The Effect of Morphological Parameters on IVF Outcomes in Single Blastocyst Transfer Cycles

Tek Blastokist Transfer Sikluslarında Morfolojik Parametrelerin IVF Sonuçlarına Etkisi

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ABSTRACT

Aim: Our aim is to evaluate the effect of three morphological parameters (Blastocoel expansion, trophectoderm (TE) morphology grade, and inner cell mass (ICM) morphology grade) on clinical pregnancy in single blastocyst transfers.
Methods: The study included 74 fresh day 5 single blastocyst transfers in a two-year period. There were 30 women with clinical pregnancy (Group 1). Women that failed to get pregnant after IVF/ICSI procedure were included in the non-pregnant group (n = 44) (Group 2). The blastocysts were graded according to Gardner and Schoolcraft. Age of the couple, body mass index, infertility duration, day 3 follicle stimulating hormone, luteinizing hormone, number of days of gonadotropin stimulation, total gonadotropin dose, estradiol level on the day of hCG administration, number of oocytes retrieved, number of metaphase II oocytes, number of 2PN, TE morphology, ICM morphology, blastocoel expansion were recorded. These outcomes were compared between the two groups. Statistical comparisons were carried out by Chi-square test and Student "t" test. Regardless of the statistical test, only a p value ≤ 0.05 was considered significant.
Results: During the study period, 702 embryo transfers were performed; of these 74 (10.5%) were on Day 5. While number of oocytes retrieved, number of metaphase II oocytes and 2PN increased in pregnant group, estradiol level on the day of hCG administration, total gonadotropin dose, blastocoel expansion were similar in both groups. ICM and TE morphology were significantly associated with pregnancy ($p<0.05$).
Conclusion: The clinical pregnancy rate seems to be affected by both ICM and TE morphology.

Key Words: Inner cell mass morphology, IVF outcomes, single blastocyst transfer, trophectoderm morphology

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ÖZET

Amaç: Biz bu çalışmada tek blastokist transferi yapılan hastalarda 3 morfolojik parametrenin (blastosöl genişlemesi, trofektoderm (TE) morfolojik evrelemesi, iç hücre kütlesi (ICM)) klinik gebelik oranlarına etkisini araştırmayı amaçladık.
Yöntem: Çalışmaya iki yıl içinde 75 taze 5. gün embriyo transferi yapılan hasta dahil edildi. IVF-ICSI işlemi sonrası klinik gebeliği olan hastalar grup 1 (n=30) ve işlem sonrası gebelik elde edilememiş hastalar grup 2 (n=44) olmak üzere ikiye ayrıldı. Blastokistler, Gardner ve Schoolcraft sınıflamasına göre evrelendirildi. Çiftlerin yaşları, vücut kitle indeksi, infertilite süresi, 3.gün folikül stimüle edici hormonu, lüteinize edici hormonu, gonadotropin uygulama süresi, toplam gonadotropin dozu, HCG uygulama günündeki östrojen düzeyi, toplanan oosit sayısı, metafaz 2'deki oosit sayısı, 2PN sayısı, TE morfolojisi, ICM morfolojisi, blastosöl genişlemesi kaydedilerek bu veriler iki grup arasında karşılaştırıldı. İstatistiksel karşılaştırmalar Ki-Kare testi ve student t test ile yapılarak, p değeri <0,05 olanlar anlamlı olarak kabul edildi.
Bulgular: Çalışma süresince 702 embriyo transferinin yapıldığı, bunlardan 74'ünün (%10,5) 5. gün embriyosu olduğu tespit edildi. Her iki grupta HCG uygulama günündeki östrojen düzeyi, toplam gonadotropin dozu, blastosöl genişlemesi açısından anlamlı fark izlenmezken, gebe kalan grupta toplanan oosit sayısı, metafaz 2'deki oosit sayısı, 2PN sayısının artmış olduğu tespit edildi. ICM ve TE morfolojisin gebelikle anlamlı düzeyde ilişkili olduğunu tespit ettik ($p<0.05$).
Sonuç: Çalışmamızda klinik gebelik oranlarının hem ICM'den hem de TE morfolojisinden etkilenmiş olduğunu saptadık.

Anahtar Sözcükler: İç hücre kütlesi, IVF sonuçları, tek blastokist transferi, trofoektoderm morfolojisi

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INTRODUCTION

Blastocyst transfer is accepted to achieve higher implantation and live birth rates compared with cleavage stage embryos (1). For blastocyst grading, Schoolcraft system is commonly used (2). Elective blastocyst transfer on day 5 could improve the implantation rate significantly compared to day 3 embryo transfer without increasing the risk of complications related with multiple pregnancies (2). Some studies have shown that timing of blastocoel development and grade of expansion are good predictors of implantation (3-5). Others have revealed a relationship between size and shape of the inner cell mass (ICM) and implantation (6,7). It is not clear that which blastocyst morphology parameter is the best indicator of clinical pregnancy.

Our aim is to evaluate the effect of three morphological parameters (Blastocoel expansion, trophectoderm (TE) morphology grade, and ICM morphology grade) on clinical pregnancy in single blastocyst transfers.

MATERIALS and METHODS

The study included 74 fresh day 5 single blastocyst transfers in a two-year period. This retrospective study was approved by local Ethics Committee. Inclusion criteria for this study were as follows: 1) the patient age was <40 years; 2) there was no evidence of an endocrinologic disorder (normal prolactin and thyroid-stimulating hormone levels); 3) the patient body mass index (BMI) was <30.0 kg/m^2. All patients underwent controlled ovarian stimulation using a recombinant follicle-stimulating hormone. When one or more follicles reached a maximum diameter of 18 mm, human chorionic gonadotropin (HCG) was administered. Transvaginal oocyte retrieval was performed 36 hours after hCG injection. There were 30 women with clinical pregnancy (Group 1). Women that failed to get pregnant after IVF/ICSI procedure were included in the non-pregnant group (n=44) (Group 2). The blastocysts were graded according to Gardner and Schoolcraft (2). Gardner and Schoolcraft developed criteria for describing blastocysts. This can help select suitable blastocysts for in vitro fertilization. Better blastocysts have a better implantation success rate. Parameters are (1) blastocyst grade, (2) inner cell mass (ICM), (3) trophectoderm. They gave six numerical scores(1-6) to blastocysts regarding the degree of blastocoel expansion and status of hatching. The early blastocystswith the beginning of blastocoel formation are scored as 1 and hatched blastocysts as 6.

Scores; 1) The blastocoel cavity represents less than half the volume of the embryo,

2) The blastocoel cavity is more than half the volume of the embryo,

3) Full blastocyst, cavity completely fills the embryo,

4) Expanded blastocyst, cavity is larger than the embryo with thinning of the Shell,

5) Hatching out of the Shell,

6) Hatched out of the shell.

ICM is only possible for assessment of full blastocysts graded 3-6. The ICM and TE were assessed each as A, B or C, where A is score for optimal morphology and C for severe irregularities observed (2).

Age of the couple, body mass index, infertility duration, day 3 follicle stimulating hormone, luteinizing hormone, number of days of gonadotropin stimulation, total gonadotropin dose, estradiol level on the day of hCG administration, number of oocytes retrieved, number of metaphase II oocytes, number of 2PN, TE morphology, ICM morphology, blastocoel expansion were recorded. These outcomes were compared between the two groups.

Distribution of parameters was tested by Kolmogorov-Smirnov test. Statistical comparisons were carried out by Chi-square test and Student "t" test. Regardless of the statistical test, only a p value ≤ 0.05 was considered significant.

RESULTS

During the study period, 702 embryo transfers were performed; of these 74 (10.5%) were on Day 5. The pregnancy rate was 40.5%. Patient and cycle characteristics compared by live birth outcome are summarized in Table 1, as are the individual scores for each morphological parameter of transferred blastocysts. Comparison of pregnancy and no pregnancy group in single blastocyst transfer cycles were performed based on blastocoel expansion grade, inner cell mass grade, trophectoderm grade and patient characteristics. While number of oocytes retrieved, number of metaphase II oocytes and 2PN increased in pregnant group, estradiol level on the day of hCG administration, total gonadotropin dose, blastocoel expansion were similar in both groups. ICM and TE morphology were significantly associated with pregnancy (p<0.05).

Table 1. Characteristics of the two groups

	Group 0 (non-pregnant)	**Group 1 (pregnant)**	**p**
Number	44(%59.5)	30(%40.5)	
Age, y	28.3 ± 3.4	28.5 ± 4.2	0.887
Paternal age, y	32.2 ± 4.5	30.9 ± 3.8	0.215
BMI (kg/m^2)	24.2 ± 4.4	24.7 ± 2.8	0.330
Infertility duration, y	4 (1-14)	4.2 (1-15)	0.964
Basal FSH (mIU/mL)	6.14 ± 1.69	6.0 ± 2.0	0.836
Basal LH (mIU/mL)	5.2 (0.7-16.7)	4.5 (0.5-34.3)	0.793
Number of days of stimulation	10 (6-22)	9 (6-13)	0.222
Total FSH dose, IU	1700 (750-4650)	1450 (825-3650)	0.108
Peak estradiol, pg/mL	2960 ± 1091	3256 ± 1253	0.292
Number of oocytes retrieved	12 (5-24)	14 (8-35)	0.01
Number of metaphase II oocytes	10 (4-21)	13 (4-34)	0.003
Number of 2PN	7 (2-15)	9 (4-15)	0.002
ICM morphology			
A	14	15	0.042
B	30	13	
C	0	2	
TE morphology			
A	6	12	0.011
B	19	13	
C	19	5	
Blastocel expansion			0.109
1	11	4	
2	6	2	
3	13	11	
4	12	7	
5	1	6	
6	1	0	

Abbreviations: BMI: Body mass index, FSH: Follicle stimulating hormone, ICM: inner cell mass, LH: Luteinizing hormone, TE: trophoectoderm.

DISCUSSION

There is still debate on the best blastocyst morphology parameter. Zaninovic et al., has reported the separate impact of each grading parameter after single embryo transfer (8) and found that grade of TE was more predictive of implantation, with no significance for ICM morphology or blastocoel expansion.

Zhang et al (9) revealed that blastocyst with good ICM morphology could increase clinical pregnancy rate in vitrified-warmed single blastocyst transfer cycles. Richter et al (10) also agreed with Zhang. Richter et al., reported that ICM was significantly related to the implantation rate (10). Also Kovacic et al., reported ICM contributed more to blastocyst quality than TE, they found blastocysts with normal ICM and non-optimal TE in comparison with the opposite-normal TE and abnormal ICM had higher pregnancy rate (11). Ahlstrom et al., found (12) that TE grade was superior over ICM for selecting the best blastocyst for embryo transfer. The strength of their study design is the large number of SET with blastocysts. This gives a unique possibility to analyze the impact of the three morphological parameters on live birth outcome with good statistical power. They found that grade of TE was more predictive of implantation, with no significance for ICM morphology or blastocoel expansion. Another finding in this study was the significance of blastocoel expansion for predicting live birth, in the absence of TE and ICM (12). Van den Abbeel et al.,(13) found that expansion and hatching stage, ICM and trophectoderm grade were all related to treatment outcome of fresh single-blastocyst transfers, but selection of the best blastocysts for transfer should first take into account the expansion and hatching stage.

