and a root mean squared error of approximation (RMSEA) below 0.08. The obtained model (Table 2) demonstrated a good fit based on the main goodness-of-fit indices: χ^2/df =1.573, CFI =0.984, GFI =0.914, normed fit index =0.958, and RMSEA =0.072.

Internal Reliability

Cronbach's alpha and composite reliability were used to assess internal reliability. Internal reliability of a measurement construct is deemed adequate when both Cronbach's alpha and composite reliability values exceed the recommended threshold of 0.70, indicating consistent and reliable measurement of the underlying latent variable. As shown in Table 3, both Cronbach's alpha and composite reliability values exceeded the recommended threshold of 0.70, indicating a high level of internal consistency among the indicators.

Table 1. Demographic profile and clinical characteristics of the patients (n=111)

		Frequency	**Percent**
Age	<30 years	3	2.7
	30-39 years	16	14.4
	40-49 years	41	36.9
	50-59 years	39	35.1
	60-69 years	12	10.8
Marital status	Married	87	78.4
	Single	12	10.8
	Divorcee	12	10.8
Ethnicity	KadazanDusun	39	35.1
	Bajau	15	13.5
	Chinese	12	10.8
	Melayu	16	14.4
	Brunei	8	7.2
	Others	21	18.9
Level of education	No formal education	8	7.2
	Primary	10	9.0
	Secondary	52	46.8
	Graduate	31	27.9
	Postgraduate	10	9.0
Occupation	Housewife	54	48.6
	Self-employed	8	7.2
	Government staff	40	35.9
	Private staff	9	8.1
Economic status	B40	87	78.4
	M40	22	19.8
	T20	2	1.8
Distance from the hospital	<25 km	46	41.4
	26-50 km	32	28.8
	51-100 km	13	11.7
	101-150 km	11	9.9
	151 km or more	9	8.1
	Total	**110**	**100.0**
		Frequency	**Percent**
Stages of cancer (during detection)	I	18	16.2
	II	48	43.2
	III	37	33.3
	IV	8	7.2
Time of presentation	3–6 months	90	81.1
	6 months–1 year	7	6.3
	> 1 year	14	12.6
	Total	**111**	**100.0**

Table 2. Goodness-of-fit for a structural model

	χ2	df	χ2/df	CFI	GFI	NFI	RMSEA	PNFI	PCFI
Recommended values*	N/A	N/A	<3	>0.9	>0.9	>0.9	<0.08	>0.5	>0.5
Model values	58.209	37	1.573	0.984	0.914	0.958	0.072	0.644	0.662

*Suki, 2014, χ^2/df, degrees of freedom.
CFI: Comparative Fit Index, GFI: Goodness-of-Fit Index, NFI: Normed Fit Index, RMSEA: Root mean square error of approximation, PNFI: Parsimony-Adjusted Normed Fit Index, PCFI: Parsimony-Adjusted Comparative Fit Index

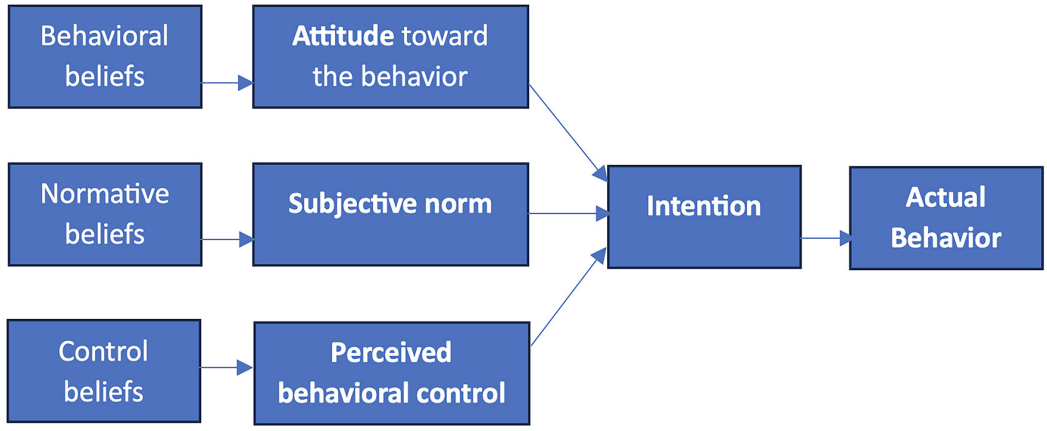

Figure 1. Theory of planned behavior Ajzen (7).

Convergent Validity

Convergent validity was assessed through standardised loading items, composite reliability, and average variances extracted (AVE). Convergent validity is established when standardised loadings and composite reliabilities exceed 0.700, and the AVE is greater than 0.500. Table 3 demonstrated that both standardised loadings and composite reliabilities exceed 0.700, Moreover, the AVE was above 0.500 in all instances, confirming successful convergent validity.

Discriminant Validity

Discriminant validity was assessed by comparing the shared variance between constructs with the square root of the AVE for each construct. Discriminant validity is established when the square root of the AVE for each construct exceeds the corresponding inter-construct correlations (shared variances). The correlation matrix revealed that the square root of the AVE for each construct was greater than the absolute value of its correlation with other constructs, thereby confirming satisfactory discriminant validity (Table 4). Since correlation coefficients were all below 0.700, multicollinearity was not a concern in this study.

Evaluation of Structural Model

The structural model underwent evaluation by scrutinising fit indices and the estimates of explained variance. Based on the assessment of the measurement model, this model demonstrated a reasonable fit. Table 5 and Figure 2 presents the standardised path coefficient of the structural model. The association between attitude and patients' intention to seek medical consultation after experiencing breast cancer symptom(s) is presented in Q1. The SEM approach revealed a positive influence of attitude on patients' intention to seek medical consultation after breast cancer symptoms (β1=0.844, p<0.001). The subsequent inquiry, Q2, hypothesised that PBC positively impacts patients' intention to seek medical consultation after experiencing breast cancer symptoms. The analysis found a positive and significant impact of PBC on patients' intention (p=0.004, with β2 =0.178). Furthermore, the examination of the impact of subjective norm on patients' intention to seek medical consultation after experiencing breast cancer symptoms is proposed in Q3. However, with a standardised path coefficient (β2) of 0.088 and a p-value of 0.199, subjective norm emerged as having an insignificant influence on patients' intention to seek medical consultation after detecting breast cancer symptoms. Consequently, Q3 appeared to have a negative influence on patients, leading to delays in their intentions to seek medical consultations after observing breast cancer symptoms.

DISCUSSION

This study investigated the behavioural intention of patients to seek medical consultation after experiencing symptoms of breast cancer using the TPB model. It elucidated how various psychosocial factors, such as the TPB constructs of attitude, subjective norm, and PBC, impact patients' intention to seek medical consultation after becoming aware of breast cancer symptoms.

In this investigation, attitude emerged as the most influential predictor of patients' intention to seek medical consultation after breast cancer symptoms, followed by PBC. The findings of this study validated the positive correlation between attitude and patients' intentions to seek medical consultation after breast cancer symptoms. These results are consistent with studies conducted by. Sun et al. (14), Wang et al. (10), and Fajriah et al. (15) have highlighted attitude as a contributing factor to women's participation in breast cancer screening programmes. However, Khazir et al. (16) found no predictive relationship between attitude and intention.

Table 3. Probable factors influencing intention of the breast cancer patients to seek medical consultation after breast cancer symptom (s) (n=110)

Item	Standardized loadings	Cronbach's alpha	Composite reliability	Average variance extracted
Attitude		0.977	0.975	0.908
attd2: Having early breast check-up is worth doing	0.919			
attd3: Having early breast check-up will detect lumps / abnormalities if I have any	0.974			
attd4: Having early breast check-up will lead to an early diagnosis of breast cancer if I have it	0.997			
attd5: Having early breast check-up will lead to an early treatment if I have breast cancer	0.919			
Subjective norm		0.826	0.826	0.704
Sn8: Most people who are important to me think I should have my breast check-up	0.836			
Sn9: If the important people around me had a breast cancer check-up, I would also carry out a check-up.	0.842			
Perceived behavioural control		0.870	0.871	0.772
PBC8: I believe I can solve the family commitment problem to get breast check-up in any healthcare facilities	0.902			
PBC9: I believe I can solve the work commitment problem to get breast check-up in any healthcare facilities	0.855			
Intention		0.922	0.922	0.798
Intent2: I have been paying attention to breast cancer check-up information	0.913			
Intent3: I am willing to promote breast cancer-related knowledge	0.887			
Intent4: I am willing to mobilize others to participate in breast cancer check-up if they have symptom(s)	0.880			

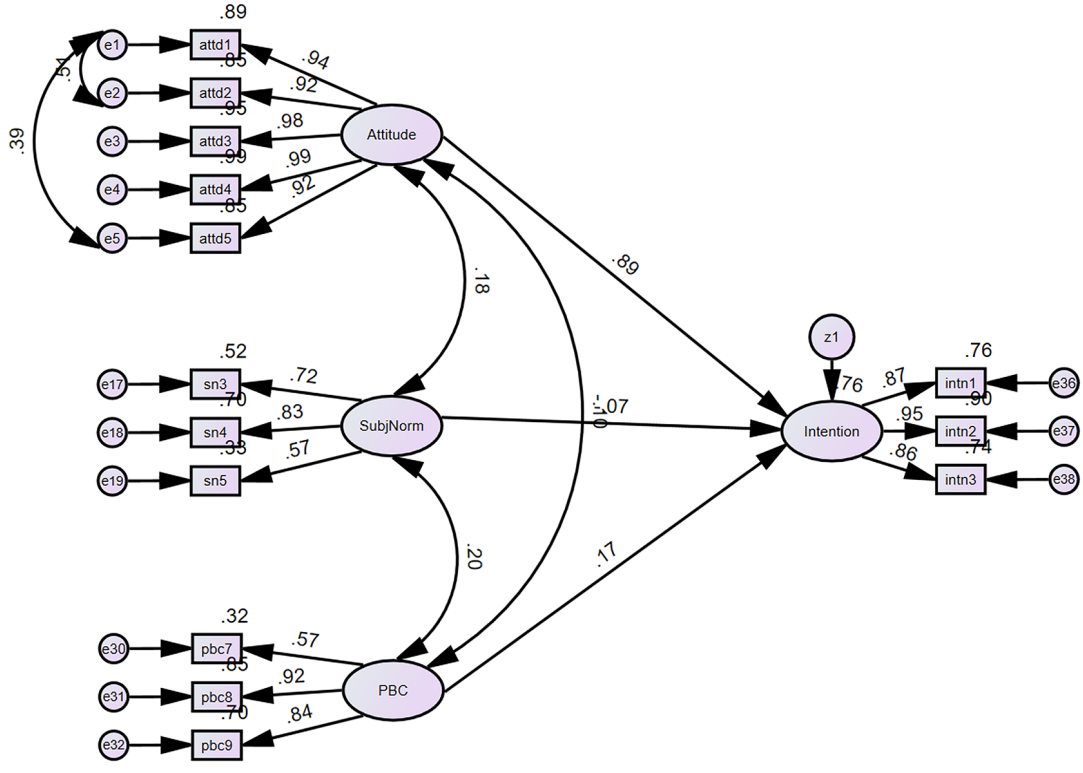

Figure 2. Final structural model of intention of patients to seek medical consultation after breast symptom (n=110)

PBC: Perceived behavioural control

Table 4. Correlations between the possible factors (n=111)

	Attitude	Subjective norm	Perceived behavioural control	Intention
Attitude	**0.953**			
Subjective norm	0.444**	**0.839**		
Perceived behavioural control	-0.117	0.049	**0.879**	
Intention	0.813**	0.438**	0.089	**0.893**

**Significant at p<0.01 level, bolded numbers represent the square root of average variances extracted (AVE).

Table 5. Relationships of late presenter patients' intention to seek medical consultation after breast cancer symptoms

	Path			Estimate	S.E.	C.R.	P
Q_1	Attitude	→	Intention	0.844	0.076	10.958	<0.001
Q_2	Perceived behavioral control	→	Intention	0.178	0.058	2.888	0.004
Q_3	Subjective norm	→	Intention	0.088	0.069	1.283	0.199

**significant at α=0.05

Similarly, perceived behavioral control exhibited a significant relationship with patients' intention to seek medical consultation after breast cancer symptoms, aligning with the findings of Rezabeigi-Khazir et al. (17) and Wang et al. (10), Rezabeigi-Davarani et al. (18), which suggested that perceived behavioral control predicts intentions and actions. However, Peyman et al. (19) found perceived behavioral control to be the weakest predictor of breast cancer screening, possibly due to variations in research settings, subjects, or data collection tools.

Contrary to attitude and PBC, subjective norm did not significantly influence patients' intention to seek medical consultation after experiencing breast cancer symptoms in this study. This suggests that individuals, important to the patients (partners, family, peers, physicians, and media), may have negatively influenced them, leading to delays in seeking medical consultations after noticing breast cancer symptoms. Despite patients' strong inclination to seek medical consultation for their breast cancer symptoms, as indicated by the positive significance of attitude and PBC, they were hindered by a lack of support, from their surroundings hindered them. This finding resonates with Keshavarzi (20), where subjective norm did not predict mammography screening intent and behaviour. However, it contradicts studies by Dezham (16) and Hatefnia (21), which showed a positive relationship between subjective norm and mammography screening intention and behaviour. Sun et al. (15), Wang et al. (10), and Jensen et al. (22) also concluded that subjective norm is the strongest predictor of screening intention.

Subjective norm is influenced by significant individuals in one's life, such as spouses, relatives, friends, health experts, and the media. One reason subjective norm did not significantly affect intention may be the lack of understanding among spouses regarding breast cancer symptoms. Men often lack clarity about breast cancer and its symptoms, leading them to pay less attention to their spouse's complaints. In a study by Khakbazan et al. (24), only 14% of women received encouragement from their husbands to seek medical consultation after experiencing breast cancer symptoms, while 31% received no encouragement from anyone.

Apart from spouses, insufficient encouragement from family members and friends may also contribute to delays in seeking medical consultations after experiencing breast cancer symptoms. Family members and friends should offer psychological support and emotional comfort to alleviate barriers to intention. Molina (24) indicated that advice from friends and family members increased the intention to undergo mammography screening. In this study, most patients were housewives with no active income, but with family commitments. Thus, increased support and encouragement from families and friends could give patients more time and courage to seek medical consultation for their symptoms. A lack of encouragement from family members and healthcare providers significantly affects women's decisions regarding breast cancer screening programmes. There is a correlation between lower levels of social support and the lack of participation in breast cancer screening (23). Stronger social support networks contribute to the development of more positive attitudes toward preventive healthcare.

Another factor noted in the study by Bonsu and Ncama (25), Moodley et al. (26), Kohler et al. (27) and Khakbazan et al. (23) is the absence of family history having breast cancer. As explained, subjective norm can be divided into injunctive normative belief and descriptive normative belief (28). An injunctive normative belief is the approves or disapproves of next of kin of the women to performing the behaviour while descriptive normative belief are beliefs as to whether important others themselves perform the behaviour. If the women having a family history of breast cancer, they are likely to experienced seeing their own family to seek medical consultation for breast symptoms, therefore this will increase women awareness of their increase susceptibility to breast cancer (29). In this study, only around 20.9% of the patients had a family history of breast cancer and only few of them (6%) having close friends that had breast cancer.

In addition to spouses, family members, and friends, health experts also play a significant role in influencing the subjective norm. In the study by Vahedin Shahrodi et al. (30), physicians and healthcare staff were the most informative sources regarding breast cancer

and screening methods. Emphasis on the importance of breast examinations and relevant knowledge by experts and primary care physicians is crucial. Intervention by community health workers and local volunteers can help alleviate women's discomfort and shyness about breast healthcare. Limited access to doctors, healthcare workers, educational resources, diagnosis, and treatment processes contributes to women's low awareness about clinical examinations and their importance. Women often neglect breast examinations until they experience severe symptoms, highlighting poor awareness about clinical examination execution and importance of clinical examinations. Most patients detected breast cancer at stage II (44%) and stage III (34%) and sought medical consultation only after experiencing multiple breast cancer symptoms.

In the study by Sun et al. (14), past experiences influence women's behaviours towards breast cancer screening. Women who had been screened before were more likely to get advice from physicians regarding breast cancer prevention Wu et al. (31). In this study, since the subjective norms found to be negatively linked with intentions, it is assumed that most women had never been screened before. This might suggest that poor communication between health care provider and the community lead to late presentation. Thus, collaboration between healthcare providers and relatives or community is essential to enhance the positive influence of subjective norm.

Subjective norms as media influence might publicize the harm of breast cancer and increase check-up intention, but it could also increase the exposure of screening process Sun et al. (14). Media can therefore give negative effects on subjective norm. Thus, patients may feel more embarrassed, especially with the male physician's involvement. Therefore, given our research findings, more intervention approaches should be taken to improve the efficacy of media influence such as television, brochures or leaflets, newspapers or magazines, and broadcast. Sun et al. (14) reported that advocacy and education has positive impact on subjective norms. Community that constantly exposed to the regular intervals of announcements or reminders of screening behaviours, and consequently, their emotions will be aroused and they will increasingly engage in the behaviours.

Study Limitations

This study marks an inaugural exploration into the inclination of late presenting breast cancer patients in Sabah to seek medical consultation after experiencing breast cancer symptoms. The TPB and SEM analytical models were employed for the purpose. This research provides valuable insight into the factors influencing delayed medical consultation, emphasizing that late presentation of breast cancer can vary based on psychosocial influences and is not solely determined by demographic or clinical factors.

Despite the substantial findings, the study encountered certain limitations. It was conducted with a limited number of participants for SEM analysis, relied on patients' recollections for recording dates, potentially introducing recall bias, and also relied on self-reporting for the questionnaire. Additionally, the absence of questions regarding negative intentions hindered the assessment of the direct impact of subjective norm on behavioural intention. However, these limitations do not undermine the overall significance of the study but rather offer suggestions for future research. To enhance data validity, it is recommended to incorporate interviews as a complement to the questionnaire, rather than solely relying on self-reported questionnaires. Moreover, combining the TPB model with other models such as the prototype willingness model could amplify explanatory power and offer a superior fit to the data compared to a single model. Furthermore, it is advisable to compare the model with other health education and health promotion models to enrich understanding and applicability.

CONCLUSION

In this study, subjective norm was seen to have a negative influence on the intentions of patients to seek medical consultation after experiencing cancer symptoms for the first time, while the patient's attitude and PBC was noted to have a positive influence on their intention to seek medical consultation after observing symptoms. Therefore, based on our research findings, additional intervention approaches should be implemented to enhance the efficacy of subjective norm influences on the public, such as awareness among partners, family members, peers, physicians, and various media. Thus, policies and programmes that encourage and mobilise the public to take preventive measures with regard to their own health should reasonably include education through the subjective norm.

Ethics

Ethics Committee Approval: Ethical approval was secured from the Medical Research and Ethics Committee (MREC) (approval number: NMRR-20-32-52564 (IIR), date: 27.02.2020), Ministry of Health Malaysia (MOH).

Informed Consent: Written consent was obtained from all those listed, and assurances were given regarding patient confidentiality.

Footnotes

Authorship Contributions

Surgical and Medical Practices: F.H., S.Z.S., N.A.S.N.K., Concept: F.H., S.Z.S., N.A.S.N.K., Design: S.Z.S., N.A.S.N.L., Data Collection or Processing: Analysis or Interpretation: D.L., M.T.H.P., Literature Search: D.L., Writing: D.L.,

Conflict of Interest: No conflict of interest was declared by the authors.

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The Effect of Depression and Stress Hormones on the Development of Gestational Diabetes Mellitus

Gebelik Diyabetinin Gelişiminde Depresyon ve Stres Hormonlarının Etkisi

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ABSTRACT

Objective: This study aims to determine the effect of depression and stress hormones on the development of gestational diabetes mellitus (GDM) in pregnant women diagnosed with GDM using serum cortisol, plasma adrenaline (A), plasma noradrenaline (NA), and the Beck Depression Inventory (BDI).

Methods: 70 pregnant women diagnosed with GDM were included in the patient group, and 70 pregnant women without GDM were included in the control group. International Association of Diabetes and Pregnancy Study Group criteria were used for the diagnosis of GDM. Single-step 75 g oral glucose tolerance test was performed at 24-28 weeks of gestation. Serum cortisol, A, and NA levels were measured. BDI was used to investigate depressive symptoms.

Results: The patient and control groups were similar in terms of age, BMI gravidity, and parity. When compared with the control group, A and NA levels were significantly higher in the patient group (p=0.016, p=0.033, respectively). BDI results in the patient group were similar to those in the control group (p=0.151). The mean A levels of 33 pregnant women with minimal depression were 110.59±35.03 pg/mL, the mean A levels of 31 pregnant women with mild depression were 126.65±22.33 pg/mL, and the mean A levels of pregnant women with moderate depression were 95.09±30.86 pg/mL. This difference was statistically significant (p=0.005).

ÖZ

Amaç: Bu çalışmanın amacı, gestasyonel diyabet mellitus (GDM) tanısı almış gebelerde depresyon ve stres hormonlarının GDM gelişimi üzerindeki etkisini; serum kortizol, plazma adrenalin (A), plazma noradrenalin (NA) düzeyleri ve Beck Depresyon Ölçeği (BDI) kullanarak değerlendirmektir.

Yöntemler: Çalışmaya, GDM tanısı almış 70 gebe kadın hasta grubuna ve GDM tanısı olmayan 70 gebe kadın kontrol grubuna dahil edilmiştir. GDM tanısı için Uluslararası Diyabet ve Gebelik Çalışma Grubu Birliği kriterleri kullanılmış ve gebeliğin 24-28. haftaları arasında tek adımlı 75 g oral glukoz tolerans testi uygulanmıştır. Serum kortizol, A ve NA düzeyleri ölçülmüş; depresif semptomları değerlendirmek amacıyla BDI kullanılmıştır.

Bulgular: Hasta ve kontrol grupları yaş, vücut kitle indeksi, gravide ve parite açısından benzer bulunmuştur. Kontrol grubuna kıyasla, hasta grubunda adrenalin ve noradrenalin düzeyleri anlamlı derecede yüksek saptanmıştır (sırasıyla p=0,016 ve p=0,033). BDI sonuçları açısından ise gruplar arasında anlamlı bir fark bulunmamıştır (p=0.151). Minimal depresyonu olan 33 gebenin ortalama A düzeyi 110,59±35,03 pg/mL, hafif depresyonu olan 31 gebenin 126,65±22,33 pg/mL, orta derecede depresyonu olan gebelerin ise 95,09±30,86 pg/mL olarak ölçülmüştür. Bu fark istatistiksel olarak anlamlı bulunmuştur (p=0.005).

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ABSTRACT

Conclusion: This study suggests that the sympathoadrenal system may play a role in the etiopathogenesis of GDM in pregnant women rather than depression. However, larger prospective studies are needed to further elucidate the relationship between depression, stress hormones, and GDM.

Keywords: Gestational diabetes, depression, adrenaline, noradrenaline, cortisol, sympathoadrenal axis

ÖZ

Sonuç: Bu çalışma, gebelerde GDM'nin etiyopatogenezinde depresyondan ziyade sempatoadrenal sistemin rol oynayabileceğini göstermektedir. Ancak, depresyon, stres hormonları ve GDM arasındaki ilişkiyi daha iyi ortaya koymak için daha geniş örneklemli ve prospektif çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Gestasyonel diyabet, depresyon, adrenalin, noradrenalin, kortizol, sempatoadrenal aks

INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized by high blood glucose levels that emerge in pregnancy and persist for the duration of the pregnancy (1). This metabolic disorder is associated with various complications, including miscarriage, fetal abnormalities, preeclampsia, stillbirth, macrosomia, and more. Moreover, individuals with GDM could face a greater risk of obesity, hypertension, and type 2 diabetes (2).

GDM is typically diagnosed through glucose tolerance testing, with the most commonly used method being the 75 g oral glucose tolerance test (OGTT). Alternatively, a two-step testing protocol, 50 g and 100 g OGTTs, can be used (3). Timely diagnosis and appropriate management play a crucial role in minimizing the potential health risks for both mother and baby.

Cortisol and plasma adrenaline (A), are released from the adrenal gland, while plasma noradrenaline (NA), comes from sympathetic nerves. During pregnancy, hormone levels, particularly cortisol, rise due to the effects of the placenta. Levels of NA and A remain stable at this time (4). Holzman et al. (5) discovered that elevated NA and A levels are linked to preterm births. Almon et al. (6) report that women with GDM show increased cortisol levels, which contribute to insulin resistance and promote GDM. Furthermore, high cortisol levels are associated with preterm births, miscarriages, and preeclampsia (6).

Social, genetic, and psychological factors are known to influence the development of GDM, placing both mothers and offspring at heightened risk for various physical and psychological complications (7,8). Mental health concerns, particularly among pregnant women at high risk for GDM, have attracted significant research interest globally. Studies conducted within this population suggest that anxiety and depression contribute substantially to the development of GDM alongside physiological factors (9). However, prior research exploring the relationship between depression and GDM has yielded conflicting results (10-14). This study aims to explore the influence of depression and stress hormones on emerging GDM, using serum cortisol, and Beck Depression Inventory (BDI) as key indicators.

MATERIALS AND METHODS

This study was approved by the Ankara Bilkent City Hospital No. 2 Clinical Clinical Research Ethics Committee (approval number: e2-23-3189, date: 18.01.2023) and complied with the principles outlined in the Helsinki Declaration. Pregnant women who attended an antenatal clinic between August 2022 and April 2023 and satisfied specific inclusion criteria were recruited. The criteria included the absence of risk factors or coexisting conditions such as hypertension, epilepsy, rheumatologic disorders, kidney disease, and thrombophilia. Furthermore, participants had no previous diagnosis of depression or history of antidepressant use, were between the 24th and 28th weeks of pregnancy, and underwent a 75 g OGTT. The study involved 70 pregnant women diagnosed with GDM as part of the patient group, while another 70 pregnant women without GDM formed the control group. Prior to participation, all individuals provided informed consent.

Demographic information (age, gender), gravidity, and parity were recorded for all participants. Height measurements were obtained without the use of headgear or footwear, using a standard height measurement scale. Weight was measured using a standard scale after removal of any accessories. Body mass index (BMI) was calculated.

After an overnight fast of 8-10 hours, venous blood samples were collected at 08:30 following a 30-minute rest period in a seated position. Serum cortisol, plasma A, and plasma NA levels were measured as stress markers at the beginning of OGTT. The samples were collected under standard conditions, for example in a quiet, temperature-controlled environment, following a rest period and without prior caffeine intake. Serum cortisol was measured using electrochemiluminescence, while plasma A and NA were assessed by radioimmunoassay. Plasma glucose levels were measured using a Roche automated biochemical analyzer.

In this trial, GDM was diagnosed using the criteria established by the International Association of Diabetes and Pregnancy Study Group (IADPSG) (15). A 75 g OGTT was performed between the 24th and 28th weeks of gestation, and GDM was defined when any of the threshold values were met or surpassed. The threshold values were defined as fasting plasma glucose (FPG) ≥92 mg/dL, 1-hour PG ≥180 mg/dL, or 2-hour PG ≥153 mg/dL. The test was administered after a fasting period of at least eight hours. Blood glucose levels were obtained before intake of the 75 g glucose solution, as well as at 60 and 120 minutes after consumption.

Following the diagnosis of GDM, depressive symptoms in participants were assessed using the BDI. Originally formulated by Aaron T. Beck in 1961 and subsequently revised in 1971, the BDI was translated into Turkish by Hisli (16) in 1988, and its validity and reliability confirmed in 1989. This inventory consists of 21 self-report items aimed at assessing depressive symptoms and associated attitudes. It employs a four-point Likert Scale, allowing respondents to choose the statement that best reflects their current state. All items are assigned a score from 0 to 3, resulting in a total score between 0 and 63. In the Turkish adaptation study, a cutoff score of 17 was

determined for identifying depression (16). Depression severity is classified into 4 categories: minimal (0-9), mild (10-16), moderate (17-29), and severe (30-63).

Statistical Analysis

Statistical analysis were performed using SPSS Statistics 20. Categorical variables were indicated as frequencies and percentages, whereas continuous variables were reported as means with standard deviations. The one-sample Kolmogorov-Smirnov test was conducted to determine the normality of the dataset. Since the data did not follow a normal distribution, the Mann-Whitney U test was used to evaluate comparisons between two groups, and the Kruskal-Wallis test was used to evaluate comparisons for multiple groups. Differences in categorical variables were assessed with the chi-square test. A p-value of less than 0.05 was accepted as statistically significant.

RESULTS

The patient and control groups demonstrated comparable characteristics regarding age and BMI (p=0.910, p=0.118, respectively). Similarly, no significant differences were found between the two groups in terms of gravidity and parity (p=0.158, p=0.464, respectively). Nevertheless, FPG, first-hour PG, and second-hour PG levels were markedly greater in the patient group than in the control group (p<0.001 for all). No statistically significant difference was found in serum cortisol levels between the groups (p=0.136). In contrast, A and NA levels were significantly higher than those of the control group (p=0.016, p=0.033; respectively). BDI scores were similar between groups (p=0.151). Additionally, no participants in the study were identified with severe depression (Table 1).

In the GDM group, OGTT values were not different among those with minimal levels and mild and moderate depression based on the BDI (p>0.05). The mean A levels of 33 pregnant women with minimal depression were 110.59±35.03 pg/ml; the mean A levels of 31 pregnant women with mild depression were 126.65±22.33 pg/mL and the mean A levels of 35 pregnant women with moderate depression were 95.09±30.86 pg/mL. This finding was statistically significant (p=0.005). Also, the difference in mean BMI between pregnant women with mild and moderate depression was statistically significant (p=0.018). The mean BMI of diabetic pregnant women with moderate depression was higher than that of others (Table 2).

DISCUSSION

In this study, the BDI was administered to pregnant women with GDM; BDI was similar between groups (p>0.05), which contrasts with previous findings in the literature. Arafa et al. (17) conducted the first study assessing the connection between depression and GDM. In their research, women with depression had a higher risk for GDM compared to those without a history of depression. Conversely, OuYang et al. (9) conducted a systematic review and concluded that additional research is needed to establish whether depression is a risk factor for GDM.

A study by Myers et al. (18) found that individuals with a variant form of the *oxytocin receptor (OXTR)* gene displayed more severe symptoms of both depression and anxiety compared to those without the variant. In a similar study, researchers examining the relationship between single nucleotide polymorphisms (SNPs) in the *OXTR* gene and psychological symptoms in Malaysian women with GDM found that certain SNPs were linked to increased stress symptoms, resulting in a 2.9-fold higher likelihood of experiencing stress (19). Another study, which aimed to evaluate the relationship between OXTR and *melatonin receptor 1B* gene SNPs and psychological symptoms in women diagnosed with GDM, found a significant difference in the frequency of gene polymorphisms in the AA and GG genotypes of OXTR rs53576 (p=0.04) (20). These findings suggest that SNPs in the *OXTR* gene could lead to depression and anxiety in pregnant women with GDM. However, there are several limitations to the studies: small sample sizes and the inclusion of

Table 1. Comparison of demographic characteristics, 75 grams oral glucose test, plasma stress hormone levels, and beck's depression inventory results between patients and control group

	Patients (n=70)	Controls (n=70)	p
Age (years)	27.51±5.64	27.23±4.72	0.910
BMI (kg/m^2)	26.48±7.83	26.73±4.46	0.118
Gravidity (n)	2.04±1.08	2.50±1.56	0.158
Parity (n)	0.87±0.96	0.74±0.87	0.464
Cortisol (µg/dL)	22.86 ± 5.19	24.74±6.74	0.136
A (pg/mL)	116±31.34	94.85±49.93	**0.016**
NA (pg/mL)	305.01±76.46	267.97±109.31	**0.033**
FPG (mg/dL)	92.86±17.11	87.37±5.28	<0.001
1-hour PG (mg/dL)	174.07±29.94	121.59±28.65	<0.001
2-hour PG (mg/dL)	152.90±28.32	102.50±21.39	<0.001
BDI			0.151
Minimal depression	33	34	
Mild depression	31	23	
Moderate depression	6	13	

BMI: Body mass index, A: Plasma adrenaline, NA: Noradrenaline, FPG: Fasting Plasma glucose, PG: plasma glucose, BDI: Beck's depression inventory

Table 2. Examination of the data between the classes formed according to the beck's depression inventory in the patient group

	Minimal depression (n=33)	Mild depression (n=31)	Moderate depression (n=6)	p
Age (years)	27.94±5.46	27.29±5.77	26.17±5.63	0.740
BMI (kg/m^2)	26.97±9.13	24.51±3.35	33.90±11.08	**0.018**
Gravidity (n)	1.94±1.07	2.16±1.08	2.00±1.15	0.655
Parity (n)	0.82±0.99	0.94±0.94	0.83±0.89	0.796
Cortisol (µg/dL)	23.09±5.50	22.61±4.17	22.90±7.58	0.947
A (pg/mL)	110.59±35.03	126.65±22.33	95.09±30.86	**0.005**
NA (pg/mL)	293.93±87.18	311.51±68.29	332.42±25.95	0.887
FPG (mg/dL)	90.79±14.44	91.35±14.92	112.00±16.82	0.172
1-hour PG (mg/dL)	170.82±32.86	121.59±28.65	180.00±39.22	0.907
2-hour PG (mg/dL)	149.91±25.81	176.39±23.52	155.00±44.76	0.648

BMI: Body mass index, A: Adrenaline, NA: Noradrenaline, FPG: Fasting plasma glucose, PG: Plasma glucose, BDI: Beck's depression inventory

only a few genetic markers due to resource constraints. In our study, genetic analyses could not be performed due to a lack of resources.

Feng et al. (21) conducted a study with 150 participants to investigate the relationship between stress hormones and GDM. This study found a slight increase in cortisol levels in the GDM group compared to the control group, although this difference was not statistically significant (p=0.09). However, the levels of A and NA were elevated in the GDM group (p=0.00, p=0.03, respectively) (21). Similarly, in our study with 140 pregnant women, serum cortisol levels did not significantly differ between the groups (p=0.136). However, A and NA were higher in the GDM group (p=0.016, p=0.033; respectively).