In the present study, different from other published studies on the subject, the clinical pregnancy rate seems to be affected by both ICM and TE morphology. This prediction shows that blastocyst selection criteria could be improved by more correctly using the morphological parameters we are grading. Of course, prospective randomized studies are needed to decide which morphological parameter is the best to improve live birth rates.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Blake DA, Farquhar CM, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. Cochrane Database Syst Rev 2007;4:CD002118.
2. Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. In: Mortimer JR, editor. Toward Reproductive Certainty: Infertility and Genetics Beyond 1999. Carnforth, UK, Parthenon Press; 1999: 378-88.
3. Dokras A, Sargent IL, Barlow DH. Human blastocyst grading: an indicator of developmental potential? Hum Reprod 1993;8:2119-27.
4. Shapiro BS, Harris DC, Richter KS. Predictive value of 72-hour blastomere cell number on blastocyst development and success of subsequent transfer based on the degree of blastocyst development. Fertil Steril 2000;73:582–6.
5. Yoon HJ, Yoon SH, Son WY, Im KS, Lim JH. High implantation and pregnancy rates with transfer of human hatching day 6 blastocysts. Fertil Steril 2001;75:832–3.
6. Richter KS, Harris DC, Daneshmand ST, Shapiro BS. Quantitative grading of a human blastocyst: optimal inner cell mass size and shape. Fertil Steril 2001;76:1157–67.
7. Shapiro BS, Richter KS, Harris DC, Daneshmand ST. A comparison of day 5 and day 6 blastocyst transfers. Fertil Steril 2001;75:1126–30.
8. Zaninovic N, Berrios R, Clarke RN, Bodine R, Ye Z, Veeck LL. Blastocyst expansion, inner cell mass (ICM) formation, and trophectoderm (TM) quality: is one more important for implantation? Fertil Steril 2001;76:S8.
9. Zhang H, Zhou Y, Li Y, Zheng Y, Xiao S, Wu Y, et al. Prediction of clinical pregnancy in vitrified-warmed single blastocyst transfer cycles by pre-freeze morphology. Iran J Reprod Med. 2014;12:567–72.
10. Richter KS, Harris DC, Daneshmand ST, Shapiro BS. Quantitative grading of a human blastocyst: optimal inner cell mass size and shape. Fertil Steril 2001; 76: 1157-67.
11. Kovacic B, Vlaisavljevic V, Reljic M, Cizek-Sajko M. Developmental capacity of different morphological types of day 5 human morulae and blastocysts. Reprod Biomed Online 2004; 8: 687-94.
12. Ahlstrom A, Westin C, Reismer E, Wikland M, Hardarson T. Trophectoderm morphology: an important parameter for predicting live birth after single blastocyst transfer. Hum Reprod 2011;12:3289–96.
13. Van den Abbeel E, Balaban B, Ziebe S, Lundin K, Cuesta MJ, Klein BM, Helmgaard L, Arce JC. Association between blastocyst morphology and outcome of single-blastocyst transfer. Reprod Biomed Online. 2013;27:353-61.

Mide Kanserli Olguların Geriye Dönük İncelenmesi

A Retrospective Study of Gastric Cancer Cases

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ÖZET

Amaç: Mide kanseri Avrupa'da kadınlarda ve erkeklerde görülme sıklığı açısından beşinci sırada yer almaktadır. Erkek kadın oranı ise 1.6:1 olarak belirlenmiştir. Mide kanseri, kansere bağlı ölümlerde ülkemizde erkeklerde ikinci, kadınlarda ise üçüncü sırada yer almaktadır. Mide kanserinin prognozu genellikle kötüdür. Bunun sebebi de tanıda gecikme ve tanı konan olguların ileri evrede olmasıdır.

Gereç ve Yöntemler: Süleyman Demirel Üniversitesi Tıp Fakültesi Genel Cerrahi Anabilim Dalı 1995 -2009 yılları arasında mide kanseri tanısı ile cerrahi işlem yapılan 204 olgu geriye dönük olarak incelendi. Bu olgulardan 131'ine küratif cerrahi rezeksiyon yapıldığı belirlendi. Olguların %80'i lokal ileri evre olgulardı (evre IIIa, IIIb ve IV). Olguların yaş, cinsiyet, başvuru semptomları, tümör lokalizasyonu, T, N, M, evre, tümör diferansiyasyonu, histolojik tipleri, tümör çapı, vasküler ve perinöral invazyon varlığı, yapılan cerrahi işlem, rezeksiyon tipi, diseksiyon tipi, diseke edilen toplam lenf nodu sayısı, metastatik lenf nodu varlığı, metastatik lenf nodu sayısı ve toplam lenf nodu sayısına oranı, komplikasyonlar, cerrahi mortalite, adjuvan kemoterapi, adjuvan radyoterapi ve diğer adjuvan tedavileri ve sağkalım süreleri belirlendi. Bu verilerin sağkalım üzerine etkileri lojistik regresyon analizi ile değerlendirildi. Sağkalım açısından 3 ve 5 yıllık sağkalım eğrileri Kaplan-Meier yöntemiyle çıkarıldı

Bulgular: Küratif rezeksiyon ve diseksiyon yapılan 131 olguda morbidite %15.2, mortalite ise %7.6 idi. Evre ve metastatik lenf nodlarının toplam lenf nodu sayısına oranı sağkalım üzerinde en önemli faktörler olarak bulundu ($p<0.05$). Evrelere göre sağkalımda 5 yıllık sağkalım oranlarının evre Ia için %79.1, Ib için %78.3, II için %61.8, IIIa için %46, IIIb için %24.8 ve IV için %25.8 olduğu görüldü.

Sonuç: Sonuç olarak olgularımızın çoğunun lokal ileri evre olgular olmasına karşın yapılan radikal cerrahi işlemlerden fayda gördüğü ve sağkalım oranlarının bu anlamda olumlu olduğunu söylemek mümkündür.

Anahtar Sözcükler: Mide kanseri, cerrahi, sağkalım

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ABSTRACT

Objective: Gastric cancer is the fifth most commen neoplasm in terms of incidence in men and women in Europa. Male to female ratio was determined as 1.6:1. Gastric cancer is the second most commen neoplasm in men and third most commen neoplasm in female in deaths due to cancer in our country. Prognosis of gastric cancer is generally reserved. The low survival rate is due to the delay in diagnosis, most cases being diagnosed in an advanced stage.

Material and Methods: Between 1995 and 2009, 204 gastric cancer patients were retrospectively evaluated who was treated surgically in Faculty of Medicine, Suleyman Demirel University. It was observed that tumors of the 131 patients were curatively resected. The great portion (80%) of these patients were in locally advanced stages (stage IIIa, IIIb and IV). Age, gender, symptoms, tumor localisation, T, N, M, stage, tumor differantiation, histologic type, occurence of vascular and perineural invasion, surgery type, resection type, dissection type, count of dissected lymph nodes, metastatic lymph nodes, ratio of metastatic lymph nodes, complications, morbidity and mortality, adjuvant chemotherapy schedules, adjuvant radiotherapy and other adjuvant therapies, and surviaval of these patients were analyzed. For detecting the effects of these factors upon survival, logictic regression analysis was performed. Survival times were analyzed with Kaplan-Meier method.

Results: It was seen that surgical morbidity was 15.2%, and mortality was 7.6% in curatively resected 131 patients. Stage and the ratio of metastatic lymph nodes were evaluated as significantly important in survival ($p<0.05$). Survival ratio for 5 years were estimated a s 79.1%, 78.3%, 61.8%, 46%, 24.8% and 25.8% according to the stages Ia, Ib, II, IIIa, IIIb and IV respectively.

Conclusion: In conclusion, it could be considered that although the great portion of gastric cancer patients were locally advanced cases, the survival rates were increased with radical resections.

Key Words: Gastric cancer, surgery, survival

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GİRİŞ

Mide kanseri ile ilgili en eski bilgiler M.Ö. 1550 yıllarına ait olduğu bilinen antik Mısır'ın Ebers papirüslerinde bulunmuştur (1). Mide kanseri Avrupa'da kadınlarda ve erkeklerde görülme sıklığı açısından beşinci sırada yer almaktadır. Erkeklerde akciğer, prostat, kolorektal ve mesane kanserinden sonra, kadınlarda ise meme, kolorektal, akciğer ve endometrium kanserlerinden sonra gelmektedir. Kadın erkek oranı ise 1,6:1 olarak belirlenmiştir (2). Mide kanseri, kansere bağlı ölümlerde ülkemizde erkeklerde ikinci, kadınlarda ise üçüncü sırada yer almaktadır (3).

Mide neoplazmlarının %95'inden fazlası adenokarsinomlar olup; geriye kalan %5'i lenfomalar, leiomyosarkomlar ve daha az sıklıkta karsinoid tümörler, karsinosarkomlar ve skuamöz hücreli karsinomlar oluşturmaktadır (4). Lenf nodu metastazı mide kanserinin prognozuna etkili en önemli faktörlerden biridir (5). Mide kanserinin prognozu genellikle kötüdür. Bunun sebebi de tanıda gecikme ve tanı konan olguların ileri evrede olmasıdır (6).

Bu çalışmamızda bir üniversite hastanesi genel cerrahi kliniğinde gerçekleştirilen mide kanseri ameliyatlarını literatür eşliğinde sunmayı amaçladık.

GEREÇ VE YÖNTEMLER

Süleyman Demirel Üniversitesi Tıp Fakültesi Genel Cerrahi Anabilim Dalı bünyesinde mide kanseri tanısı ile 01.01.1995 ile 31.05.2009 yılları arasında çeşitli cerrahi müdahaleler gerçekleştirilen 204 olgu geriye dönük olarak değerlendirdi. Olguların yaş, cinsiyet, başvuru semptomları, tümör lokalizasyonu, T, N,M, evre, tümör diferansiyasyonu, histolojik tipleri, tümör çapı, vasküler invazyon ve perinöral invazyon varlığı, yapılan cerrahi işlem rezeksiyon tipi, diseksiyon tipi, diseke edilen toplam lenf nodu sayısı, metastatik lenf nodu varlığı, metastatik lenf nodu sayısı, metastatik lenf nodlarının toplam lenf nodlarına oranı, cerrahi komplikasyonlar, cerrahi mortalite, adjuvan kemoterapi, adjuvan radyoterapi, diğer adjuvan tedaviler, primer kemoterapi, radyoterapi, sağkalım süreleri değerlendirildi. Elde edilen bu bulguların sağkalım üzerine etkisi istatistiksel olarak belirlendi, sağkalım eğrileri (3 v e 5 yıllık) elde edildi. Olguların sağkalım süreleri hem tıbbi kayıtlardan hemde çeşitli iletişim araçları kullanarak belirlendi. Bu açıdan takip dışı olgu olmadı.

İstatiksel analizler "SPSS 15.0 for Windows" paket istatistik programı kullanılarak gerçekleştirildi.

BULGULAR

Cerrahi müdahale yapılan 204 olgunun 129'u erkek, 75'i kadındı (1,7/1). Olguların yaş ortalaması 62.3 (28-87) yıl olarak belirlendi. Yaşların dekadlara göre dağılımında yığılmanın özellikle 6., 7. ve 8. dekadda olduğu görüldü. Bu olgulardan 131'ine adenokarsinom tanısı ile küratif rezeksiyon yapılırken, 59'una tanısal veya palyatif cerrahi işlemler yapıldığı görüldü, geri kalan 14 olguda ise histolojik tanıların gastrointestinal stromal tümör (GİST), lenfoma ve karsinoid tümör olduğu görüldü.

Küratif Rezeksiyon yapılan 131 olgunun değerlendirilmesinde 85'inin erkek, 46'sının kadın olduğu belirlendi (1,9/1). Bu olguların yaş ortalaması 67,8 idi (28-87). En sık gözlenen semptomlar ise karın ağrısı ve kilo kaybı idi (Tablo 1). Tümör 55 olguda antrumda, 45 olguda korpusta, 23 olguda kardiyada ve 8 olguda ise diffüz olduğu gözlendi. Tümör çapına bakıldığında ortalama çapın 5,9 cm (0,5-17,0) olduğu belirlendi. Küratif rezeksiyon yapılan olguların 65'ine total gastrektomi, 61'ine distal subtotal gastrektomi, 3'üne hemigastrektomi ve 2'sine ise proksimal distal gastrektomi yapıldı. Ameliyat ve patoloji raporlarının incelenmesinde cerrahi teknik açısından rezeksiyon işlemlerine standart olarak omentektominin eklendiği, lenf nodu diseksiyonunun cerrahlara bağımlı olduğu bu açıdan klinikte standart bir cerrahi tekniğin oluşmadığı, diseksiyon tiplerininde cerrahtan cerraha değiştiği, cerrahi işlemlerin standardize edilmediği gözlenmiştir. Tümörün organ invazyonu yaptığı olgularda rezeksiyon genişletilmiş, total gastrektomiye standart olarak splenektomi eklenmemiştir. Rekontrüksiyon kousunda da aynı şekilde standardize edilmiş bir yaklaşım yoktur. Olgulardan 85'inde D1 diseksiyon, 46'sında ise D2 diseksiyon yapılmış, ancak D2 diseksiyon yapılan olguların çoğunda lenf nodu bölgeleri işaretlenmemiş ve patolojik açıdan da bu yönde bir inceleme ve raporlama yapılmamıştır. Yapılan rezeksiyonların 112'si R0, 19'u ise R1 rezeksiyondur. Küratif rezeksiyon yapılan olguların histopatolojik incelemesinde ise 106 olguda adenokarsinom, 12 olguda taşlı yüzük hücreli karsinom, 9 olguda tübüler tip karsinom, 3 olguda müsinöz tip karsinom, 1 olguda ise papiller tip karsinom gözlenmiştir. Küratif rezeksiyon yapılan olgular diferansiyasyon durumuna göre değerlendirildiğinde ise 26 olgunun iyi diferansiye, 33 olgunun orta diferansiye, 69 olgunun az diferansiye ve 3 olgunun indiferansiye olduğu gözlenmiştir.

Küratif rezeksiyon yapılan olgular invazyon durumuna göre değerlendirildiğinde ise 85 olguda perivasküler invazyon, 46 olguda perinöral invazyon gözlenmiştir. Ortalama diseke edilen lenf nodu sayısı 18,6 (2-41) olarak belirlenirken, lenf nodu metastazı saptanan olgularda ortalama metastatik lenf nodu sayısı 7,4 (1 -36) idi (Tablo 2). Cerrahi işlemler sonrasında 20 (%15,2) olguda komplikasyon gözlendi. En sık gözlenen komplikasyonlar yara yeri enfeksiyonu, anastomoz kaçağı ve alt solunum yolu enfeksiyonu idi (Tablo 3). Cerrahi mortalite (post-op 30 gün içinde) ise 10 (%7,6) olguda görüldü. Anastomoz kaçağı görülen 6 olguda, alt solunum yolları enfeksiyonu gelişen 3 olguda ve MOF gelişen 1 olguda postoperatif dönemde exitus gelişti.

Olguların 57'sine postoperatif adjuvan kemoterapi uygulandı. Toplam 42 olguya 5-FU + leucoverin, 11 olguya 5- FU + cisplatin, 4 olguya ise 5-FU + Mitomycin-C uygulandı. Olgulardan 2'sinde adjuvan radyoterapi uygulandığı belirlendi. Bu iki olguda distal cerrahi sınırda mikroskopik tümör saptanan olgulardı. İleri evre 2 olguda da intraperitoneal hipertermik kemoterapinin uygulandığı belirlendi.

İncelenen verilerin sağkalıma etkisini incelemek için gerçekleştirilen Lineer Lojistik Regresyon analizinde "evre" ve "metastatik lenf nodunun toplam lenf nodlarına oranı" sağkalıma istatistiksel olarak anlamlı etki yapan faktörler olarak belirlendi ($p<0,05$). Küratif rezeksiyon yapılan 131 olgunun 3 yıllık ve 5 yıllık sağkalım oranları incelendiğinde; 3 yıllık sağ kalım oranı %52,7, 5 yıllık sağ kalım oranı %41,3 olarak gözlenmiştir (Tablo 4).

Palyatif veya tanısal işlem yapılan 59 olgu incelendiğinde ise bu olguların 40'ının erkek 19'unun ise kadın olduğu belirlendi (2,1/1). Bu olguların yaş ortalaması 61,6 (37-85) yıl idi. Bu olgulardan 21'ine beslenme amacıyla ostomi, 16'sına ileus nedeniyle laparotomi, 13'üne bypass cerrahisi, 9'una ise diğer işlemler uygulandı. İzlemde bu olguların ortalama sağkalımının 5 (1-27) ay olduğu belirlendi. Olguların 13'üne kemoterapi uygulandığı gözlendi. Olgulardan 18'inde (%30,5) postoperatif komplikasyon gözlenirken, en sık görülen komplikasyon cerrahi alan enfeksiyonu idi (10 olgu, %16,9). Olgulardan 4'ünde (%6,7) oluşan komplikasyonlar nedeniyle postoperatif dönemde exitus gözlendi.

Diğer tanılarla opere edilen olgulardan 8'i GİST tanısıyla, 1'i karsinoid tümör ve 5 olgu da lenfoma tanısı ile opere edilmişti. GİST tanısıyla opere edilen 8 olgudan 4'üne wedge rezeksiyon, 3'üne total gastrektomi ve 1'ine distal gastrektomi uygulandı. Karsinoid tümör tanısıyla opere olan 1 olguya total gastrektomi, lenfoma tanısıyla opere edilen 5 olguya da total gastrektomi uygulandı.

Tablo 1. Başvuru semptomları

Semptomlar	Sıklık	%
Karın Ağrısı	69	33.8
Kilo Kaybı	38	18.6
Kusma	21	10.3
Kanama	18	8.8
Halsizlik	13	6.4
Bulantı	12	5.9
İştahsızlık	10	4.9
Yanma	9	4.5
Şişkinlik	9	4.5
Yutma Güçlüğü	8	3.9
Hazımsızlık	3	1.5
Sırt Ağrısı	3	1.5
Ekşime	3	1.5
İshal	2	0.9
Ağız Kokusu	1	0.5
Geğirme	1	0.5
Ağza Ekşi Su Gelmesi	1	0.5

Tablo 2. Metastatik lenf nodlarının toplam diseke edilen lenf nodlarına oranı (MLN/TLN)

MLN/TLN	Olgu Sayısı
≤%30	37
%30-60	29
≥%60	45

MLN: Metastatik lenf nodu
TLN: Total lenf nodu

Tablo 3. Komplikasyonlar

Komplikasyonlar	Olgu Sayısı
Cerrahi Alan Enfeksiyonu	12
Yara Ayrışması (Eviserasyon)	1
Alt Solunum Yolları Enfeksiyonu	6
İntraabdominal Abse	1
Anastomoz Kaçağı	7
MOF	1
Böbrek Yetmezliği	1
Derin Ven Trombozu	1

Tablo 4. Olguların 3 ve 5 yıllık sağkalım oranları

Evre	Olgu sayısı	3 yıllık	5 yıllık
Ia	2	98.6	79.1
Ib	11	91.3	78.3
II	12	77.7	61.8
IIIa	44	63.8	46
IIIb	30	41.3	24.8
IV	32	41.6	25.8
Genel	131	52.7	41.3

TARTIŞMA

Gastrik adenokanserler hastalığın erken döneminde özgün belirtiler göstermezler. Hastalar genelde hafif epigastrik rahatsızlık veya hazımsızlığı kansere yormadıklarından önemsemezler ve tanı incelemesinden önce benign hastalık bulgusuyla 6 -12 hafta tedavi alırlar. Hızlı kilo kaybı, iştahsızlık ve kusma genelde ilerlemiş hastalığın belirtisidir. Bu özellikler basitçe kısmen tıkayıcı (mekanik veya fizyolojik) bir lezyonun varlığına bağlıdır.

Epigastrik ağrı benign ülser ağrısına benzer ve yemekle azalır, ancak anjinayı taklit edebilir. Disfaji genelde kardiya veya gastroözofageal bileşke tümörlerine bağlıdır. Antral tümörler mide çıkışındaki tıkanmaya bağlı belirtilere neden olabilir. Nadir de olsa, transvers kolonu tutan büyük tümörler kolon obstrüksiyonu ile başvurabilir. Hastaların %30 kadarında fizik muayenede kitle ele gelebilir (7,8). Bizim çalışmamızda da en sık gözlenen semptomlar ise karın ağrısı ve kilo kaybı idi.

Son yıllarda kardiya dışı mide kanserlerinin görülme sıklığı düşmüştür. Kardiya ve alt özofagus tümörleri ise Avrupa ve Kuzey Amerika'da son dönemde artış göstermektedir. Midenin distal kısımına lokalize tümörlerin görülme sıklığındaki düşüşün en olası nedeni H. pylori eradikasyonu olsa gerekir. Üst 1/3 tümörlerindeki artışın nedeni ise reflü özofajit görülme sıklığındaki artıştan kaynaklanıyor olmalıdır (9). Bu değişim hastalığın yaşla ilişkisini de değiştirmektedir: distal yerleşimli tümörler daha çok yaşlılarda görülürken, proksimal yerleşimli tümörler sıklıkla gençlerde görülmektedir (10). Bizim çalışmamızda da tümörün 55 olguda antrumda, 45 olguda korpusta, 23 olguda kardiyada ve 8 olguda ise diffüz olduğu gözlendi.

Midenin proksimal 1/3'ünde ve 1/3 orta kısmında yer alan lezyonlarda cerrahi tedavi seçeneği total gastrektomi olmalıdır. Tüm mideyi tutan diffüz tip gastrik kanserlerde de total gastrektomi yapılmalıdır. Her ne kadar 1/3 distal yerleşimli tümörlere de total gastrektomi yapılmasını önerenler olsada bu lokalizasyonlar için total gastrektominin sağkalım avantajı gösterilememiştir (2). Yapılan prospektif randomize çalışmalar bu konuda yeterli kanıt sağlamaktadırlar (11-13). Proksimal subtotal gastrektomi, yeterince radikal bir rezeksiyona imkan vermeyişi, gastroözofageal reflü ve regürjitasyonun yol açtığı özofajit ve total gastrektomiye göre daha uygun bir restorasyon sağlamayışı nedeniyle pek tercih edilmemektedir (14). Midenin distal 1/3 yerleşimli tümörleri için ise distal subtotal gastrektomi yeterli görülmektedir (2). Bizim çalışmamızda ise küratif rezeksiyon yapılan olguların 65'ine total gastrektomi, 61'ine distal subtotal gastrektomi, 3'üne hemigastrektomi ve 2'sine ise proksimal distal gastrektomi yapıldı.