Interestingly, among women with GDM, those with moderate depression had the lowest mean adrenaline levels, despite having the highest BMI. One possible explanation is the physiological blunting of the adrenergic response in the context of chronic stress or depressive states. Moreover, higher BMI may influence hormonal feedback mechanisms. These complex interactions warrant further investigation.

Cortisol, A, and NA are established biological markers of stress, and their levels typically increase in response to stress (23,24). In another study, Feng et al. (24) found significantly elevated levels of A, NA, and glucagon in women with GDM, suggesting that stress could contribute to the pathophysiology of GDM. Furthermore, stress hormones are recognized as factors that promote hyperglycemia, potentially worsening insulin resistance and elevating blood glucose levels (25,26). Prolonged oxidative stress can also contribute to increased insulin resistance and disrupt glucose metabolism. A previous study indicated that enhanced oxidative stress could impair stress habituation and elevate levels of A, NA, and cortisol (27,28). Cortisol, a key stress hormone, can increase the release of glucose from the liver, impair β-cell function, and reduce insulin secretion, all of which may facilitate hyperglycemia (29,30). In our study, cortisol levels did not significantly increase in the GDM group compared to the control group, which is similar to the findings of studies by Da Costa et al. (31) and Feng et al. (24). A study found that anxiety and depression can trigger chronic hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in excessive secretion of cortisol and insulin resistance, which in turn raises the risk of GDM (32). The absence of a significant elevation in cortisol in our study may be because no participants had severe depression, with most exhibiting minimal or mild depression. This likely prevented chronic HPA axis hyperactivity and a significant rise in cortisol levels.

Our research has several limitations, such as the small sample size and evaluating maternal serum cortisol, A, and NA levels, as well as the BDI, only at the time of initial diagnosis (measured once), without follow-up data on these parameters prior to pregnancy or during later gestational weeks.

The relationship between GDM and depression remains inconclusive, and there may be other mechanisms underlying hyperglycemia in women with GDM. Although our hypothesis is to explore the effect of depression and stress hormones on the development of GDM, our findings show that cortisol levels were similar between groups. The mean levels of A and NA were higher in the GDM group. (p=0.016, p=0.033; respectively). This suggests that activation of the sympathoadrenal system, rather than the HPA axis, may have a more significant role in the etiopathogenesis of GDM. Additionally, the comparable BDI scores in both groups may imply that the influence of depression on the development of GDM in pregnant women is minimal. These findings could contribute to the interpretation of GDM in clinical practice, and further research may explore whether stress hormone levels could serve as an additional or early test in GDM diagnosis, particularly when the 75 g OGTT is inconclusive.

Study Limitations

This study has several limitations. First, its cross-sectional design limits the ability to establish causality between stress hormones and the development of GDM. Second, the sample size, although adequate for initial analysis, may not fully capture the variability in hormonal and psychological responses among a broader population. Third, the assessment of depression was based solely on the BDI, which, while validated, is a self-reported measure and may be subject to reporting bias. Additionally, other potential confounding factors, such as sleep quality, socioeconomic status, or pre-existing mental health conditions, were not evaluated. Lastly, hormone levels were measured at a single time point, which may not reflect long-term or fluctuating levels during pregnancy. Larger studies with

more participants are needed before these findings can be applied routinely.

CONCLUSION

Our study highlights that the sympathoadrenal system might play a more important role than depression in pregnant women with GDM. However, additional large-scale prospective studies are necessary to better understand the relationship between depression, stress hormones, and GDM. Early detection and intervention for GDM could help prevent the risk of maternal and fetal complications associated with the condition.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee (approval number: e2-23-3189, date: 18.01.2023) and complied with the principles outlined in the Helsinki Declaration.

Informed Consent: Consent form was filled out by all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.Ö., Y.O., E.A., Concept: A.Ö., Y.O., H.Y., Design: Y.O., H.D., H.Y., Data Collection or Processing: A.Ö., Analysis or Interpretation: A.Ö., E.A., H.D., Literature Search: A.Ö., E.A., E.Ü., Writing: A.Ö., E.A., E.Ü.

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Evaluation of Genomic Variants in Non-syndromic Congenital Heart Disease in Turkish Pediatric Group

Non-sendromik Konjenital Kalp Hastalığı Tanısı Almış Pediyatrik Türk Grupta Genomik Varyantların Değerlendirilmesi

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ABSTRACT

Objective: Congenital heart disease (CHD) is the most common congenital malformation in the population, and so far, all the known genetic factors could explain only 20-25% of the cases.

Material and Methods: In this study, microarray analysis was performed, and next-generation sequencing of *Myosin Heavy Chain 6 (MYH6)*, *NK2 Homeobox 5 (NKX2-5)*, *GATA Binding Protein 4 (GATA4)*, *Notch Receptor 1 (NOTCH1)*, and *T-Box Transcription Factor 1 (TBX1)* genes, which are known to be involved in the etiology of non-syndromic CHD, was performed in 40 patients with isolated cardiac defects between the ages of 0-18 and 40 age-matched controls.

Results: In microarray analysis, 9 novel copy-number variations (CNVs) that were not reported in population databases, and included *OMIM* genes were detected in 1.5% (6/40) of the patients. Even though the detected CNVs had not been previously associated with CHDs and were classified as Variant of Uncertain Significance (VUS), overall CNV count burden in the patient group was significantly higher than in the control group. Also, there were no pathogenic/likely pathogenic sequence variants in *MYH6, NKX2-5, GATA4, NOTCH1*, and *TBX1* genes. The c.700C>T [p.(Arg234Cys)] and c.5949C>G [p.(Asn1983Lys)] in the *NOTCH1* gene were classified as VUS and have not been detected in the control group.

Conclusion: Although microarray technologies and candidate gene sequencing are useful diagnostic tools, routine genetic testing of sporadic non-syndromic CHD patients is controversial. We believe

ÖZ

Giriş: Konjenital kalp hastalıkları (KKH) toplumda en sık görülen malformasyonlar olmakla birlikte bilinen genetik faktörler tüm olguların yaklaşık %20-25'lik kısmını açıklayabilmektedir.

Gereç ve Yöntem: Bu çalışmada 0-18 yaş arasında izole KKH tanısı alan 40 olgu ve benzer yaş grubundaki 40 kontrol bireyin periferik kan örneklerinde, KKH etiyolojisinde rol oynadığı bilinen *Miyozin Ağır Zincir 6 (MYH6), NK2 Homeobox 5 (NKX2-5), GATA Bağlayıcı Protein 4 (GATA4), Notch Reseptörü 1 (NOTCH1)* ve *T-Box Transkripsiyon Faktörü 1 (TBX1)* genlerinin yeni nesil dizi analizi yöntemleriyle dizilenmesi ve mikrodizin analizleri çalışılmıştır.

Bulgular: Mikrodizin analizi sonucunda olguların %1,5'unda (6/40) *OMIM* geni içeren ve popülasyon veritabanlarında daha önce bildirilmemiş 9 kopya sayısı değişimi tespit edilmiştir. Tespit edilen kopya sayı varyasyonları daha önce KKH ile ilişkilendirilmemiş ve klinik önemi belirsiz (KÖB) varyant olarak sınıflandırılmış olsa da, hasta grubundaki toplam CNV yükü kontrol grubuna kıyasla anlamlı derecede daha fazla olarak değerlendirilmiştir. İlave olarak olgu grubunda dizi analizi ile değerlendirilen *MYH6, NKX2-5, GATA4, NOTCH1* ve *TBX1* genlerinde patojenik/olası patojenik olarak sınıflandırılan herhangi bir sekans varyantı saptanmamıştır. *NOTCH1* geninde, kontrol grubunda bulunmayan ve KÖB olarak sınıflandırılan c.700C>T p.(Arg234Cys) ve novel c.5949C>G p.(Asn1983Lys) varyantları tespit edilmiştir.

Sonuçlar: Mikrodizin teknolojileri ve aday genlerin dizilenmesi önemli tanısal araçlar olmasına rağmen, sporadik vakalarda geniş kapsamlı

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ABSTRACT

that still remains a challenge to interpret the variants detected in multifactorial CHD with complex etiology, and further studies are needed.

Keywords: Next-generation sequencing, microarray analysis, congenital heart disease

ÖZ

genetik testlerin rutin tanıda uygulanması tartışmalıdır. Kompleks etiyolojiye sahip multifaktöriyel kalıtım gösteren KKH'larda saptanan varyantların klinik öneminin yorumlanması oldukça güçtür ve ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Yeni nesil dizi analizi, mikrodizin analizi, konjenital kalp hastalıkları

INTRODUCTION

Congenital heart disease (CHD) is the most common congenital malformation in the population with an estimated frequency of 1-2%. Every year approximately 1.35 million new patients are born with the condition, and it still remains one of the most important causes of childhood mortality and morbidity in well-developed countries (1).

Most of the patients, approximately 75-80%, have isolated cardiac defects without any extra-cardiac malformation or global developmental delay, intellectual disability. The rest manifest the cardiac defects as a part of a syndrome such as aneuploidies, monogenic, or microdeletion/duplication syndromes (2).

Large-scale epidemiological studies revealed that a genetic or environmental cause can be identified in approximately 20-30% of CHDs (3). Among the genetic causes, chromosomal aneuploidies and gross structural chromosome aberrations that could be detected by conventional cytogenetic analyses are responsible for 20-25%; submicroscopic chromosomal rearrangements such as 22q11.2 deletion syndrome and Williams syndrome are responsible for 10-12%; and monogenic syndromes such as Kabuki syndrome and Noonan syndrome are responsible for 3-5% (4). It becomes even more compelling in the context of the non-syndromic CHD group. Positive family history increases the risk of recurrence depending on the type of defect (3.4 to 79.1 times) in siblings, and monozygotic twin studies show a higher concordance than the rest of the population (5,6). Also, consanguinity of the parents has been shown to increase the risk (7). Non-syndromic cardiac defects are mostly diagnosed as sporadic multifactorial malformations resulting from complex genetic mechanisms or environmental factors. To further define the genetic contributions to the causes, developmental steps in the embryonic and fetal period are studied in particular, and several genes coding transcription factors, cardiac structural proteins, or molecules responsible for the signal transduction pathways are evaluated (8).

In recent studies, it is estimated that copy-number variations (CNV), which involve the loss and gain of genomic material more than 1 kb, is responsible for approximately 3-10% of the non-syndromic CHD patients. Similarly, next-generation sequencing (NGS) of the candidate genes revealed that sequence variants are responsible for 2% (9). Many genes have so far been identified as responsible for the non-syndromic CHDs, such as *Myosin Heavy Chain 6 (MYH6), NK2 Homeobox 5 (NKX2-5), GATA Binding Protein 4 (GATA4), Notch Receptor 1 (NOTCH1), and T-Box Transcription Factor 1 (TBX1)* (10-12).

Over the past decade, with the development of massive parallel sequencing techniques and microarray technologies, it has become possible to elucidate the monogenic pathogenic variants contributing to specific phenotypes and identify candidate genes. Therefore, this study aimed to perform microarray analysis and NGS on previously implicated genes in Turkish pediatric cases of non-syndromic CHD.

MATERIALS AND METHODS

Study Group

A group of 40 patients was included in the study. All patients were diagnosed by echocardiography at Pediatric Cardiology clinics and then evaluated at Medical Genetics clinics for syndromic traits and pedigree analysis. Inclusion criteria for the patients were not to have extra-cardiac anomaly or global developmental delay or intellectual disability. All 40 age-matched (8±4.6) control samples, consisting of 18 females and 22 males, were examined by echocardiography before being included in the study. The control group was included in the microarray analysis to assess the contrast in size and CNV count burden between two groups. Written informed consent was obtained from legal representatives before collecting blood samples in accordance with the Declaration of Helsinki. The study was approved by the Non-interventional Clinical Research Ethics Committee of Eskişehir Osmangazi University (decision number: 18, date: 25.06.2019).

Microarray Method

DNA was extracted from peripheral blood samples of all cases using the A.B.T. DNA Purification Kit (TM), according to the manufacturer's recommendations. For microarray analysis, the Agilent Comparative Genomic Hybridization + Single Nucleotide Polymorphism Microarray Kit (4x180K) was used. DNA digestion, labeling, purification, and hybridization procedures were performed according to the manufacturer's instructions, and after post-hybridization washes, the scanning process was performed.

Next-Generation Sequencing

Peripheral blood samples were collected from all patients, and by the Magna Pure Compact LC (Roche Applied) extraction kit gDNA was isolated according to the manufacturer's recommendations. Amplification of all exons and exon/intron boundaries of *NKX2-5, TBX1, NOTCH1, MYH6*, and *GATA4* genes in each pool of 51 amplicons was done by TaqDNA polymerase using a Thermal Cycler (Thermo Fisher Scientific Inc.). Library preparation was done with NEXTERA XT Index Kit V2, and sequence analysis on a micro flow cell was performed with the MiSeq instrument (Illumina, Inc.).

Analysis of Sequence Variants

Sequence alignment to the reference genome and the quality filter were performed by MiSeq Reporter v2.3 software. Single nucleotide variants, small insertion variants, and deletion variants were filtered. Coverage depth and quality scores were controlled using

the integrative genomics viewer database. The variant classification according to the American College of Medical Genetics (ACMG) criteria was done using the platforms such as Varsome and Franklin (13). Non-synonymous variants that were not reported in population databases (GnomAD) or had a minor allele frequency of <0.01 were noted as rare variants. The functional impact of missense variants was assessed using *in silico* prediction tools: MutationTaster (http://www.mutationtaster.org/), Prediction of Effects of Human nsSNPs (http://genetics.bwh.harvard.edu/pph2/), Scale-Invariant Feature Transform (http://sift.jcvi.org/), and Human Splicing Finder (http://www.umd.be/HSF/). The dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) databases were used for literature review.

Analysis of Copy-Number Variations

Feature Extraction 3.0.2.11 and Agilent Cytogenomics 3.0.2.11 software were used for data extraction and analysis, respectively. Conservative log2 ratio thresholds were taken as 0.3 and -0.3, respectively. Genomic regions with at least 5 probes were included for analysis. DECIPHER database v11.12 (https://www.deciphergenomics.org/), the Database of Genomic Variant (http://dgv.tcag.ca/dgv/app/home), and PubMed were used as resources to assess the variants detected. Franklin Genoox database (https://franklin.genoox.com/clinical-db/home), and ClinGen CNV Interpretation Calculator (https://cnvcalc.clinicalgenome.org/cnvcalc/) were used to classify the variants according to ACMG/ClinGen Technical Standards for CNVs (14).

Statistical Analysis

Data analysis was performed with IBM SPSS 21.0. Relationships between means of a continuous variable were evaluated by Independent Samples t-test analysis.

RESULTS

In this study, a total of 40 patients were included: 14 males and 26 females. The mean age of the patients was noted as 6±5.1. According to the diagnostic criteria, 13 septal defects (32.5%), 12 left ventricle outflow defects (30%), 7 conotruncal anomalies (17.5%), 4 complex cardiac anomalies (10%), 2 right ventricle outflow defects (5%), 1 atrioventricular canal defect (2.5%), 1 pulmonary venous return anomaly (2.5%) were detected. The cardiac anomalies of the patients are given in Table 1. The incidence of consanguinity among the parents of the patients was 7.5% (3/40).

Results of Next-generation Sequencing

Patients with non-syndromic congenital heart defects were included in the study; previously defined structural cardiac protein-coding and transcription factor genes were sequenced by NGS methods. Mean coverage was 152x for 98.10% of the targeted amplicons of the *NOTCH1* gene, 134x for 98.40% of the targeted amplicons of the *MYH6* gene, 171x for 99.30% of the targeted amplicons of the *GATA4* gene, 117x for 99.70% of the targeted amplicons of the *NKX2-5* gene and 105x for 99.10% of the targeted amplicons of the *TBX1* gene. There were no pathogenic or likely pathogenic variants detected in this study, but in 5% (2/40) of patients, variants of uncertain significance (VUS) that might be clinically significant were detected. The detected VUS variants and clinical findings of the variant carriers are summarized in Table 2.

The c.700C>T, [p.(Arg234Cys)] variant in the *NOTCH1* gene has been detected in case 34, who was a 9-month-old female patient. She was born to healthy, non-consanguineous parents. There was no prenatal exposure to teratogens during the pregnancy, nor was there a family history of CHD. She was diagnosed with secundum ASD and pulmonary stenosis at 6 months of age. The variant was classified as VUS according to the ACMG criteria. Segregation analysis revealed the variant occurred de novo.

c.5949C>G [p.(Asn1983Lys)] variant in the *NOTCH1* gene has been detected in case 40, who was a 14-year-old male patient. He was born to healthy, non-consanguineous parents. There was no prenatal exposure to teratogens during the pregnancy or family history of CHD. He was diagnosed with bicuspid aortic valve and aortic stenosis after the birth. This variant was a novel change that was not previously reported in databases, and the allele frequency was not available at the GnomAD database. The variant was classified as VUS according to the ACMG criteria. Segregation analysis revealed, the variant occurred *de novo*.

Table 1. Clinical features of the patients

Cardiac anomaly type	n
Septal defects	13
Left ventricle outflow defects	12
Conotruncal anomalies	7
Complex cardiac anomalies	4
Right ventricle outflow defects	2
Atrioventricular canal defects	1
Pulmonary venous return anomaly	1
Total	40

Table 2. Variations detected in uncertain significance in this study

Case No	Gene transcript	cDNA protein	Zygosity Rs Identification	GnomAD allele frequency	Pathogenicity prediction (SIFT, mutationtaster)	Type of congenital heart defect
34	NOTCH1 NM_017617.5	c.700C>T [p.(Arg234Cys)]	Heterozygous rs567890045	0.00000688	Damaging, disease causing	Pulmonary stenosis and secundum atrial septal defect
40	NOTCH1 NM_017617.5	c.5949C>G [p.(Asn1983Lys)]	Heterozygous (-)	(-)	Damaging, disease causing	Bicuspid aorta, aortic stenosis

Results of Microarray Analysis

There were a total of 62 CNVs detected in the patient group and 27 CNVs in the control group. Among the 62 CNVs, five were detected in both control and study groups. Among the patient and control groups, CNV detection rates were 82% (33/40) and 22.5% (9/40), respectively (odds ratio: 16.2381 95% confidence interval: 5.3905 to 48.9145, p<0.0001). The burden of CNV counts (total CNVs/ total individuals) was significantly higher (p<0.001) in the patient group compared to the control group, Among the CNVs detected in patients, 45.1% (28/62) were copy-number losses and 54.8% (34/62) were copy-number gains. 14.51% (38/62) of the CNVs were previously reported in DGV more than once, therefore classified as ''benign''. Also, 12.9% (8/62) of CNVs did not have any genes included in the region. Pathogenic or likely pathogenic variants were not detected in our study, but we have identified VUS CNVs in 15% (6/40) of the patient group. 14.5% (9/62) were are summarized in Table 3.

Case 13 was a 1-year-4-month-old female patient who was diagnosed with perimembranous VSD. She had a 55.749 kb gain at the region 6p25.3 and a 272.22 kb gain at the region 17q25.3. Neither of the aberrations has been previously reported in CHD. Gain of the 6p25.3 region included the *FOXCUT* and *FOXC1* genes. The *FOXC1* gene was reported to be haploinsufficient and was previously associated with Anterior segment dysgenesis 3, multiple types (OMIM #301631) and Axenfeld-Rieger syndrome, type 3 (OMIM #602482). The *FOXCUT* gene encodes a long non-coding RNA, which was suggested to be a regulator of the *FOXC1* transcript and involved in the proliferation and migration processes of tumor cells. Gain of the related region was not reported in the DECIPHER database. The 17q25.3 (79,638,223-79,910,442) region included 17 *OMIM* genes *(CCDC137, HGS, MRPL12, SLC25A10, GCGR, MCRIP1, P4HB, ARHGDIA, ALYREF, ANAPC11, PCYT2, NPB, SIRT7, MAFG, PYCR1, NOTUM, ARL16)*. *GCGR, P4HB, ARHGDIA,* and *PYCR1* were morbid genes in the region, but they have not been previously associated with congenital heart defects. This gain was not reported in the DECIPHER database.

Case 23 was a 7-year-5-month-old male patient who was diagnosed with aortic coarctation and bicuspid aortic valve. He had a 358,299 kb gain at 3q13.31 and an 81.09 kb gain at the 8q24.11 region. The gain at the 3q13.31 region includes the *OMIM* gene, *TUSC7*. In the DECIPHER database, a patient (DECIPHER ID: 384018) who has autism was reported with a gain of a similar region, but it was classified as likely benign and had been maternally inherited. Gain at the 8q24.11 region includes the *EXT1* gene, which encodes the exostosin-1 protein. Loss of the *EXT1* gene causes multiple exostoses type 1, but gain of the gene is not associated with any CHD.

Case 26 was a 4-year, 3-month-old male patient who was a diagnosed with d-TGA. He had a 166,784 kb gain at 4q32.1. The region includes three OMIM genes (*ASIC5, TDO2* and *CTSO*) but they have not been reported as morbid genes. Gain of a similar region was reported in DECIPHER database, previously in a patient with intellectual disability (DECIPHER ID: 345189) but not reported in a patient who was diagnosed with CHD.

Case 28, was a 14-year-3-month-old male patient who has been diagnosed with TOF. He had a 638.515kb gain at Xq21.31. The region included the *PCDH11X OMIM* gene. The *PCDH11X* gene belongs to the protocadherin gene family and has not been associated with any phenotype to date. It was reported to be expressed mainly in brain, and ovary tissues. In addition to that, it is implicated in cell-cell communication and dendritic synaptic plasticity, and is suggested as a candidate gene for dyslexia. Gain of a similar region was reported previously in the DECIPHER database in a patient with intellectual disability and obesity (DECIPHER ID: 258856) who did not have any cardiac findings.

Case 35 was a 17-year-old female patient, who was diagnosed with a bicuspid aortic valve. She had 169.936 kb loss at 19p13.3. The region included three *OMIM* genes (*TLE2, TLE6* and *ZNF77).* The *TLE2* gene has not been previously associated with any phenotype but is known to act as a corepressor in the negative regulation of the canonical Wnt signaling pathway (15).

Table 3. CNVs that were not reported previously in DGV and included genes

Case No	Locus	Size (kb)	Aberration type	Aberration	OMIM genes included	ACMG classification
13	6p25.3	55.749	Gain	arr[GRCh37]6p25.3(1,556,504_1,612,252)x3	*FOXCUT, FOXC1*	VUS
	17q25.3	272.22	Gain	arr[GRCh37]17q25.3(79,638,223_79,910,442) x3	*CCDC137, ARL16, HGS*	VUS
23	3q13.31	358.299	Gain	arr[GRCh37]3q13.3(116,229,141_116,587,439)x3	*TUSC7*	VUS
	8q24.11	81.09	Gain	arr[GRCh37]8q24.1(118,757,607_118,838,696)x3	*EXT1*	VUS
26	4q32.1	166.784	Gain	arr[GRCh37]4q32.1(156,740,213_156,906,996)x3	*ASIC5, TDO2, CTSO*	VUS
28	Xq21.31	638.515	Gain	arr[GRCh37]Xq21.3 (91,632,904_92,271,418) x3	*PCDH11X*	VUS
35	19p13.3	169.936	Loss	arr[GRCh37] 19p13.3 (2,876,148_3,046,083)x1	*ZNF77, TLE2, TLE6*	VUS
36	6q27	98.904	Gain	arr[GRCh37] 6q27 (168,954,929_169,053,833)x3	*SMOC2*	VUS
	13q21.32	95.998	Gain	arr[GRCh3713q21.3 (67,459,885_67,555,883)x3	*PCDH9*	VUS

VUS: Variant of Uncertain Significance, ACMG: American College of Medical Genetics

TLE6 gene is associated with preimplantation embryonic lethality, autosomal recessive (OMIM #612399). It regulates spermatogonia proliferation and cell cycle progression, but is not associated with the pathogenesis of congenital heart defects. *ZNF77* gene has not been previously associated with any phenotype and is predicted to enable DNA-binding transcription repressor activity.

In Case 36, a 10-year-3-month-old male patient diagnosed with a bicuspid aortic valve, a 98.904 kilobases gain at 6q27 and a 95.998 gain at the 13q21.32 regions were detected. gain at the 6q27 region includes *SMOC2* gene. This gene Dentin dysplasia, type I, characterized by microdontia and misshapen teeth, and with autosomal recessive (OMIM #125400), but not with any CHD. The gain at the 13q21.32 region included the *PCDH19* gene. This gene is associated with developmental and epileptic encephalopathy type 9, X-linked (OMIM #300088), but not with any CHD.

DISCUSSION

Congenital heart defects are the most common birth defects all around the world. It remains one of the most important reasons for childhood mortality and morbidity (16). Given the multifactorial nature of the condition, it is challenging to identify the genetic etiology, thereby making it difficult to provide proper genetic counseling to patients. However, thanks to advances in molecular technologies, information on the molecular pathology of the disease has begun to emerge (17).

This study aimed to perform microarray analysis and NGS of *NKX2-5, MYH6, GATA4, NOTCH1* and *TBX1* genes in the Turkish pediatric group with non-syndromic CHD. Microarray analysis is the most important tool for identifying CNVs in routine practice. Among other genetic conditions, it has emerged as a useful tool for the diagnosis of both isolated and syndromic CHDs (18). More recently, in addition to common pathogenic microdeletion syndromes that include extracardiac abnormalities such as 22q11.2 deletion, CNVs that include dosage-sensitive genes important for cardiogenesis, or include regulatory elements, have been identified in isolated CHDs. It is known that CNVs are the underlying mechanism of 3-25% of syndromic CHDs while they account for 3-10% of the non-syndromic CHDs.

In our study, we did not detect any pathogenic or likely pathogenic CNVs, but we have identified CNVs in 15% (6/40) of the patient group. Previous studies described in Table 4 show that pathogenic/likely pathogenic CNVs are detected in the patient cohorts, including both syndromic and non-syndromic CHDs (20-24). Since our patient group included only non-syndromic CHDs and there was no positive family history, we assumed that there were no pathogenic/likely pathogenic CNVs detected.

Besides gene content and pathogenicity classification, researchers also suggest that the total number and size of the CNVs are higher in CHD patients than in control groups. Several studies indicate that the overall CNV count burden is likely higher in CHD patients compared to the control group. Similar to previous studies in the literature, CNV counts were detected significantly higher in the patient group compared to the control group in our study (25,26).

Since the advent of massive parallel sequencing technologies, our understanding of the genetics of CHDs has rapidly expanded (27). Targeted or non-targeted sequencing (whole exome sequencing, whole genome sequencing) technologies have been applied to both syndromic and non-syndromic forms of CHD. Causative variants are mostly identified in familial non-syndromic patients, but due to the multifactorial nature of the CHDs it is not possible to identify a pattern presuming Mendelian inheritance (28,29).

NKX2-5, GATA4, TBX1, NOTCH1, and *MYH6* genes have been identified as strong candidates for non-syndromic CHDs in the past decades (4,30,31). In our study, we have detected a total of 103 variants in the five genes by the NGS technique. There were no pathogenic or likely pathogenic variants detected in this study; however, in 5% (2/40) of patients, VUS were detected. Dong et al. (32) have evaluated 73 CHD probands from consanguineous Turkish families with whole exome sequencing and detected causative genetic alterations in 13.7% of the patients. It was assumed that the detection rate of this study was high because of the consanguinity among the parents and the contribution of recessive variants (9.6%), which is lower in contrast to our study (32). Blue et al. (33) reported pathogenic variants at 57 genes that have been previously associated with both syndromic and non-syndromic CHDs in five

Table 4. Microarray studies in CHDs in the literature

Studies	Patient included	CHD type	Array platform	Pathogenic CNVs detected
Erdogan et al. (20) 2008	105	Non-syndromic	244K Agilent	5/105 (4.7%)
Greenway et al. (21) 2009	114	Non-syndromic syndromic	Affymetrix SNP 6.0	11/114 (9.6%)
Breckpot et al. (22) 2011	46	Non-syndromic syndromic	Affymetrix SNP 6.0	2 /46 (4.3%)
Goldmuntz et al. (23) 2011	58	Syndromic	Affymetrix 100K	12/58 (20.7%)
Tomita-Mitchell et al. (24) 2020	945	Non-syndromic syndromic	Affymetrix SNP 6.0	35/945 (4.3%)
Our study	40	Non-syndromic	Agilent, 180K	-ca

CNV: Copy-number variation, CHD: Congenital heart disease

probands (5/16) with positive family history. It was noted that the high prevalence of the pathogenic variants in the study was due to the inclusion of exceptionally familial cases. Similar to that, Jia et al. (34) evaluated 36 CHD patients from 13 families by targeted NGS analysis of 57 CHD-related genes and detected potentially disease-causing variants in 46% (6/13) of the families. Also, in a study done by Pulignani et al. (35) 68 non-syndromic CHD patients (57 sporadic and 11 familial) were evaluated by next-generation sequencing of 16 candidate genes. They have detected 20 possible disease-causing variants out of 68 patients. We believe that the lower detection rate in our study, compared to previously reported ones, is due to the inclusion of both familial and syndromic cases in other studies. Also, it might differ because of the distinct variant classification criteria that are used and the different number of genes covered in targeted NGS panels.

The *NOTCH1* gene is located at the 9q34 region and consists of 34 exons. It encodes a protein that belongs to an essential intracellular signaling receptor family that has important functions such as cell proliferation, cell death, and cell fate decisions during embryogenesis (36). It was reported that during cardiogenesis, activation of the NOTCH1 receptor function in the epithelial-mesenchymal transition of the endocardial cushion development and the formation of the semilunar valves (37). It is well known that truncating variants of the *NOTCH1* gene are associated with the development of CHDs, especially BAV and left ventricular outflow tract malformations. The c.700C>T [p.(Arg234Cys)] variant was previously reported (rs567890045), but has not been associated with any phenotype. It is located at codon 234 in the extracellular EGF-like domain of the protein. Since the variant is located adjacent to the O-glycosylation site of 232 amino acids, it was predicted to alter posttranslational modification and intracellular interactions of the protein (38). c.5949C>G [p.(Asn1983Lys)] is a novel variant that was reported in the literature. The variant is present in the ankyrin repeat domain of the protein, and there have been several functional studies indicating that such variants might disrupt the proper folding of the protein.

Study Limitations

Although it was designed as a case-control study, and age-matched controls with normal echocardiography have been included, we believe that one of the limitations of the study that might have influenced the results is the limited number of patients who were involved in our study. Second, in the NGS analysis, only five autosomal dominant candidate genes, which have the strongest association, were included; therefore, future studies with a large number of genes might be more informative.

CONCLUSION

The objective of this study is to identify genetic etiology among pediatric patients with non-syndromic congenital heart defects. We believe that one of the challenges is to interpret the VUS and give proper genetic counselling. To further identify the genetic background of the CHDs, genome-wide analysis done in larger populations, might be more effective than targeted tests.

Ethics

Ethics Committee Approval: The study was approved by the Non-interventional Clinical Research Ethics Committee of Eskişehir Osmangazi University (decision number: 18, date: 25.06.2019).

Informed Consent: Written informed consent was obtained from legal representatives before collecting blood samples in accordance with the Declaration of Helsinki.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.K., T.A., E.S., B.U., Concept: S.K., B.Ö., S.A., T.A., B.U., O.Ç., Design: E.S., Supervision: E.E.G., B.D.A., Resources: B.Ö., S.A., Material: S.A., Data Collection or Processing: S.K., S.A., Analysis or Interpretation: E.E.G., Literature Search: B.D.A., S.A., Writing: T.A., E.S., Critical Review: O.Ç., B.U.

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Evaluation of the Vascular Effects of Iloprost in Patients with Thromboangiitis Obliterans (Buerger's Disease)

Tromboanjitis Obliterans (Buerger Hastalığı) Olgularında İloprostün Vasküler Etkilerinin Değerlendirilmesi

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ABSTRACT

Objective: Thromboangiitis obliterans (TAO) is a common vascular pathology that occurs as a result of increased cigarette smoking in individuals with a genetic predisposition. Due to the incomplete understanding of its pathogenesis, the condition's diagnosis, treatment, and prevention processes still present challenges.

Methods: In this study, 15 patients (1 female, 14 male), aged between 20 and 55, diagnosed with TAO, were treated with iloprost, a stable PGI2 analogue. Each patient received intravenous iloprost for six hours daily over 28 days. The dose titration was as follows: treatment started with 0.5 ng/kg/min for the first three days. The dose was increased by 0.5 ng/kg/min every 30 minutes until a maximum dose of 2.0 ng/kg/min was reached and maintained for six hours. Before and after treatment, 20 mCi Tc-99m pyrophosphate injections were administered, and lower extremity perfusion rates were assessed through scintigraphy in both resting and exercising positions. A significant increase in perfusion rates was observed after treatment ($p<0.05$).

Results: In this study, iloprost treatment accelerated ischemic ulcer healing, increased walking distance, alleviated pain at rest, and improved the ankle-brachial index in patients with TAO. These positive effects of treatment persisted for up to six months. Changes before and after treatment were statistically significant, as shown both subjectively and scintigraphically ($p<0.05$).

Conclusion: The aim of this study was to evaluate the effect of PGI2 analogues, such as iloprost, on tissue perfusion reserve in patients with TAO at the microvascular level using scintigraphy.

Keywords: Iloprost, thromboangiitis obliterans, scintigraphy, prostacyclin (PGI2)

ÖZ

Amaç: Tromboanjitis obliterans (TAO), sigara içen bireylerde genetik yatkınlık nedeniyle ortaya çıkan yaygın bir vasküler patolojidir. Patogenezinin tam olarak anlaşılamaması nedeniyle tanı, tedavi ve önleme süreçleri hala zorluklar sunmaktadır.