Mide rezeksiyonu komplikasyonları ve bunların rölatif sıklığı şöyledir: solunum %3 -55, enfeksiyöz %2-22, anastomotik %3-21, kardiyak %1-10, renal %1-8, kanama %0,3-5 ve pulmoner emboli %1-4 (7). Özellikle solunum yolları enfeksiyonları ilk sırada yer alması ve sonuçları nedeniyle üzerinde önemle durulması gereken sorunlardır (15-17). En tehlikeli komplikasyon hastaların %3-12'sinde görülen anastomoz kaçaklarıdır. Kaçak geç dönemde de olabileceğinden erken postoperatif dönemdeki sağlam bir anastomoz komplikasyonsuz gidişin garantisi değildir (7). İntraabdominal abseler ve enfeksiyonlar diğer önemli komplikasyon başlığını oluştururlar.

Bunların önemi mortalite oranının artmasına neden olamalarıdır. Lo ve ark.'nın (18) yayınladığı 2076 olguluk radikal gastrektomi serisinde, intraabdominal abse gelişen olguların %18,7'sinin mortal seyrettiği bildirilmiştir. Önemli bir başka konuda gastrektomi ve lenf nodu diseksiyonuna ek olarak organ rezeksiyonu yapılan olguların komplikasyon oranıdır. Özer ve ark.'nın (19) yaptığı bir çalışmada bir organ ve birden fazla organın standart cerrahi işleme ek olarak çıkarıldığı olgularda mortalite ve morbiditenin arttığı gözlenmiştir. Birden fazla organın çıkarıldığı olgularda mobidite %37,5 ve mortalite de %12,5 olarak bildirilmiştir. Bizim çalışmamızda da en sık gözlenen komplikasyonlar yara yeri enfeksiyonu, anastomoz kaçağı ve alt solunum yolu enfeksiyonu idi.

Avrupa verileri incelendiğinde sağkalım sonuçlarının yüz güldürücü olduğunu söylemek mümkün değildir: 1990-1994 arasında her iki cins için sağkalım 1 yıl için %42, 5 yıl için ise % 23 olarak saptanmıştır (2). Bu oranlar bölgesel farklılıklar, cinsiyet ve yaşla ilgili farklılıklar göstermekle birlikte oldukça düşüktür. Genel sağkalım oranlarına bakıldığında Çin ve Japonya'da bu oranların daha yüksek olduğu söylenebilir. Beş yıllık sağkalım oranları Çin için %30-57,1 arasında değişirken, Japonya'da %63,8-77,2 arasındadır (20). Oranların yüksek oluşu muhtemelen bu ülkelerdeki tarama programlarına ve erken mide kanseri oranının yüksekliğine bağlı olduğu düşünülebilir. İngiltere ve Galler'i içine alan bir analizde gelişen teknoloji, evrelemede daha hassas davranılması, cerrahi öncesi ve sonrası tedavilerin etkinliği gibi nedenlerle 1980'den bu yana mide kanserinde sağkalım oranlarında belirgin iyileşme olduğu belirtilmektedir (21). Bizim çalışmamızda da küratif rezeksiyon yapılan 131 olgunun 3 yıllık ve 5 yıllık sağkalım oranları incelendiğinde; 3 yıllık sağ kalım oranı %52,7, 5 yıllık sağ kalım oranı %41,3 olarak gözlenmiştir.

Mide kanserinde önemli prognostik faktörden biri metastatik lenf nodu sayısının toplam çıkarılan lenf nodu sayısına oranıdır (2). Yapılan sağkalım analizlerinde metastatik lenf nodu oranı bağımsız bir faktör olarak sağkalımda etkili bulunmuş ve çalışmaların çoğunda N sınıflamasının bu orana göre yeniden düzenlenmesi önerilmiştir (22-33). Bizim çalışmamızda ise incelenen verilerin sağkalıma etkisini incelemek için gerçekleştirilen Linear Lojistik Regresyon analizinde "evre" ve "metastatik lenf nodunun toplam lenf nodlarına oranı" sağkalıma istatistiksel olarak anlamlı etki yapan faktörler olarak belirlendi ($p<0,05$).

Yaptığımız çalışmadaki kısıtlamalarımız çalışmanın retrospektif bir çalışma olması, geniş bir zaman dilimine yayılması ve exitus gelişen hastaların yakınları aracılığıyla bilgi alınmasıdır.

SONUÇ

Mide kanserli olgularımızın geriye dönük incelenmesinde belirgin olarak gözlenen unsurlar olguların büyük kısmının lokal ileri evre olduğu, bu olgulara yapılan radikal cerrahi girişimlerin morbidite ve mortalitesinin yüksek olduğu, ancak sağkalım sonuçlarının da her şeye rağmen hastalar lehine olumlu olduğunu söylemek mümkündür. Gelecekte diğer solid tümör gruplarında olduğu gibi mide kanserli olgularımızda da gerek preoperatif, gerek operatif, gerekse postoperatif tüm işlemlerimizi önceden belirlenmiş standartlar çerçevesinde yürütmemiz gerektiği anlaşılmaktadır.

Çıkar Çatışması
Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

KAYNAKLAR

1. Lochhead P, El-Omar EM. Gastric cancer. Br Med Bull. 2008;85:87-100.
2. Catalano V, Labianca R, Beretta GD, Gatta G, de Braud F, Van Cutsem E. Gastric cancer. Crit Rev Oncol Hematol. 2005;54:209-241.
3. Yalcin S. Gastric cancer in Turkey-a bridge between west and East. Gastrointest Cancer Res. 2009;3:29-32.
4. Şahin M, Tanrıkulu Y, Erel S, Bayraktar K, Akkuş MA. Mide Kanserinde Gastrektomi Deneyimlerimiz. Bidder Tıp Bilimleri Dergisi 2010;2:20-26.
5. Xu DZ, Geng Q, Long ZJ, et al. Positive lymph node ratio is an independent prognostic factor in gastric cancer after D2 resection regardless of the examined number of lymph nodes. Ann Surg Oncol 2009;16:319-26.
6. Lazar D, Taban S, Sporea I, Dema A, Cornianu M, Lazar E, Goldiş A, Vernic C. Gastric cancer: correlation between clinicopathological factors and survival of patients (II). Romanian Journal of Morphology and Embryology 2009, 50(2):185-194.
7. Al-Refaic WB, Abdalla EK, Ahmed SA, Mansfield PF. Gastric cancer. In: Feig BW, Berger DH, Fuhrman GM, editors. M.D. Anderson Hand Book of Surgical Oncology. Philadelphia: Lippincott Williams & Wilkins; 2006. p.205-240.

8. Mercer DW, Robinson KE. Stomach. In: Courtney MT, Beauchamp RD, Evers BM, Mattox KL, editors. Sabiston Textbook of Surgery 18th ed. Philadelphia: Saunders Elsewier; 2008. p.1223-7.
9. Bertuccio P, Chatenoud L, Levi F, Praud D, Ferlay J, Negri E, Malvezzi M, La Vecchia C. Recent patterns in gastric cancer: A global overview. Int J Cancer. 2009;125(3):666-673.
10. Wilkinson NW, Howe J, Gay G, Patel-Parekh L, Scott-Conner C, Donohwe J. Differences in the pattern of presentation and treatment of proximal and distal gastric cancer: results of the 2001 gastric patient case evaluation. Ann Surg Oncol. 2008;15:1644-50.
11. Gouzi JL, Huguier M, Fagniez Pl, Launois B, Flamant Y, Lacaine F, Paquet JC, Hay JM. Total versus subtotal gastrectomy for adenocarcinoma of the gastric antrum. AFrench prospective controlled study. Ann Surg 1989; 209:162-6.
12. Robertson CS, Chung SC, Woods SD, Griffin SM, Raimes SA, Lau JT, Li AK. A prospective randomized trial comparing R1 subtotal gastrectomy with R3 total gastrectomy for antral cancer. Ann Surg 1994;220:176-82.
13. Bozzetti F, Marubini E, Bonfanti G, et al. Total versus subtotal gastrectomy: surgi cal morbidity and mortality rates in a multicenter Italian randomized trial. The Italian Gastrointestinal Tumor Study Group. Ann Surg 1997;226:613-20.
14. Karaayvaz M, Kocaoğlu H, Demirci S. Mide Kanserleri. In: Abdülmuttalip Ünal editor. Klinik Cerrahi Onkoloji. Ankara: Türkiye Klinikleri Yayınevi; 1997. p.468-78.
15. Sah BK, Zhu ZG, Chen MM, Yan M, Yin HR, Zhen LY. Gastric cancer surgery and its hazards: post operative infection is the most important complication. Hepatogastroenterology. 2008;55:2259-63.
16. Tsujimoto H, Ichikura T, Ono S, Sugasawa H, Hiraki S, Sakamoto N, Yaguchi Y, Yoshida K, Matsumoto Y, Hase K. Impact of postoperative infection on long-term survival after potentially curative resection for gastric cancer. Ann Surg Oncol. 2009;16:311-8.
17. Mohri Y, Tonouchi H, Miki C, Kobayashi M, Kusunoki M; Mie Surgical Infection Research Group. Incidence and risk factors for hospital-acquired pneumonia after surgery for gastric cancer: results of prospective surveillance. World J Surg. 2008;32:1045-50.
18. Lo CH, Chen JH, Wu CW, Lo SS, Hsieh MC, Lui WY. Risk factors and management of intra- abdominal infection after extended radical gastrectomy. Am J Surg. 2008;196:741-5.
19. Ozer I, Bostanci EB, Orug T, Ozogul YB, Ulas M, Ercan M, Kece C, Atalay F, Akoglu M. Surgical outcomes and survival after multiorgan resection for locally advanced gastric cancer. Am J Surg. 2009;198:25-30.
20. Li ZX, Kaminishi M. A comparison of gastric cancer between Japan and China. Gastric Cancer. 2009;12:5-53.
21. Rao S, Cunningham D. Survival from cancer of the stomach in England and Wales up to 2001. Br J Cancer. 2008;99 Suppl 1:S19-20.
22. Xu DZ, Geng QR, Long ZJ, Zhan YQ, Li W, Zhou ZW, Chen YB, Sun XW, Chen G, Liu Q. Positive lymph node ratio is an independent prognostic factor in gastric cancer after d2 resection regardless of the examined number of lymph nodes. Ann Surg Oncol. 2009;16:319-26.
23. Huang CM, Lin BJ, Lu HS, Zhang XF, Li P, Xie JW. Prognostic impact of metastatic lymph node ratio in advanced gastric cancer from cardia and fundus. World J Gastroenterol. 2008 Jul 21;14:4383-8.
24. Ozgüç H, Sönmez Y, Yerci O. Metastatic/resected lymph nodes ratio-based classification in gastric cancer. Turk J Gastroenterol. 2008;19:2-7.
25. Saito H, Fukumoto Y, Osaki T, Yamada Y, Fukuda K, Tatebe S, Tsujitani S, Ikeguchi M. Prognostic significance of the ratio between metastatic and dissected lymph nodes (n ratio) in patients with advanced gastric cancer. J Surg Oncol. 2008;97:132-5.
26. Liu C, Lu P, Lu Y, Xu H, Wang S, Chen J. Clinical implications of metastatic lymph node ratio in gastric cancer. BMC Cancer. 2007;7:200.
27. Yu JW, Wu JG, Zheng LH, Zhang B, Ni XC, Li XQ, Jiang BJ. Influencing factors and clinical significance of the metastatic lymph nodes ratio in gastric adenocarcinoma. J Exp Clin Cancer Res. 2009;28:55.
28. van der Schoot J, Van Hee R, Philipsen T, De Bock D, Delvaux V, Weyler J. The prognosis of patients operated on for gastric cancer in relation to the percentage of lymph nodes invaded by tumour. Acta Chir Belg. 2008;108:308-12.
29. Yu J, Yang D, Wei F, Sui Y, Li H, Shao F, Li Y. The staging system of metastatic lymph node ratio in gastric cancer. Hepatogastroenterology. 2008;55:2287-90.
30. Hidaka H, Eto T, Maehara N, Jimi S, Hotokezaka M, Chijiiwa K. Comparative effect of lymph node metastasis classified by the anatomical site or by the number of nodes involved on prognosis of patients with gastric cancer. Hepatogastroenterology. 2008;55:2269-72.
31. Isik A, Okan I, Firat D, Yilmaz B, Akcakaya A, Sahin M. A new prognostic strategy for gastric carcinoma: albumin level and metastatic lymph node ratio. Minerva Chir. 2014;69:147-53
32. Isık A, Demiryilmaz I, Yılmaz İ, Firat D,Cimen O, Eken H Effectiveness of Manual Knotting at Laparoscopic Appendectomy Gazi Medical Journal 2016;27:19-20
33. Isik A, Eken H, Demiryilmaz İ,Yılmaz İ, Fırat D, Çimen P, Peker K, Güven H Rectal Lymphoma Kolon Rektum Hast Derg 2015;25:106-108