Yöntemler: Bu çalışmada, TAO teşhisi konulan 15 hasta (1 kadın, 14 erkek), 20-55 yaş aralığında, iloprost ile tedavi edildi. İloprost, bir PGI2 analogudur. Her hasta, 28 gün boyunca günde altı saat intravenöz iloprost aldı. Doz titrasyonu, ilk üç gün için 0,5 ng/kg/dak ile başladı ve her 30 dakikada 0,5 ng/kg/dak artırarak maksimum 2,0 ng/kg/ dak dozuna ulaşıldı ve altı saat boyunca sürdürüldü. Tedaviden önce ve sonra, 20 mCi Tc-99m pirofosfat enjeksiyonları uygulandı ve alt ekstremite perfüzyon oranları, hem dinlenme hem de egzersiz pozisyonlarında skintigrafi ile değerlendirildi. Tedaviden sonra önemli bir perfüzyon artışı gözlemlendi ($p<0,05$).

Bulgular: Bu çalışmada, iloprost tedavisi, TAO'lu hastalarda iskemik yara iyileşmesini hızlandırdı, yürüme mesafesini artırdı, dinlenme ağrısını hafifletti ve ayak bileği-kol kan basıncı indeksini iyileştirdi. Tedaviye bağlı olumlu etkiler altı aya kadar sürdü. Tedaviden önce ve sonra yapılan değişiklikler, hem subjektif hem de sintigrafik olarak istatistiksel olarak anlamlıydı ($p<0,05$).

Sonuç: Bu çalışmanın amacı, skintigrafi kullanarak mikrovasküler düzeyde TAO'lu hastalarda PGI2 analogları gibi iloprostun doku perfüzyon rezervi üzerindeki etkisini değerlendirmekti.

Anahtar Sözcükler: İloprost, tromboanjitis obliterans, sintigrafi, prostaglandin (PGI2)

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INTRODUCTION

Buerger's disease or thromboangiitis obliterans (TAO) is one of the peripheral obstructive vascular disorders presenting as a non-atherosclerotic, segmental, inflammatory condition commonly occurring in lower extremities and rarely in upper extremities, affecting small or medium arteries and adjacent veins. Peripheral endothelium-dependent vasorelaxation is impaired in patients with TAO (1). TAO is distinguished from other vasculitides by the predominantly inflammatory thrombus formation leaving relatively intact areas on the vascular walls (2). The treatment of this common disorder generally consists of antiaggregant drugs, vasodilator agents, and, recently, prostacyclin analogues (3,4). Prostacyclin I2 (PGI2) is a prostacyclin analogue exerting its effects by regulating microcirculation, and is disrupted in ischaemia, inflammatory tissue lesions, vasospasm, and cold stress intolerance. The favourable effects of PGI2 provide dilated precapillary vessels, reduced flow resistance, increased digital blood flow, and prevent microthrombus formation by decreasing leukocyte adhesion in small capillary channels. The agent administered to the patients in the present study was iloprost, a stable PGI2 analogue providing the aforementioned effects. The aim of the present study is to evaluate the therapeutic effects of iloprost, a PGI2 analogue, which is one of the medical treatment options for TAO and for which various treatment protocols are used, including surgical reconstruction, sympathectomy, and medical treatment.

MATERIALS and METHODS

Informed consent forms approved by the Ethics Committee, designated by Atatürk University, were obtained from patients and relatives/caregivers prior to the start of the study (decision number: 1, date: 11.02.2004). Patients were informed that they could withdraw from the study at any time without giving any reason, and that they could be withdrawn from the study at the discretion of the study doctor if the treatment was deemed not indicated or potentially harmful for the patient. A total of 15 patients with Buerger's disease and Fontaine stage IV (claudication, rest pain and/or ischaemic ulcerated wound) were included in the study. One male and one female patient withdrew from the study on iloprost treatment by their own will. Two male patients with Buerger's disease replaced these patients. The general characteristics of the patients are summarized in Table 1.

None of the patients had any chronic disease such as coronary or congestive heart failure, diabetes mellitus, malignancy, or chronic obstructive pulmonary disease. Patients diagnosed with Buerger's disease who were potential candidates for major amputation and who had not undergone any vascular reconstruction or any intervention for lumen enlargement (angioplasty) or fibrinolytic therapy were included in this clinical study. Exclusion criteria of the present study included pregnancy, lactation, abnormal platelet count, bleeding-coagulation disorder, and advanced renal or hepatic dysfunction.

To avoid confounding factors, anticoagulants, pentoxifylline, and low or high molecular weight heparin were not used during the study period. Intensive wound management was continued.

Routine systemic examination, non-invasive vascular investigations (Doppler ultrasound, ankle/brachial index measurements), and haematological and biochemical investigations were performed for the 15 patients prior to the start of the study. Furthermore, results of the scintigraphic extremity assessment were recorded on individual patient follow-up forms. A self-monitoring system was implemented by keeping patients in the same room for as long as possible. One ampoule of iloprost was mixed in a 5% dextrose solution and administered to the patients via an intravenous route with an infusion pump every day. The drug solution was prepared daily. The clinical study group consisted of patients receiving intravenous iloprost over 6 hours for 28 days. Following the initial dose of 0.5 ng/kg/min for three days, the individual dose was titrated every thirty minutes, increasing by increments of 0.5 ng/kg/min to achieve the maximum dose of 2.0 ng/kg/min given intravenously over 6 hours from the forearm vein. The individual tolerable dose was determined by the occurrence of side effects, including flushing, headache, abdominal pain, and nausea. The findings are summarized in Table 2.

Treatment efficacy was evaluated according to the following criteria in this clinical study:

1. Decrease and/or relief of rest pain (using visual analogue pain scale)

2. Wound shrinkage and/or healing in patients with wound in extremities

3. Change in claudication distance

4. Changes in Ankle Brachial Index (ABI).

Patients were monitored closely for arterial blood pressure (BP), and study treatment was initiated after normalization of BP in patients with systolic BP over 140 mmHg. Iloprost therapy was planned as four weeks of treatment (28 days), and 6 months of post-treatment follow-up. On day 1-3-7, and 28 after the initiation of treatment, rest pain was assessed with the visual analogue pain scale and ischaemic wound changes were evaluated. The modified grading scale for Buerger's recommended by SVS/ISCVS (Society for Vascular Surgery/International Society for Cardiovascular Surgery) was used at 6 months, and ankle/brachial index and scintigraphic findings were recorded in patient follow-up forms.

Using a scintigraphic method, the present study aims to evaluate whether iloprost affects perfusion reserves and its effects at the microvascular level. Patients received intravenous Tc-99m pyrophosphate at 20 mCi for the scintigraphic assessment. Foot exercise was performed prior to scintigraphic imaging, and after the Tc injection. The first imaging was performed 15 minutes after Tc injection, and the second imaging was performed following a one-hour rest, after the first imaging. The same scintigraphic imaging was repeated on day 28 of treatment and at 6 months, and scintigraphic images were obtained in anterior and posterior positions. Total counts (Cts) were obtained from the regions of interest (ROIs) of the posterior images, and perfusion rates were calculated using a formula. A double-detector gamma camera (Siemens ECAM) and the computer integrated with this system were used in the present study. A low-energy, high-resolution collimator with parallel holes was selected, and the images were obtained at 140 keV photo peak (the energy peak of Tc-99m) with an energy window of 20%. The images were evaluated with the computers integrated into the gamma camera. Total Cts were calculated from ROIs of the scintigraphic images of the extremity at the posterior position before and after

treatment with iloprost, and these total Cts were recorded. The total Cts of the posterior images were used to calculate the perfusion rates according to the following formula:

$$\% \text{ perfusion reserve} = \frac{\text{PreT (PostT) Ct 15 min.-PreT (PostT) Ct 1 hour x 100}}{\text{PreT (PostT) Ct 1 hour}}$$

*PreT: Pre-treatment *PostT: Post-treatment *15 min: exercise *1 hour: Rest

Statistical Analysis

Statistical analysis of the data was performed using Statistical Package for the Social Sciences (SPSS) version 11.0, the Wilcoxon test, and the t-test. P-values less than 0.05 were considered statistically significant (Table 3).

RESULTS

The iloprost tolerance and side effects were monitored during the clinical study by recording physical findings and investigating patients' complaints. These findings are summarized in Table 2. The patients exhibited no severe side effects requiring discontinuation from the study. The symptoms observed in these patients resolved following a dose reduction by 0.5 ng/kg/min every thirty minutes. Blood samples were obtained from the patients included in this study prior to treatment, on day 28 of treatment, and at the end of the follow-up of six months, and no direct effects were detected in the haematological or biochemical (hepatic and renal function tests) tests. The difficulty in evaluating the objective parameters led to the assessment of more subjective parameters such as rest pain,

Table 1. Demographic characteristics of the patients

Demographic characteristics	n (number of patients)	% (percentage of patients)
Age (years)		
20-30	2	13.3
31-40	3	20
41-55	10	66.7
Gender		
Male	14	93.3
Female	1	6.7
Time from diagnosis		
1-5 years	11	73.4
6-10 years	2	13.3
11-20 years	2	13.3
Sympathectomy		
Yes	6	40
No	9	60
Level of sympathectomy		
Lumbar	4	67
Thoracic	1	16.5
Lumbar+thoracic	1	16.5
Claudication distance		
<100 metres	3	20
100-200 metres	4	26.7
201-300 metres	2	13.3
301-500 metres	5	33.3
>500 metres	1	6.7
Mean ankle/brachial index	0.51±0.18	
Smoking	15	100
Ischaemic wound		
Lower extremity	13	86.7
Upper extremity	2	13.3
Amputation level		
Upper extremity (below elbow)	1	25
Lower extremity (below knee)	2	50
Lower and upper extremity (below knee and below)	1	25

history, related parameters, and use of analgesics. The changes in treatment efficacy parameters before and after iloprost treatment are presented in Table 4.

The evaluation at six months showed (i) further reduction in rest pain during the post-treatment period, (ii) no persistent or new wounds (Figure 1 and Figure 2), (iii) further improvement in claudication distance compared to the increase at 28 days, and (iv) no reduction in ABI rates compared to the rates observed 6 months earlier. The statistical comparison between pre- and post-treatment periods regarding ABI on day 28 and at 6 months, change in claudication distance, recovery rate in ischaemic wounds, and change in rest pain showed that the post-treatment findings were significantly different from the pre-treatment findings ($p<0.05$). While the change in rest pain was not very significant on day 1, and 3, the reduction in rest pain was statistically significant on day 7 ($p<0.05$). The rest pain showed a highly significant reduction on day 28 and at 6 months ($p<0.001$). While the change in claudication distance was not very significant on day 1, 3, and 7, the change was statistically significant on day 28 ($p<0.05$). The difference was highly significant at the end of 6 months ($p<0.001$). To minimize the physician bias during the assessments in this clinical study, simultaneous evaluations were carried out by an external, independent observing physician before and after, (on day 28 and at 6 months), the treatment. These simultaneous evaluations revealed consistent findings.

Table 2. The symptom and severity findings of the patients

Symptoms	Severity-no. of patients		
	Mild	Moderate	Severe
Flushing	2	1	0
Nausea	1	1	1
Head ache	1	1	1
Abdominal pain	0	1	0

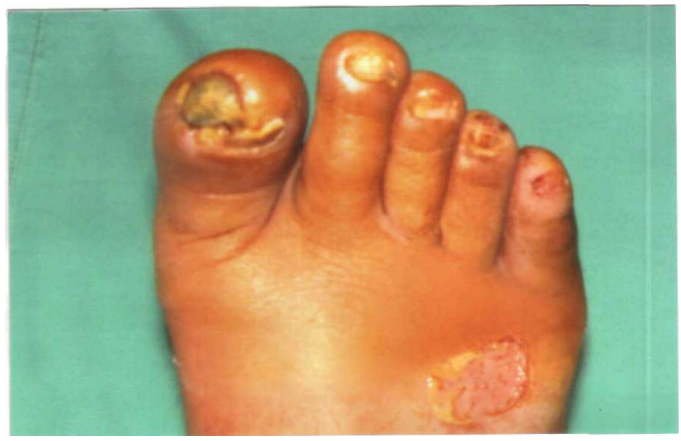

Figure 1. Before treatment with iloprost.

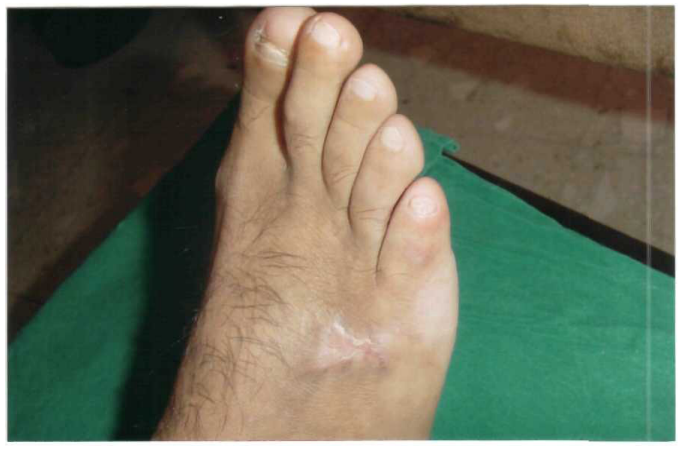

Figure 2. Six months after treatment with iloprost.

Table 3. Comparison of scintigraphic % perfusison outcomes in both extremities before and after treatment with iloprost (t-test)

	Right before treatment	Right after treatment	Left before treatment	Left after treatment	Difference right	Difference left	Right 100	Left 100
Minimum	12.44	17.63	13.16	19.78	-27.07	-37.07	-156.75	-153.5
Maximum	78.84	90.25	75.6	92.68	1.12	6.75	5	5.6
Mean	29.7087	42.454	28.6587	40.287	-12.7453	-11.623	51.8719	48.0114
Standard deviation	16.2323	18.283	14.8494	18.2	8.0862	9.1985	42.0949	43.6744

Table 4. Changes in therapeutic efficacy parameters before and after treatment with iloprost

	Day 1	Day 3	Day 7	Day 28	6 months
Reduction in rest pain	Ø	10-15%	30%	98%	98%
Wound shrinkage	Ø	1-3%	10-15%	75%	100%
Increase in claudication distance	Ø	Ø	3%	80%	95%
Ankle/Brachial Index	Ø	Ø	3%	65%	80%

Ø: No change was observed

DISCUSSION

The disorder commonly associated with smoking or tobacco consumption has also been linked to an aetiology involving genetic factors, hypercoagulability, vascular endothelial structure, and immunological mechanisms (3,4). In 1879, von Winiwarter identified an obstructive arterial disease apart from atherosclerosis and termed the disease "endarteritis obliterans". Pathological investigations revealed intimal proliferation, thrombosis and fibrosis. von Winiwarter was the first to identify that endarteritis and endophlebitis differed from atherosclerosis. In 1908, Leo Buerger termed the disorder "TAO", and provided a detailed description of clinical and pathological characteristics of the disease. Austrian surgeon Alexander von Winiwarter (April 22, 1848-October 31, 1917), born in Vienna, was the first to describe endarteritis and endophlebitis as distinct from atherosclerosis. Leo Buerger, who was born in Vienna and raised in German culture, and was of Jewish descent, described in detail and accurately the clinical and pathological features of the disease he named "TAO" in 1908 (5).

Buerger's definition is based on his observations, which confirmed that the symptoms of the disease are not only in the arteries but in the veins, where the first signs of the disease often appear. Buerger recorded all his experiences and opinions in his book "Über die Kreislaufstörungen der Extremitäten einschliesslich Brand, vasomotorische und trophische Störungen.", published in German. In 1917, F. Parkes Weber of the German Hospital in London reported a series of cases that confirmed Buerger's view. He proposed the name "Nicht syphilitische Arteriitis obliterans der Juden". Although it was generally confirmed that the disease was rare in women, as Buerger had previously stated, it was later decided not to define it as specific to Jews (6).

Following Buerger's description, Allen and Brown reported cases with TAO, the majority of whom were heavy-smoking males of Jewish descent. They reported claudication in the feet, gangrenous ulceration in the toes, or gangrene affecting the toes or the whole foot in these cases. Although Allen and Brown suggested the hypothesis that TAO was an infectious disease, their pathological description was consistent with the original report by Buerger (2). One of the interesting features of Buerger's disease is the geographic distribution. Among peripheral arterial diseases, the incidence of Buerger's disease is less than 1% in the USA. The incidence is 0.5-5.6% in European countries, approximately 10% in our country, and about 50% in the Far East (3). TAO, or Buerger's disease, is an inflammatory vasculitis that causes non-atherosclerotic involvement in small and medium arteries, adjacent veins, and neighbouring nerves of extremities (2-7). Histopathology is generally diagnostic in the acute phase of the disease and is characterized by acute inflammation involving all layers of the vascular wall. In terms of arteriographic and pathological aspects, TAO is a partial, (i.e. characterized by both affected and unaffected regions), vascular entity. It is typically seen in young males (aged 20-50 years) heavy smokers or consume tobacco products. The patients included in the present study exhibited heavy smoking habits.

TAO is a panarteritis with several controversial treatment options. Approximately 76% of patients have ischaemic ulcerations at the time of presentation (8). When revascularization is not possible in patients with critical extremity ischaemia and ischaemic ulcerations, the last option to provide pain relief is often amputation.

Extremity amputations are historically considered one of the oldest surgical interventions. Such procedures are generally evaluated negatively, and indications for amputations performed for reasons other than trauma are delayed and often rejected by the patient and their relatives. This situation, which creates a psychological and emotional void in addition to the physical loss in the individual, can be exemplified in German literature as a traumatic reflection of the internal struggle experienced by a 19-year-old soldier, who became an amputee in Erich Maria Remarque's work "Im Westen nichts Neues" (9).

Major amputation rates as high as 70% have been reported (10,11). This rate was 26.7% in the present study (Table 1). Trophic changes and wounds were present in the lower extremities of 13 patients (86.7%) and the upper extremities of two patients (13.3%). 73% of the patients were diagnosed with Buerger's disease within 5 years.

Because the disorder primarily affects distal small arteries, reconstructive surgery is rarely possible or successful. When vessel reconstruction and sympathectomy is insufficient and/or unsuccessful in patients with TAO, rest pain and trophic lesions occur as notable problems. Currently, there is no medical treatment option generally considered effective in TAO. Drugs with different mechanisms of action have been tried in recent years, and among them, the importance of prostanoids such as PGE1 and PGI2 has been studied and discussed (10-12). Iloprost treatment is reported to provide favourable effects on trophic lesion healing, relief of rest pain, decrease amputation rates, and reduce overall mortality. These favourable effects are associated with the increased microcirculation induced by iloprost. Furthermore, iloprost provides a protective effect on endothelial cells by decreasing the production of adhesion molecules and coagulation end-products. The therapeutic potential of PGI2 has been investigated in several conditions under the scope of peripheral arterial diseases (such as atherosclerotic obliterative disease, diabetic angiopathy, TAO of inflammatory/immunological origin, or Raynaud's phenomenon) starting from the demonstration of its robust effects on platelets and vascular wall. However, the use of PGI2 remains limited due to its chemical instability. The main problem regarding the use of PGI2 is the short half-life of the drug. Clinical trials have focused on a more stable PGI2 analogue. Using, a PGI2 analogue showing the aforementioned properties, is used in treatment protocols for 21 and 28 days. Several centres in Germany and France report favourable effects on lesion occurrence and amputation rates in patients with TAO, diabetes mellitus, and obstructive arterial disease, as well as in patients with disease-related rest pain, ulcerations, and gangrene lesions and in patients at the point of amputation (13,14). The findings of the present study are consistent with the literature.

Despite the ongoing advances in lumen enlargement methods and bypass surgeries, which have been used for decades in peripheral arterial diseases, these interventions are still associated with high rates of failure. The PGI2-like iloprost has been comprehensively studied in patients with critical leg ischaemia, and the results of the studies have shown a possible reduction in amputation rates with this drug (15,16). Although the present study does not reflect a study with grafts, the intra-graft iloprost injections administered at the end of peripheral arterial bypass intervention in patients with peripheral arterial disease have been shown to increase continuous

blood flow in femorodistal vascular grafts. They show favourable effects on bypass outcomes by reducing distal vascular resistance, providing endothelial protection, and regulating microcirculation at the capillary level (14-17). Amputation rates are higher with aspirin therapy, compared to iloprost therapy. Furthermore, intravenous iloprost is significantly more effective in reducing rest pain and promoting trophic lesion healing in patients with TAO compared to low dose oral aspirin (14-18). The present study also demonstrates improved rest pain and wound healing with iloprost treatment. Clinical success rates as high as 96% have been reported following iloprost therapy (19). Higher response to iloprost therapy (improvement in rest pain and ulcerations) has been reported with the treatment duration of 4 weeks (28 days) than with 2 weeks (14 days) or 3 weeks (21 days) (17-22). The treatment duration selected to evaluate the therapeutic efficacy, of iloprost in the present study was 28 days, and the findings are consistent with the literature.

Iloprost has been shown to provide beneficial effects compared to placebo in patients with ulcers or gangrene. The therapeutic effects are maintained up to 6 months following completion of therapy and result in lower amputation rates. In addition to improving clinical symptoms, several controlled studies have shown the favourable effects of iloprost on amputation rates and mortality (20-22). In the present study, patients exhibited significant improvement in terms of clinical presentation, and none of the patients underwent amputation following the treatment. Iloprost treatment was used for 14-21 days in a multi-centre study in patients undergoing below-knee amputation. The wounds healed in 59% of these patients without requiring amputation at upper levels. Furthermore, the probability of recovery has been reported to increase by twofold in patients previously managed with revascularization via surgical or radiological methods (16-23). This outcome is associated with the ability of iloprost to prevent platelet aggregation, induce vasodilation, and fibrinolysis, prevent leukocyte activation through its effects at the microcirculatory level (24). Iloprost also provides a protective effect on endothelial cells by decreasing the production of adhesion molecules and coagulation end-products (25,26) and shows therapeutic effects by regulating microcirculation through normalisation of the disrupted mechanisms without changing the total blood flow in extremities (27).

According to the studies on iloprost, the possible side effects which may occur in the beginning of treatment include headache, flushing, nausea, and vomiting (28). The side effects of iloprost are commonly associated with its vasodilator effect. These side effects can be managed by decreasing infusion rate or discontinuing treatment for short periods in the majority of the cases. The mild side effects do not require dose reduction. None of the patients included in the present study experienced severe side effects requiring discontinuation of treatment. According to the comments of the TASC study group on studies using iloprost therapy in patients with peripheral arterial disease, iloprost is the drug that has been investigated in randomised controlled studies with the largest number of patients in advanced, critical extremity ischaemia. There is no available method to predict which patients would respond to treatment; however, the relative safety of iloprost is considered potentially beneficial in all patients until early amputation is inevitable (29-31).

In the present study, the pre- and post-treatment assessment of the effects of iloprost therapy using Tc-99m pyrophosphate showed a statistically significant difference in post-treatment perfusion rates in 13 (87%) patients ($p<0.05$), and no significant difference in 2 (13%) patients (Table 2 and Table 3). This outcome indicates that iloprost improves perfusion in regions with previously disrupted blood flow at distal parts of the obstruction, and this can be assessed using scintigraphic methods. Currently, intravenous prostaglandins or prostacyclin analogues seem the most widely used medications in patients with Buerger's disease with critical limb ischemia. We believe that it may also be used as an adjunctive follow-up method to evaluate the treatment outcome in similar peripheral arterial disorders. The findings of the present study are consistent with the results of similar studies in the literature (32,33).

Although pre-surgery signs and symptoms of the disease reoccurred in the short or long term in patients undergoing sympathectomy among the patients included in this clinical study, the durable reduction or improvement of symptoms observed during the post-treatment period compared to the pre-treatment period, led to the question whether iloprost therapy may be an alternative treatment option to sympathectomy. Sympathectomy is an invasive intervention, while iloprost therapy is a non-invasive method.

CONCLUSION

Iloprost showed favorable clinical efficacy and safety in the fifteen TAO patients included in the present study. We believe that, to gain general acceptance in relevant indications, it may be used as a beneficial treatment option to gain general acceptance in relevant indications, and may also for the scintigraphic assessment of treatment efficacy. In our opinion, these results require further studies to ethically support using different treatment approaches in larger patient populations.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ethics Committee of Atatürk University (approval number: 1, date: 11.02.2004).

Informed Consent: Informed consent were obtained from patients and relatives/caregivers prior to the start of the study.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: M.A.K., Concept: M.A.K., Design: M.A.K., Data Collection or Processing: M.A.K., Ş.K., Analysis or Interpretation: M.A.K., Ş.K., Literature Search: M.A.K., Ş.K., Writing: M.A.K., Ş.K.

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Comparison of Preoperative and Postoperative Anti-Mullerian Hormone Levels in Patients Operated for Ovarian Torsion

Over Torsiyonu Tanısı ile Opere Olan Hastalarda Preoperatif ve Postoperatif Anti-Müllerian Hormon Değerlerinin Karşılaştırılması

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ABSTRACT

Objective: This study aimed to investigate how surgical treatment affects ovarian reserve in patients with ovarian torsion who desire fertility, by comparing preoperative and postoperative serum anti-Mullerian hormone (AMH) levels. Additionally, it explored clinical and procedural factors potentially influencing AMH fluctuations, including the surgical method, side of torsion, and type of operation.

Methods: A total of 23 patients who underwent surgical management for ovarian torsion between 2020 and 2022 and had both pre-and postoperative AMH levels recorded were included. All hormone measurements were conducted using the same Beckman Coulter assay at a single institutional laboratory. Postoperative AMH assessments were performed at least three months following the intervention. Subgroup analyses were conducted based on operative features, including detorsion with or without cystectomy, oophorectomy status, surgical technique (laparotomy vs. laparoscopy), and laterality of torsion (right vs. left ovary).

Results: Although an overall upward trend in AMH levels was noted postoperatively, this increase did not reach statistical significance across the entire sample. A significant postoperative rise in AMH was observed among patients who did not undergo oophorectomy (p=0.014). When analyzed by surgical technique, a statistically

ÖZ

Amaç: Bu çalışmada, over torsiyonu nedeniyle opere edilen ve fertilite arzusu bulunan hastalarda cerrahi müdahalenin over rezervine etkisinin serum anti-Müllerian hormon (AMH) düzeyleri üzerinden değerlendirilmesi amaçlanmıştır. Ayrıca, AMH düzeylerindeki değişime neden olabilecek cerrahi yaklaşım, torsiyon tarafı ve uygulanan operasyon tipi gibi klinik faktörler incelenmiştir.

Yöntemler: Çalışma, 2020-2022 yılları arasında over torsiyonu tanısıyla opere edilen ve preoperatif ile postoperatif AMH düzeyleri ölçülmüş olan 23 hastanın ile gerçekleştirilmiştir. AMH ölçümleri Beckman Coulter cihazı ile aynı kit kullanılarak yapılmıştır. Postoperatif AMH düzeyleri cerrahiden en az üç ay sonra değerlendirilmiştir. Detorsiyon ± kistektomi, ooferektomi varlığı, cerrahi yaklaşım (laparotomi/laparoskopi) ve torsiyon tarafına göre alt grup analizleri yapılmıştır.

Bulgular: Genel hasta grubunda postoperatif AMH düzeylerinde artış eğilimi gözlenmiş, ancak bu fark istatistiksel olarak anlamlı bulunmamıştır. Ooferektomi yapılmayan hastalarda AMH artışı anlamlıydı (p=0,014). Laparotomi uygulanan hastalarda postoperatif AMH düzeylerinde anlamlı artış saptanırken (p=0,021), laparoskopi grubunda bu fark anlamlı değildi. Sol over torsiyonu olan hastalarda postoperatif AMH düzeylerindeki artış anlamlıydı (p=0,039).

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ABSTRACT

significant increase was found in the laparotomy group (p=0.021), while no significant change occurred in the laparoscopy group. Additionally, patients with left-sided torsion showed a significant improvement in AMH levels after surgery (p=0.039).

Conclusion: Detorsion may serve as a viable surgical strategy for preserving ovarian reserve in women concerned about future fertility. The choice of surgical approach and the side of torsion appear to play a role in hormonal recovery and should be considered during preoperative planning.

Keywords: Ovarian torsion, AMH, ovarian reserve, detorsion

ÖZ

Sonuç: Detorsiyon işlemi, özellikle fertilite arzusu taşıyan hastalarda over rezervinin korunmasında etkili bir yöntem olabilir. Cerrahi yaklaşım ve torsiyonun tarafı gibi değişkenler AMH düzeylerini etkileyebileceğinden, tedavi planlamasında bu faktörlerin dikkate alınması önerilmektedir.

Anahtar Sözcükler: Over torsiyonu, AMH, over rezervi, detorsiyon

INTRODUCTION

Ovarian torsion, a gynecological emergency, is when the ovary rotates around the utero-ovarian and infundibulopelvic ligaments. Ovarian vascular flow is compromised by this rotation, which first impairs lymphatic and venous outflow before gradually compressing the infundibulopelvic ligament and impairing arterial circulation. The vascular disruption may cause ischemia and necrosis that results by reducing the ovarian tissue's ability to receive blood flow. While venous congestion occurs more quickly, arterial perfusion usually lasts longer because arterial walls are thicker and more elastic than venous walls. Significant ovarian enlargement and stromal edema may result from this mismatch. About 2.7% of all emergency gynecologic procedures are due to ovarian torsion (1). It is most often detected in women of reproductive age, especially those with underlying ovarian masses; however, it may occur at any age, including during pregnancy and the neonatal period (2,3). Because of the pelvis's physical structure, which includes a shorter left utero-ovarian ligament and a sigmoid colon on the left side that supports the neighboring ovary structurally right-sided torsion is more often documented (4). An ovarian mass is shown to be a contributing cause in about 85% (5) of cases of torsion. Therefore, to maintain ovarian viability and reproductive potential, prompt diagnosis and surgical intervention are crucial (6).

In clinical settings, ovarian torsion usually manifests as moderate to severe unilateral or widespread pelvic pain that starts suddenly and is often accompanied by nausea and vomiting, particularly in individuals who have adnexal masses (2,7). Laboratory results are not pathognomonic. Because of hemorrhagic and ischemic alterations, the afflicted ovary may appear larger, edematous, and heterogeneous on ultrasound imaging compared to the contralateral side. Comparative assessment with the opposite ovary is necessary because Doppler ultrasonography may show normal, reduced, or nonexistent blood flow in the torn ovary (8,9). Furthermore, a "whirlpool sign" that indicates a twisted vascular pedicle may be seen, and in a small number of studies, this sign has shown a diagnostic sensitivity of over 90% (10).

For the diagnosis and treatment of ovarian torsion, surgery is still the gold standard since it allows for prompt detection and therapy. Depending on the clinical situation, either a laparotomy or a laparoscopy may safely accomplish detorsion (11). A black, enlarged ovary was often thought to be necrotic, but newer research indicates that ovarian activity may frequently be maintained in these situations (12,13).

The amount and quality of a woman's accessible oocytes are both included in her ovarian reserve, which is a reflection of her reproductive potential (14). Clinicians usually use a mix of biochemical markers and ultrasonographic studies to assess ovarian reserve, even though there isn't a single, well-recognized test for this purpose. Anti-Mullerian hormone (AMH) is one of the frequently used markers among these (15). AMH, which is produced by preantral and early antral follicles, is thought to be a sign of general ovarian activity and provides insights into the size of the primordial follicle pool (16). Its clinical utility is enhanced by its reduced vulnerability to environmental variations and relative stability during the menstrual cycle (17). However, high and fluctuating AMH concentrations are common in people with polycystic ovarian syndrome. AMH levels gradually drop with age as the primordial follicle pool shrinks, and they often stop being detectable around menopause (18,19). In clinical settings, AMH screening is often used to evaluate ovarian reserve in patients undergoing ovarian surgery, infertility testing, or therapies like chemotherapy or radiation therapy that carry a risk of gonadal damage (20).

By measuring blood AMH levels in patients with ovarian torsion, this research sought to determine the possible impact of detorsion surgery on ovarian reserve and to investigate potential clinical and surgical variables that may be involved in these hormonal alterations.

MATERIALS AND METHODS

The Department of Obstetrics and Gynecology at the Şahinbey Research and Training Hospital, which is connected to Gaziantep University Faculty of Medicine, conducted this retrospective observational research. Patients with an ovarian torsion diagnosis who had surgery and had their blood AMH levels assessed both before and after the procedure. Patient anonymity was preserved throughout the entire data analysis process, and all clinical data were obtained via the hospital's secure electronic health record system.

Patient Selection and Data Collection

Serum AMH levels were measured during the clinical course of patients with a desire for fertility who had surgery at the Department of Obstetrics and Gynecology, Şahinbey Research and Training Hospital, Gaziantep University Faculty of Medicine, between 2020 and 2022 after being diagnosed with ovarian torsion. Patients whose procedures were carried out by the same skilled gynecologic surgical team were chosen as the research population.

Measurements taken at least three months after surgery were used to assess postoperative AMH levels. Age between 18 and 44, a desire for future fertility, and the availability of both preoperative and postoperative AMH readings. Exclusion criteria included being older than 44, lacking preoperative and/or postoperative AMH data, not attending postoperative follow-up, and having had further ovarian surgery after detorsion. The medical records of 33 individuals who received treatment for ovarian torsion, within the allotted time frame, were examined. Ten individuals were eliminated due to lack of hormonal data or surgical follow-up. The research also included oophorectomy patients, who were assessed independently in a subgroup analysis.

Serum AMH levels were measured at the hospital's hormone laboratory using the same assay kit and equipment (Beckman Coulter) for all patients receiving treatment at the same facility. AMH1" refers to AMH levels recorded before surgery, while "AMH2" refers to levels tested at least three months after surgery. The hospital's electronic medical record system was used to gather demographic and clinical data, such as age, obstetric history, preoperative ultrasound results, and operation type. Finally, 23 patients who met the inclusion criteria were included in the final analysis. The patient selection process is summarized in Figure 1.

This study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of Gaziantep University (decision number: 2022/380, date: 07.12.2022).

Statistical Analysis

The R programming language was used to conduct statistical studies and create graphical representations (version 2024.04.2+764). While continuous variables were presented using descriptive statistics as such as mean ± standard error (mean ± standard deviation) or median (minimum-maximum), if applicable, categorical data were shown as percentages (%). The Shapiro-Wilk test was used to evaluate the data's distribution. The Wilcoxon Signed-Rank Test was used to analyze differences between preoperative and postoperative AMH levels for paired data that did not have a normal distribution.

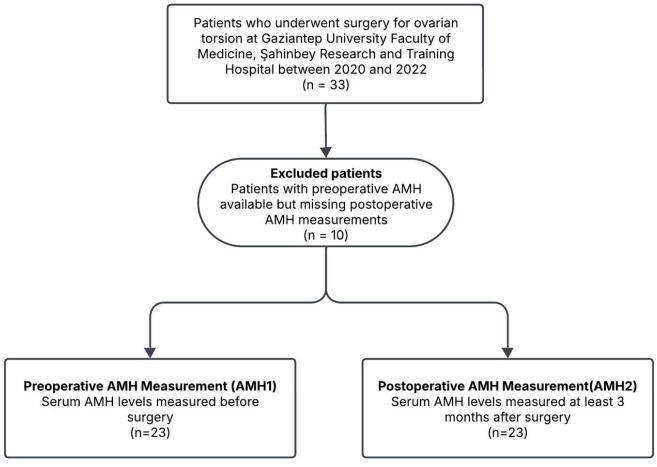

Figure 1. Flowchart of the patient selection process for inclusion in the study.

AMH: Anti-Mullerian hormone

Two independent groups were compared for differences using the Mann-Whitney U test. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

This research assessed a total of 23 individuals who had surgery to treat ovarian torsion. The research cohort's demographic and surgical characteristics are reported in Table 1. The median age of the participants was 28, and their ages varied from 18 to 44. 26.1% were unmarried, whilst 73.9% were married. Parity ranged from 0 to 5, and gravidity ranged from 0 to 9. Left ovarian torsion occurred in 34.8% of cases, whereas right ovarian torsion was observed in 65.2% of cases. Regarding surgical method, laparotomy (L/T) was performed on 56.5% of patients, while laparoscopy (L/S) was performed on 43.5% of patients. Before surgery, the mean blood AMH content was 3.22±2.4 ng/mL; after the operation, it rose to 4.51±2.1 ng/mL (Table 1).

Figure 2 presents the individual changes in AMH levels observed in the study. Most patients demonstrated increased postoperative

Table 1. Clinical and demographic characteristics of patients included in the study

Variables	n=23
Age, Median(min.-max.)	28 (18-44)
Single, n (%)	6 (26.1%)
Married, n (%)	17 (73.9%)
Gravidity, median (min.-max.)	2 (0-9)
Parity, median (min.-max.)	1 (0-5)
Side of torsion-right, n (%)	15 (65.2%)
Side of torsion-left,n (%)	8 (34.8%)
Type of surgery-laparotomy, n (%)	13 (56.5%)
Type of surgery-laparoscopy, n (%)	10 (43.5%)
Preoperative AMH, (mean ± SD), ng/mL	3.22±2.4
Preoperative AMH, (mean ± SD), ng/mL	4.51±2.1

AMH: Anti-Mullerian hormone, SD: Standard deviation, min.-max.: Minimum, maximum

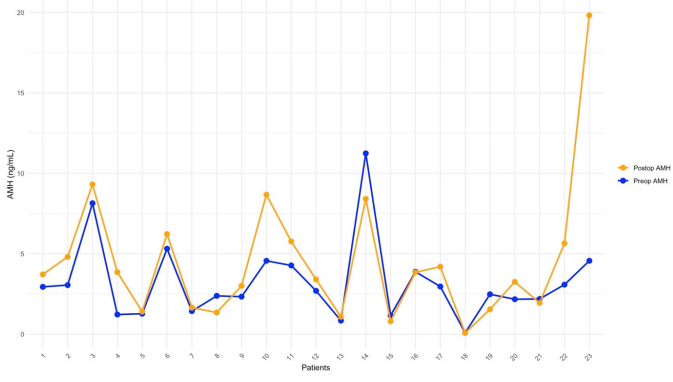

Figure 2. Individual preoperative and postoperative AMH levels of patients undergoing detorsion surgery.

AMH: Anti-Mullerian hormone

AMH levels compared to their preoperative measurements, with several individuals exhibiting a marked rise following surgery. However, a decline in AMH levels was observed postoperatively in a few patients compared to their preoperative measurements.

The variations in AMH levels according to the type of surgery performed are shown in Table 2. Postoperative AMH levels exhibited an increasing tendency in instances when detorsion was followed by cystectomy; however, the rise was not statistically significant (p=0.098). Patients who had detorsion without cystectomy, likewise, showed a comparable non-significant increase in outcomes (p=0.135). AMH readings before and after surgery did not significantly vary in the subset of individuals who had oophorectomy (p=0.500). On the other hand, postoperative AMH levels increased significantly among individuals who did not have an oophorectomy (p=0.014).

Changes in AMH levels according to the surgical approach and side of torsion are presented in Table 3. A statistically significant increase in postoperative AMH levels was observed in patients who underwent laparotomy (L/T) (p=0.021). In contrast, no significant difference was found between preoperative and postoperative AMH levels in the group that underwent laparoscopy (L/S) (p=0.375). Among patients with right-sided torsion, the increase in AMH levels was not statistically significant (p=0.187), whereas a significant increase in postoperative AMH levels was observed in those with left-sided torsion (p=0.039) (Table 3).

DISCUSSION

This study assessed the influence of surgical management on ovarian reserve by measuring serum AMH levels before and after surgery in patients with ovarian torsion. It also evaluated a range of clinical and intraoperative factors that could potentially influence hormonal changes. As a well-established biomarker of ovarian reserve, is closely related to the number of remaining primordial follicles (21,22). Despite the absence of a universally accepted standard for evaluating ovarian function, AMH remains widely used in clinical practice due to its stability throughout the menstrual cycle and relative resistance to external influences (23). Studies have demonstrated that AMH is a superior predictor of ovarian response to stimulation compared to other markers such as age, follicle-stimulating hormone, estradiol, and inhibin B, and its diagnostic performance is considered comparable to that of antral follicle count (AFC) (24).

In our cohort of 23 patients, there was no statistically significant change in overall AMH levels when comparing preoperative (AMH1) and postoperative (AMH2) values measured at least three months after surgery. Nonetheless, 16 patients (69.5%) showed an increase in AMH following surgery, while 7 patients (30.5%) exhibited a decrease. Several factors may explain the observed postoperative reductions in AMH, including excessive use of electrosurgical devices, inflammatory damage, accidental excision of healthy ovarian tissue, or trauma related to the surgical technique (25,26). Additionally, the literature highlights the role of reperfusion injury-alongside ischemia-as a contributor to follicular damage. Reactive oxygen species generated during the reperfusion phase may cause cellular injury in already compromised ovarian tissue (27). Some reports have suggested that detorsion itself may exacerbate tissue injury (28). However, such detrimental effects were not clearly observed in our study; on the contrary, most patients demonstrated a postoperative increase in AMH. This finding may reflect the restoration of blood flow to previously edematous ovarian tissue, allowing for greater AMH release into circulation.

Subgroup analyses further revealed that patients who underwent detorsion combined with cystectomy exhibited a postoperative increase in AMH, though the difference did not reach statistical significance (p=0.098). Similarly, in patients treated with detorsion alone, no statistically significant change was noted (p=0.135). Previous studies have reported a postoperative decline in AMH levels following cystectomy, with some showing recovery by the

Table 2. Comparison of preoperative and postoperative AMH levels according to the type of surgical intervention

Surgical group	Preoperative AMH (median, min.-max.)	Postoperative AMH (median, min.-max.)	p
Detorsion + cystectomy	2.94 (1.43-5.31)	3.71 (1.54-8.67)	p=0.098
Detorsion without cystectomy	2.35 (0.08-11.24)	3.54 (0.07-19.82)	p=0.135
Oophorectomy performed	1.27 (1.16-2.19)	1.40 (0.80-1.94)	p=0.500
No oophorectomy	2.95 (0.08-11.24)	3.84 (0.07-19.82)	**p=0.014**

Data are presented as median (min.-max.); p-values were calculated using the Wilcoxon Signed-Rank Test. Statistically significant p-values (p<0.05) are shown in bold.
AMH: Anti-Mullerian hormone, min.-max.: Minimum-maximum

Table 3. Comparison of preoperative and postoperative anti-Mullerian hormone levels based on surgical method and side of torsion

Variable	Preoperative AMH (median, min.-max.)	Postoperative AMH (median, min.max.)	p
Laparotomy	2.34 (0.84-5.31)	3.71 (1.08-19.82)	**0.021**
Laparoscopy	2.96 (0.08-11.24)	3.60 (0.07-9.31)	0.375
Right-sided torsion	2.69 (0.08-11.24)	3.4 (0.07-9.31)	0.187
Left-sided torsion	2.57(1.22-5.31)	3.78 (1.40-19.82)	**0.039**

Data are presented as median (min.-max.); p-values were calculated using the Wilcoxon Signed-Rank Test. Statistically significant p-values (p<0.05) are shown in bold.
AMH: Anti-Mullerian hormone, min.-max.: Minimum-maximum

third month (29), while others have demonstrated a more prolonged reduction, particularly in patients with endometriomas and higher preoperative AMH levels (30). Our results support this observation, as most patients had AMH levels that exceeded baseline values by three months postoperatively, suggesting that cystectomy may not have long-lasting detrimental effects on ovarian reserve.

Although AMH levels declined after oophorectomy, this change was not statistically significant (p=0.500). In contrast, patients who did not undergo oophorectomy experienced a significant increase in postoperative AMH levels (p=0.014). This finding supports the negative effect of oophorectomy on ovarian reserve, though conclusions should be drawn cautiously given the limited sample size in this subgroup.

Regarding the surgical approach, a statistically significant change in postoperative AMH levels was observed in patients who underwent laparotomy (L/T) (p=0.021). In contrast, no significant difference was found in those who underwent laparoscopy (L/S) (p=0.375). While this difference may reflect variations in surgical exposure or intraoperative handling, it should be interpreted with caution due to the small sample size and lack of objective perfusion assessment. Nevertheless, the wide variation in AMH levels within the laparoscopy group suggests that the surgical approach alone may not be the sole determinant of outcome.

Previous studies have shown that laparoscopic detorsion can effectively preserve ovarian reserve without significantly impairing AMH levels or AFCs, supporting the efficacy of minimally invasive techniques when performed by experienced surgeons (31). Notably, previous reports have emphasized that experienced laparoscopic surgeons can perform atraumatic and effective detorsion even with limited operative fields, suggesting that operator skill may outweigh the choice of surgical method. Additionally, it has been highlighted that surgical expertise, intraoperative decision-making, and the severity and duration of torsion are critical factors influencing ovarian reserve preservation, rather than the surgical approach itself (32). Moreover, the extent of ischemic injury caused by prolonged or severe torsion is a critical determinant of ovarian functional recovery, regardless of the surgical technique used. These considerations suggest that the higher postoperative AMH levels observed in the laparotomy group in our study may reflect multifactorial influences rather than the surgical technique's inherent advantages.

Furthermore, it is essential to recognize that postoperative changes in AMH levels do not necessarily translate into reduced fertility potential. Prospective data have demonstrated that although AMH levels may decrease after ovarian surgery, the likelihood of achieving pregnancy and live birth remains comparable over long-term follow-up (33). Therefore, while AMH dynamics offer valuable insights into ovarian function recovery, they should be interpreted within a broader clinical context, particularly in fertility-desiring women undergoing detorsion.

When analyzed according to the side of torsion, a significant increase in postoperative AMH levels was observed in patients with left-sided torsion (p=0.039), whereas the change was not substantial in right-sided cases (p=0.187). This may be explained by the anatomical and physiological advantages of the left ovary, including better venous drainage and lymphatic flow. The literature also notes that the left ovary is more resistant to torsion due to support from the sigmoid colon and a shorter utero-ovarian ligament, which may potentially result in less tissue damage during torsion events (4). These anatomical features may contribute to better functional recovery.

Even when the ovary appears dark or bluish-black intraoperatively, detorsion has been regarded as a safe and effective fertility-preserving option in patients wishing to retain reproductive potential, as previously noted in the literature (34). The present study supports this perspective, as postoperative AMH levels were frequently found to be elevated. These findings suggest that while ovarian reserve may experience a temporary reduction during the acute phase of torsion, timely and appropriate surgical management can allow for the preservation of ovarian function.

A key strength of this study lies in its exclusive focus on women with fertility desire who underwent surgical treatment specifically for ovarian torsion. Moreover, various surgical approaches, such as detorsion, cystectomy, and oophorectomy, and their evaluation through subgroup analyses, provide a comprehensive view rarely explored in similar research. By investigating patient-specific fluctuations in AMH levels, this study offers valuable insights into the potential for preserving ovarian function following torsion.

Study Limitations

However, several limitations should be acknowledged. The relatively small number of patients limits the generalizability of the results. Additionally, although AMH values were recorded both before and after surgery as part of routine clinical monitoring, the study's retrospective nature may reduce the strength of causal interpretations. Ideally, a prospective study design would have enabled stronger conclusions. Furthermore, postoperative AMH was measured at a single time, three months after surgery, which may not adequately capture long-term changes in ovarian reserve. Another limitation is the absence of intraoperative Doppler ultrasonography or other standardized techniques to assess ovarian blood flow objectively before and after detorsion. This limits the ability to definitively interpret the relationship between surgical approach and postoperative AMH dynamics. Nevertheless, all hormonal assessments were performed within the same institution using identical assay kits and equipment, ensuring consistency and reliability in laboratory measurements.

CONCLUSION

In conclusion, this study's findings demonstrate that serum AMH levels are largely preserved-or even increased-in patients who undergo detorsion. Surgical approaches other than oophorectomy, particularly when performed on time, appear to support the maintenance of ovarian function. The observed effects of surgical technique and torsion laterality on ovarian reserve further emphasize the importance of conservative management. Therefore, in cases of ovarian torsion where fertility preservation is a priority, detorsion can be safely performed using an appropriate surgical approach.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of Gaziantep University (decision number: 2022/380, date: 07.12.2022).

Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Surgical and medical practices: E.Y., İ.T., S.S., Concept: E.Y., İ.T., S.S., A.I.K., Design: E.Y., A.I.K,. Data Collection and Processing: E.Y., D.A.A., F.Ç., Analysis or interpretation: İ.T., Y.S., Literature search: İ.T., S.S., Y.S., Writing: F.Ç., A.I.K, İ.T.

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A Rare Complication Years After Abdominal Surgery: Incarcerated Drain-site Hernia

Abdominal Cerrahiden Yıllar Sonra Nadir Bir Komplikasyon: Inkarsere Dren Yeri Fıtığı

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ABSTRACT

Drain-site hernia is an exceptionally rare complication, primarily occurring shortly after the removal of drains. Comorbid conditions that hinder wound healing, along with factors contributing to increased intra-abdominal pressure, are identified as risk factors. Notably, the use of drains with a diameter larger than 10 mm has been specifically linked to this complication. In this report, we present a case of drain-site hernia that developed 40 years after intra-abdominal surgery, and progressed with small bowel incarceration. The main objective is to question the routine utilization of drains and explore critical factors influencing drain selection, given that this case highlights an extremely rare and delayed complication associated with drain use. A selective approach is more appropriate than the routine use of drains after intra-abdominal surgery.

Keywords: Abdominal drain, drain-site hernia, small bowel incarceration

ÖZ

Dren yeri fıtığı oldukça nadir görülen bir komplikasyondur. Vakaların çoğunda dren çekildikten sonra erken dönemde ortaya çıkar. Karın içi basıncı artıran faktörlerin yanı sıra yara iyileşmesini bozan yandaş hastalıklar da risk faktörlerindendir. Özellikle çapı 10 mm'nin üzerinde olan drenlerin kullanımı ile ilişkilendirilmiştir. Bu makalede abdominal cerrahiden 40 yıl sonra gelişen ve ince barsak inkarserasyonu ile seyreden bir dren yeri fıtığı olgusu sunulmuştur. Dren ilişkili bu çok nadir geç komplikasyon aracılığı ile drenlerin rutin kullanımının sorgulanması ve dren seçiminde dikkat edilmesi gereken faktörlerin tartışılması amaçlanmıştır. İntraabdominal cerrahi sonrası rutin dren kullanımındansa seçici bir yaklaşım daha uygundur.

Anahtar Sözcükler: Abdominal dren, dren yeri fıtığı, ince barsak inkarserasyonu

INTRODUCTION

Intra-abdominal drains remain an integral part of numerous abdominal surgical procedures. However, their use can lead to various complications, such as infection, hemorrhage, intestinal perforation, and in rare cases, drain site evisceration (1-3). These complications typically manifest during the early postoperative period. Conversely, drain-site hernia is an exceptionally rare and late complication associated with the use of drains. In this report, we present a unique case of drain-site hernia that emerged four decades after an intra-abdominal surgery and led to small bowel incarceration.

CASE REPORT

A 77-year-old male patient presented with a complaint of painful swelling in the abdomen following a coughing episode. The patient's medical history includes hypertension, diabetes mellitus,

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and had undergone emergency laparotomy 40 years ago due to a traffic accident, involving a segmental bowel resection. The patient mentioned that until the day he sought medical attention, there had been no swelling or mass in his abdomen. Upon examination, a painful and tender mass was palpated in the left lower quadrant. Notably, a 2 cm scar was observed on the skin above the mass, which the patient identified as the site of the drain used during the operation four decades ago.

Ultrasonographic examination revealed a 2 cm diameter fascial defect on the abdominal wall. Furthermore, a hernial sac containing hyperechoic omental fatty tissue and an intestinal loop was visualized (Figure 1). The afferent bowel wall exhibited significant edema. As a result, the patient underwent emergency surgery to address the issue. Although the defect was small, the hernia sac was remarkably large (Figure 2). During the procedure, the hernia sac, which contained the omentum and a loop of the small intestine, was carefully opened. Although the tissues in the hernia sac were highly edematous, there were no signs of strangulation. To facilitate reduction, the defect was slightly enlarged, and the intestine and omentum were successfully reduced into the abdomen. Subsequently, a primary repair was performed, and a self-gripping polypropylene mesh, with a diameter of 10x10 cm, was applied. The patient was discharged on the second day following the operation. Written and informed consent for publication was obtained from the patient.

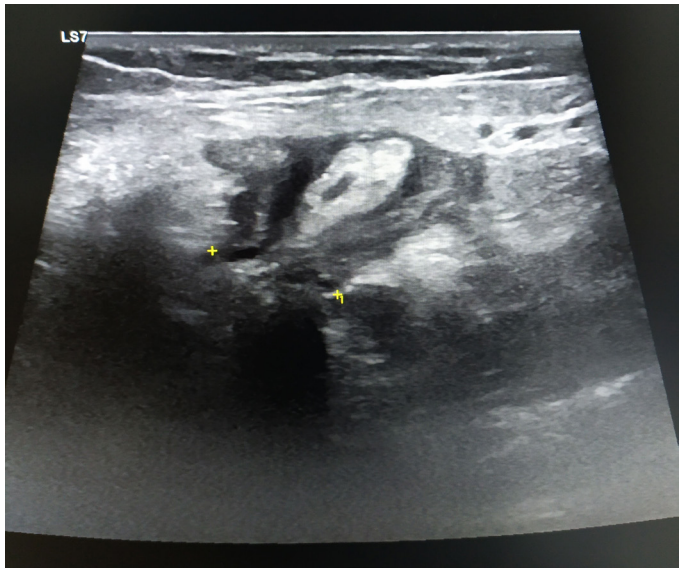

Figure 1. Ultrasonographic image of incarcerated hernia sac.

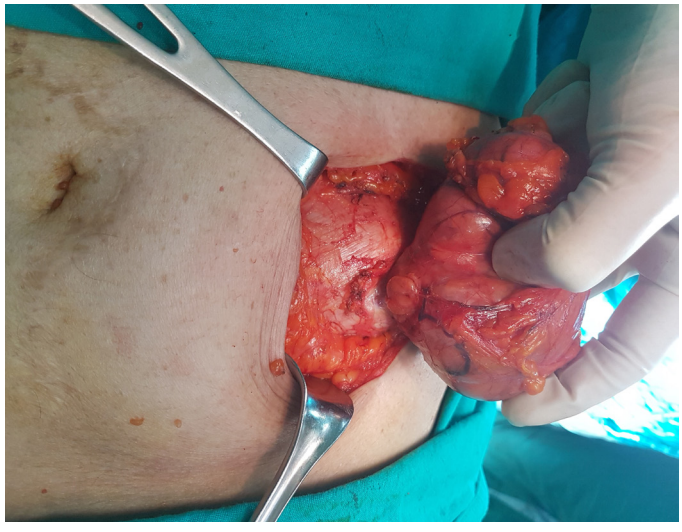

Figure 2. Intraoperative view of the drain-site hernia sac.

DISCUSSION

The debate on intraabdominal drainage is still ongoing. Several studies have highlighted potential complications associated with prophylactic drain use, including infection, evisceration, adhesion, intestinal erosion, anastomotic separation, bleeding, increased postoperative abdominal pain, and decreased pulmonary function (1-4). The lack of consensus on the routine use of drains in various surgical procedures is evident, and no standardized algorithm exists concerning the features of drains, such as diameter, vacuum, and material. In clinical practice, the surgeon's experience and preference often play a role in determining the choice of drainage after abdominal surgery, while institutional facilities may also influence the characteristics of the selected drain.

Drain site evisceration following abdominal surgery is an infrequently reported complication. Notably, in most of the reported cases, the diameter of the drain used has been found to be 10 mm or more (1,2). Although we lack information regarding the diameter of the drain used in our patient, the presence of a 2 cm skin scar suggests the use of a large-diameter drain was used.

Typically, hernias tend to occur during the early postoperative period, often within hours after drain removal (1,2). Several factors contribute to the development of hernias, including conditions that increase intra-abdominal pressure, such as post-operative vomiting, cough, constipation, mechanical ventilation, and post-operative ileus, as well as factors that impede wound healing, such as poor nutritional status, diabetes mellitus, and steroid therapy (1-3,5). In our case, the incarcerated hernia occurred a remarkably 40 years after drain removal. The fact that it developed after a cough indicates that a sudden increase in intra-abdominal pressure can provoke a fascial defect or weaken the drain site, even after an extended period.

Various studies propose strategies to decrease complications related to drain usage following abdominal surgery. These strategies encompass the use of drains with a diameter below 10 mm, positioning drains at an oblique angle through the abdominal wall, gradual removal of the drains, and employing a purse string suture for closure post-removal (2,5,6). Furthermore, it is strongly advised to exercise caution when deciding to use drains, ensuring they are employed only when necessary (1,4). Several studies indicate that routine drain use is not imperative for many intra-abdominal surgical procedures (4,7).

CONCLUSION

A selective approach is more appropriate than the routine use of drains after intra-abdominal surgery. By preferring a small-diameter drain and careful placement and removal, reduce drain-related

complications. It should be kept in mind that a hernia may develop even many years after the drain is removed.

Ethics

Informed Consent: Written and informed consent for publication was obtained from the patient.

Footnotes

Authorship Contributions

Surgical and Medical Practices: R.K., M.E., Concept: R.K., Design: M.E., Supervision: R.K., Resources: M.E., Material: M.E., Data Collection or Processing: M.E., Analysis or Interpretation: R.K., Literature Search: M.E., Writing: R.K., M.E., Critical Review: R.K.

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The Embedded U-Suture Technique for Better Cosmetic View in Patients Underwent Endoscopic Thoracic Sympathectomy

Endoskopik Torasik Sempatektomi Uygulanan Hastalarda Daha İyi Kozmetik Görünüm İçin Gömülü U-Sütür Tekniği

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ABSTRACT

In endoscopic thoracic sympathectomy (ETS) surgery, a chest drain is inserted into the pleural space after the procedure for lung expansion and is quickly removed after lung hyperinflation. A horizontal mattress suture, also named U-suture, is quickly knotted around the chest drain to prevent air from entering the pleural space. The U-suture may cause poor cosmetic appearance because of excessive scar tissue development. In this study, we aimed to investigate the new embedded U-suture technique using polyglactin suture, which is an absorbable material, and its cosmetic results. In this retrospective study, we collected data on patients who underwent ETS performed with a new technique. A total of 30 patients were included in the study. Neither serious wound complications nor excessive scar formation was observed in the follow-ups of the patients. The embedded U-suture technique in ETS surgery has some advantages such as good cosmetic results, no need for the removal of sutures, and applicability in both genders.

Keywords: Endoscopic thoracic sympathectomy, hyperhidrosis, uniportal, VATS, sympathectomy

ÖZ

Endoskopik torasik sempatektomi (ETS) ameliyatında, işlemden sonra akciğer ekspansiyonu sağlamak için plevral boşluğa göğüs dreni yerleştirilir ve akciğer hiperinflasyonundan sonra hızla çıkarılır. Plevral boşluğa hava girmesini önlemek için horizontal matress sütür, U sütür olarak da adlandırılır, göğüs dreni etrafına hızla düğümlenir. U sütür, aşırı skar dokusu gelişimi nedeniyle kötü kozmetik görünüme neden olabilir. Bu çalışmada, emilebilir bir materyal olan poliglaktin sütür kullanılarak yapılan yeni gömülü U sütür tekniğini ve kozmetik sonuçlarını araştırmayı amaçladık. Bu retrospektif çalışmada, yeni bir teknikle ETS uygulanan hastalara ait verileri topladık. Çalışmaya toplam 30 hasta dahil edildi. Hastaların takiplerinde ciddi yara komplikasyonları veya aşırı skar oluşumu gözlenmedi. ETS ameliyatında gömülü U sütür tekniğinin iyi kozmetik sonuçlar, dikiş alınmasına gerek olmaması ve her iki cinsiyette de uygulanabilirlik gibi bazı avantajları vardır

Anahtar Sözcükler: Endoskopik torasik sempatektomi, hiperhidrozis, uniportal, VATS, sempatektomi

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INTRODUCTION

Endoscopic thoracic sympathectomy (ETS) surgery, defined for various indications, is often applied to primary focal hyperhidrosis and facial blushing. Today, in parallel to advances in minimally invasive surgery, the ETS procedure is performed successfully with video-thoracoscopic single port technique, in many centers. Ipsilateral lung collapse is technically created through double lumen intubation, and sympathetic blockade is performed through sympathectomy or nerve clipping at the appropriate level according to the indication of ETS (1). Also, some modifications such as single lumen intubation and apneic periods, intrapleural CO_2 insufflation, or awake video-assisted thoracic surgery (VATS) were reported in various publications (2-3). Ipsilateral tube thoracostomy and underwater seal drainage are performed after the sympathetic interruption and are removed after lung re-expansion with hyperinflation, by the anesthetist. A U-suture, which was previously placed around the chest drain, is quickly tied while the tube is removed to prevent pneumothorax due to intrapleural negative pressure. In the conventional technique, the U-suture is thrown over the skin and knotted after tube removal (Figure 1a). This technique may lead to poor cosmetic appearance due to both suture marks and possible scar tissue suture technique in long-term follow-ups (Figure 1b). In this study, we aimed to describe the intramuscular embedded U-suture technique and its cosmetic results in patients who underwent ETS surgery.

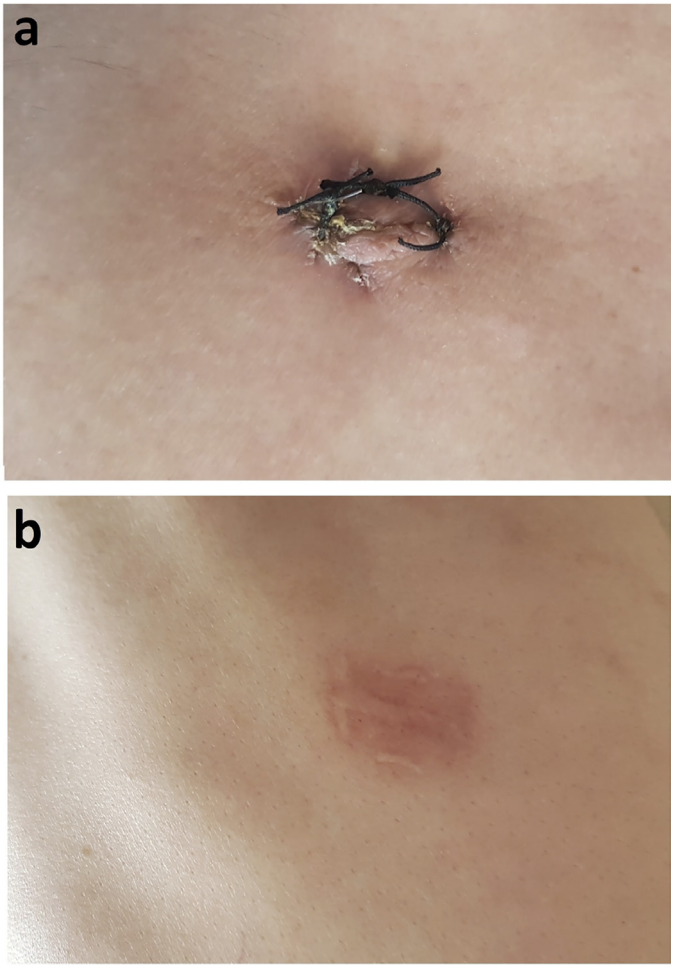

Figure 1. a) Figure shows closure of port incision with silk suture material and conventional horizontal mattress (U-suture) technique. **b)** Poor cosmetic results due to scar tissue can occur in long-term follow-ups in the conventional method

CASE REPORT

Surgical Technique

Patients are placed in the Semi-Fowler position after the double lumen intubation. A skin incision of about 1 centimeter in length is made at the axillary region. The serratus anterior, intercostal muscles, and parietal pleura layers were passed after ipsilateral lung collapse, and the hemithorax was entered through the 3rd intercostal space. The thorax is explored with a 5 mm 30-degree optic, and instruments such as a hook, clip applier, or energy devices are inserted into the thorax adjacent to the optic. After providing sympathetic blockage at the appropriate level according to indication of ETS, a chest drain is inserted into the thoracic cavity through the same hole, and connected to the underwater drainage. A U-suture is placed in the muscle fibers surrounding the chest drain with a 2-0 round polyglactin suture material (Figure 2a). Ipsilateral lung hyperinflation is performed by the anesthesiologist, and the surgeon observes the air leakage. When air leak stops, the chest drain is removed and U-suture is knotted as quickly as possible to prevent the pneumothorax (Figure 2b). The skin layers are closed subcuticular with an absorbable monofilament suture material (Figure 2c and Figure 2d). The scar is minimal, and the cosmetic appearance is satisfactory in this technique (Figure 2e). After the approval of the local ethics committee, the records of patients who underwent ETS surgery with the embedded U-suture technique between January 2019 and April 2022 were retrospectively reviewed. Data on the patients such as age, gender, hyperhidrosis areas, presence of postoperative complications, and wound site problems were collected. A total of 30 patients were included in the study. The median age was 23 (16-44) years. The clinicopathologic characteristics of patients were given in the table (Table 1). All patients included in the study underwent bilateral ETS. The most common sympathectomy level was R3-4 for primary palmar hyperhidrosis in 19 patients (63%). The most common postoperative complication was chest pain, which improved with non-steroidal anti-inflammatory drugs, occurring in 21 patients (70%). Unilateral minimal pneumothorax was detected in 3 patients (10%), and bilateral minimal pneumothorax in only one patient (3.3%). Tube thoracostomy was not required in any of the patients. Neither serious wound complications nor excessive scar formation were observed in the follow-ups of the patients.

We aimed to present a new embedded U-suture technique and its cosmetic advantages in this study. U-suture, used in conventional methods, has some disadvantages. It has a bad cosmetic appearance, can cause excessive scar tissue, and needs to be removed again. Various physicians conducted studies to prevent poor cosmetic appearance, due to VATS incision. Kim defined a subcutaneous suture technique in patients undergoing uniportal VATS performed for pneumothorax (4). Kesler et al. (5) reported a peri areolar incision technique for good cosmetic results in patients who underwent ETS surgery. Although good cosmetic results were reported in this peri areolar technique, its undesirable feature is that it cannot be applied in female patients. Chen et al. (6) demonstrated advantages of

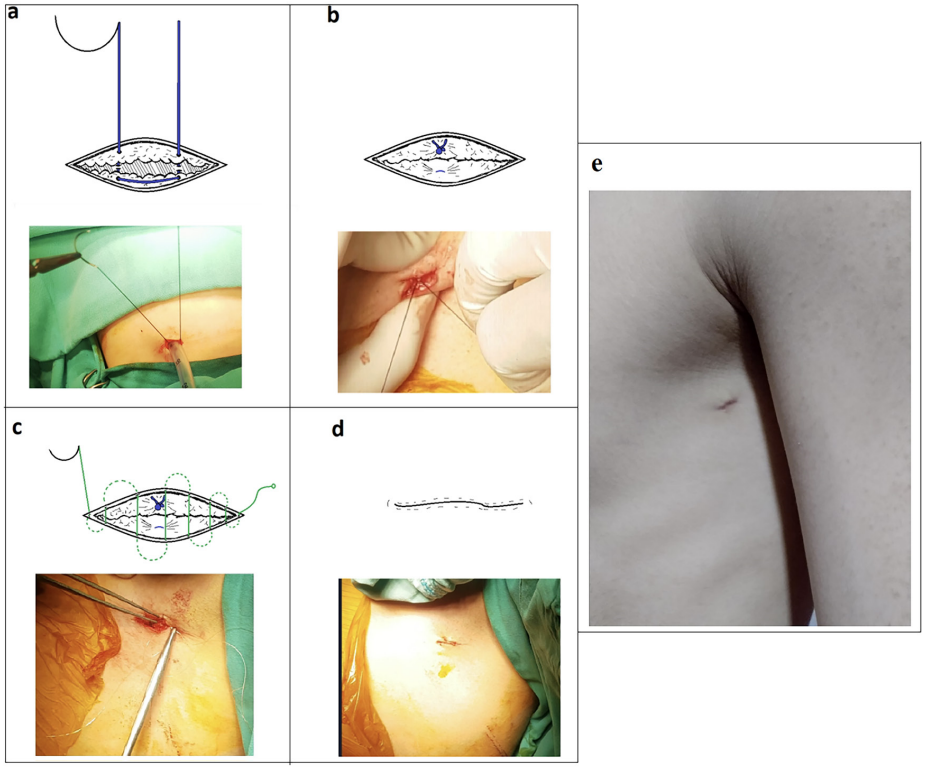

Figure 2. a) A U-suture (2-0, round, polyglactin) is placed in the muscle fibers surrounding the chest drain. **b)** U-suture is knotted after removing the chest drain. **c)** The skin layers are closed subcuticular with an absorbable monofilament suture material. **d)** Figure shows post closure view of port access incision. **e)** In long-term follow-ups, better cosmetic appearance can be obtained with the new embedded U-suture technique than with previous methods

Table 1. Characteristics of patients included in the study, n=30

		n	%
Age, median (range)	23 (16-44) years		
LOS, median (range)	1 (1-3) days		
Gender			
	Female	16	53.3
	Male	14	46.7
Hyperhidrosis area and symphaticotomy level			
	Palmar/R3-4	19	63.3
	Axillary/R4-5	2	6.7
	Craniofacial hyperhidrosis & blushing/R3-4-5	2	6.7
	Palmar and axillary	6	20
	Craniofacial and palmar / R2-3-4	1	3.3
Postoperative complication			
	Chest pain	21	70
	Dyspnea	12	40
	Pneumothorax (unilateral)	3	10
	Pneumothorax (bilateral)	1	3.3

LOS: Length of stay, SD: Standard deviation

needlescopic periareolar ETS technique in a prospective randomized study, but it is applicable only to male patients. However, the embedded U-suture technique described in this article applies to both genders and yields good cosmetic results. This study was approved by the Gazi University Ethics Committee (approval number: 2022-915, date: 26.07.2022). Informed consent regarding publication were obtained from the patients.