Kantaron Ekstresinin Gentamisinin Neden Olduğu Ototoksisite Üzerinde Koruyucu Etkisi

Protective Effect Of *Hypericum Perforatum* Extract On Gentamicin Induced Ototoxicity

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ÖZET

Amaç: Hypericum perforatum tarla, yol ve orman kıyılarında, tepelerde ve çayırlarda Temmuz'dan Eylül'e kadar çiçeklenen ve ülkemizde, sarı kantaron, kanotu, kılıçotu, mayasilotu ve yaraotu gibi adlara sahip ve bazı hastalıklara karşı yöresel insanlar tarafından kullanılan bitkidir ve yapılan çalışmalarda anti inflamatuar ve antioksidant etkinliği gösterilmiştir. Bu nedenle kantaron bitkisinin gentamisin ile oluşturulan ototoksisite üzerinde koruyucu etkinliği olup olmayacağını planladık.
Yöntem: Bu amaçla ferelere 9 gün 100 mg/kg gentamisin ve gentamisinle birlikte 70 mg/kg kantaron ekstresi verildi. 9. ve 10. günlerde gentamisinin ve kantaron ekstraktının farelerin motor koordinasyonunu üzerine etkisini değerlendirmek için rotarod testi uygulandı.
Bulgular: Gentamisin uygulaması farelerin düşüş süresini azalttı. Gentamisin ile birikte kantaron ekstaktı verilmesi bu süreyi uzattı.
Sonuç: Çalışmamız gentamisinin neden olduğu ototoksisiteyi önlemede yararlı olacağını göstermiştir.

Anahtar Sözcükler: Gentamisin, ototoksisite, kantaron, rotarod ve motor koordinasyon

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ABSTRACT

Objective: Hypericum perforatum, which have various names locally such as "sarı kantaron, kanotu, kilicotu, mayasilotu, yaraotu", is a plant which blooms between july and september at farms, borders of roads and woods, top of hills and grasslands, whose anti-inflammatory and antioxidant effects are shown in various studies. Due to this reason, we planned a study to examine the protective effects of Hypericum perforatum on ototoxicity caused by gentamicin.
Methods: For this purpose, 100 mg/kg gentamicin and 70 mg/kg Hypericum perforatum extract are administered to mice for 9 days. On the 9th and 10th days rotarod performance was assessed to evaluate the Gentamicin and Hypericum Perforatum extract on motor coordination of mice.
Results: Gentamicine treatment decreased fall latency of mice and Gentamicine together Hypericum Perforatum extract treatment increased fall latency of mice.
Conclusion: Our study showed that Hypericum Perforatum extract will be usefull to prevent gentamicin induced ototoxicity

Key Words: Gentamicin, ototoxicity, Hypericum perforatum, rotarod and motor coordination

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GİRİŞ

Aminoglikozid antibiyotikler gram (-) aerobik enfeksiyonların tedavisi ve kontrolünde yaygın olarak kullanılmaktadır(1-2). Gentamisin bunlar arasında en yaygın kullanım alanı bulmasına rağmen nefrotoksik ototoksik etkilerinden dolayı kullanımları sınırlanmıştır(3). Bu antibiyotiği kullananların %25-33 arasında duyma kaybı yaşanmakta ve %15'inde vestibular toksisites gelişmektedir. Bu ilaç gelişmekte olan ülkelerde yaygın olarak kullanıldığından bu ülkelerdeki sağır dilsizliğin %66 sorumlu olduğu bildirilmiştir(4). Bu ilacın kullanımından en çok koklea, vestibular aparat ve böbrekler etkilenmektedir(5-7).

Denge bozukluğundan vestubular aparattaki hasardan kaynaklanmaktadır. Ototoksisite sonucu bu aparatta var olan saç hücrelerinin kaybı denge bozukluklarına neden olmaktadır(7-11).

Hypericum perforatum tarla, yol ve orman kıyılarında, tepelerde ve çayrlarda Temmuz'dan Eylül'e kadar çiçeklenen ve ülkemizde, sarı kantaron, kanotu, kılıçotu, mayasılotu ve yaraotu gibi adlara sahip ve bazı hastalıklara karşı yöresel insanlar tarafından kullanılan bitkidir(12-14). Yapılan çalışmalar bu bitkini çok güçlü bir antiinflamatuar olduğu bildirilmiştir. Bir çalışmada kantaron uygulamasının LSP ile indüklenmiş siklooksijenaz-2 ve iNOS enzimlerini inhibe ettiği gösterilmiştir(15). Ayrıca kantaronun biyoaktif maddelerinden hiperforin güçlü bir siklooksijenaz-1 ve 5-lipooksijenaz inhibitörü olduğu ortaya konmuştur(16). Ayrıca kantaron uygulaması prostaglandin inflamasyonda rol oynayan prostaglandin sentezini azaltmaktadır(17).

Klinikte sedasyon, uyanıklığın belirlenmesi, dikkat, bilgi işlemi ve motor becerilerin de dahil olduğu bir dizi kognitif ve psikomotor testler kullanılarak ölçülmektedir. Ancak, bu testlerin subjektif olmaya meyilli olması nedeniyle bunların hayvan modellerine uyarlanması güç olmaktadır. Sonuç olara; sedasyon ve motor koordinasyon defisitlerinin ayrıldığı hayvan modelleri neredeyse imkânsızdır. En yaygın kullanılan, pahalı olmayan teçhizatı, basit onaylı protokolleri ve basit girişimiyle tutarlı sonuçlar sağlayan, rotarod yöntemidir. Genel olarak yeni ilaçların oluşturduğu motor kordinasyonu değerlendirmede kullanılmaktadır. İlaç verildikten sonra hayvan cihaza yerleştirilir ve düşene kadar geçen süre düşme latensi olarak kaydedilir(18).

Çalışmamızda gentamisinin neden olduğu ototoksisite'nin göstergesi olan denge bozukluğu üzerinde kantaron ekstresinin nasıl bir etki oluşturacağını araştırmayı planladık.

YÖNTEMLER

Deneylerde Çukurova Üniversitesi Tıbbi Bilimler Deneysel Araştırma ve Uygulama Merkezi (TIBDAM)'dan sağlanan 8 haftalık balb/c albino türü erkek fareler kullanıldı.

Fareler kontrol, gentamisin ve gentamisin ile birlikte kantaron ekstresi uygulanan olarak üç gruba ayrıldı. Gentamisin grubuna 9 gün 100 mg/kg günde bir kez intraperitonal gentamisin uygulaması yapıldı. Gentamisin ile birlikte kantaron ektresi uygulanan gruba 9 gün 100 mg/kg günde bir kez intraperitonal gentamisin uygulaması yapılacak ve gavaj ile 70mg/kg kantaron ekstresi uygulandı. Kontrol gruplarına da aynı deneysel koşullar sağlandı ve intraperitonal fizyolojik serum uygulandı. 9. gün ve 10. gün (gentamisin verilmedi) farelerin motor denge ve koordinasyonu rotarod testi ile değerlendirildi. Rotarod testi için, hayvanlar grup grup çalışıldı. Her hayvan 4 pençesiyle, dakikada 12 tur dönen yerden 25 cm yükseklikteki 2,5 cm çaplı barın üzerine yerleştirildi. Her hayvan için bar üzerinde durma süresi kaydedildi.

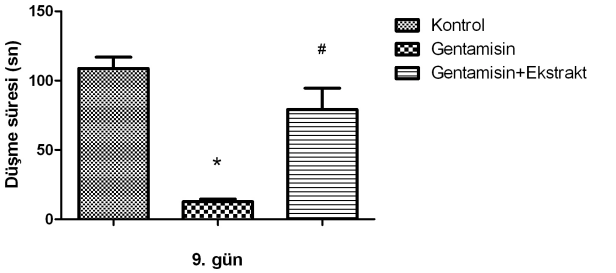
Sonuçların değerlendirilmesi

Dokuların gevşeme yanıtları kasılmaların yüzdesi olarak ifade edildi. standart hataları ile birlikte (± SEM) gösterildi. Grafiklerin çizimi ve istatistiksel analiz için bilgisayar ortamında Graph-Pad Prism (CA, USA) programı kullanıldı. İstatistiksel karşılaştırmalar için tek yönlü varyans analizi (ANOVA) ve *post hoc* testi olarak Bonferoni kullanıldı. 0.05'den küçük P değerleri anlamlı olarak kabul edildi.

BULGULAR

9. gün rotarod test sonuçları

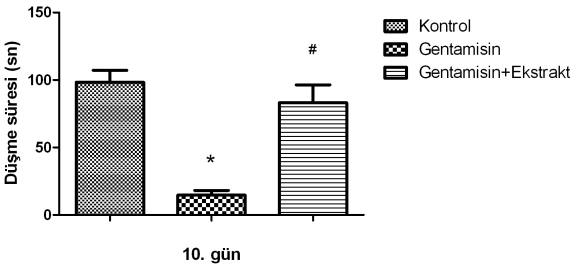
Gentamisin uygulamasının 9. gününde gentamisin uygulanan farelerin rotarod testinde düşme süreleri istatistiksel olarak anlamlı bir şekilde azalmıştır. Kantaron ekstresi uygulaması bu bu süreyi anlamlı olarak artırmıştır(Şekil 1).



9. gün

10. gün rotarod test sonuçları

Gentamisin uygulamasının 10. günde gentamisin uygulanan(9 gün uygulandı) farelerin rotarod testinde düşme süreleri istatistiksel olarak anlamlı bir şekilde azalmıştır. Kantaron ekstresi uygulaması bu süreyi anlamlı olarak artırmıştır(Şekil 2).