CONCLUSION

As a result, the embedded U-suture technique in ETS surgery has some advantages such as good cosmetic results, no need to remove sutures, and applicability in both genders. Additionally, a significantly increased risk of pneumothorax is not expected in this technique.

Ethics

Ethics Committee Approval: This study was approved by the Gazi University Ethics Committee (approval number: 2022-915, date: 26.07.2022).

Informed Consent: Informed consent regarding publication were obtained from the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.S., I.A., M.T.A., A.K., G.A., A.Ç., Concept: M.S., I.A., M.T.A., A.K., G.A., A.Ç., Design: M.S., I.A., M.T.A., A.K., G.A., A.Ç., Supervision: M.S., I.A., M.T.A., Material: M.S., A.Ç.,

Data Collection or Processing: M.S., I.A., M.T.A., Analysis or Interpretation: M.S., I.A., M.T.A., A.K., G.A., A.Ç., Literature Search: M.S., I.A., M.T.A., Writing: M.S., I.A., M.T.A., A.K., G.A., A.Ç., Critical Review: M.S., I.A., M.T.A., A.K., G.A., A.Ç.

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Unraveling the Enigma of Ogilvie Syndrome's Acute Colonic Pseudo-Obstruction Complicated with Multiple Comorbidities: A Case Report

Çoklu Komorbiditelerle Karmaşıklaşan Akut Kolonik Psödo-obstrüksiyonlu Ogilvie Sendromunun Gizemini Çözmek: Bir Olgu Sunumu

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ABSTRACT

Ogilvie syndrome, an uncommon condition characterized by acute colonic pseudo-obstruction. This presents unique diagnostic and management challenges. It is particularly difficult because it usually occurs in patients with a complicated medical history and is associated with variable outcomes.

A 61-year-old gentleman with cervical fracture-induced paraplegia, gastritis, and hemorrhoids presented with a distinct manifestation of Ogilvie syndrome. The patient presented with symptoms of abdominal distension, fever, shortness of breath, and a 2-week history of no bowel movements. The presence of comorbidities and the common occurrence of constipation complicate the diagnostic process. Radiological imaging revealed extensive colonic dilation from the cecum to rectosigmoid junction. The patient's management included a conservative approach involving intravenous fluids, intermittent aspiration, antibiotic therapy, and vigilant monitoring. As a result, the patient displayed improved clinical parameters, reduced abdominal distension, and gradual return of bowel function. However, the patient's intricate medical history continues to pose ongoing challenges, necessitating long-term follow-up. Previous cases of Ogilvie syndrome were retrieved from PubMed to characterize the clinicopathological features and identify prognostic factors of Ogilvie syndrome.

This case highlights clinical acumen in distinguishing Ogilvie syndrome, which presents symptoms of acute emergency abdomen and intestinal obstruction, as opposed to constipation with chronic and non-specific abdominal discomfort.

Keywords: Ogilvie's syndrome, acute colonic, pseudo-obstruction, paraplegia, multiple comorbidities, case report

ÖZ

Ogilvie sendromu, akut kolonik psödo-obstrüksiyon ile karakterize nadir bir durumdur. Bu durum, tanı ve yönetim açısından özel zorluklar sunar. Genellikle karmaşık tıbbi öyküsü olan hastalarda görüldüğü ve değişken sonuçlarla ilişkili olduğu için tanısı zordur.

Servikal kırığa bağlı parapleji, gastrit ve hemoroid öyküsü olan 61 yaşındaki erkek hasta, Ogilvie sendromunun belirgin bir klinik tablosu ile başvurdu. Hasta, karın şişliği, ateş, nefes darlığı ve iki haftadır dışkılamama şikayetleri ile başvurdu. Komorbiditelerin varlığı ve kabızlığın sık görülmesi tanı sürecini zorlaştırmaktadır. Radyolojik görüntülemede, çekumdan rektosigmoid bileşkeye kadar uzanan yaygın kolonik dilatasyon saptandı. Hastanın tedavisi; intravenöz sıvılar, aralıklı aspirasyon, antibiyotik tedavisi ve dikkatli izlem içeren konservatif bir yaklaşımı içerdi. Sonuç olarak, hastanın klinik parametrelerinde iyileşme, karın şişliğinde azalma ve bağırsak fonksiyonlarının kademeli olarak geri dönmesi gözlendi. Ancak hastanın karmaşık tıbbi geçmişi, uzun dönem takip gerektiren sürekli zorluklar oluşturmaya devam etmektedir.

PubMed'den daha önce bildirilen Ogilvie sendromu vakaları taranarak sendromun klinikopatolojik özellikleri ve prognostik faktörleri belirlenmiştir.

Bu olgu, Ogilvie sendromunun tanısında klinik sezginin önemini vurgulamakta; akut karın ve bağırsak tıkanıklığı belirtileriyle kendini gösteren bu acil durumu, kronik ve özgül olmayan karın rahatsızlıkları ile seyreden kabızlıktan ayırt etmenin gerekliliğine dikkat çekmektedir.

Anahtar Sözcükler: Ogilvie sendromu, akut kolonik, psödo-obstrüksiyon, parapleji, çoklu komorbiditeler, olgu sunumu

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INTRODUCTION

Massive colonic dilatation without mechanical blockage serves as a characteristic hallmark of Ogilvie syndrome, alternatively referred to as acute colonic pseudo-obstruction, which is an uncommon and challenging gastrointestinal illness (1,2). We present the case of a 61-year-old Malay male patient who developed Ogilvie syndrome alongside multiple underlying conditions, including a cervical fracture with paraplegia, gastritis, and hemorrhoids. This case sheds light on the complex interplay between the underlying health issues and other factors, and highlights the challenges in diagnosing and managing this condition (2).

Ogilvie syndrome often mimics mechanical colonic obstruction, making its diagnosis particularly challenging (3,4). Considering the patient's medical history and presenting clinical symptoms, this case report is significant due to the difficulty in teasing out the diagnosis in the context of constipation due to paraplegia. This also highlights the importance of a multidisciplinary approach for managing such cases. This case report aims to contribute to the medical literature by providing insights into the diagnostic intricacies and treatment strategies of Ogilvie syndrome in the context of a complex medical scenario. To avoid poor outcomes and mortality, emphasis has been placed on awareness, early diagnosis, and detection. Colonoscopic decompression can be considered an option, as it can avoid intestinal ischemia, perforation, and peritonitis, although sometimes conservative treatment is performed (5).

CASE REPORT

A 61-year-old Malay male presented to our facility with a complaint of abdominal pain that had persisted for two weeks. Symptoms started 2 weeks prior to admission. The patient reported mild diffuse abdominal pain. The pain was associated with distension and vomiting. The patient's medical history included paraplegia from a cervical fracture for the past 2 years, as well as gastritis and hemorrhoids in the past year. Notably, he had relied on rectal enemas for daily bowel movements in the past year due to chronic constipation. Otherwise, he was not on any other treatment, including antacids, proton pump inhibitors, or micronized purified flavonoid fractions. The patient denied fever, chest pain, or abnormal urinary patterns prior to admission. There was no rectal bleeding, loose stool, altered bowel habits, or tenesmus. The lack of previous abdominal surgery was also notable. The patient denied a family history of malignant tumors, including colorectal cancer, and denied a family history of tuberculosis.

On physical examination, the vital signs were as follows: body temperature, 36.5 °C; blood pressure, 125/83 mmHg; heart rate, 88 beats per minute; and respiratory rate, 20 breaths per minute. Abdominal examination revealed abdominal distension and mild diffuse abdominal tenderness, with no palpable masses. Digital rectal examination indicated mucous discharge, a lax anus with poor rectal tone, and no palpable masses. Notably, the patient exhibited lower-limb weakness and sensory loss on neurological examination. Symmetrical lower-limb weakness was characterized by severe flexor and extensor weakness (Medical Research Council grade 1). There was the lower-extremity sensory loss. In addition, the reflexes over the upper and lower limbs were normal, and the plantar responses were bilateral, demonstrated by flexion.

Laboratory investigations showed leukocytosis (total white blood cell count: 13 g/L; normal range: 4-11 g/L) in the full blood count and hyponatremia (Na: 133 mmol/L; normal range: 135-145 mmol/L) in the renal function test, with accompanying metabolic acidosis (pH, 7.33; HCO_3^-, 18 mmol/L; PO_2, 87 mmHg; PCO_2, 35 mmHg) upon venous blood gas analysis. Liver function tests showed a cholestatic picture with elevated alkaline phosphatase (alkaline phosphatases, 215 IU/L; normal range: 44-147 IU/L) and high total bilirubin (24 mmol/L; normal range: 3-17 mmol/L), as well as a direct bilirubin of 17 mmol/L (normal range: 0-3 mmol/L). Other blood test results were normal.

Considering the patient's condition, mechanical intestinal obstructions secondary to colorectal carcinoma and chronic constipation were initially considered on the 1st day of admission. However, after abdominal computed tomography (CT) was performed, the patient's presentation was deemed unlikely to be due to mechanical intestinal obstructions such as colorectal carcinoma, sigmoid volvulus, and constipation, based on specific clinical features and radiological findings. These findings indicated the absence of mechanical obstruction, suggesting Ogilvie syndrome.

The initial impression of Ogilvie syndrome was based on the absence of mechanical obstruction on CT and clinical features, including the degree of abdominal distension and absence of bowel sounds. The condition was complicated by community-acquired pneumonia (CAP), which then precipitated congestive heart failure. The patient complained of sudden onset of fever, occasional cough, and shortness of breath, especially while lying supine. Ischemic heart disease was ruled out after cardiac biomarkers, including troponin T, and electrocardiography showed normal results. On day 4 of admission, an incidental finding on serial abdominal CT revealed choledocholithiasis within the common bile duct. However, this patient was asymptomatic.

An initial impression of gallstone ileus was made; however, serial abdominal CT ruled out the cause. The definitive treatment, colonoscopic (endoscopic) decompression, was suggested to the patient; however, the patient refused and opted for conservative management. Surgery was advised if conservative or colonoscopic (endoscopic) decompression failed or if the patient had peritonitis or perforation. On day 7 of admission, a decreasing trend of urine output was noted based on the common bile duct, and further evaluation showed acute kidney injury (AKI) secondary to a stone at the left pelvic ureteric junction and proximal ureter, causing unilateral moderate hydronephrosis. The patient was medically managed.

On day 10 of admission, the patient developed AKI and hyponatremia secondary to overdiuresis. On day 12 of admission, he developed acute coronary syndrome, complaining of sudden onset of central chest pain associated with worsening shortness of breath, which was confirmed with a positively high troponin T level. However, during this time, the patient refused further management and finally opted for discharge (at his own risk) on day 13 of admission.

Imaging Examinations

Chest radiography revealed significant cardiomegaly without air under the diaphragm (Figure 1). Non-contrast abdominal and pelvic CT of the patient in the coronal view (Figure 2A), sagittal view (Figure 2B), axial view (Figure 2C), and oblique view (Figure 2D) revealed extensive diffuse large bowel distension. Choledocholithiasis with common bile duct dilation was also incidentally noted. Otherwise,

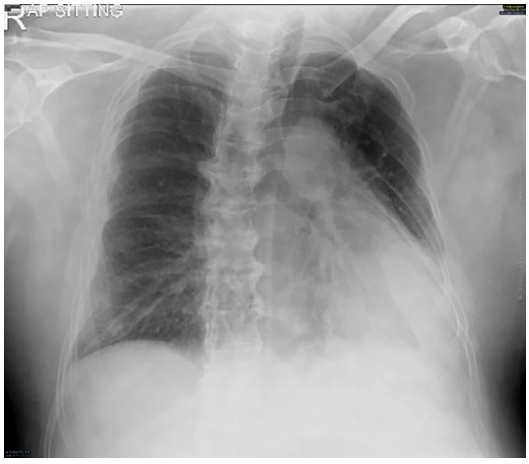

Figure 1. X-ray imaging of the Ogilvie syndrome. Chest X-ray in a posteroanterior view revealed significant cardiomegaly without air under the diaphragm.

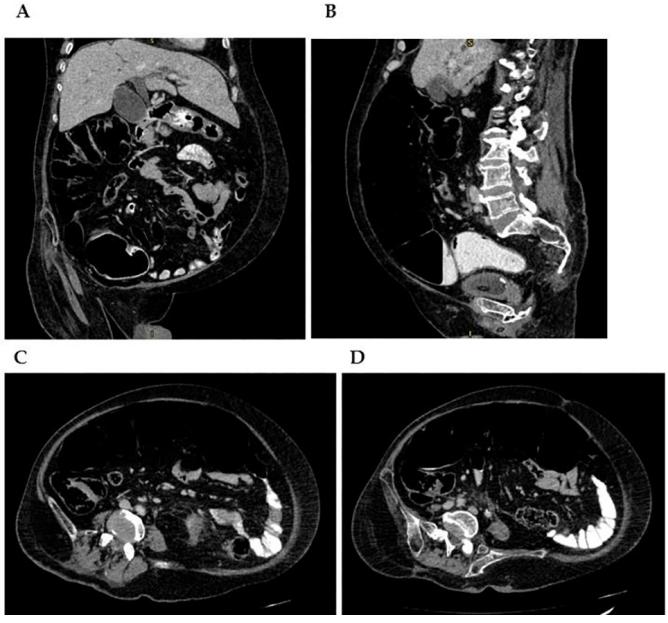

Figure 2. Computed tomography imaging of the Ogilvie syndrome. (A) Coronal view; B) Sagittal view; C) Axial view; D) Oblique view. General description for the four views of the computed tomography showed that there was segmental dilatation of the large bowel loops, one located and extending from the proximal sigmoid colon until the rectosigmoid junction.

there were no intraluminal/extraluminal masses, adhesions, or volvulus. Ultrasound of the kidney, ureter, and bladder revealed a stone at the left pelvic ureteric junction and proximal ureter with moderate hydronephrosis.

Final Diagnosis

The Ogilvie syndrome was diagnosed based on clinical presentation and complemented by initial CT findings to rule out any mechanically caused intestinal obstruction.

Treatment

The patient presented with Ogilvie syndrome and underwent a comprehensive treatment. Initially, intravenous fluids, antibiotics, and proton-pump inhibitors were administered. Simultaneously, conservative measures, such as strict I/O charting, elevated position, and nothing-by-mouth status, were implemented. Intermittent aspiration of 500 mL of fluid was performed as part of the therapeutic approach. Serial abdominal examinations were conducted to detect peritonism and promptly ensure vigilant monitoring.

During the course of treatment, the patient exhibited several complications, such as compensated acute heart failure, AKI, and CAP secondary to *Klebsiella pneumonia*, despite gradual improvement in symptoms of Ogilvie syndrome, with a decrease in abdominal distension and improved bowel sounds. Follow-up tests demonstrated improvements in laboratory parameters and reduced colonic dilation.

The prescribed regimen for Ogilvie syndrome, including neostigmine administration, nothing by mouth, two pints (1000 mL) of normal saline every 24 h, and intravenous cefoperazone and metronidazole administration, contributed to the overall positive outcome. Additional interventions such as fleet enema, 1/1 twice daily, were administered to alleviate constipation. Intravenous glycopyrrolate 1 amp diluted in 100 mL normal saline solution every 4 h was used to treat the respiratory secretions. Other interventions, such as intravenous paracetamol (1 g QID), intravenous bromhexine, saline nebulization, intravenous pantoprazole, tablet potassium citrate, and intravenous magnesium sulfate (1 amp in 100 mL normal saline per hour) were then administered to treat the comorbidities and complications.

Outcome and Follow-up

After 1 week of hospitalization, the patient's condition improved despite multiple complications; however, the patient was discharged on request. Telephone follow-ups were performed. Through regular telephone follow-ups, supplemented by a symptom diary, we tracked his overall well-being. The patient complained of residual abdominal distension and pain. Upon review in the clinic 2 weeks after discharge, our patient was active and had no residual gastrointestinal symptoms, except mild abdominal distension. The patient did not have any site-specific infections. The patient remained compliant with the medications for other comorbidities. One month after discharge, he did not complain of abdominal symptoms.

DISCUSSION

The presented case of Ogilvie syndrome in a 61-year-old male of Malay descent underscored the complex clinical presentation and diagnostic challenges associated with this condition. Ogilvie syndrome, characterized by colonic dilation without mechanical obstruction, poses diagnostic challenges owing to its clinical resemblance to mechanical bowel obstruction (6). Ogilvie syndrome has been reported to mimic mechanical obstruction in approximately 40%-50% of cases, making early differentiation crucial (7,8). Ogilvie syndrome is an uncommon entity, with <70 cases reported in the literature.

The precise pathophysiological mechanisms underlying Ogilvie syndrome remain the subject of ongoing investigation. These include

potential disruptions to the autonomic nervous system due to various factors such as trauma, spinal anesthesia, and pharmacological agents. Additionally, interruption of parasympathetic fibers spanning from S2 to S4 may play a pivotal role in the pathogenesis of this condition (9). These factors collectively lead to distension of the colon, which is characterized by an increase in colonic diameter. Consequently, this increased tension on the colonic wall gives rise to the hallmark clinical presentation of Ogilvie syndrome, primarily characterized by gradual abdominal distension occurring over 3-7 d, although rapid onset within 24-48 h is also possible. Patients with this syndrome may manifest symptoms such as abdominal pain, nausea, vomiting, and a combination of constipation and diarrhea.

The rate of cecal perforation (1%-3%) is associated with a high mortality rate (50%-71%) (2,10). Statistical data from prior research has shed light on the characteristics and associated risk factors of Ogilvie syndrome. Reports indicate that this syndrome primarily affects older individuals with underlying medical conditions, particularly chronic diseases such as kidney, lung, or heart disease; brain or nervous system disorders (such as paraplegia in this case); and severe pulmonary disease. (3,4,6). Our patient's male sex and age of 61 years align with the typical demographic profile of Ogilvie syndrome (6,11). The occurrence of pneumonia (as in this case) and sepsis is also considered common infections associated with Ogilvie syndrome (1,4,7), along with prolonged immobility in bed or poor underlying functional status (4-7,10).

Managing Ogilvie syndrome in a 61-year-old male with cervical fracture-induced paraplegia, gastritis, hemorrhoids, left pelvic ureteric junction and proximal ureter stones, moderate hydronephrosis, choledocholithiasis with common bile duct dilation, extended-spectrum β-lactamase-producing *Klebsiella pneumonia* pneumonia, AKI, ischemic heart disease with heart failure, and reduced ejection fraction presented multifaceted challenges (Table 1).

The management of Ogilvie syndrome includes conservative measures, endoscopic decompression, and surgery in severe cases (7). Initial interventions included nasogastric decompression, bowel rest, electrolyte correction, and discontinuation of exacerbating medications, especially opioids. If no improvement occurs within 24-48 h, neostigmine, an anticholinesterase, may be considered (2,12). Unresponsive cases may benefit from alternatives such as endoscopic decompression and cecostomy (6,7). The comprehensive approach of this case emphasizes the advantages of integrating medical, surgical, and radiological interventions by a multidisciplinary care team for complex Ogilvie syndrome cases. The patient's paraplegia from a cervical fracture introduced a unique dimension, potentially contributing to colonic dysmotility and dilation observed in up to 20% of Ogilvie syndrome cases (11,12).

The challenges in managing this complex case stemmed from the intricate interplay between Ogilvie syndrome, paraplegia, and gastrointestinal, urological, respiratory, and cardiovascular issues. Each comorbidity introduces a layer of complexity that requires a nuanced understanding of the underlying pathophysiology and tailored interventions to optimize patient outcomes. The delicate balance in addressing these multifaceted challenges underscores the need for a comprehensive and multidisciplinary approach for managing such intricate clinical scenarios.

While this case report provides a detailed account of the patient's condition, its single-case nature limits its generalizability. Nevertheless, it underscores the need for vigilant diagnosis of Ogilvie syndrome, particularly in patients with pre-existing conditions that may influence its development (2,13). The presence of gallstones, renal stones with moderate hydronephrosis, heart failure with reduced ejection fraction, and Ogilvie syndrome signifies a complex medical scenario with the potential for multiple complications.

Table 1. Challenges in managing Ogilvie syndromes in a complex case: A 61-year-old male with multiple comorbidities

Challenges	Key points
Mobility limitations	Cervical fracture-induced paraplegia restricted mobility, hindering conservative strategies like early ambulation.
Physiological response impairment	Paraplegia affected the patient's ability to respond to physiological cues, complicating bowel function.
Gastritis	The symptoms exacerbated fluid and electrolyte imbalances, potentially worsening colonic dilation and leading to AKI.
Hemorrhoids	Caused discomfort during bowel movements, necessitating pain management to prevent straining and exacerbation of colonic distension
Renal compromise	The presence of a left PUJ obstruction and a proximal ureter stone, with moderate hydronephrosis, demanded cautious fluid management to prevent AKI.
Choledocholithiasis with CBD dilation	Required careful management to prevent biliary complications and ensure optimal organ function Challenges whether the cause of intestinal obstruction was due to gallstone ileus
ESBL-producing *Klebsiella pneumonia*	Complicated respiratory status and antibiotic choices, increasing the risk of septicemia and lengthening hospitalization
Fluid dynamics (over diuresis)	AKI secondary to over-diuresis highlighted the complexity of fluid management in Ogilvie syndrome and associated conditions
Cardiovascular compromise	IHD with heart failure and reduced ejection fraction increased the risk during interventions involving neostigmine, necessitating careful medication and fluid management.

AKI: Acute kidney injury, IHD: Ischemic heart disease, PUJ: Pelvic ureteric junction, CBD: Common bile duct, IHD: Ischemic heart disease

CONCLUSION

In conclusion, this case report offers a comprehensive view of Ogilvie syndrome in a patient with a complex medical history. This underscored the importance of a multidisciplinary approach, vigilance in diagnosis, and the potential impact of neurological dysfunction on the development of the syndrome. The statistical data from prior research provided valuable insights into the demographics and risk factors associated with Ogilvie syndrome, aiding its clinical understanding and management. Although this case may not be fully generalizable, it is a valuable reference for clinicians addressing similar complex scenarios. Although Ogilvie syndrome associated with paraplegia is clinically rare, doctors should make a positive and accurate diagnosis and provide humane care and treatment because patients experience both mental and physical problems.

Ethics

Informed Consent: Informed written consent was obtained from the patient for the publication of this report and any accompanying images.

Footnotes

Authorship Contributions

Surgical and Medical Practices: T.H.K., A.D.Z., Concept: T.H.K., A.D.Z., Design: T.H.K., Supervision: A.D.Z., Resources: T.H.K., Material: T.H.K., A.D.Z., Data Collection or Processing: T.H.K., Analysis or Interpretation: T.H.K., A.D.Z., Literature Search: T.H.K., Writing: T.H.K., Critical Review: A.D.Z.

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The Undescended Thyroglossal Duct Cyst: A Rare Clinical Encounter of Thyroglossal Duct Cyst in the Base of Tongue

İnmemiş Tiroglossal Kanal Kisti: Dil Tabanında Nadir Görülen Bir Tiroglossal Kanal Kisti Klinik Görünümü

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ABSTRACT

The thyroglossal duct cyst (TDC) commonly presents as an anterior neck swelling. TDCs are a congenital disorder that are usually asymptomatic. It rarely occurs the tongue or at the base of the tongue. The patient usually presents with upper airway obstruction symptoms that need immediate attention. We report a case of an infant who presented with respiratory distress symptoms, requiring immediate intubation. The computed tomography scan images showed a well-circumscribed hypodense collection located at the base of the tongue. Direct laryngoscopy and excision of the cyst were performed under general anaesthesia. The infant recovered after the procedure, and there has been no recurrence since surgery.

Keywords: Case report, thyroglossal duct cyst, laryngoscopic excision

ÖZ

Tiroglossal kanal kisti (TDK) genellikle ön boyun şişliği olarak ortaya çıkar. TDK'ler genellikle asemptomatik olan konjenital bir hastalıktır. Nadiren dilde veya dilin tabanında görülür. Hasta genellikle acil müdahale gerektiren üst solunum yolu tıkanıklığı semptomlarıyla gelir. Acil entübasyon gerektiren solunum sıkıntısı semptomlarıyla gelen bir bebek vakasını bildiriyoruz. Bilgisayarlı tomografi tarama görüntüleri dilin tabanında iyi tanımlanmış hipodens bir koleksiyon gösterdi. Genel anestezi altında doğrudan laringoskopi ve kistin eksizyonu yapıldı. Bebek işlemden sonra iyileşti ve ameliyattan bu yana tekrarlama olmadı.

Anahtar Sözcükler: Olgu sunumu, tiroglossal kanal kisti, laringoskopik eksizyon

INTRODUCTION

Incomplete obliteration of the thyroglossal duct is the root cause of thyroglossal duct cyst (TDC), a congenital anomaly. Clinically, 20% to 25% at the level of suprahyoid, 15% to 20% at the hyoid, and 25% to 60% at the infrahyoid level (1). The incidence of TDC at the base of the tongue, or the lingual TDC, is uncommon (2). It is a rare condition and may require urgent interventions. The lesion clinically manifests during infancy, and it commonly presents with obstructive symptoms. Management will require a different approach than the other TDCs mentioned. We report a case of lingual TDC presenting with acute upper airway obstruction. The infant was subjected to airway, and surgical intervention. The patient responded well to the treatment, and no recurrence was observed.

CASE REPORT

A 1-month-old boy was brought to the casualty by his parents for recurrent choking episodes for the past 2 weeks associated with cyanosis. He has no history of foreign body ingestion, no fever, and no history of prior intubation. Antenatal and postnatal history

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were insignificant. Upon assessment, the patient was stridorous, tachypnoeic, and appeared lethargic with the presence of chest recessions. There was no noticeable neck swelling. He developed type I respiratory failure. The patient was intubated with an appropriate-sized endotracheal tube with no difficulty during the intubation process. He was diagnosed with aspiration pneumonia, and intravenous antibiotics were commenced. Attempts to wean the infant from intubation had failed as he developed stridor and respiratory distress following each extubation.

A computed tomography (CT) scan was performed. Imaging showed a non-enhancing cystic lesion at the base of the tongue, measuring 1.0 x 0.7 x 1.1 cm. No septation or calcification was seen. There was no extension or involvement of adjacent structures (Figure 1a and Figure 1b). The thyroid gland was visualised and appears normal.

He was referred to the ear, nose, and throat team for a formal upper airway assessment. He was subjected to a direct laryngoscopy, examination under anaesthesia, and excision of the lesion. Intraoperatively, a cystic mass was present at the base of the tongue, displacing the epiglottis posteriorly (Figure 2). The cyst was aspirated, yielding one mL of mucoid content. The cyst wall was excised using cold instruments, and the residual cyst wall was cauterised using diathermy (Figure 3). Tracheoscopy showed no abnormality from the glottis until the carina. Post-operatively, the patient was given intravenous dexamethasone 0.1 mg/kg three times daily (TDS) for a total of 3 days. He was successfully extubated three days later without any upper airway obstruction symptoms or respiratory distress.

The cyst wall's reported tissue consisting of stratified squamous epithelium and sporadic mucous glands with scattered lymphoplasmacytic cells. This finding is consistent with TDC. He was discharged one week later without any complications. Upon follow-up, he has no recurrence of his symptoms and is thriving well. A flexible nasopharyngolaryngoscopy examination was performed during the visit, and no residual mass or cystic lesion was seen.

DISCUSSION

TDC is a congenital cervical anomaly that accounts for around 7% of the population worldwide. Embryologically, the thyroid primordium originates from the foramen caecum at the posterior third of the tongue. It descends towards the anterior neck to reach its final position at the pre-tracheal level in the seventh week of gestation (1). During the tenth week of gestation, the thyroglossal duct will involute. Failure of involution will lead to TDC formation (3).

The common site of presentation is an anterior midline neck swelling around the hyoid region (1). The cyst rarely occurs at the tongue base, also termed a lingual TDC. The incidence of lingual TDC is very low, reported as being between 2.1%-8.3%. Burkart et al. (2) reported that the incidence of lingual TDC was around 8.5%, while other studies suggested an incidence of 2.1% (2,4). Clinically, the presentation depends on the lesion's site. TDC located in the midline of the neck is usually asymptomatic. Some may be diagnosed as epidermoid cysts, plunging ranulas, lymphangioma, or recurrent neck abscesses, such as infected TDC (5). Acute obstructive symptoms such as stridor, apnea, dysphagia, and respiratory distress are more commonly seen in lingual TDC. Burkart et al. (2) reported that 44% of the lingual TDC patients presented with upper airway obstruction. In this case, the patient presented with multiple choking episodes with cyanosis. He was intubated for airway protection, and a CT scan was performed, confirming the diagnosis of lingual TDC. In patients with less acute symptoms, nasoendoscopy can be performed to exclude other pathologies, such as laryngomalacia or choanal atresia (3).

Preoperative imaging is essential to ensure the presence of the thyroid gland and to determine the site of the lesion. Ultrasound assists in determining the presence of normal thyroid gland tissue, thereby avoid accidentally removing an ectopic thyroid, which can be mistaken for TDC (6). It is a cheaper modality and is more readily available across many centres, but it requires skillful operators, and cannot rule out other anomalies if present. A CT scan or MRI is beneficial for assessing the anatomy, location, concurrent lesion, and extension of the TDC in preparation for surgery. However, a

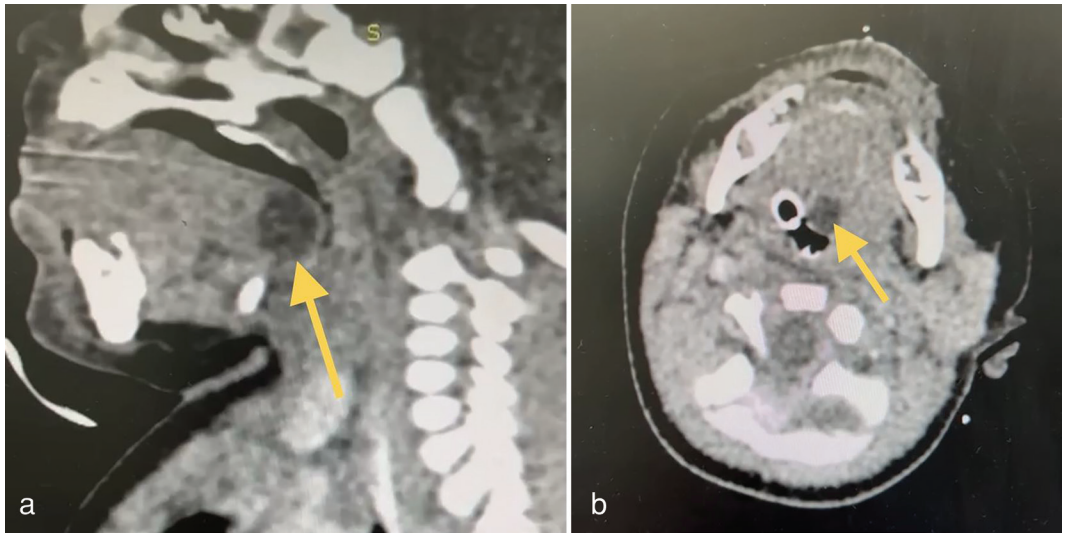

Figure 1. a) and b) a CT scan showing the cystic lesion at the base of the tongue (arrow).
CT: Computed tomography

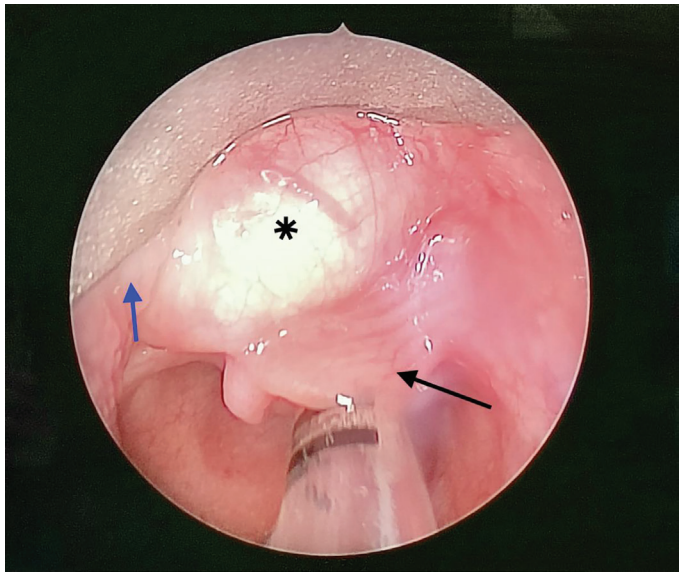

Figure 2. Direct laryngoscopy view demonstrating a cystic mass (asterisk) at the base of the tongue (blue arrow) pushing the epiglottis (black arrow) posteriorly.