10. gün

TARTIŞMA

Yapılan çalışmalarda gentamisinin neden olduğu ototoksisitede artan oksidatif stresinde rolü olduğu gösterilmiştir(19). Çalışmamızda kullandığımız kantaron ektresinin içerdiği flavanoid ve fenolik bileşiklerden dolayı potansiyel antioksadatif özelliği vardır(20-21). Kantaron ekstresinin çalışmamızda gösterdiğimiz gentamisinin neden olduğu ototoksisite üzerinde koruyucu etkisi bu özelliğinden kaynaklanmış olabilir. Ayrıca Bu bitkinin güçlü bir antiinflamatuar özelliği vardır. Yapılan çalışmalarda gentamisinin neden olduğu diğer bir toksik etkisi olan nefrotoksisitede inflamatuar medyatörlerin artışı da eşlik ettiği gösterilmiştir(22-24). Bu mediyatörler de gentamisinin neden olduğu ototoksisiteye katkı yapmış olabilir bunun için ileri çalışmalara ihtiyaç vardır. Ayrıca bu bitkinin içerdiği hiperisin, flavonoidler ve hiperforin gibi antioksidan maddelerde antiinflamatuar etkiye sinerjistik katkı sağlamaktadır. Yapılan çalışmalar hiperisinin serbest radikal yakalayıcı olduğu, 5- ve 12-lipoksijenaz yolağı üzerinden fosfolipidlerden araşidonik asit salınımını ve IL-1a ve IL-12 formasyonunu inhibe ettiği gösterilmiştir. Bundan başka inflamatuar mediyatörlerin düzenleyicisi olan NF-kB 'nin hiperisin tarafından inhibe edildiği bildirilmiştir(25,26). Diğer bir etken madde olan hyperforinin serbest oksijen radikallerinin formasyonunu lökositlerden elastaz salınımını, siklooksijenaz-1'i, 5-lipooksijenazı ve IL6 salınımını inhibe ettiği gösterilmiştir(27,28). Flavanoid olan hiperosid ve izokuarsitrin inflamasyon patogenezinde rol alan nötrofil elastazı inhibe ettiği, hiperosid nitrik oksit sentazı inhibe ederken izokuarsitrin'in de prostoglandin biyosentezini ve salınımını inhibe ettiği gösterilmiştir(29,30). Buna ilaveten flavanoid olan amentoflanon'un siklooksijenaz-2, fosfolipaz A2, iNOS'u ve nötrofillerden araşidonik asit salınımını ingibe ettiği gösterimiştir(31-36).

Sonuç olarak çalışmamız gentamisinin neden olduğu ototoksisiteyi önlemede yararlı olacağını göstermiştir.

Çıkar Çatışması

Yazarlar arasında herhangi bir çıkar çatışması bulunmamaktadır.

KAYNAKLAR

1. Pruika EM, Schacha J. Formation of free radicals by gentamicine and iron and evidence for an iron/gentamicine complex. Biochem Pharmcol 1995;17:1749–52.

2. Rizzi MD, Hirose K. Aminoglycoside ototoxicity. Curr Opin Otolaryngol Head Neck Surg 2007;15:352–7.

3. Chen Y, Huang W-G, Zha D-J, et al. Aspirin attenuates gentamicin ototoxicity from the laboratory to the clinic. Hear Res 2007;226:178–82.

4. Rybak LP, Touliatos J, Campbell K. Ototoxicity. Otolaryngology Basic Science and Clinical Review. New York: Thieme. 2006:129–36.

5. Unal OF, Ghoreish SM, Atas A, Akyurek N, Akyol G, Gursel B. Prevention of gentamicin induced ototoxicity by trimetazidine in animal model. Int Ped Otorhinolarynngol 2005;69:193–9.

6. Liu HY, Chi FL, Gao WY. Taurine attenuates aminoglycoside ototoxicity by inhibiting inducible nitric oxide synthase expression in the cochlea. Neuroreport 2008;8:117–20.
7. Kim YS, Ahn JC, Baek IY, Rhee CK, Jung JY. Minocycline Protects Vestibular Hair Cells from Neomycin-Induced Ototoxicity by Inhibiting Reactive Oxygen Species Production and Caspase-3 Activity. Korean J Otolaryngol-Head Neck Surg. 2006;49:1159-64.
8. Tsuji K, Velazquez-Villasenor L, Rauch SD, Glynn RJ, Wall C, 3rd, Merchant SN. Temporal bone studies of the human peripheral vestibular system. Meniere's disease. The Annals of otology, rhinology & laryngology.2000;181:26–31.
9. Kawamoto K, Izumikawa M, Beyer LA, Atkin GM, Raphael Y. Spontaneous hair cell regeneration in the mouse utricle following gentamicin ototoxicity. Hearing research. 2009;247:17–26.
10. Warchol ME, Lambert PR, Goldstein BJ, Forge A, Corwin JT. Regenerative proliferation in inner ear sensory epithelia from adult guinea pigs and humans. Science. 1993;259:1619–22.
11. Zheng JL, Keller G, Gao WQ. Immunocytochemical and morphological evidence for intracellular self-repair as an important contributor to mammalian hair cell recovery. J Neurosci. 1999;19:2161–70.
12. Umek A, Kreft S, Kartnig T, Heydel B. Quantitative phytochemical analyses of six hypericum species growing in slovenia. Planta medica 1999;65:388–90.
13. Tatsis, EC, Boeren S, Exarchou V, Troganis AN, Vervoort J. Gerothanassis IP. Identification of the major constituents of Hypericum perforatum by LC/SPE/NMR and/or LC/MS. Phytochemistry 2007;68:383-93
14. Schwob I, Bessière JM, Viano J. Composition of the essential oils of Hypericum perforatum L. from southeastern France. C R Biol. 2002;325:781-5.
15. Raso GM, Pacilio M, Di Carlo G, Esposito E, Pinto L, Meli R. In-vivo and in-vitro anti-inflammatory effect of Echinacea purpurea and Hypericum perforatum. J Pharm Pharmacol. 2002;54:1379-83.
16. Albert D, Zündorf I, Dingermann T, Müller WE, Steinhilber D, Werz O. Hyperforin is a dual inhibitor of cyclooxygenase-1 and 5-lipoxygenase. Biochem Pharmacol. 2002;5:1767-75.
17. Kumaz V, Singh PN, Bhattacharya SK. Anti inflamatory and analgesic activity of Indian Hyericum perforatum L. IJEB. 2001;39:339-343.
18. Stanley JL, Lincoln RJ, Brown TA, McDonald LM, et al. The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. J. of Psychopharmacology 2005;19:221-227
19. Chang J, Yang JY, Choi J, Jung HH, Im GJ. Calcium imaging in gentamicin ototoxicity: increased intracellular calcium relates to oxidative stress and late apoptosis. Int J Pediatr Otorhinolaryngol. 2011;75:1616-22.
20. Cakir, A., Mavi, A., Yildirim, A., Duru, M. E., Harmandar, M., Kazaz, C. Isolation and characterization of antioxidant phenolic compounds from the aerial parts of Hypericum hyssopifolium L. by activity-guided fractionation. J. Ethnopharmacol. 2003;1:73–83.
21. Malis CD, Bonventre JV. Mechanism of calcium potentiation of oxygen free radical injury to renal mitochondria. A model for post-ischemic and toxic mitochondrial damage. J Biol Chem. 1986 ;25:14201–8.
22. Rordorf G, Koroshetz WJ, Bonventre JV. Heat shock protects cultured neurons from glutamate toxicity. Neuron. 1991;7:1043-51.
23. Verity MA. Mechanisms of phospholipase A2 activation and neuronal injury. Ann. NY Acad. Sci. 1993;679:110–120
24. Mattace RG, Meli R, Di CG, Pacilio M, Di CR. Inhibition of inducible nitric oxide synthase and ycloxygenase- 2 expression by ¯ avonoids in macrophages J774A. Life Sci. 2001;68:921-31
25. Panossian, AG, Gabrielian E, Manvelian V, Jurcic K, Wagner H. Immunosuppressive effects of hypericin on stimulated human leukocytes: inhibition of the arachidonic acid release, leukotriene B4 and interleukin-1a production, and activation of nitric oxide formation. Phytomedicine 1996;3:19–28
26. Kang BY, Chung SW, Kim TS. Inhibition of interleukin-12 production in lipopolysaccharide-activated mouse macrophages by hypericin, an active component of Hypericum perforatum. Planta Med. 2001;67:364–6
27. Bork PM, Bacher S, Schmitz ML, Kaspers U, Heinrich M. Hypericin as a non-oxidant inhibitor of NF-kB. Planta Med. 1999;65:297–300
28. Feisst, C., Werz, O. Suppression of receptor-mediated Ca2+ mobilization and functional leukocyte responses by hyperforin. Biochem. Pharmacol. 2004;67:1531–9
29. Albert, D., Zündorf, I., Dingermann, T., Müller, W. E., Steinhilber, D., Werz, O. Hyperforin is a dual inhibitor of cyclooxygenase- 1 and 5-lipoxygenase. Biochem. Pharmacol. 2002;64:1767–75
30. Gobbi M, Moia M, Funicello M, Riva A, Morazzoni P, Pennini T. In vitro effects of the dicyclohexylammonium salt of hyperforin on interleukin-6 release in different experimental models. Planta Med. 2004;70:680–2
31. Mora A, Paya M, Rios JL, Alcaraz MJ. Structureactivity relationships of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation. Biochem. Pharmacol. 1990;40:793–7
32. Tordera M, Ferrandiz ML, Alcaraz MJ. Influence of anti-inflammatory flavonoids on degranulation and arachidonic acid release in rat neutrophils. Z. Naturforsch. 1994;49:235–40
33. Banerjee T, Van der Vliet, AG, Ziboh VA. Downregulation of COX-2 and iNOS by amentoflavone and quercetin in A549 human lung adenocarcinoma cell line. Prostaglandins Leukot. Essent. Fatty Acids 2002;66:485–92
34. Banerjee T, Valacchi G, Ziboh VA, Van der Vliet A. Inhibition of TNFalpha-induced cyclooxygenase-2 expression by amentoflavone through suppression of NF-kappaB activation in A549 cells. Mol. Cell. Biochem. 2002;238:105-10
35. Kim HP, Mani I, Iversen L, Ziboh VA. Effects of naturally-occurring flavonoids and bioflavonoids on epidermal cyclooxygenase and lipoxygenase from guinea-pigs. Prostaglandins Leukot. Essent. Fatty Acids 1998;58:17–24
36. Kim HK, Pham HT, Ziboh VA. Flavonoids differentially inhibit guinea pig epidermal cytosolic phospholipase A2. Prostaglandins Leukot. Essent. Fatty Acids 2001;65:281-6

Increasing Factors of Depression Among School Children Aged 10-15 Years

10-15 Yaş arası Okul Çocuklarında Depresyon Faktörlerinde Artış

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ABSTRACT

Objective: Major depressive disorder is an escalating problem in both developed and under developed countries. Similar to the adolescents, depressive symptoms have been diagnosed at an extensive level among school going children. The study is based around investigating the increasing factors of depression among school going children of Jordan.
Methods: A sample of 1650 school going students from Amman has been considered, which was assessed on behalf of two instruments. The age group of 10-15 years was identified for recruiting students from five government and five private schools. The collected data was analyzed through SPSS version 20.
Results: Findings of the study reflected that depression in childhood is strongly associated with ecological factors and psychiatric co-morbidities. Furthermore, the results revealed that age, gender, living arrangements, and domestic violence are also associated with childhood depression. The results further identified that the prevalence rate of depression is higher in girls as compared to the boys.
Conclusion: Depression can become more severe, if it is not managed at the early stage. Similarly, depression in childhood is likely to continue in adulthood if it is not properly treated at initial stages. Moreover, the rate of depression vary across the globe due to the different social and economic factors of each country.