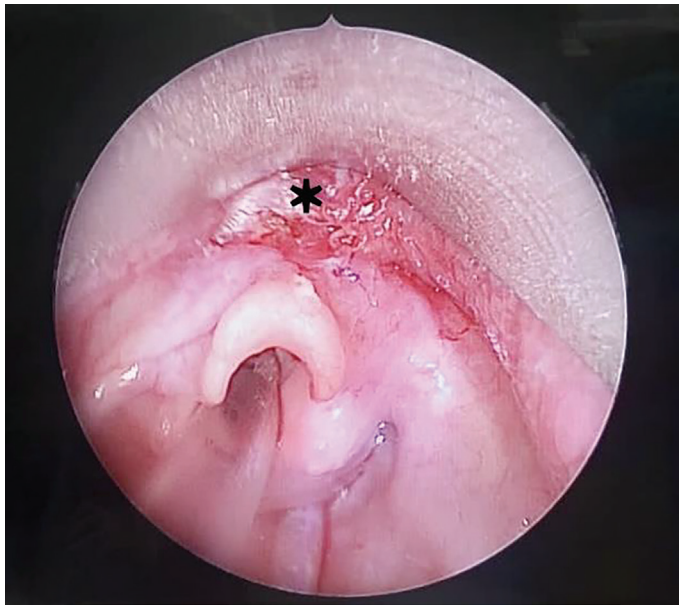

Figure 3. Post excision of the base of tongue cystic mass (asterisk) via direct laryngoscopy.

CT scan is more costly, has higher radiation exposure and requires sedation for paediatric patients (7).

Urgent surgical intervention is indicated, especially in lingual TDC cases complicated by acute upper airway obstruction. Endotracheal intubation is required if the patient develops respiratory distress or has an impending airway collapse. Difficult intubation may necessitate other interventions, such as a laryngeal airway mask, needle cricothyroidotomy, or tracheostomy (8).

Sistrunk remains the gold standard surgical procedure for TDC and has the lowest recurrence rate (6). For lingual TDC, management involves a different approach, and intervention is needed more urgently. Several surgical techniques were described in the literature. In cases of a large cyst that obstructs the airway, puncture and aspiration of the cyst's contents via direct laryngoscopy are performed to reduce the size of the cyst (9). According to Bai et al. (9) this method has effectively treated obstructive symptoms. Close monitoring is required, and if recurrence sets in, a second-look surgery and lesion excision should be performed in a controlled setting. Histopathological examination should be performed to confirm the diagnosis, and malignancy should also be excluded, although the incidence is less than one percent (10).

Another method, described by Urao et al. (11) is endoscopic marsupialisation of the cyst with direct laryngoscopy under general anesthesia. This method is complicated, as suturing the cyst wall is tedious. Endoscopic excision of the cyst using electrocautery, microscissors, or microdebriders is the most effective method (3). The cyst wall needs to be removed completely to prevent recurrence. A study applying this method reported no recurrence was encountered during the follow-up of all patients (2).

In this case, direct laryngoscopy was performed, and cyst excision was done using cold instruments. A tracheoscopy was performed to confirm the absence of other airway pathologies. IV Dexamethasone at a 0.1-0.5 mg/kg TDS dosage for 3 days can be administered to reduce laryngeal oedema post-operatively (12).

CONCLUSION

A lingual TDC is a rare condition that can present as an upper airway emergency. Urgent airway intervention is warranted should the patient develop respiratory distress secondary to upper airway obstruction. Direct laryngoscopy and examination under anaesthesia are a diagnostic and therapeutic method used to secure the airway. A complete cyst wall excision should be performed to prevent recurrence.

Ethics

Informed Consent: Consent was obtained from the parent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.H.B.M.S., A.M.A., S.A.B., A.F.J., Concept: M.H.B.M.S., A.M.A., S.A.B., A.F.J., Design: M.H.B.M.S., A.M.A., S.A.B., A.F.J., Data Collection or Processing: M.H.B.M.S., A.M.A., S.A.B., A.F.J., Analysis or Interpretation: M.H.B.M.S., A.M.A., S.A.B., A.F.J., Literature Search: M.H.B.M.S., Writing: M.H.B.M.S., A.M.A., A.F.J.

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Helicobacter Pylori: Background, Diagnostic Methods and Nutritional Aspects

Helicobacter Pylori: Arka Plan, Tanı Yöntemleri ve Beslenme Yönleri

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ABSTRACT

Helicobacter pylori (*H. pylori*) bacteria have infected one-half of the world's population. The subset of *H. pylori* colonization with persistent inflammation is associated with an increased risk of developing gastritis, peptic ulcer disease, gastric cancer, and iron-deficiency anaemia. There are extra-gastric diseases that potentially can weaken the immune system and thus expose the host cells to bacterial infection. Accurate diagnosis of *H. pylori* infection is crucial for proper eradication treatment. There are several invasive and non-invasive methods available for *H. pylori* detection, and each of the methods has its advantages and disadvantages. In addition, nutritional status plays an important role in the progression of the *H. pylori* infection, which is further discussed in this review. We believe that there will be continuous improvements in the diagnostic methods and pharmaceutical treatments in the management of *H. pylori* infection. The emphasis on nutritional intake in the individual diet should also be implemented in the primary healthcare setting to reduce the incidence of *H. pylori* infection.

Keywords: Diagnosis, diet, eradication treatment, gastritis

ÖZ

Helicobacter pylori (*H. pylori*) bakterileri dünya nüfusunun yarısını enfekte etmiştir. *H. pylori* kolonizasyonunun kalıcı enflamasyona sahip alt kümesi, gastrit, peptik ülser hastalığı, mide kanseri ve demir eksikliği anemisi geliştirme riskinin artmasıyla ilişkilidir. Bağışıklık sistemini zayıflatabilecek ve böylece konak hücrelerini bakteriyel enfeksiyona maruz bırakabilecek gastrik dışı hastalıklar vardır. *H. pylori* enfeksiyonunun doğru teşhisi, uygun eradikasyon tedavisi için çok önemlidir. *H. pylori* tespiti için çeşitli invaziv ve non-invaziv yöntemler mevcuttur ve bu yöntemlerin her birinin avantajları ve dezavantajları vardır. Ek olarak, beslenme durumu *H. pylori* enfeksiyonunun ilerlemesinde önemli bir rol oynar ve bu incelemede daha ayrıntılı olarak ele alınmıştır. *H. pylori* enfeksiyonunun yönetiminde tanı yöntemlerinde ve farmasötik tedavilerde sürekli iyileştirmeler olacağına inanıyoruz. Bireysel diyette besin alımına vurgu, *H. pylori* enfeksiyonunun sıklığını azaltmak için birincil sağlık hizmeti ortamında da uygulanmalıdır.

Anahtar Sözcükler: Tanı, diyet, eradikasyon tedavisi, gastrit

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INTRODUCTION

Approximately one-half of the world's population is infected with *Helicobacter pylori (H. pylori)* (1). It has been estimated that *H. pylori* bacteria infect 90% of patients with duodenal ulcers and 80% of patients with gastric ulcers. *H. pylori* bacterium is a spiral gram-negative bacterium in a group called Epsilon proteobacteria class that can colonize the human stomach, especially the upper gastrointestinal tract site (2). The milestone discovery of *H. pylori* by Australian doctors, Barry J. Marshall and Robin Warren, has altered the diagnosis and treatment of stomach-related diseases (3). Although the curved bacteria had been reported, it was not until 1982 that Warren and Marshall identified the bacteria as the cause of chronic gastritis.

Initially, Marshall failed to develop an animal model for the experiments, so he decided to do a self-experiment by drinking a culture of the bacteria obtained from his patient (4). He developed nausea, vomiting, and achlorhydria 3 days after drinking the culture, and on day 8, the repeated endoscopy and biopsy results showed gastritis and positive *H. pylori* infection. He recovered with antibiotic and bismuth treatment, which eventually fulfilled Koch's third and fourth postulates for the role of *H. pylori* and gastritis. Koch's postulates were established to assess the microorganism that causes a disease (5). The bacteria were initially recognized as *Campylobacter pylori*, which was then renamed to *H. pylori,* as it has a helical physical structure and is mostly found in the pyloric region of the stomach (6).

H. pylori bacteria enter the body through oral and travel to the digestive system for colonization (7). It starts with a small subset of colonization and develops more serious outcomes over time, and massive colonization of *H. pylori* results in peptic ulcer, duodenal ulcer, or gastric cancer (8). The routes of transmission of *H. pylori* remain unclear. The most common routes of transmission are person-to-person, faecal-to-oral and environmental exposures (9). In developing countries, plausible environmental factors include untreated water, poor sanitation, and crowded living places that contribute to the spread of *H. pylori* bacteria (10).

Early research explained that person-to-person transmission occurs through two modes, namely, vertical and horizontal transmissions (11). The researchers explained that vertical transmission is spread among the same family members, while horizontal transmission involves contact from outside of the family or contaminated environments. The clustering of the *H. pylori* infection among family members is suggested to be due to genetic predisposition, close interpersonal contacts, and the same socioeconomic status (9). Dental plaque and saliva are typical means of transmission between family members (12).

The fact that *H. pylori* can survive in a pH environment as low as 1 and continue to create inflammation demonstrates its resilience (13). Figure 1 illustrates the anatomical structure of *H. pylori* bacteria and its function to induce inflammation in host cells. It can produce its cytoplasmic urease for enzymatic activity to convert urea to ammonia and carbon dioxide (14). The ammonia is responsible for neutralizing the acidic environment in the stomach to prevent the acid inhibition or damage of *H. pylori* bacteria. The presence of ammonia increases the pH in the gastric environment to protect the bacteria from gastric acid.

In contrast, *H. pylori* bacteria are unable to survive without urea at the extreme acidity (15). Due to urease activity, the gastric lining becomes less viscous, thus, allowing bacteria to adhere to the cells and colonize (16). *H. pylori* is a responsible agent that causes gastrointestinal diseases such as gastritis, peptic ulcer, duodenal ulcer, and stomach cancer (17). The severity of the disease depends on the immune system of the individual, the type of *H. pylori* strains, and environmental factors (18).

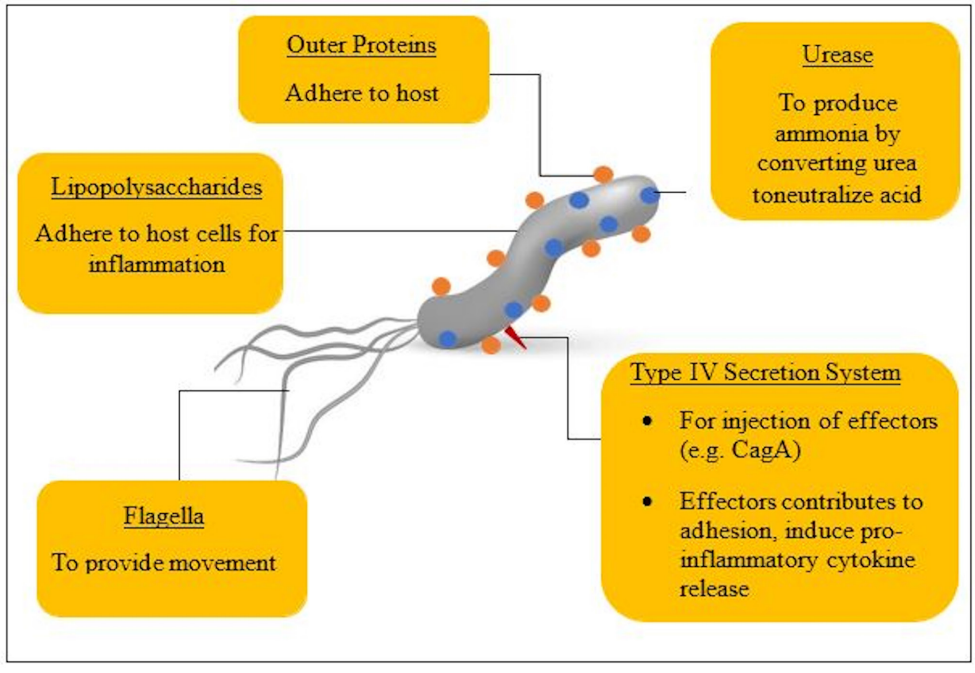

Figure 1. The anatomical structure of H. pylori bacteria and its function on host cells.
H. pylori: Helicobacter pylori

Diagnostic Test for *H. pylori* Infection

There are several methods to diagnose *H. pylori* infection. Each method has its advantages and disadvantages. An invasive test is biopsy-based that includes a rapid urease test (RUT), histological evaluation, and direct detection of *H. pylori* using polymerase chain reaction (PCR). On the other hand, there is also non-invasive treatment which includes serological tests, urea breath tests (UBT), and stool antigen tests (SAT). The comparison of the methods is provided in detail as shown in Table 1.

Rapid Urease Test

RUT can present the result in 1 hour, making it convenient for clinicians who need faster results (19). As mentioned, the principle of the RUT is based on the urease activity, which converts urea into ammonia and CO_2 to neutralize the gastric environment for its survival, increasing gastric pH. Most of the *H. pylori* specimens will become red or pink color after 50 minutes of exposure to the medium, indicating the production of the ammonium ion and increased pH (20). Overall, the specificity of the UBT is more than 95%, but sensitivity is more than 85%.

Table 1. The comparison of the invasive and non-invasive method in the detection of *H. pylori* infection

Type	Method	Features	Advantages	Disadvantages
Invasive	RUT	Sensitivity: >85% Specificity: >95%	• Easy, rapid and inexpensive • Sensitivity increases with increasing number of biopsies preferably from both gastric antrum and corpus • High accuracy in patient with peptic ulcer bleeding	• False-positive result as it can influence urease activity produce by other bacteria • False-negative result in patient with PPIs, bismuth, antibiotic and achlorhydria • Sensitivity decreases in the presence of the formalin contamination in the specimen
	Histology	Sensitivity: 60-90% Specificity: 75-100%	• The accuracy of the test increases with multiple biopsies (at least 2 biopsies) from the antrum and corpus • Able to detect the severity of the inflammation	• False-negative result in patients with PPIs and antibiotic • Unable to detect patchy *H. pylori* colonization
	PCR	Sensitivity: 80% Specificity: 100%	• Detect *H. pylori* instantly • Able to identify antimicrobial resistance	• Expensive and require high technology • False-positive resultas it can detect DNA in dead bacteria • False-negative result as it unable to detect *H. pylori* in coccoid form • Less accurate in the presence of heavy metals, proteins and polysaccharides complex
	Serological	Sensitivity: 80-90% Specificity: 80-90%	• Easy, rapid and inexpensive • High sensitivity in patient presented with gastric atrophy or on ump-proton inhibitors (PPIs) and antibiotic	• Unable to differentiate past and present infection • Less accurate in low prevalence region • False-positive result because of the influence by the positive predictive-value of antibody
Non-invasive	UBT	Sensitivity: >95% Specificity: >93%	• Widely available, cheap and easy to use • Convenience for elderly, children and pregnant women as it is requiring less radiation procedure • Gold standard to detect in asymptomatic individual	• Lack of data on antibiotic resistance • False-positive result that cause by other bacteria through urease activity • Less accuracy in patient on pump-proton inhibitors PPIs
	SAT	Sensitivity:>95% Specificity:>95%	• Less expensive and become second option when UBT no available • Not requiring high technology • Convenience as sample can be taken at home by the patient	• Less accurate if patient on antibiotic, altered bowel movement and underwent distal gastrectomy • Less accurate when the sample taken from unformed or watery stool

SAT: Stool antigen tests, UBT: Urea breath tests, *H. pylori: Helicobacter pylori*, PCR: Polymerase chain reaction, rapid urease test

The sensitivity of the test can be increased when more than 1 specimen is taken, preferably from the antrum and corpus of the gastric region (21). Additionally, RUT can be used in patients presenting with peptic ulcer bleeding. However, there are several limitations of RUT. First, RUT is influenced by bacterial urease concentration. Some cocci bacteria, such as *Staphylococci and Streptococci*, are involved in the production of urease activity, thus interfering with the detection of *H. pylori* bacteria (22). Secondly, RUT is influenced by antibiotics, pump-proton inhibitors (PPIs), bismuth, and achlorhydria, which can yield false-negative results (23). Hence, it is advisable to discontinue PPIs 2 weeks before, and antibiotics and bismuth 4 weeks before a UBT test (24). Finally, the RUT becomes less sensitive in the presence of formalin contamination in biopsy specimens.

Histological Evaluation

A histological test is the first method to detect *H. pylori*. It provides the assessment of the presence of *H. pylori* bacteria to the degree of inflammation caused by the infection (25). The use of the compound called Giemsa, hematoxylin, and eosin stains is sufficient to detect the *H. pylori* bacteria and the severity of the inflammation. However, several factors influence the accuracy of the test, including the site and number of biopsies (23). An increase in the number of biopsies can decrease the result of false-negative because histological tests are unable to detect patchy *H. pylori* colonization. Another factor that can yield a false-negative result is the use of PPIs and antibiotics, as these medications can change the form of *H. pylori* to its cocci form, making it unable to detect *H. pylori* (26).

Polymerase Chain Reaction

PCR is used to detect bacteria and to characterize bacterial genes and antimicrobial resistance (22). It allows researchers to detect *H. pylori* DNA in biopsy samples, and the PCR reaction mixture is prepared according to the manufacturer (27). PCR samples are not only limited to the biopsy but PCR have also been improved by using stool and saliva specimens. Modification of PCR technology has increased the sensitivity to 80% and specificity up to 100% (21). PCR has several advantages. It can detect *H. pylori* bacteria in a short period; hence, it offers convenience in a large sample size. Since the PCR can be used in the detection of antibiotic resistance, it can guide clinicians in suggesting antibiotic therapy for the eradication of *H. pylori* bacteria (28).

However, PCR tests cannot be used for daily detection of *H. pylori*, like UBT and SAT, because of high prices and the requirement of high-tech equipment. Since the principle of PCR is based on genetics, it also might produce false-positive results because it can detect the DNA from dead bacteria (29). On the other hand, a PCR test also can result in a false-negative as the test is unable to detect *H. pylori* in coccoid form. *H. pylori* can mutate the genes by changing its shape to a coccoid form to survive under environmental conditions such as extreme pH, very low or high temperature, and oxygen tension (26). Last but not least, the specificity of the PCR is reduced with the presence of heavy metal, protein, and polysaccharides complexes (30).

Serological Test

A serological test is used for the detection of anti-*H. pylori* IgG antibody for the diagnosis of *H. pylori* infection. Individuals infected with *H. pylori* usually present with specific antibodies such as IgG, IgA, and IgM, which can be detected via serological tests (9). IgG predominantly responds to the presence of chronic *H. pylori* infection (22). Serological tests are widely used in epidemiological studies because they are easy, rapid, and affordable. It also offers sensitivity and specificity in the range of 80% to 90%, and it depends on the individual's immune response, the duration of the exposure to the infection, nutritional status, and other antigen-related bacteria (22).

This rapid method also has high sensitivity in patients presenting with atrophy and is not influenced by antibiotics and PPIs; therefore, patients can continue taking the medications before the test (31). Serological tests have several disadvantages that limit their usefulness. This test cannot differentiate the past and present of the *H. pylori* infection; hence, it cannot be used to monitor the effectiveness of the eradication treatment (19). In fact, the serology result remains positive 3 years, after the treatment. The accuracy of the test is also lower in the low prevalence area which might produce false-positive result, thus further test by doing another diagnostic test such as histology, RUT and SAT to confirm the *H. pylori* infection (32).

Urea Breath Test

UBT, which is characterized by its high sensitivity and specificity, is widely available (9). However, the specificity of the UBT decreases among infants younger than 2 years old, as it requires active cooperation and often yields false-positive results (33). The first UBT test was reported 7 decades ago by American biochemist when they did experimental study by injecting C urea intravenously into anaesthetized cats that were not secreting gastric juice. They found out that the amount of C- CO_2 decreased in the breath and concluded that the colonization of *Helicobacter felis* bacteria was the culprit in the cat (34). The urease activity was abated after being treated with a mixture of penicillin, Terramycin, and sulphaguanidine in the cats.

Bacteria other than *H. pylori* are not present in the stomach except in patients with achlorhydria (35). As mentioned, *H. pylori* bacterium is involved in the mechanism that breaks down urea into ammonia and CO_2 (14). CO_2 enters the bloodstream and is subsequently exhaled in the lungs. Therefore, the CO_2 with the labelled C is measurable in the diagnosis of the *H. pylori* infection. Before conducting the UBT, a tablet containing urea is swallowed, and the amount of the trapped labelled-CO_2 is measurable in the exhalation. UBT offers a sensitivity of more than 95% and a specificity of more than 93%, with an affordable price (36). Besides, it can detect *H. pylori* bacteria in asymptomatic individuals, making it the gold standard for the diagnosis of *H. pylori* infection, especially among the elderly, children, and pregnant women, due to less radiation exposure (37). Although UBT has high sensitivity, there is a lack of data on antibiotic resistance that is important for eradication treatment (38). There is a chance of UBT yielding false-positive results that are induced by bacteria other than *H. pylori*, such as *Helicobacter heilmannii,* which is responsible for the process of urease activity (39). Patients who are on PPIs are also required to stop 2 weeks prior to UBT test because it can influence the results (31).

Stool Antigen Test

SAT is a non-invasive method that was introduced after the UBT test. The test is designed to detect the antigen associated with *H. pylori* in the stool (40). There are 2 types of SAT; one is based on enzyme immunoassay (EIA) and the other is based on immunochromatography

(ICA). Both tests yield high accuracy results, but in general, ICA provides less reliable results as compared to EIA (41). Early SAT used EIA based on polyclonal antibodies, which was then further improved by changing to monoclonal antibodies because they are more accurate with high sensitivity (42-43). The guidelines created by the Japanese researchers suggested that SAT has a sensitivity of more than 96% and a specificity of more than 97% before the eradication (44).

SAT is a relatively inexpensive non-invasive method as it requires fewer high-technology equipment to detect *H. pylori* bacteria in the stool sample (45). it offers convenience as patients can take the stool sample at home. SAT does not require discontinuation of PPI as does UBT because some monoclonal antibodies are not affected by PPIs. The SAT has disadvantages. Since the principle of the SAT is dependent on the antigen reaction, hence, it is less accurate when the sample is obtained from watery or unformed stool because the consistency of *H. pylori* specific antigen called serum pepsinogen I is diluted (46). SAT also has less accuracy in patients who underwent distal gastrectomy, with specificity decreased to 90.5%, altered bowel movements, and those on antibiotics and PPIs (21,47).

H. pylori Infection-Related Disease Outcomes

H. pylori bacteria are known to cause inflammation in the stomach region. This inflammation can lead to the development of diseases such as chronic gastritis, peptic ulcer disease, gastric cancer, iron-deficiency anaemia and diabetes mellitus (T2DM).

Chronic Gastritis

The primary infection of the *H. pylori* bacteria resulting in chronic gastritis is accompanied by epigastric pain and is sometimes asymptomatic (48). Gastritis is defined as inflammation of the gastric lining and can manifest as acute or chronic (49). Acute gastritis is temporary inflammation followed by clinical manifestations such as nausea, vomiting, and indigestion. *H. pylori* bacteria favor the antrum region of the stomach to start the colonization before migrating to another region (50). It often develops with the involvement of hypochloridria and neutrophil inflammatory cell infiltration (51). Researchers suggest that neutrophils are the marker in the development of gastric cancer, which has been examined with fluorescence RNA in situ. Chronic gastritis is divided into 2 categories: atrophic and non-atrophic, with the primary causal factor being *H. pylori* infection (52-53). Non-atrophic gastritis could progress to atrophic gastritis if proper treatment is not received by patients.

Peptic Ulcer

An early study found that 15% of *H. pylori*-infected individuals developed peptic ulcer disease (54). Symptoms of the peptic ulcer include nausea, vomiting, weight loss, and stomach bleeding (55). A peptic ulcer is a subset of the stomach and duodenum which extends into the submucosa (56). *H. pylori* bacteria cause inflammation, degeneration and injury to epithelial cells. Patients diagnosed with peptic ulcer disease are recommended to proceed with diagnostic tests for *H. pylori* bacteria because peptic ulcer can also be induced by non-steroidal anti-inflammatory drugs that are commonly used to relieve pain and reduce inflammation (57,58).

Gastric Cancer

H. pylori infection is one of the strongest factors in the development of gastric cancer. *H. pylori* bacteria are persistent in producing inflammation in the stomach, which weakens the immune response if left untreated (59). Prolonged exposure to the inflammatory response predisposes gastric cells to become cancer (60). The inflammatory compounds attack healthy tissue and cause alteration in the DNA transcription hence resulting in DNA damage (61). This is the initial stage of gastric cancer. Further steps in the pathway are to promote cell proliferation and produce more reactive oxygen species that further damage the DNA and decrease the efficiency of DNA repair, resulting in cancer cell development (62).

Type 2 Diabetes Mellitus

A meta-analysis found that *H. pylori* bacteria increased the risk of T2DM by 27% (63). T2DM is characterized by insulin deficiency or insulin resistance (64). Insulin is a hormone that is secreted by the pancreas to maintain glucose homeostasis. In the cross-sectional study conducted in Cameroon, central region of Africa, about 73% of the subjects with *H. pylori* infection were diagnosed with T2DM (65). Infected patients with body mass index (BMI) of more than 25kg/m^2 are more prone to develop T2DM compared to infected patients with normal BMI in the range of 18.5 to 24.9kg/m^2. Symptoms vary and include dysphagia, early satiety, nausea and vomiting (66).

The pathogenesis of *H. pylori* in relation to T2DM is interesting. Gastritis related to *H. pylori* alters the secretion of hormones called gastrin and somatostatin (67). Gastrin stimulates the production of insulin while somatostatin decreases the production of insulin. *H. pylori* acts to promote the production of somatostatin and reduce gastrin, causing a decrease in the production of insulin and increasing the susceptibility of insulin to oxidative stress and inflammatory mediators (68-69). Other evidence indicates that T2DM itself can increase the risk of *H. pylori* infection because T2DM can impair the immune system of host cells, thus, increasing the susceptibility of infection (70). Besides, gastric motility is often disrupted in patients with T2DM, thereby increasing the risk of *H. pylori* colonization and inducing inflammation (71).

Iron-deficiency Anemia

Chronic gastritis related to *H. pylori* infection is closely associated with impairment of iron absorption, resulting in iron deficiency, anemia, and vitamin B12 deficiency (72). This clinical manifestation resulted from hypochlorhydria in the stomach, which subsequently impairs the absorption of dietary iron, which is necessary to metabolize ferric into ferrous form. *H. pylori* uses iron for its colonization and increases the production of hepcidin (73). The hormone hepcidin is released by the liver for iron metabolism. A reduction in the intragastric pH affects iron absorption from the diet (74). This has been demonstrated in the study conducted among school-aged children with *H. pylori* infection, who presented with elevated hepcidin levels and iron deficiency, compared to non-infected children (75).

Treatment For *H. pylori* Infection

H. pylori infection is often treated with antibiotics for a minimum of 2 weeks (76). The treatment also usually will include medication such as PPIs and bismuth subsalicylate, to help in the healing process by coating the ulcer base and protecting the stomach from further acid exposure (26,77).

H. pylori Infection and Nutrition

Nutrition plays an important role in maintaining ecology in the gastric environment (2). The health ecology in the gastric environment is

highly influenced by the host's diet. The contribution of the nutrients to *H. pylori* is explained in detail as follows:

Salt

High intake of salt is associated with an increase in the risk of *H. pylori* infection and development of pre-malignancy (78). The consumption of salt in high amounts can destroy the gastric mucosal barrier, resulting in inflammation and, thus, favor colonization by *H. pylori* bacteria (79). One of the potential pathways is that high salt intake alters the viscosity and integrity of gastric mucosa, thus, promoting the colonization of *H. pylori* colonization and persistent inflammation forming gastric proliferation and mutation in DNA (80). This has been demonstrated well in an early animal study whereby a high salt diet modifies the structure of gastric mucosa via an inflammatory process in mice infected with *H. pylori* (81). The loss of parietal cells, which secrete gastric acid, creates an achlorhydria environment, exacerbates the colonization of *H. pylori,* and enhances the process of carcinogenesis (82). Another interesting animal experimental study was conducted where researchers introduced a diet with a high concentration of salt, 87.5% sodium chloride, to Mongolian gerbils (83). 87.5% of sodium chloride is the starting concentration, approximately equivalent to 3 to 20% of salt in salted fish, 25% of salt in pickles, and 19% of salt in soy sauce. This mixture was given to the gerbil with no variation over a prolonged period. Over 60% of the gerbils were infected with cagA+ *H. pylori* strain within 4 months of interventions.

Zinc

Zinc plays a pivotal role in the maintenance of DNA integrity, synthesis, repair and cell division (84). It is also involved in the defense mechanism to protect DNA from oxidative stress. Deficiency of zinc can impede DNA damage through the production of oxidative stress mediators and impair its role as a DNA repair agent. Previous studies clearly demonstrated the relationship between zinc deficiency and the role of *H. pylori* infection in the human mechanism (85). This has been confirmed in the case-control study whereby the *H. pylori*-infected group, presented with gastritis, peptic ulcer, and gastric cancer, having a lower level of serum zinc compared to the healthy control group (86).

H. pylori bacteria require zinc for nutrition to support its growth and colonization; thus, it needs to compete with calprotectin (87). Calprotectin is a zinc-binding protein that can be found in neutrophils, and the presence of calprotectin in the stomach region is closely related to the inflammatory response (88). It is released into the gut to chelate with zinc in response to bacterial infection. In response to calprotectin, the *H. pylori* bacterium alters its structure to decrease cell surface hydrophobicity to enhance its fitness in the presence of calprotectin to response when there is availability of the zinc (89). In the state of zinc deficiency, this increases susceptibility to inflammatory response, gastric inflammation, and DNA damage in the host cells.

Vitamin C

Adequate intake of vitamin C acts as a defence mechanism against infection. It is believed that high intake of vitamin C from fruits and vegetable sources reduces the risk of H. pylori infection (90). The antioxidant role of vitamin C is to protect the gastric mucosa from the inflammation caused by *H. pylori* infection, and it also reduce the production of carcinogens to prevent the colonization of *H. pylori* bacteria (91). Vitamin C has been shown to play a crucial role in inhibiting the activity of neutrophils induced by *H. pylori* infection (92). It has been demonstrated well in the study that the level of vitamin C in gastric acid and serum is lower in *H. pylori*-infected patients with gastritis and peptic ulcers (93).

In addition, vitamin C has been used as part of treatment to eradicate *H. pylori* (94). Whether increased vitamin C levels during the eradication period from intake of natural fruits and vegetables, along with the addition of vitamin C supplementation, increase the effectiveness of therapy is still a controversial issue. Normal dietary intake of vitamin C, about 40mg per day from fruits and vegetables, allows vitamin C to play its role as an antioxidant and enzymatic cofactor (95). Thus, nutritional management and education emphasizing a high daily intake of fruits and vegetables are important, especially among *H. pylori*-infected populations.

Probiotics

Probiotic bacteria called Lactobacillus and Bifidobacterium can be found widely in probiotic yoghurt drinks. The supplementation of probiotics during the *H. pylori* eradication period has improved the rate of success (96). Supplementation of probiotics by consuming yoghurt drinks two times a day for 6 months demonstrates a decrease in *H. pylori* urease compared to the infected group that only supplemented with milk (97). A meta-analysis examined The effect of Lactobacillus bacteria and reported that it improves by approximately 10 percent over placebo before antibiotic drug therapy (98). It plays a role as an antioxidant and an anti-inflammatory agent that later explains its role in decreasing stabilizing the gastric barrier (99).

The mechanism of probiotics against the severity of *H. pylori* infection is still unclear. However, a few researchers have suggested that probiotics may help in producing mucin to strengthen the gastric barrier and compete with *H. pylori* bacteria for adhesion receptors, as well as stabilize the gut barrier to prevent bacterial colonization (100). Moreover, Lactobacillus displays similar characteristics to *H. pylori* as both can survive in low pH environments in the stomach and protect the gut from inflammation induced by *H. pylori* bacteria (101). The longer the duration of yoghurt consumption, the better the effect in decreasing the density of *H. pylori* colonization in the gut (100). Intake of yoghurt in the long term was found to be a safe and effective strategy to prevent an infection.

CONCLUSION

The development of the current methods to detect *H. pylori* bacteria allows more accurate diagnosis of *H. pylori* infection to combat the disease outcomes associated with it. An accurate diagnosis helps to guide clinicians in choosing the appropriate eradication treatment for infected *H. pylori* individuals. The selection of the method depends on the sample size, the location, the clinical manifestation of the infected individual, and the pros and cons of the methods. The selection of more than one method is advisable in certain circumstances to further confirm the *H. pylori* diagnosis. Importantly, the nutritional status of the individual should not be overlooked as it undoubtedly influences disease outcomes related to *H. pylori* infection. We believe that there would be continuous improvement in the diagnostic method and

pharmaceutical treatment in the management of *H. pylori* infection. The emphasis on nutritional intake in the individual diet also should be implemented in the primary healthcare setting to reduce the incidence of *H. pylori* infection.

Footnotes

Authorship Contributions

Surgical and Medical Practices: F.H., N.A.S.N.L., Concept: S.S.S.A.R., Design: Data Collection or Processing:Analysis or Interpretation:Literature Search: F.H., S.E.H.T., Writing: V.U.K.