Key Words: Depressive disorder syndrome, depression, factors, Jordan, school children

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ÖZET

Amaç: Majör depresif bozukluk, hem gelişmiş hem de gelişmemiş ülkelerde tırmanan bir sorundur. Ergenlerle benzer şekilde, depresif belirtiler okula devam eden çocuklara kapsamlı bir düzeyde teşhis konulmuştur. Bu çalışma, Ürdün'de okula devam eden çocuklar arasındaki depresyonun artan faktörlerini araştırmaya odaklanmıştır.
Yöntem: Amman'tan gelen 1650 öğrenci örneği iki araç ile değerlendirildi. Beş devlet ve beş özel okulda okuyan öğrenci seçimi için 10-15 yaş grubu belirlendi. Toplanan veriler SPSS sürüm 20 ile analiz edildi.
Bulgular: Araştırmanın bulguları, çocukluk çağındaki depresyonun ekolojik faktörler ve psikiyatrik hastalıklara eşlik eden hastalıklarla güçlü bir şekilde ilişkili olduğunu yansıtıyordu. Ayrıca, sonuçlar yaş, cinsiyet, yaşam düzenlemeleri ve aile içi şiddetin çocukluğun depresyonuyla ilişkili olduğunu ortaya koydu. Sonuçlar ayrıca, depresyon prevalans oranının kızlarda erkeklerden daha yüksek olduğunu ortaya koymuştur.
Sonuç: Depresyon, erken evrede tedavi edilmezse daha şiddetli olabilir. Benzer şekilde, çocukluk çağındaki depresyonun ilk aşamalarda uygun şekilde tedavi edilememesi durumunda yetişkinlikte devam etmesi muhtemeldir. Dahası, her ülkenin farklı sosyal ve ekonomik faktörlerinden dolayı depresyon oranı dünyada farklılık göstermektedir.

Anahtar Sözcükler: Depresif bozukluk sendromu, depresyon, faktörler, Ürdün, okul çocukları

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INTRODUCTION

Major depressive disorder (MDD) is widely linked with the extensive complicated symptoms in regards of physical and psychological systems. The early treatment of disorder may decrease the serious consequences of disorder; however, the problem seems to be extremely common across the globe, especially in the developing countries. The study aimed to comprehend the increasing factors of depression among school children aged 10-15 years. Therefore, it has contributed to the fields of psychology and physiology to highlight the prevalence of depressive disorders among the individuals so that the patients can be treated by the improved ways.

Depression is the seventh major cause of diseases in the low income countries, which is tending to be untreated, inveterate, and persistent. Similarly, depression will become the second major cause of disease by the end of 2020 in developing countries. Different studies have revealed that prevalence of depression among adolescents is higher in females as compared to males. The differences in diagnosis have been reported between 13-15 years of age among both genders (1, 2).

According to past researches, MDD is persistent and common in both adults and children. Major depressive disorders (MDD) have increased the risks of suicide, bullying, and substance use in children and adults. The prevalence rate of MDD during the whole life is 1.5%. Similarly, the prevalence rate of depression has been identified in between the ranges of 6% to 20%. The lowest depression rate was found in China i.e. 6%; whereas, the highest depression rate has been found in USA i.e. 20%. The survey conducted in United Arab Emirates revealed that the depression rate in the life-time for females is 9.5%; whereas, for males is 2.5%. The survey also found that the depression rate is higher in women; accordingly, the ratio of depression among both females and males is 4:1 (3, 4).

Another study conducted in Saudi Arabia, used the sample of patients from psychiatric hospital, which revealed that the prevalence rate of depression in Saudi Arabia was 17% with the increased risk of symptoms among females. As per the survey conducted in Pakistan, it was revealed that the depression rate in men was 15%; whereas, the depression rate in women was 46%. Such findings clearly depicts that the depression rate in women is much higher as compared to men. The prevalence rate of depression is also higher in Jordan; evidence from one study revealed that the depression rate is higher than 30% in the females of Jordan (1, 5,6).

In order to decrease the severe consequences of major depressive disorder (MDD); developed countries have used different medical treatments, which permanently reduced the symptoms of depression among the individuals of different age groups. Through medical treatments, developed countries have reduced the consequences of depression up to 80%; however, the situation is completely different with the Middle Eastern countries, especially Jordan. The reason behind the depression in adolescents of Jordan is differences in the socio-cultural settings, inappropriate management settings of medical, and lack of resources. Moreover, Jordan adolescents are highly dependent on their parents; due to which, they experience abnormal psychological and social reactions during their personal and professional life (3, 4).

Depression amongst adults is common and unrelenting. However, the complicating factors in childhood have also increased the prevalence of co-morbid psychiatric problems. The major occurrence of co-morbid psychiatric disorders include anxiety, substance abuse, and attention-deficit/hyperactivity disorder. The prevalence rate of co-morbid psychiatric disorder among adolescents and children is 40-70%. Symptoms of psychotic disabilities are commonly seen in children and adolescents that are experiencing pediatric depression (1, 7, 8).

High depression is linked with enormous social challenges and poor behavior of health. Moreover, increased risk of suicide among youth leads to greater risk of psychological disorders such as anxiety and substance abuse. Those who are experiencing high depression are most likely to involve in endangered sexual practices and other behaviors. Depression that occurs during the age of late childhood will continue to adulthood in most of the cases; however, in certain cases, depressive disorders are diminished with the growing age (9, 10). Another study has revealed, that 75% of the adults, who are experiencing Major depressive disorders (MDD) during their adult age, have experienced their first episode of depression during the age of childhood. Furthermore, highly depressed adolescents and youth face the problem of communication among peers, and are most likely to be involved in physical fights with peers (1, 11, 13).

The symptoms of depression among children are different from that of adults. The depression of children includes irritable mood, anger, and poor academic performance. Children, who are facing the problem of depression, may also demonstrate psychomotor agitation, symptoms of apprehension (anxiety disorder), irrational fear, regressive behavior, and separation anxiety. Vegetative and somatic complaints can also be linked with the depression in children (9, 14).

The studies revealed that major risk factors for depression among children are substance use and bullying. The association between substance use and depression is also common among the individuals, having emotional disorder. Among children, bullying is recognized as the common practice at personal settings or within school territories. Different studies have indicated that bullying is associated with low self-esteem, psychosomatic complaints, symptoms of apprehension and depression, and desires of suicidal attempt (12; 13, 15). Similar case is with the youth; children who are highly depressed are engaged in bullying, both as perpetrators and victims. They are prone to counter the problem of emotional adjustments and poor psychosocial aspects, which in turn results in increased seclusion and social isolation. Due to high prevalence of bullying, as both perpetrators and victims, the association between depression and bullying should be examined. Almost 10-20% of adolescents and children are rapidly engaged in bullying; and as compared to girls, boys are more involved in the practices of bullying (10, 15, 16).

This study has mainly focused on the major factors of the depression and the prevalence of symptoms of depression among children. Alongside, it has performed an investigation closer to the certain areas, which includes the prevalence of depressive symptoms in Jordanian children between the ages of 10-15 years. It has also evaluated the increasing risk factors of depression and its association with different causative agents.

METHODS

This study has been conducted in the capital city of Jordan i.e. Amman. Five districts of Amman, which include Jubeiha, Al-Abdali, Na'our, Marj Al-Hamam and Al-Muwaqqar, were identified as the selected schools for the research. Random sampling method has been used during the selection stage of districts. However, equal selection of the children was done from five districts and their schools. Kish's formula has been introduced to conduct this study, which is commonly used for cross-sectional studies. Each school has been categorized in five districts by using the dataset of school authority. Multistage sampling method has been used in order to obtain the sample size of school. In the first stage, five districts of Amman have been selected randomly; and in the second stage, schools were categorized into two groups. The first group is comprised of government schools and the second group is comprised of private schools. Five schools of government and five private schools have been selected randomly from each district.

The list of schools was prepared in order to select the students that lie between the ages of 10-15 years. During the scrutiny process, 2000 students have been found who were liable to be aligned with the aforementioned criteria. The researcher then scrutinized the students by using the mental health test. A sample paper has been provided to 2000 students, which was comprised of 10 different IQ questions. Those students who got marks below 5 were selected for further study. After completing the scrutiny process, 300 students have been excluded who have scored marks above 5. Moreover, 50 students were also excluded who were reluctant to take part in the study. Finally, 1650 students were selected as the final sample for the study.

Two assessment tools have been selected for assessing the psychological concepts of the selected students. The selected tools were M.I.N.I.-KID (Mini International Neuropsychiatric Interview for Children and Adolescents) and SDQ (Strengths and Difficulties Questionnaire). Neuropsychiatric interviews were conducted from children through the first tool; however, the difficulties and strengths were identified through SDQ tool. In order to assess the behavioral and emotional problems of school going children, the SDDQ (Susceptibility to Driver Distraction Questionnaire) has also been used in this study. This instrument is based on 25 item questionnaire, which identified the possible psychological distress among children. This instrument is valid for both developed and developing countries, and it mainly covers the areas of behavioral and emotional difficulties. This instrument has used the scoring range of Likert scale.

M.I.N.I.-KID has been utilized as instrument in the study, which holds the DSM IV (Diagnostic and Statistical Manual of Mental Disorders) criteria for assessing the children and adolescents mental disorder. The mental judgment evaluated in this study are panic disorder, episode of depression, substance use disorder, social phobia, dysthymia, generalized anxiety disorder, manic episode, and pervasive development disorder. Syndrome recognition for the purpose of analysis has been grouped as follows:

Depressive syndrome (depressive episode, dysthymia): obstinate feelings of insignificance and sadness.
Anxiety syndrome disorder (separation and generalized anxiety disorder, social phobia): Anxiety is a worry regarding any event. These syndromes can lead to shakiness and fast heart rate.
Disorder of psychotic syndrome (manic episode): diminished association with reality, which leads to delusions and hallucinations.
Disorder of behavioral developmental syndrome (pervasive development disorder): involves autism, and use of substance.

In order to analyze the mental problems of children, three questions have been asked from each student;

1) Have you ever tried to die due to feeling bad or depressed?
2) Have you ever attempted to harm yourself?
3) Have you ever endeavored to kill yourself?

These questions were helpful in evaluating the mental state of the children due to the fact that, the depressed souls often think of attempting suicide, or seek for the ways that might hurt them. They believe that practicing these values would help them to get rid of all the negative thoughts.

The variables includes age, gender, level of education, district, place of living, mental illness history, living arrangements, total income of the family, housing nature and attendance in the facility of mental health. Moreover, other variables that have been considered during the study were domestic violence, bullying, substance use and suicide. Data was analyzed through SPSS Version 20, which has applied regression and correlation analysis to investigate the strength of relationship. Backward elimination regression model has been used to investigate the association of socio demographic determinants. All ethical concerns were taken into consideration by taking the approval from the Ministry of Health of Jordan. The complete activity of mental disorder was performed in the school setting to provide more familiar and relaxed environment to the students as shown in Table 1.

RESULTS

Out of 2000 students, the final sample size was comprised of 1650 students. Out of the total sample of 1650 students, the proportion of males and females was between 700 males and 950 females. All of the selected participants were aged between 10-15 years. Equal selection was done from government and private schools to avoid any bias in the study. Comprehensive demographic information has been placed in Table 1. In this study, the prevalence of depressive syndrome disorder was 9.5%. Evidence from the study also revealed that the prevalence rate was slightly higher in females as compared to males. The prevalence rate of depressive disorder syndrome in females was 10%; whereas, it was 9% in males.

Table 2 has been categorized in both males and females to identify the prevalence of depression rate in both genders. The prevalence of depressive episode was 8% and dysthymia was evaluated as 4%.