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The Peril of Macular Degeneration: A Challenge to Vision

Makula Dejenerasyonu Tehlikesi: Görme İçin Bir Zorluk

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ABSTRACT

Age-related macular degeneration (ArMD) significantly contributes to the prevalence of blindness on a global scale. ArMD has emerged as the third most prevalent cause of vision impairment worldwide with the substantial increase in the elderly population. The key variables contributing to geographical atrophy (GA) and neovascular ArMD are ArMD, key forms of ArMD, both leading to significant vision impairment in older persons. GA is also known as late-stage macular degeneration. Cigarette smoking, dietary components, cardiovascular diseases, inherited markers, genes that control angiogenic pathways, lipids, and complement were shown to be important risk factors. In the last two decades, numerous studies have demonstrated that the percentage of macular degeneration cases has decreased. This may be attributed to the introduction of novel approaches to diagnosis and treatment. This review comprehensively explores ArMD, covering recent developments and classifications. It covers diagnosis methods, grading systems, and prevention strategies. It highlights advancements in treatment, including ankyrin repeat proteins, gene therapy via recombinant adeno-associated virus, and offering promising avenues for ArMD management.

Keywords: Age-related macular degeneration, vascular endothelial growth factor, geographic atrophy, scaffold, 3D bioprinting

ÖZ

Yaşa bağlı makula dejenerasyonu (ArMD), küresel ölçekte körlüğün yaygınlığına önemli ölçüde katkıda bulunur. Yaşlı nüfusun önemli ölçüde artmasıyla ArMD, dünya çapında görme bozukluğunun üçüncü en yaygın nedeni olarak ortaya çıkmıştır. Coğrafi atrofi (GA) ve neovasküler ArMD'ye katkıda bulunan temel değişkenler, her ikisi de yaşlı kişilerde önemli görme bozukluğuna yol açan ArMD'nin temel formları olan ArMD'dir. GA, geç evre makula dejenerasyonu olarak da bilinir. Sigara içmek, diyet bileşenleri, kardiyovasküler hastalıklar, kalıtsal belirteçler, anjiyojenik yolları kontrol eden genler, lipitler ve tamamlayıcının önemli risk faktörleri olduğu gösterilmiştir. Son yirmi yılda, çok sayıda çalışma makula dejenerasyonu vakalarının yüzdesinin azaldığını göstermiştir. Bu, tanı ve tedaviye yönelik yeni yaklaşımların getirilmesine bağlanabilir. Bu inceleme, son gelişmeleri ve sınıflandırmaları kapsayarak ArMD'yi kapsamlı bir şekilde incelemektedir. Tanı yöntemlerini, derecelendirme sistemlerini ve önleme stratejilerini kapsamaktadır. Ankrin tekrar proteinleri, rekombinant adeno-ilişkili virüs yoluyla gen terapisi ve ArMD yönetimi için umut verici yollar sunulması da dahil olmak üzere tedavi alanındaki ilerlemeleri vurgulamaktadır.

Anahtar Sözcükler: Yaşa bağlı maküler dejenerasyon, vasküler endotelyal büyüme faktörü, coğrafi atrofi, iskele, 3D biyobaskı

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INTRODUCTION

Age-related macular degeneration (ArMD) is a long-lasting eye ailment that significantly contributes to permanent blindness in the geriatric population globally. The condition has grown due to the significant increase in life expectancy worldwide. The macula is a distinct rounded area in the central part of the back of the eye, measuring about 5.5 mm in diameter. It is positioned roughly 0.53-0.8 mm below the center of the optic disc. The center of the macula contains a small central depression known as the fovea centralis. The macula contains a high concentration of cone photoreceptors and is the region with the greatest visual acuity. The fovea is responsible for the sharp and detailed central vision. The macula is particularly vulnerable to damage caused by direct exposure to light. As a result, it is the primary cause of significant and gradual vision deterioration in individuals aged 55 and above (1). In ArMD, the macula undergoes changes in its deeper retinal layers and the surrounding blood vessels, resulting in a decline in central vision. The impact on the quality of life of the aged population will be severe, resulting in a considerable monetary and social challenge in health care. The phenomenon of aging is rapidly emerging as a looming epidemic on a, especially in highly industrialized and affluent nations. According to the United States of America (USA) projections, individuals aged 61 and beyond are expected to triple, reaching an estimated 2 billion. Similarly, the population of individuals aged 81 and above would also increase, surging five times by 2060, (2,3). This age group would make up 33% of the population in the industrialized world. The significant shift in the population's age distribution is a cause for concern due to the rise of health issues closely linked to aging (4). Our literature analysis provides a clear explanation of the factors that increase the risk of ArMD, the different stages of the disease, and the methods used for diagnosis and treatment. The prevalence of ArMD is 7.45% among Asians, 13.44% among Europeans, and 7.61% among individuals of African descent (5). Asia, with its population accounting for over 50% of the global population, is projected to experience 113 million cases of ArMD by the year 2040 (1). A study conducted in Germany revealed a 23% rise in early ArMD patients and a 36% increase in late ArMD cases over a 15-year period from 2002 to 2017 (6). The United States had a prevalence of 18.34 million cases of early-stage ArMD among individuals aged 40 and above in 2019, accounting for 11.64% of the population. The frequency of late-stage ArMD was 1.49 million, which accounted for 0.94% of the population. ArMD is responsible for 55.6% of cases of visual impairment and 23.9% of cases of blindness among Caucasian persons in the USA. Approximately 11 million people worldwide experience visual loss and blindness as a result of advanced macular degeneration. The World Health Organization reports that the majority of individuals with visual impairment and blindness are over the age of 50 (7). Approximately 8 million cases of vision deficiency and blindness can be attributed to ArMD, which is more prevalent in high-income nations. According to a study conducted in 2015, ArMD was responsible for blindness in 5.8% of blind individuals worldwide, making it the fourth most prevalent cause. It was the third most significant factor leading to moderate to severe vision impairment (MSVI), impacting 3.9% of persons with visual impairments. Recently, the application of treatment techniques has increased, such as the direct administration of vascular endothelial growth factor (VEGF) inhibitors via ocular injections, which has significantly reduced the likelihood of blindness. However, no notable decrease in the occurrence of MSVI was observed (8-10). Although there have been significant advancements in precautionary treatments, the number of primary-stage ArMD cases is projected to increase rapidly due to the expanding growth of the geriatric population; the estimated rise in ArMD blindness might be two to three times higher without treatment. This review article comprehensively explores ArMD, covering recent developments and classifications. It covers diagnosis methods, grading systems, and preclusion strategies. It highlights advancements in treatment, including developed ankyrin repeat proteins (DARP), gene therapy via recombinant adeno-associated virus, and scaffold-based retinal implants, offering promising avenues for ArMD management.

ArMD Categories and Classifications

Several grading systems are available to categorize ArMD for diagnostic and prognostic purposes. Two primary forms of ArMD are typically distinguished: dry ArMD and Wet ArMD. They can be divided into phases and subclasses according to their progress and ocular symptoms (11). Dry ArMD, or non-exudative macular degeneration, is characterized by drusen, which are small yellow deposits between the retinal pigment epithelium (RPE) and Bruch's membrane (BrM). The initial signs of macular degeneration are the extracellular deposits, which can vary in size and density. ArMD is primarily classified into early, middle, and advanced stages. Early-stage ArMD typically exhibits no noticeable symptoms and is mostly diagnosed through a clinical eye examination. Stage 1 of ArMD is characterized by typical age-related changes, including drusen smaller than 63 µm, commonly known as drupelets, and no abnormalities in pigmentation (12). In early ArMD (second stage), drusen have a diameter ranging from 63 to 124 µm and do not cause any irregularities in the RPE cells. Intermediate ArMD is characterized by large drusen measuring at least 125 µm in diameter, together with accompanying abnormalities in the RPE (13). Drusen are frequently accompanied by changes in the RPE, leading to localized areas of reduced or increased pigmentation. The RPE pigmentary changes serve as indicators for more severe phases, and hence have a role in possible vision loss. A widely used grading system employed by numerous scholars and clinicians defines the progressive stage of dry ArMD by the presence of geographical atrophy (GA), characterized by the degeneration of the choriocapillaris and the permanent photoreceptor cell depletion (14). The range of ArMD includes the neo-vascular form, often known as the exudative or wet type of ArMD. This form is characterized by the fast growth of new blood vessels into the sub-RPE or sub-retinal locations. Choroidal neovascularization (CNV) enlargement results in the emergence of atypical blood vessels, which may lead to bleeding and have significant consequences such as the detachment of the RPE, ultimately causing rapid vision deterioration (15). Uncommon serous pigment epithelial detachment (PED) may cause substantial vision impairment. This process occurs when fluid accumulates in the spaces underneath the retina, causing the RPE to rupture and degeneration of. The progression of degeneration and cellular demise in ArMD is characterized by a gradual pace, typically devoid of any discernible symptoms during the initial phases. Macular morphology is commonly used to evaluate clinical symptoms, while genetic biomarkers are not widely employed for disease screening. Valid functional endpoints are necessary for accurately assessing

visual changes in ArMD, in addition to the physical manifestations observed in the macula.

Best-corrected visual acuity (BCVA) assesses the capacity to see retinal features at a specific distance after correcting refractive defects. It is commonly used as a reliable indicator to monitor the decline in vision and, consequently, the impact on life (16). The result of the gradual and continuous decline of eyesight, lasting for several months to years. The eye is suitable for implementing advanced therapy medicinal products (ATMPs) based on genes, tissues, or cells. The compact size of the organ is suitable for effective therapy with a minimal amount of ATMPs (17). The segmented anatomical structure can restrict the spread of therapeutic drugs to regions that are not intended to be targeted. Presently, expanding an ATMP for ocular diseases can facilitate innovation. It can reveal innovative therapy approaches for previously considered untreatable eye diseases (17). Cell therapy is being developed as a treatment for ArMD using the immune-privileged property seen in the sub-retinal area. Potential approaches include either regenerating or fixing the impaired RPE cells, or introducing cells that have a beneficial influence on the function and survival of photoreceptors via paracrine signaling. The primary focus of this research is to identify the most effective transplantation targets, including RPE, photoreceptors, and choroidal endothelial cells. The optimal time and techniques for administering these transplants are currently being explored. Much research has investigated the efficacy of administering cell-based therapy directly into the eye's vitreous fluid, but the findings have been inconsistent. In laboratory conditions, delivering cells to the back part of the eye by directly injecting cell suspensions or cultivated cells under the retina in laboratory conditions is also a promising strategy. Administering RPE cells in a suspended condition has many disadvantages, including the loss of their unique qualities, the formation of rosettes, and the migration of cells. These issues are absent in implants that use scaffolds. Implanting viable RPE cells onto precisely designed biomimetic scaffolds has the potential to accurately replicate the structure of natural tissues and assist in the recovery of visual capabilities (18). An optimal scaffold should be non-immunogenic, display exceptional mechanical strength, and be adequately thin to facilitate the passage of nutrients and metabolites between the retina and choriocapillaris. Through a scaffold-based method, cells may attain and sustain basal and apical polarization using tight junctions before implantation (19). Transplantations using different scaffolds have shown increased cell viability and better arrangement of RPE cell populations. To transport RPE-scaffold materials to the subretinal region, it is necessary to employ meticulously developed tools to minimize any potential damage. The SMAD family member 3 pathway has been observed to alter the phenotypic behavior of RPE cells, leading to the creation of scars through microglia on different scaffolds (20). In addition, some scaffolds have yet to be tested in living organisms, and it can be difficult to control the variability between different batches and the biomechanical properties of these scaffolds. An important obstacle in retinal tissue engineering (TE) is the establishment of suitable neuronal influences between the RPE implant and its adjacent cellular milieu. Advancements in biomaterials science and stem cell research, as well as knowledge gathered from clinical trials, may aid in overcoming the difficulties related to creating high-quality cell therapy medical products for ArMD (21).

Diagnosis

A dilated fundus examination is recommended for individuals aged 55 or older to detect macular degeneration. To analyze ArMD, the assessor should observe the presence of drusen deposits, pigmentary abnormalities, GA, bleeding, exudation of fluid, formation of scars, and development of fibrosis (22). The distribution, dimensions, and abundance of drusen are all factors that need to be considered. A comprehensive eye examination is conducted to eliminate the possibility of any simultaneous ocular pathological conditions. While the examination is important for disease staging, several imaging modalities are increasingly essential for connecting inspection findings and guiding therapy (23). Significant progress has been made in retinal imaging techniques. Preferential hyperacuity perimetry (PHP) quantifies the intensity of visual irregularities, such as metamorphopsia and scotoma, that occur in the middle 15° of the visual field. This is assessed using the very acute visual ability known as hyperacuity. PHP has shown an appreciable sensitivity (83%) and specificity (88%) in properly distinguishing between recent-onset CNV and intermediate phases of ArMD. In addition, PHP has been suggested to assess the efficacy of photodynamic and anti-VEGF therapy in treating neovascular nArMD (24,25). Traditionally, fluorescein angiography (FA) has been the established method for detecting CNV in ArMD. A patient's vein is injected with fluorescein dye, and images of the chorioretinal circulation are captured over an extended period. This invasive imaging technique may be used to detect the presence of leaks from different types of neovascular lesions. Indocyanine green angiography (ICG) may be used in certain situations. Indocyanine green dye is used in ICG procedures to evaluate the blood circulation in the choroid and identify hidden CNV problems. Optical coherence tomography (OCT), a frequently used noninvasive method, has significantly revolutionized the understanding and management of ArMD. It provides a thorough depiction of the various strata of the retina (26). OCT employs light to provide a precise image of the 10 layers of the retina and the underlying choroid. This method has similarities with ultrasonography in its ability to accurately identify the specific layers impacted by ArMD. The photographs aid the clinician in providing a more precise representation of the disease stage and CNV activity while facilitating the differentiation between wet ArMD and dry ArMD. OCT may be used to assess the effectiveness of therapy over time and provide insights for future treatment decisions. OCT imaging can accurately detect the presence of fluid in both the retina and underneath layers, a characteristic feature of wet ArMD. OCT angiography, sometimes called OCT angiography (OCT-A), is a modern imaging technique. OCT-A is an advanced technology that improves the ability to see the choroid's complex circulation system without invasive treatments (27). This approach aids in understanding the changes that occur in the small blood vessels of neovascular ArMD (nArMD) when abnormal new blood vessels are growing in the choroid (CNV lesions). Furthermore, OCT-A can promptly identify the development of fresh blood vessels, allowing for more comprehensive monitoring and, if needed, faster intervention. OCT-A has supplanted FA and ICG in the majority of clinical situations. Nevertheless, OCT lacks the sensitivity to detect first indications or identify individuals with a higher likelihood of ArMD. This is due to its subpar spatial resolution and insufficient intrinsic information about the retinal and RPE cells as well as the

microvasculature (28,29). Hence, innovative diagnostic methods are being developed to enhance accuracy for detecting distinct indications of disease progression. The present focus of research is on adaptive optics retinal imaging, which offers improved resolution and the ability to distinguish detailed outlines of microstructural features in microvasculature, retinal nerve fibers, and photoreceptor cells. Resonance raman spectroscopy is a highly capable technique that accurately quantifies the concentrations of carotenoids and xanthophylls in the macular area of the human retina (30).

Grading of Age-related Macular Degeneration

Multiple grading methods are available to categorize ArMD for analytical purposes and prognosis determination. Two primary forms of ArMD are often distinguished: dry ArMD and wet ArMD. Figure 1 represents symptoms of macular degeneration.

Both may further be classified by the ocular symptoms and course of the disease, which are used to categorize it into subclasses and stages. Dry ArMD, sometimes called non-exudative macular degeneration, is distinguished by the presence of drusen. Drusen are small yellow deposits located in the space between the RPE and BrM (31). The early signs of macular degeneration are extracellular deposits, which can vary in size and density. ArMD is primarily classified into early, middle, and advanced stages. The initial stage of ArMD typically lacks noticeable symptoms and is mostly identified through a clinical eye examination. Stage 1 of ArMD is characterized by typical aging changes with drusen size smaller than 63 µm, known as drupelets, and no pigment abnormalities. In early ArMD (second stage), drusen have a diameter ranging from 63 to 124 µm and do not cause any irregularities in the RPE cells. Intermediate ArMD is characterized by large drusen measuring at least 125 µm in diameter, along with accompanying abnormalities in the RPE (32).

Drusen are frequently accompanied by changes in the RPE, leading to localized areas of reduced or increased pigmentation. The presence of RPE pigmentary alterations indicates advancing to more severe stages and hence plays a role in the possibility of visual impairment. A widely used grading system defines the advanced stage of dry ArMD as the presence of GA, described by the choriocapillaris degeneration, and the gradual, irreversible damage to the cells responsible for detecting light, known as photoreceptor cells (33). The spectrum of ArMD includes the neovascular variant, referred to as the exudative or wet variation of ArMD. This type is distinguished by the rapid proliferation of new blood vessels in the sub-RPE or subretinal regions. CNV enlargement results in the leakage of aberrant arteries, potentially leading to bleeding and severe consequences such as the detachment of the RPE, ultimately culminating in rapid vision loss. While uncommon, severe PED has the potential to cause substantial vision impairment. This disorder occurs when fluid accumulates in the spaces under the retina, causing the RPE to rupture and the outer retina to degenerate (34). The progression of disintegration and cellular demise in ArMD is characterized by a gradual, progression, typically devoid of any discernible symptoms during the initial phases. Evaluating clinical symptoms usually involves the examination of macular morphology, and using genetic indicators for disease screening is not a common practice. Valid functional endpoints are necessary for accurately assessing visual changes in ArMD, in addition to the physical signs observed in the macula. BCVA is a measure of the capacity to see details of an object at a exact distance after correcting refractive abnormalities. It is often used as a reliable indicator to track the decline of vision and its impact on quality of life (35). The result of the non-neovascular type is a gradual but consistent decline in eyesight, lasting several months, to years. Initially, the presence of drusen alone was the clinical indication; however, the distinctive structural features were linked to the swift advancement to the advanced stages of ArMD. The nonexudative type ultimately results in irreversible deformation of the RPE and GA. However, when exudative alterations occur, there is a sudden decline in visual potential, posing a significant threat to the individual's quality of life within weeks (36). Regardless of the specific form, if either atrophic or exudative changes impact the fovea, it may lead to significant visual impairment due to central vision loss. Figure 2 shows fundus photography (top) and its corresponding spectral domain optical

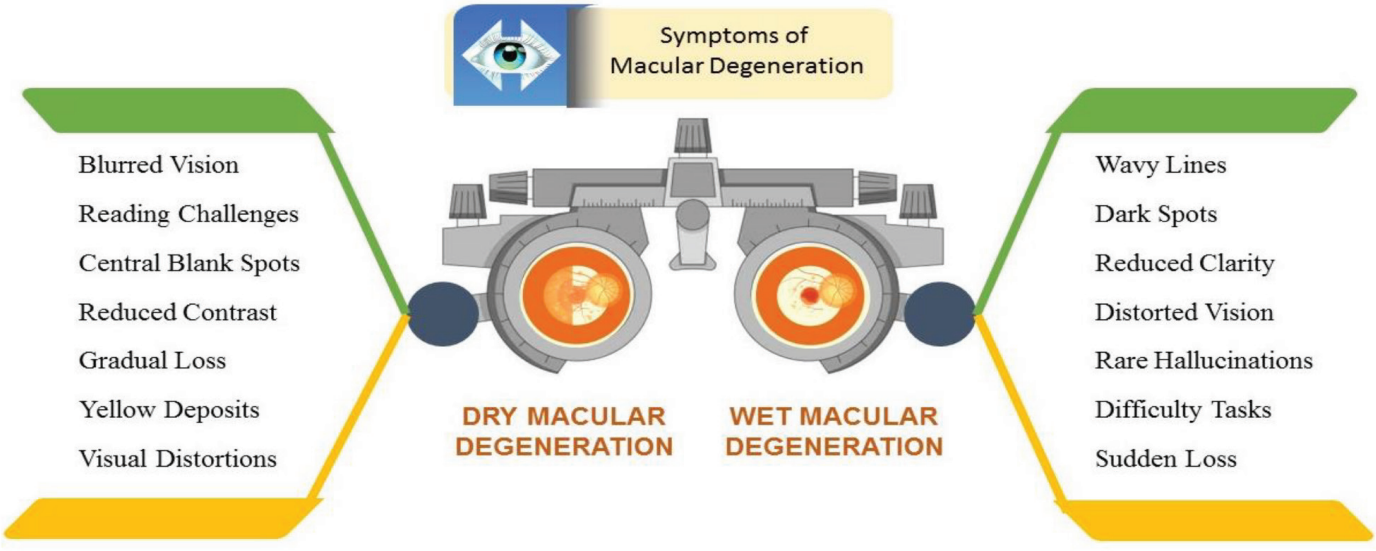

Figure 1. Symptoms of macular degeneration.

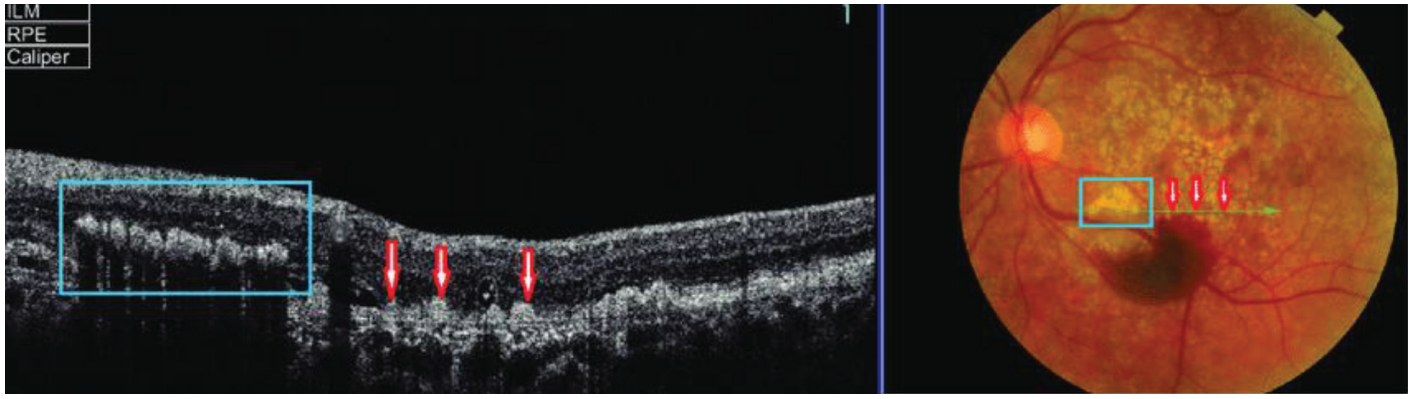

Figure 2. The fundus image on the right displays two distinct types of deposits: exudates (shown by the blue rectangle) and drusen (highlighted by the red arrow) in the patient's left eye, who has wet ArMD. The width of the B-scan line in the fundus image is equivalent to that of the B-scan SD-OCT image on the left. ArMD refers to ArMD, whereas SD-OCT stands for spectral domain optical coherence tomography (37).

ArMD: Age-related macular degeneration, SD-OCT: Spectral domain optical coherence tomography

coherence tomography (SD-OCT) image (bottom), demonstrating a B-scan of the two different types of deposit: exudates (blue square) and drusen (red arrow).

Preclusion

Lutein and zeaxanthin, which are isomers, are the primary components of the macular pigment in the human retina. Their attention is concentrated on the foveal area. The primary role is to safeguard the photoreceptor's membrane system from harm caused by light and radiation (38). The Age-Related Eye Disease Study (AREDS) identified a 25% reduction in progressive ArMD and formulated specific guidelines for using vitamin supplements. Dietary supplements mostly consist of zinc, vitamin C, vitamin E, lutein, and zeaxanthin. Due to the increased susceptibility to lung cancer in those who smoke beta-carotene was replaced with lutein and zeaxanthin in the AREDS formulation. Supplementation is recommended for those who have intermediate ArMD or early ArMD in one eye and severe ArMD in the other eye. Nevertheless, there is a lack of scientific evidence to support its efficacy in treating early ArMD in both eyes. Supplementation is not recommended for preventing ArMD in the general population or for those with a verified family history of the illness. The case-control study on eye illness also showed a 40% decrease in the probability of advanced AMD, in those who took more than 6 mg of carotenoid daily (39). Current research focuses on a scleral iontophoresis device that delivers liquid carotenoid formulations directly into the retina (40). Patients, particularly those with early ArMD in one or both eyes, should implement essential modifications to their lifestyle. Aside from weight reduction and smoking cessation, these alterations may require adjusting one's dietary intake to include antioxidant-rich foods and fish containing omega-3 and omega-6 fatty acids. The lipid profile and blood pressure are two modifiable risk factors. Although the research on the correlation between exposure to ultra-violet radiation and the development of ArMD remains inconclusive, it is worth considering minimizing sun exposure as a possible lifestyle modification.

Treatment of Age-related Macular Degeneration

Anti-VEGF Refers to Drugs or Treatments That Target VGEF

Before the development of potent anti-VEGF medications, other treatments were used for ocular treatment of wet ArMD. The initial therapies for nArMD, such as photodynamic treatment, angiostatic steroids, irradiation, and laser photocoagulation, successfully prevented significant vision impairment resulting from nArMD. Nevertheless, they failed to provide substantial improvements regarding visual function (41). Anti-VEGF drugs function by directly interacting with either VEGF or VEGF receptors. These drugs have outperformed all previous treatment methods, improving visual clarity. Currently, it is the most favored treatment for wet ArMD. Unfortunately, no preventive or therapeutic measures are now available for this condition (42). The current intravitreal VEGF inhibitors include ranibizumab, bevacizumab, aflibercept, and brolucizumab. The recommended intervals for administering ranibizumab and bevacizumab are four to eight weeks. Aflibercept is delivered every 12 weeks, and brolucizumab is given every 8 to 12 weeks. "Pro re nata (PRN)" and "treat and extend" are two different distinct dosage strategies for anti-VEGF therapy. The procedures begin by delivering a predetermined monthly dose for three months. The decision to provide or delay as-needed (PRN) medication is made weekly, based on the OCT results indicating either active exudation, or stability. Despite the lack of disease activity, the treat-and-extend protocol method requires administering intravitreal injections every visit. If the retinal fluid is shown to be suppressed or eliminated on OCT, the interval between visits is extended by a preset duration. If fluid reoccurrences, the drug is delivered, and the frequency of visits is decreased (43).

Developed Ankyrin Repeat Proteins

DARP molecules are derived from ankyrin repeat proteins, prevalent binding proteins in the human genome. Abicipar polyethylene glycol (Pegol) is a 34-kilodalton recombinant protein that has undergone polyethylene glycol modification. Currently, research is being conducted to evaluate the therapeutic potential of this

medication for diabetic macular edema and nArMD. It has shown strong inhibitory effects on VEGF-A (44). Abicipar has a reduced molecular weight, a 90-fold higher binding affinity for human VEGF-A165, and almost double the duration of effect inside the eye, compared to ranibizumab. The phase 2 research of the Registration, Evaluation, Authorization and Restriction of Chemicals program found that abicipar, when given in doses of either 1 or 2 mg, exhibited a long-lasting impact that was comparable to ranibizumab at a dose of 0.5 mg (45). This benefit was shown in terms of the mean enhancement in BCVA and the decrease in central retinal thickness. The research did not uncover any safety concerns. The phase 3 Sequoia and Cedar investigations demonstrated that receiving six to eight administrations of abicipar had better visual outcomes than receiving 13 administrations of ranibizumab at week 52. The abicipar regimens, administered either every eight weeks and twelve weeks, were more effective than monthly ranibizumab in achieving consistent visual acuity. All abicipar dosage frequencies maintained visual benefits in the ensuing year, as reported in the first year. Phase 2b MAPLE research evaluated the safety and effectiveness of abicipar, utilizing an innovative manufacturing technology. The goal was to decrease the presence of pollutants that can cause inflammation in the formulation. The results indicated no endophthalmitis or retinal vasculitis sequelae, and the incidence of severe intraocular infections was 1.6% (46).

Gene Therapy Using Recombinant Adeno-associated Virus

Recombinant adeno-associated virus (RAAV) vectors provide long-term treatment for ArMD by inducing recipient cells to create chemical substances that inhibit VEGF. The phase 1 experiment on nArMD confirmed the safety of a single subretinal injection of an RAAV particle that carries the gene, named Fms Related Receptor Tyrosine Kinase 1 (FLT1). This transcript includes a naturally occurring chemical that hinders the activity of VEGF. The presence of sFLT-1 (soluble FLT-1) was mostly seen in the specific tissue and did not negatively affect the eyes or the whole body (47). The clinical study lasted for 52 weeks and included 32 patients who were randomly allocated to receive ranibizumab PRN (with or without subretinal RAAV). No notable disparities were seen between the groups regarding BCVA or center point thickness when using the sFLT-1 gene therapy. The researchers found a minimal level of efficacy in terms of improvements in BCVA and a decrease in fluid levels over the three-year monitoring period of the phase 1/2b study. The researchers observed that the true impact of RAAV and sFLT-1 may have been concealed due to the limited number of participants. They also noted that the trial initially aimed to assess safety and included patients who had received previous treatments (48).

Thermal Laser Photocoagulation

Thermal laser photocoagulation is a medical procedure that uses a laser to coagulate or clot blood vessels to treat certain conditions. The continuous-wave thermal laser photocoagulation might prevent the accumulation of drusen deposits in the outer layers of the retina prior to the availability of anti-VEGF drugs for treating neovascular non-arteritic anterior ischemic optic neuropathy (49). However, further clinical trial findings revealed that removing drusen by laser-induced methods could not effectively prevent the progression of advanced ArMD. In addition, laser photocoagulation often leads to a reduction in BCVA and the formation of scars. Researchers have just developed an innovative laser method that produces short bursts of light.

Clustered Regularly Interspaced Short Palindromic Repeats-Associated Protein 9 (Cas9)

Cas9 is a protein associated with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). The CRISPR-Cas9 enzyme operates by cleaving DNA strands in a manner analogous to molecular scissors (50). Researchers are now evaluating the feasibility of using a CRISPR-Cas9 method, delivered by an AAV vector, to permanently decrease the levels of VEGF in the RPE layer of eyes affected with nArMD. This approach aims to decrease angiogenesis in the eyes. The concept of eliminating the need for periodic anti-VEGF injections is attractive. This technique resulted in a 26% decrease in VEGF-A levels and a 31% inhibition of CNV in a mouse model of laser-induced CNV (51). In contrast to intravitreal therapeutic techniques, CRISPR can be utilized to selectively target certain types of cells, reducing the probability of systemic side effects. While the CRISPR technique has potential as a treatment for nArMD, it is crucial to thoroughly evaluate it due to the susceptibility of CRISPR-based genome editing to unforeseen outcomes, and its irreversible nature (52).

Brimonidine

Brimonidine has alpha-2 adrenergic agonist properties, demonstrating cytoprotective and neuroprotective benefits in cultured cells and animal models. In a phase 2 trial to assess the effectiveness of brimonidine as a drug delivery system, a biodegradable implant was inserted into the eye to halt the progression of GA. Participants were given one of two doses of brimonidine, either 132 μg or 264 μg, instead of a placebo. By the time the participants reached 12 months, the group that received a larger dosage implant experienced a 28% drop in GA growth compared to the group that received a placebo (53). Before commencing clinical trials, it is necessary to conduct a more thorough evaluation of the features of this chemical taking into account the differences seen among species. In dry ArMD, drugs need to be administered topically to the posterior part of the eye (54). Achieving this task using eye drop formulations is a challenge. Recent investigations involving initial and early-stage clinical studies have shown that using these cells as a substitute for RPE has led to excellent safety profiles and potential effectiveness. Findings from preclinical studies suggest that reestablishing photoreceptors can reinstate visual capability, even when the outer retina has completely deteriorated.

Scaffold-Based Retinal Implants

Despite the immense potential of cell and gene therapies, their successful implementation in clinical practice faces major obstacles. TE provides an alternative method for treating ArMD by creating TE products (TEP) that may replace damaged RPE and restore its functioning. This effectively stops the course of the sickness (55). TE for the regeneration of RPE necessitates scaffolds with certain characteristics that facilitate cellular connections, development, and specialization while simultaneously inhibiting unwanted immunological reactions and inflammation from the host. Creating a three-dimensional framework with appropriate structural characteristics and bioactivity is necessary to achieve this objective. This crucial scaffold should be able to imitate the biological role of

the natural extracellular matrix in certain tissues, providing structural reinforcement and serving as a temporary matrix. Scaffolds may be infused with various cell types, such as stem cells, progenitor cells, differentiated cells, or can be directly implanted to accelerate functional tissue regeneration in regions that are not functioning properly (56). It is crucial to methodically include scaffold properties and their regulatory functions in scaffold design to effectively enhance tissue regeneration at the implantation site.

Advances for ArMD Treatment

Central vision loss caused by ArMD affects the individual's capacity to read, drive, recognize individuals, and perform essential everyday tasks. The phenotypic presentation of the infection exhibits considerable variation, from initial or midway ArMD with few symptoms to late-stage disease that can result in visual distortion and reduced center visual acuity. Scotomas refer to regions in the visual field with partial or total absence of vision. Total loss of central vision refers to the full lack of vision in the center area of the visual field (3). By using multimodal imaging techniques to examine the ultrastructure of the retina, retinal imaging professionals can now assess the severity of the disease and identify ArMD at an early stage with greater precision. Conducting a comprehensive fundus assessment in patients without symptoms to identify drusen is becoming recognized as a screening technique. The visual results of individuals with advanced neovascular ArMD have improved due to the advancement of anti-VEGF medications targeting VEGF. The efficacy of anti-VEGF medication varies across individuals and does not guarantee favorable outcomes for every patient (43). In addition, the effectiveness of the therapy tends to decrease with time, and the need for frequent injections places a considerable burden on the healthcare system, both for patients seeking medical care and those responsible for administering it. The human retina is an intricate tissue that plays a vital role in the visual and central nervous systems. Due to the lack of a natural reservoir of stem cells in the retina, any damage to retinal cells, particularly photoreceptor cells, results in irreversible vision loss. ArMD is a medical condition defined by the progressive deterioration of the retina. Therapeutic interventions are available for the neovascular type to prevent ArMD and halt the progression of the illness. Advanced therapies are a developing area of study that aims to provide cell and gene-based treatments for degenerative diseases like ArMD, with the potential to provide a cure. Various treatments based on cellular and genetic methods are presently undergoing clinical trials to treat ArMD and GA. Scaffold-based approaches for managing retinal degenerative diseases have shown promise as an avenue to impede or delay the progression of ArMD. Nevertheless, this approach necessitates the utilization of noxious organic solvents during the processing stage, which can detrimentally impact the survival of cells (18). 3D-bioprinting is a scaffold construction technology that offers several advantages over freeze drying. It has superior physical qualities, promotes better cell adhesion, and allows for precise enhanced mechanical characteristics by regulating scaffold microstructures. However, to achieve ideal mechanical and porous building features in 3D bioprinting, it is necessary to perform multi-objective optimizations (20). These optimizations involve identifying suitable printing settings such as print speed, pressure, stacking, and spacing, and determining the appropriate material composition, namely the ratio of cells to bioink. This necessitates a significant amount of time dedicated to conducting lengthy experiments, which requires substantial resources (56).

CONCLUSION

ArMD's central vision loss impairs reading, driving, recognizing people, and daily duties. Intermediate or early-stage ArMD illness may exhibit few symptoms. Still, advanced-stage disease may result in visual distortion, decreased central visual acuity, scotomas, and complete loss of central vision. Using several imaging techniques simultaneously, which capture precise pictures of the retina's inner structure, allows retinal imaging experts to can assess the disease's severity and discover ArMD sooner and correctly. Medical experts prefer optical coherence tomography for diagnosis; however, screening asymptomatic fundus for drusen is becoming increasingly popular. Lutein, other vitamins, and carotenoids have prevented severe ArMD progression. However, not all patients benefit from anti-VEGF treatment. Its effectiveness decreases with time, and repeated injections burden the healthcare system, including those who get medical treatment and those who provide care. Research is carried out to alleviate this load, enhance VEGF inhibition, and create gene and regenerative therapies. Unfortunately, patients with severe ArMD have few GA therapy options. Raising awareness and decreasing ArMD's harmful effects require public education on modifiable risk factors and beneficial supplements.

Footnotes

Authorship Contributions

Concept: N.R., S.R., P.M., Design: S.R., V.B.A., Supervision: S.R., Resources: P.M., Material: V.B.A., Data Collection or Processing: N.R., P.M., Analysis or Interpretation: S.R., M.S., V.B.A., Literature Search: N.R., V.B.A., Writing: N.R., S.R., M.S., P.M., V.B.A., Critical Review: P.M.

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Cyclooxygenases and Lipoxygenases in Cancer Drug Resistance

Kanserde İlaç Dirençliliğinde Siklooksijenaz ve Lipooksijenazlar

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ABSTRACT

Cancer drug resistance is a critical factor restricting the success rate of chemotherapeutics. Alterations in both cellular and systemic mechanisms could induce drug resistance in cancer. At the cellular level, numerous pathways have been associated with cancer drug resistance depending on the tumor origin and anti-cancer agent. Amongst other pathways including overexpression of multidrug resistance proteins, lipid modifier enzymes have also been linked to drug resistance in cancer via regulation of multidrug resistance protein levels, cell death mechanisms, and reactive lipid and oxygen species. Here the involvement of cyclooxygenases (COXs) and lipoxygenases (LOXs) as members of lipid modifier enzymes in cancer drug resistance should be emphasized. In this review, we summarize the studies examining the vital role of COXs/LOXs in the development and modulation of cancer drug resistance and cancer treatment.

Keywords: Cyclooxygenases, lipoxygenases, cancer drug resistance

ÖZ

Kanserde ilaç dirençliliği kemoterapötiklerin başarı oranını kısıtlayan kritik faktörlerden bir tanesidir. Hücresel ve sistemik mekanizmaların bozulması, ilaç dirençliliğini tetikleyebilir. Hücresel düzeyde, tümör orijinine ve anti-kanser ajana bağlı olarak birçok farklı yolak kanserde ilaç dirençliliği ile ilişkilendirilmiştir. Çoklu ilaç dirençliliği proteinin aşırı ekspresyonu gibi mekanizmalara ek olarak lipid düzenleyici proteinler de çoklu ilaç dirençliliği protein seviyelerini, hücre ölüm mekanizmalarını ve reaktif lipid ve oksijen türlerini regüle ederek kanserde ilaç dirençliliği ile ilişkilendirilmiştir. Bu noktada, lipid düzenleyici enzimlerin birer üyesi olarak siklooksijenazların (COX) ve lipooksijenazların (LOX), ilaç dirençliliğindeki rolü önem arz etmektedir. Sunulan derleme çalışmada, COX ve LOX'ların kanserde ilaç dirençliliğinin gelişimindeki ve modülasyonundaki ve kanser tedavisindeki hayati rollerini inceleyen çalışmalar özetlenmiştir.

Anahtar Sözcükler: Siklooksijenaz, lipooksijenaz, kanserde ilaç dirençliliği

INTRODUCTION

Cancer drug resistance is a critical phenomenon that limits the efficacy of cancer treatment. It is defined as the reduction in effectiveness of the anticancer drugs, due to several cellular and/or systemic alterations. Numerous studies elucidated the molecular mechanisms of cancer drug resistance and how a wide range of fundamental molecular pathways, such as cell death, cellular metabolism, and inflammation, have been affected by cancer drug resistance (1-3).

One of the major drug resistance mechanisms is the utilization or shaping of prostanoid and leukotriene (LT) biosynthetic pathways (4). The significance of these metabolites is immense in adjusting the tumor microenvironment by controlling angiogenesis, inflammation, and immunosuppression. Inflammation is a crucial response in organisms against a wide variety of threats. The process ensures the defense against pathogens, tissue repair, regeneration, and homeostasis (5). The inflammatory response governs the disease progression in cancer, including the stages of initiation, promotion,

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malignant conversion, invasion, and metastasis (6). Although the relation between inflammation and cancer has been on the table since 1863 with Virchow's hypothesis, the mechanism is yet to be elucidated (7). Eicosanoids, including prostaglandins (PGs) and LTs, are active lipid-based substances that regulate inflammatory processes (4). PGs and LTs are synthesized from AA, an omega-6 polyunsaturated fatty acid (PUFA), by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, respectively (8), implying that these enzymes could be decisive for the development of cancer drug resistance.

In the present review article, we summarize the studies focusing on the involvement of COXs and LOXs in cancer drug resistance. We regard that concentrating on these enzymes may provide a better understanding of cancer drug resistance in terms of lipid modification and inflammatory regulations.

Cyclooxygenases

The COX family, also known as PG G/H synthases (PGHS), is one of the well-studied enzymes due to its involvement in a wide variety of mechanisms including regulating inflammatory response, adjusting tumor microenvironment, and altering the response to anticancer agents. COX-1 (PGHS-1) isoform is continuously expressed in the majority of tissues to sustain homeostasis by regulating PGs (9). However, COX-2 (PGHS-2) is an inducible gene and is upregulated in inflammatory sites and cancer cells (10,11). Therefore, targeting COX-2 is a promising approach for better prognosis and treatment of cancer (12). The last isoform of COXs is COX-3, a splice variant of COX-1 producing a longer protein (633 amino acids long) (13,14).

COX-1 activity has been shown to be at a basal level in general and to increase when the free arachidonic acid (AA) increases. Contrary to COX-2, our knowledge of the role of COX-1 in cancer has been limited. In cooperation with COX-2, COX-1 has been reported to be deregulated in diverse cancers including renal cell carcinoma, skin, head and neck, esophageal, colorectal, breast, cervical, endometrial, and ovarian cancer and hematological tumors. Accordingly, selective inhibition of COX-1 has been proposed for cancer treatment (15). Selective COX-1 inhibitor SC-560 reduced tumor growth in the ovarian cancer grafts through inhibition of prostacyclin (PGI2), which is the major PG produced by COX-1 (16).

COX-2 was discovered in 1991 (17,18). The cDNA sequence of COX-2 was reported to be highly similar to that of COX-1, and its expression was shown in mouse fibroblasts, cultured rat mesangial cells, in RAW 264.7, and in rat alveolar macrophages as well as the ovary (19). Functional analyses showed that although there were remarkable sequence similarities between COX-1 and COX-2, they demonstrate diverse chromosomal organizations and tissue expression profiles. More importantly, the COX-2 promoter was found to have critical elements that were switched on by glucocorticoids and off by cellular stress, while COX-1 was regarded as a housekeeping gene (19,20).

COX-2 converts AA to PGs, specifically PGE2, and regulates diverse physiological mechanisms such as platelet disaggregation, inflammation, vasodilation and bone resorption. However, any disruptions in COX-2 activity could result in pathophysiological conditions, including cancer (21,22).

The role of COX-2 in carcinogenesis is well-documented. COX-2 is generally overexpressed in cancer cells and the contribution of COX-2 to the carcinogenesis is associated with the function of its main product, PGE_2 (23,24). The relationship between COX-2 and colorectal (24,25), breast (26-28), skin (29), pancreatic (30), cervical (31), lung (32), gastric (33), intestinal (34), and prostate (35) cancers has been reported. Overexpression of COX-2 caused increased genomic instability in the non-tumorigenic breast epithelial cell line MCF10A (27) and the breast adenocarcinoma cell line MCF7 (28).

COX-1 and Cancer Drug Resistance

The association of COX-1 with cancer drug resistance has not been studied in detail. It was shown that COX-1 inhibition by indomethacin elevated vincristine and doxorubicin cytotoxicity in T98G malignant glioma and doxorubicin-resistant K562 myeloid leukemia cells through downregulation of multidrug resistance protein 1 (MRP1) (36,37). On the other hand, Radilova et al. (38) showed that COX-1 downregulation in human cervical cells caused an upregulation in (MRP4; ABCC4), which was explained by the role of MRP4 in the transport of the prostanoids (38). In another study, cisplatin conditioning was shown to trigger resistance to different platinum analogs, including cisplatin, oxaliplatin and carboplatin in mesenchymal stem cells by preventing apoptosis. The researchers identified the resistance mechanism was the production of PUFAs 12-oxo-5,8,10-heptadecatrienoic acid and hexadeca-4,7,10,13-tetraenoic acid [16:4(n-3)] by cisplatin preconditioning, which was reversed by the inhibition of COX-1 and thromboxane synthase (TXAS) (39). Bottone et al. (40) also reported that selective COX-1 inhibitor SC-560 elevated the expressions of NAG-1, NAG-3, ATF3 and C/EBPB while downregulating the expressions of INSIG-1, MSX1, MAD2 and NRG-1, implying a chemopreventive effect in HCT-116 human colorectal carcinoma cells (40). On the other hand, COX-1 was shown not to be expressed in either sensitive or taxane-resistant ovarian cancer cells (41). However, the limited number of studies cannot allow us to draw comprehensive conclusions about the function of COX-1 in cancer drug resistance.

COX-2 and Cancer Drug Resistance

The effects of the alterations in COX-2 expression and activity have frequently been observed in drug resistance, in addition to its role in carcinogenesis (42). Several studies reported that COX-2 expression is enhanced in drug resistance to various chemotherapeutics. Lu et al. (43) showed that COX-2 expression was significantly elevated in cetuximab-resistant DiFi colorectal cancer cells compared to cetuximab-sensitive parental cell lines (43). Stable COX-2 transfection in LNCaP prostate cancer cells caused 3-fold resistance to carboplatin by repressing apoptotic response through upregulation of survivin, Bcl-2, and AKT (44). Krysan et al. (45) revealed that constitutive COX-2 expression stabilized survivin and caused the development of resistance to apoptosis in non-small cell lung cancer cell lines, A549 and H157 (45). Tang et al. (46) reported that COX-2 transfection conferred resistance to TRAIL-induced apoptosis in HCT-15 colon cancer cells via the transcriptional suppression of DR-5, upregulation of Bcl-2, and inhibition of the activation of caspase 3, 8, and 9 (46). The COX-2-TXA2 signaling pathway was shown to inhibit apoptosis in gefitinib-resistant non-small cell lung cancer cells (47). Recently, COX-2 upregulation through the activity of yes-associated protein 1 (YAP) has been shown to confer resistance by co-expression of myeloid cell leukemia-1 (MCL-1), multidrug resistance

(MDR), and survivin. The use of a YAP and COX-2 inhibitor reversed the resistance to taxol in colorectal cancer cells (48). In another study, COX-2 expression has been positively correlated with increased levels of P-gp and phosphorylated c-Jun, and COX-2 downregulation promoted resensitization of HCT8/V colorectal cancer cells against vincristine (49). In a similar manner, overexpression of COX-2 led to the development of acquired cross-resistance to cisplatin and doxorubicin in TRAIL-resistant H460 and H1568 lung cancer cells (50). Janakiraman et al. (51) showed that overexpression of COX-2 blocked the cleavage of caspase 3 and HuR, thereby inhibiting apoptotic pathways in paclitaxel-resistant oral squamous cell carcinoma. COX-2-deficient HT-29 colon cancer cells were reported to exhibit resistance to apoptosis when COX-2 was overexpressed. At the same time, 3-hydroxy-30,4,40,50-tetra-methoxy-chalcone (3-HTMC, an open chain flavonoid chalcone) was proven to be more potent in terms of apoptosis induction in COX-2-deficient HCT-116 cells than COX-2-sufficient HT-29 cells (52). The main product of COX-2, PGE2, acts as a cytoprotective agent (24). The stimulation of PGE2 release from stromal cells due to overexpression of COX-2 rendered prostate cancer cells resistant to the tetracycline derivative COL-3, and docetaxel (53). Similarly, Sun et al. (54) showed that overexpression of COX-2 and subsequent PGE_2 production alleviated the induction of apoptosis by 5-fluorouracil in colon cancer cells by increasing Bcl-2 at mRNA and protein levels, attenuating caspase 3/9 activation and inhibiting cytochrome c release (54).

COX-2 expression also showed positive correlation with tumorigenesis, as well as poor prognosis and increased drug resistance in cancer patients. Singh et al. (28) showed that MCF-7/COX-2 mammospheres displayed enhanced colony-forming ability. COX-2 positivity was higher in primary advanced ovarian cancer patients who were non-responding to the platinum-based chemotherapy (55). In a similar manner, patients with advanced head and neck squamous cancer displayed higher tumor recurrence due to cisplatin resistance caused by increased COX-2 expression (56). COX-2 expression was reported to be markedly increased in breast cancer-associated macrophages and correlated with poor prognosis in breast cancer (57). Similarly, a strong correlation between the expression levels of COX-2 and BCRP was reported in non-treated non-Hodgkin's lymphoma patients who displayed poor response to chemotherapy, and shorter progression-free survival time (58).

COX-2 and Cellular Drug Transporters

A correlation between high COX-2 expression and MDR1/P-gp expression was reported. Several studies have shown that MCF7 cells expressing COX-2 exhibited increased MDR1/P-gp expression and resistance to doxorubicin coupled with increased Bcl-2 expression (28,59). Li et al. (57) demonstrated that ectopic expression of COX-2 triggered cellular proliferation and survival of breast cancer cells by stimulating P-gp expression. Similarly, cisplatin-resistant SGC-7901 gastric cancer cells were found to display increased COX-2 and P-gp expression (60). Lee et al. (41) reported that COX-2 was significantly expressed in taxane-resistant SKOV3.ip1 ovarian carcinoma cells that exhibited upregulated P-gp expression (41). Likewise, TT medullary thyroid carcinoma (an extremely chemoresistant malignant neoplasia) cell line was reported to express elevated levels of COX-2 and P-gp (59). Imatinib-resistant K562 chronic myeloid leukemia (CML) cells were shown to exhibit increased histone deacetylase expression and activity, which, in turn, elevated CREB activation, COX-2 expression, and consequently, MDR1 expression and decreased intracellular doxorubicin accumulation. In the same cell line, the overexpression of COX-2 increased ABC transporter (MRP1-5, ABCG2, ABCA2) expression at both mRNA and protein levels, which was downregulated upon celecoxib treatment due to inhibition of WNT and ERK signaling pathways (60). Liu et al. (61) stated that after the interaction with Shc3, triggers, where it stimulates COX-2 expression by binding to the COX-2 promoter, which, in turn, mediated P-gp expression, resulting in decreased sensitivity to chemotherapy. It was also reported that COX-2 expression was correlated with higher MDR1/P-gp expression in primary invasive breast (62), ovarian (54,62) and non-small cell lung cancer cases (62), which were coupled with shorter progression-free survival time and decreased treatment response. Not only does COX-2 expression affect MDR1/P-gp-mediated drug resistance. MRP1 expression and subsequent chemoresistance to cisplatin were reported after COX-2 overexpression in HCT-15 colon cancer cells, which were normally lacking COX-2 (63). Kochel et al. (64) reported that in triple-negative breast cancers, high COX-2 activity was associated with increased MRP4 and decreased PG transporter (PGT) activities, which contributed to poor prognosis. Similarly, the upregulation of COX-2, stimulated MRP4 expression in the A549 non-small cell lung cancer (65). Kalalina et al. (66) showed that TPA-induced COX-2 expression in mitoxanthrone-resistant MCF7 (MCF7-MX) cells resulted in higher ABCG2/BCRP activity. COX-2 catalyzes the production of PGE2, which is imported by PGT and exported by MRP4 (67). Chung et al. (68) showed that TGFβ1 promoted COX-2 expression via the activation of the p38-MAPK-ERK pathway through galectin-1 interaction with Ras. The knockdown of galectin-1 resensitized A549 lung cancer cells to cisplatin and repressed cellular migration and invasion by suppressing COX-2 activity.

Inhibition of COX-2 in Resistant Cancer Cells

Inhibition of COX-2 is a frequently used strategy due to the involvement of COX-2 in cancer initiation and progression. Moreover, the efficacy of COX-2 inhibitors in halting multidrug resistance by downregulating efflux pumps has been confirmed (69). Selective inhibition of COX-2 gains more attention because of advantages in avoiding the undesired side effects of non-selective therapies, such as non-steroidal anti-inflammatory drug (NSAID) conjugates (70). COX-2 inhibitor NS-398 was shown to be effective at resensitizing MCF7 cells by impairing P-gp expression and function (59). Treatment with another specific COX-2 inhibitor, celecoxib, reversed the effects of high ABCG2 (BCRP) activity in MCF7-MX cells (66). Similarly, celecoxib was shown to increase apoptosis by downregulation of survivin and upregulation of glutathione S-transferase pi (GSTpi) in carboplatin-resistant prostate cancer cells (44). Additionally, celecoxib decreased stroma-induced resistance to COL-3 and docetaxel, and triggered in situ prostate cancer cell death by increasing the levels of active caspases 3 and 9 (53). Fantappie et al. (71) demonstrated that celecoxib alleviated drug resistance by decreasing the expression levels of P-gp, Bcl-xL and Bcl-2, enhancing the translocation of Bax to the mitochondria and promoting the release of cytochrome c into the cytoplasm in multidrug-resistant hepatocellular carcinoma cells. It was also reported that celecoxib treatment decreased P-gp expression in doxorubicin-resistant HT-29 colorectal

cancer cell lines (71). In the same cell line, the use of celecoxib along with imatinib resulted in decreased cell viability through diminished COX-2 and increased caspase 3 levels (72). Lee et al. (41) showed that COX-2 inhibition resulted in decreased MDR1/P-gp expression and enhanced paclitaxel cytotoxicity in taxane-resistant ovarian carcinoma. COX-2 was found to be downregulated in C-Phycocyanin-treated (a biliprotein obtained from Spirulina platensis)-treated HepG2 hepatocellular carcinoma cells. The downregulation of COX-2, in turn, decreased the expression of MDR1 and enhanced the intracellular accumulation of doxorubicin and subsequently the chemosensitivity of the cells (73). In a similar fashion, indomethacin (NSAID) and SC236 (selective COX-2 inhibitor) caused elevated doxorubicin cytotoxicity due to higher drug accumulation and retention through partial decrease in P-gp and MRP1 expressions in HepG2 cells (74). Specific COX-2 inhibitors, NS-398, rofecoxib, and celecoxib, reduced MDR1 expression, decreased drug efflux, triggered apoptosis, and increased the efficacy of doxorubicin and vinorelbine in medullary thyroid carcinoma (59,75). Celecoxib or NS398 treatment increased sensitivity to vinblastine or paclitaxel in multidrug resistant subline of KB oral squamous carcinoma cells (76). It was also reported that celecoxib sensitized renal cancer cells to sorafenib by stabilizing COX-2 mRNA in stress granules (77). Xu et al. (69) revealed that celecoxib inhibited PGE2 release, downregulated P-gp expression, and displayed synergism with cisplatin. It also promoted apoptosis through enhanced p53 expression, caspase 3 activation, and a low Bcl-2/Bax ratio in cisplatin-resistant gastric cancer cells (69). Similarly, another COX-2 inhibitor, meloxicam, was found to downregulate MDR1 expression in HL-60 acute myeloid leukemia cell lines (78). In a similar manner, JTE 522 (selective COX-2 inhibitor) was suggested as effective in resensitizing colon cancer cells to cisplatin (63). Zrieki et al. (79) pointed out that the non-specific COX inhibitor indomethacin heptyl ester could reverse the upregulation of P-gp expression and activity, which was induced due to COX-2 stimulation by 2,4,6 -trinitrobenzene sulfonic acid in colorectal cancer (79). Peng et al. (80) reported that COX-2 inhibitor NS-398 promoted the expression of cyclin dependent kinase inhibitors p21WAF1 and p27Kip1, and enhanced chemotherapy-induced apoptosis in hypopharyngeal cancer cells. In lung cancer, the inhibition of COX-2 activity repressed the resistance to apoptosis by enhancing the proteasomal degradation of survivin (45) and upregulating anti-apoptotic MCL-1 (50). The induction of apoptosis after treatment with COX-2 inhibitors was recorded in Caco-2 colorectal adenocarcinoma cell lines, which are known to be intrinsically resistant to cetuximab (43). Moreover, Neumann et al. (81) utilized cisplatin-COX-2 inhibitor conjugates to benefit from the tumor-inhibiting capacity of COX-2 inhibitors and to maximize the apoptosis-inducing effect of cisplatin in colon, ovarian, triple-negative breast and squamous cell cancers.

The use of NSAIDs was also effective against COX-2-mediated drug resistance. Tang et al. (46) showed that sulindac sulfide restored the expression of death receptor 5 (DR-5) and augmented the efficacy of TRAIL in colon cancer cells. Another NSAID, etodolac, was found to have additive effects with oxaliplatin, inhibiting the growth of RKO colon cancer cells and promoting cell death by diminishing survivin (82). Additionally, nimesulide was shown to downregulate P-gp expression and activity, providing chemoprevention and chemosensitization to CaCo-2 colorectal cancer cells (79).

In addition to small molecule inhibitors, COX-2 downregulation by genetic manipulations has also been studied in cancer drug resistance. COX-2 is a direct target of miR-101, which was significantly downregulated in cisplatin-resistant T24 human bladder cancer cells. The overexpression of miR-101 augmented the anti-proliferative effects of cisplatin and helped the induction of apoptosis (83). MiR-216a-3p was also reported to directly bind to the 3' UTR of COX-2 mRNA, and the ectopic expression of miR-216a-3p was efficient in the suppression of colorectal cancer cells (84). In BRAF and MEK inhibitor-resistant melanoma cells, increased miR-146-5p expression downregulated COX-2, resulting in elevated drug sensitivity, decreased cellular proliferation, and increased apoptosis by hampering NF-kB signaling (85). The depletion of COX-2 sensitized PARPi-resistant BRCA2-deficient pancreatic Capan-1 cells, increasing their sensitivity by 70% (86). Similarly, siRNA-mediated inhibition of COX-2 expression resulted in the induction of apoptosis in 3-HTMC-treated colon cancer cells (52). Stable knockdown of COX-2 led to partial sensitization of cetuximab-resistant DiFi cells to the agent (43). Likewise, in doxorubicin-resistant MCF7 breast cancer cells, inhibition of COX-2 by siRNA decreased the aggressiveness of the resistant cells (87).

COX-2 expression affects proliferation and resistance status not only monolayer cell lines, but also of 3D cultures and cancer patients. Ben-Batalla et al. (88) demonstrated that COX-2 inhibitors showed additive effects with anti-VEGFR-2 antibodies or the VEGFR inhibitor sunitinib in 4T1 tumors. The use of COX-2 inhibitors decreased the levels of pro-angiogenic HGF and FGF2, the migratory capacity of cancer-associated fibroblasts (CAFs) and the infiltration of CAFs into tumors (88). The specific COX-2 inhibitor, celecoxib, increased the sensitivity of the multicellular spheroids of HepG2 hepatocellular carcinoma cells to 5-fluorouracil, sorafenib, and gefitinib (89). Kim et al. (90) showed that NS-398 decreased both the expression and activity of P-gp and triggered apoptosis by downregulating Bcl-2 protein in monolayer cell cultures derived from COX-2-positive ependymoma patients (90). Additionally, Pi et al (91) reported that inhibition of COX-2 reversed resistance to anti-PD-1-mAB therapy in B16F10-R tumors in which knockout of the PTSG2 gene restored sensitivity.

Inhibition of COX-2 not only sensitizes the cancer cells to chemotherapy but also to radiation. Celecoxib was found to be effective in sensitizing HeLa cells to radiation by decreasing COX-2 mediated AKT phosphorylation (92). Moreover, Sun et al. (93) revealed that aspirin downregulated COX-2 by irreversibly disrupting the chromatin looping in COX-2 locus and increased the survival of A549 and H1299 lung cancer cells (93).

Lipoxygenases

LOXs are enzymes catalyzing the PUFAs, specifically AA and linoleic acid. The metabolic products of LOXs (5-LOX, 12-LOX, 15-LOX-1 and 15-LOX-2) are involved in several physiological and pathophysiological conditions, including inflammation and cancer (94). Due to having a wide range of roles, LOXs are studied exclusively by numerous therapeutic strategies, including screening new inhibitors and activators of LOXs, designing or repurposing drugs (95, 96). Particularly, development of LOX inhibitors is one of the promising approaches to regulate LOX enzymes' metabolites in cancer therapy as well as for the treatment of inflammation,

allergy, and cardiovascular disease (96). The importance of LOXs in cancer drug resistance should be addressed in detail because of not only their role in the production of bioactive lipids but also their ability in membrane modification and balancing redox states (97). Nevertheless, the limited number of studies in the literature have mostly been restricted to 15-LOX-1.

5-Lipoxygenase

5-Lipoxygenase (5-LOX) was shown to be involved in the modulation of angiogenesis, cellular viability and proliferation, higher metastasis and invasive capacity, lower survival rates in cancer patients, and disruption of chemopreventive efforts (98). The inhibition of 5-LOX was associated with reduced growth and increased apoptosis in gastric, esophageal, bladder, and hepatocellular cancers (99-101). Edderkaoui et al. (102) showed that 5-LOX controls reactive oxygen species production by NADPH oxidase by modulating fibronectin. The inhibition of 5-LOX resulted in decreased ROS accumulation, leading to caspase stimulation and eventual DNA fragmentation in pancreatic cancer (102). Ding et al. (103), stated that the 5-LOX pathway was overactivated in pancreatic cancer cells and the combined use of COX-2 inhibitor celecoxib and 5-LOX inhibitor MK886 significantly decreased the growth of SW1990 cells (103). Roos et al. (104) reported that selective 5-LOX inhibitor CJ13,160 inhibited WNT signaling and disrupted stem cell capacity in acute myeloid leukemia (104). Similarly, Wang et al. (105), showed that synthetic dl-nordihydroguaiaretic acid compound (dl-NOGA or Nordy) inhibited 5-LOX, resulting in a decrease in self-renewal, abrogation of clonogenicity, as well as induced differentiation in glioma stem-like cells that were resistant to radio- and chemotherapy. Additionally, 5-LOX inhibitor, auranofin, was reported to induce apoptosis in cisplatin-resistant ovarian cancer (106). On the other hand, Catalano et al. (107) showed that 5-LOX interfered with p53-induced apoptosis by decreasing the relocalization of p53 within promyelocytic leukemia protein (PML)-nuclear bodies, which induced transcription of pro-apoptotic genes.

12-Lipoxygenase

Yin et al. (108) showed that cancer stem cell-like populations isolated from DU-145 prostate cancer cell line exhibited upregulated 12-Lipoxygenase (12-LOX) and ABCG2. Similarly, Lövey et al. (109) reported that upregulation of 12-LOX resulted in resistance to radiation in pancreas cells, and 12-LOX inhibitors baicalein or BMD122 sensitized these cells to radiation. The combined use of 12-LOX inhibitors and radiation displayed a synergistic effect, leading to decreased colony formation capacity of LNCaP and PC3 cells as well as reduced tumor growth *in vivo* (109). In cisplatin-resistant SKOV3 ovarian cancer cells, targeting 12-LOX or SP1 (the mutual transcription factor for 12-LOX, COX-1, MRP1 and MRP4) inhibited EMT and metastasis and reversed cisplatin resistance (110).

15-Lipoxygenase-1 and Cancer Drug Resistance

The studies reporting the involvement of Arachidonate 15-Lipoxygenase (ALOX15)/ 15-Lipoxygenase-1 (15-LOX-1) expression and/or activity in carcinogenesis and cancer drug resistance are not only limited but are also controversial.

Kim et al. (111) showed that overexpression of 15-LOX-1 promoted growth arrest and decreased cellular viability in HCT-116 colorectal cancer cells through the upregulation of p21WAF1/CIP1 and MDM2, and the induction of p53 phosphorylation. In gastric cancer, COX-2 inhibitor SC-236 promoted apoptosis by upregulating 15-LOX-1 expression and the production of 13-S-HODE (112). Similarly, it was reported that 15-LOX-1 overexpression in the same cell line led to enhanced indomethacin-induced apoptosis, and the treatment of the cells with exogenous 13-S-HODE further strengthened this apoptosis (113). Wolff et al. (114) showed that three different 15-LOX-1 metabolites, 13-HpOTrE, 13-HpODE and 15-HpETE, reduced the viability of monolayer skin cancer cells and decreased the release of interleukin-6 to the level of tumor-free samples in 3D skin cell cultures. In K562 CML cells, the hydroperoxy metabolite of 15-LOX-1, 13-S-HpODE, inhibited cellular growth and induced apoptosis via the generation of reactive oxygen species and the activation of caspase 3 (115). However, in leukemia stem cells (LSCs), both the deletion of ALOX15 and the chemical inhibition of 15-LOX-1 impaired the function of LSCs, inhibited cell division, triggered apoptosis, and consequently attenuated CML. Additionally, combining the specific 15-LOX-1 inhibitor, PD146176, with imatinib was proven to be more effective in decreasing the number of LSCs (116). Similarly, Kelavkar et al. (117) demonstrated that the increased expression of 15-LOX-1 promoted the tumorigenesis of PC3 prostate cancer cells by increasing VEGF and Factor VIII levels as well as cellular proliferation. In our study, we found that *ALOX15* was transcriptionally downregulated in doxorubicin-resistant MCF7 breast cancer and HeLa cervical cancer cell lines. Although the ectopic expression of ALOX15 resensitized both resistant cell lines to doxorubicin, the mechanisms by which ALOX15 overexpression exerted its effects differed. The ALOX15 overexpression stimulated apoptosis via the activation of PPARγ and increased intracellular doxorubicin accumulation by altering membrane dynamics in doxorubicin-resistant MCF7 cells. In contrast, increased apoptosis was monitored in 13-S-HODE-treated doxorubicin-resistant HeLa cells (118). Downregulation of ALOX15 by miR-522 packed into exosomes and secreted by CAFs, upon de-ubiquitination of hnRNPA1 by ubiquitin-specific protease 7 (USP7), was shown to increase chemoresistance in gastric cancer cells. Zhang et al. (119) reported that downregulated ALOX15 decreased lipid-ROS accumulation in the cells, which led to the inhibition of ferroptosis, and consequently, decreased chemosensitivity.

Shureiqi et al. (120) found that NSAIDs induced 15-LOX-1 expression during apoptosis in esophageal cancers, which otherwise exhibited lower 15-LOX-1 expression. In contrast, Yoshinaga et al. (113) reported a significant decrease in 15-LOX-1 expression in colorectal surgical samples of NSAID users compared to NSAID non-users. However, in both cases, the increased expression or activity of 15-LOX-1 stimulated apoptosis, indicating its enhancing effect on NSAID-induced apoptosis.

CONCLUSION

In this review, we have summarized numerous studies that emphasized the importance of COXs and LOXs in cancer drug resistance. These studies suggest that COXs and LOXs directly and/or indirectly are involved in the development, progression, and modulation of cancer drug resistance and could be vital candidates in overcoming cancer drug resistance, and possibly, in more personalized cancer treatments. However, the involvement of these enzymes in the modulation of cancer drug resistance appears to be

in a cell/tumor origin-specific and drug-specific manner. Additionally, it is important to note that those enzymes participate in other vital functions including the regulation of inflammatory pathways, cellular redox balance, cell death mechanisms, and cell membrane modifications that affect the membrane dynamics. Unfortunately, we still have limited knowledge about the widespread effect of COX and LOX family enzymes on the regulation of these cellular processes in drug-resistant cancers. The lack of information reveals the need for further studies on the role of COXs and LOXs in the regulation of these cellular processes, as well as their effects on cancer drug resistance.

Footnotes

Authorship Contributions

Concept: H.H.K., Ç.U.M., Design: H.H.K., Ç.U.M., Analysis or Interpretation: Supervision: Ç.U.M., Literature Search: H.H.K., A.Ç.Ö., Ç.U.M., Writing: H.H.K., A.Ç.Ö., Ç.U.M., Critical Review: H.H.K., A.Ç.Ö., Ç.U.M.

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