Table 1: Demographic Data

Measure	Items	Frequency
Gender	Male	700
	Female	950
Age	10-15	1650
Level of Education	Private Schools	825
	Government Schools	825
District	Jubeiha	330
	Al-Abdali	330
	Na'our	330
	Marj Al-Hamam	330
	Al-Muwaqqar	330
Mental illness history	panic disorder	215
	episode of depression	847
	substance use disorder	71
	social phobia	89
	dysthymia	126
	generalized anxiety disorder	207
	manic episode	80
	pervasive development disorder	15

Table 2: Depression and relationship with gender

Categories	Boys	Girls	Total
DDS	81 (9%)	115 (10%)	196 (9.5%)
	(5.8%–11%)	(6.0%–11.5%)	(8%–12%)
(a) Current major depressive episode	42 (8%)	50 (8%)	92 (8%)
	(7%-8.6%)	(6%-8%)	(6%-8%)
(b) Dysthymia	12 (4%)	20(4%)	32 (4%)
	(0.1%-3%)	(2%-4.5%)	(2%-4%)

Table 3 has depicted the prevalence of Depressive Disorders (DDs) in two categories i.e. psycho-social and psychiatric characteristics. Results from the study revealed that the prevalence rate was higher in those students who have experienced domestic violence as compared to those who have not experienced any type of domestic violence during their life. Depressive disorder syndrome showed more prevalence in both genders as compared to other psychiatric problems, emotional and behavioral developmental disorder problem. Prevalence rate of DDs was higher in those children whose parents were not alive.

Table 3: Psychosocial determinants and psychiatric problems among children

Categories	Extent	Total	Percentage of Depression
Factors			
Domestic violence	No	1215	100 (8.5%)
	Yes	253	30 (10%)
Family history of mental illness	None	1215	110 (7.3%)
	First Degree relative	140	12 (6.9%)
	Other relative	230	18 (7%)
Bullying	No	1130	80 (9.5%)
	Yes	540	65 (12%)
Parents alive	No	1000	50 (5%)
	Yes	540	92 (18%)
History of mental illness (attendance at facility)	No	1525	138 (9.1%)
	Yes	100	5 (7.5%)
Psychiatric Complications			
Anxiety syndrome	No	1140	65 (6%)
	Yes	480	89 (20%)
Psychotic disorder syndromes	No	1600	138 (8.9%)
	Yes	20	12 (45%)
Suicidality	No	1540	90 (6.5%)
	Yes	89	60 (70%)
substance abuse disorders	No	1642	112 (7.7%)
	Yes	20	5 (30%)
Motor disorder syndromes	No	1500	134 (11%)
	Yes	10	10 (7%)
Behavioral & developmental disorder syndromes	No	1520	135 (9%)
	Yes	60	6 (11%)
Eating disorders	No	1600	120 (9%)
	Yes	12	8 (60%)
Number of DSM disorders	0	1000	35 (4.2%)
	1	450	60 (14%)
	2 or more	70	32 (48%)
Emotional problems (assessed by SDQ scores)	SDQ < 16	900	50 (6.5%)
	Case (SDQ ≥ 16)	600	80 (12.5%)

The results generated from the model of multiple logistic regression clearly depicted that age, arrangements of district and place of living is significantly linked with depressive disorder syndrome among children. Evidence from the results also suggested that the factors of psychiatric disorder and emotional distress are also linked with DDs. The disorders of anxiety, eating and motor syndrome are slightly associated with DDs. Moreover, the students with developmental and behavioral disorder syndrome showed less possibility to have the depressive disorder syndrome.

Table 4 clearly depicted that students who are living with both father and mother have less possibility to have DDs as compared to those who are living with grandfathers and caretakers. Those students who are living in the district of Jubeiha had excessive DDs as compared to the other districts of Amman. Results from the study revealed that psychiatric and psychosocial factors, socio-demographic and ecological factors were independently and positively associated with the depression of children. In this study, districts were categorized as ecological factors. Indication from the study revealed that nature of arrangement of living and domestic violence was significantly associated with the depression of children.

Table 4: Determinants and depression in children

		Odd ratios	**Likelihood ratio**
Districts	Jubeiha	1 (Reference level)	<0.0002*
	Al-Abdali,	0.2(0.06;0.5)	
	Na'our,	3.95(2.5;8)	
	Marj Al-Hamam	0.55 (0.17;2)	
	Al-Muwaqqar	0.68 (1.5;3)	
Age group	10-15	1.58 (0.6;3.5)	0.24
Living Style	With Parents	1	<0.0003*
	Only Mother	2.5 (1.3;4)	
	Only Father	3 (1.18;9)	
	Grandparents	6.8 (4;13)	
	other	4 (3;12.5)	
Domestic Violence	No	1	0.04*
	Yes	2 (1.09;4)	
Emotional Distress	SDQ < 16	1	<0.0002*
	SDQ ≥ 16	4 (2;5)	
Motor disorder complications	No	1	0.08
	Yes	11 (2;116)	
Eating disorders	No	1 (Reference level)	0.039*
	Yes	13 (2;145)	
Behavioral disorders	No	1 (Reference level)	0.03*
	Yes	0.3 (0.05;2)	
Anxiety	No	1 (Reference level)	0.05
	Yes	2 (1.04;2.95)	
Suicidality	No	1 (Reference level)	<0.0002*
	Yes	25.2 (12;60)	

No overwhelming evidence was found that depicted the independent and significant association between the childhood depression and deprivation of socio economic factors (family income, housing nature, and educational level of parents/caretakers). It is possible that all these factors are the risk determinants of depression of childhood. Results from the study revealed that the prevalence of depression among school going children is high in Jordan. Ecological factors, the existence of psychiatric co-morbidities and the quality of relationship of child-principle caregiver are the most essential independent variables of depression in childhood. The use of substance and bullying are also significantly associated with the depression of children. The study has not found any significant association between the orphan hoods, deprivation of socio-economic factors and depression of children.

CONCLUSION

Major depressive disorder is considered as the second most important cause of death globally. Depression is escalating in both developed and developing countries; however, the prevalence of depression is higher in developing countries. It can be observed that the trend of depression has vastly shifted from old age people to young adolescents. The study mainly focused on the prevalence of depression in school going children between the ages of 10-15 years. The extent of disability is caused due to major physical disorders including heart disease, stroke, and headache. As treatment of depression is highly receptive, it is deteriorated by the gratitude and diagnosis of low rates. MDD often starts during the period of late childhood or early adolescence. Studies revealed that 20% to 50% of adolescents experience the problem of high level of depression. In order to reduce depression and to prevent its negative outcomes, it is obligatory to recognize it as early as possible. Similarly, early diagnosis of depression would also prevent severe consequences of depressive symptoms (1, 2).

In order to conduct the study, five districts of Amman were selected by random sampling method. Two assessment tools have been used to assess the mental health of 1650 students. Findings of the study revealed that age, gender and living arrangements are significantly associated with the childhood depression. Moreover, there was no evidence found related to the association between childhood depression and social deprivation (nature of housing, parents education, and income of the family). But all these factors may indirectly affect the childhood depression. Results suggested that the most important independent variables of childhood depression were disturbed ecological factors, psychiatric co-morbidities, and quality of relationship of child-principal caregiver. The study concluded that those students, who faced the problem of depression in childhood, may likely to experience severe depression in their adulthood. However, the early recognition of depression may prevent children from severe consequences of depression. The prevalence rate of depression in school going children is high in Jordan.

The problem of depression is higher in females as compared to males, and comparatively developing countries have higher rate of depression among females than the developed countries. Moreover, the difference in the rate of depression in school going children is different across the countries due to the different social and economic factors present in each country. Most of the studies have been conducted on the symptoms of depression among high school students. However, further investigation is needed to be conducted on the prevalence and risk determinants of depression among young children.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Brooks TL, Harris SK, Thrall JS, Woods ER. Association of adolescent risk behaviors with mental health symptoms in high school students. J Adolesc Health. 2002; 31: 240-6.
2. Birmaher B, Ryan ND, Williamson DE, Brent DA, Kaufman J, Dahl RE, Perel J, Nelson B. Childhood and adolescent depression: a review of the past 10 years. Part I. J Am Acad Child Adolesc Psychiatry. 1996; 35: 1427-39.
3. Nasir LS, Al-Qutob R. Barriers to the diagnosis and treatment of depression in Jordan. A nationwide qualitative study. The Journal of the American Board of Family Practice. 2005; 18: 125-31.
4. Dilsaver SC, Benazzi F, Akiskal HS, Akiskal KK. Post-traumatic stress disorder among adolescents with bipolar disorder and its relationship to suicidality. Bipolar disorders. 2007; 9: 649-55.
5. Kessler RC, Demler O, Frank RG, Olfson M, Pincus HA, Walters EE, Wang P, Wells KB, Zaslavsky AM. Prevalence and treatment of mental disorders, 1990 to 2003. New England Journal of Medicine. 2005; 352: 2515-23.
6. Yehuda R. Post-traumatic stress disorder. New England Journal of Medicine. 2002; 346: 108-14.

7. Hirschfeld RM, Keller MB, Panico S, Arons BS, Barlow D, Davidoff F, Endicott J, Froom J, Goldstein M, Gorman JM, Guthrie D. The National Depressive and Manic-Depressive Association consensus statement on the undertreatment of depression. JAMA. 1997; 277: 333-40.
8. Rushton JL, Forcier M, Schectman RM. Epidemiology of depressive symptoms in the National Longitudinal Study of Adolescent Health. J Am Acad Child Adolesc Psychiatry. 2002; 41: 199-205.
9. Degenhardt L, Hall W, Lynskey M. Exploring the association between cannabis use and depression. Addiction. 2003; 98: 1493-504.
10. Kovacs M, Goldston D, Obrosky DS, Bonar LK. Psychiatric disorders in youths with IDDM: rates and risk factors. Diabetes care. 1997;20:36-44.
11. Weissman MM, Wolk S, Goldstein RB, Moreau D, Adams P, Greenwald S, Klier CM, Ryan ND, Dahl RE, Wickramaratne P. Depressed adolescents grown up. JAMA. 1999; 281: 1707-13.
12. Shaffer D, Craft L. Methods of adolescent suicide prevention. J Clin Psychiatry. 1999.
13. Kovacs M, Goldston D, Obrosky DS, Bonar LK. Psychiatric disorders in youths with IDDM: rates and risk factors. Diabetes care. 1997; 20: 36-44.
14. Klomek AB, Marrocco F, Kleinman M, Schonfeld IS, Gould MS. Bullying, depression, and suicidality in adolescents. JAACAP. 2007; 46: 40-9.
15. Kaltiala-Heino R, Rimpelä M, Marttunen M, Rimpelä A, Rantanen P. Bullying, depression, and suicidal ideation in Finnish adolescents: school survey. Bmj. 1999; 319: 348-51.
16. Boulton MJ, Underwood K. Bully/victim problems among middle school children. Br J EducPsychol. 1992; 62: 73-87.

Questionnaire to test IQ of children aged between 10 – 15 years

Questions	Options
1. The word "racecar" is spelled the same forwards and backwards	True False
2. Two of the following numbers add up to seventeen.	6 -13 -12-7-14
3. Which one of the five is least like the other four	Shark Deer Cow Dog Lion
4. If you arrange the letters "ANICH", you would have the name of a/an	Country Ocean State City Animal
5. Ralph travels four blocks north, then then two blocks east, then three blocks south and finally two blocks west. How many blocks is Ralph from his starting place?	One block Two block Three block Four block
6. The entire following sentence makes sense if the word toog is understood to mean the same as the word start: I tooged the car and turned on the radio just in time to hear the announcer say, "The marathon is over as the first runner crosses the toog line."	True False
7. If you rearrange the letters "TOOKY", you would have the name of a/an	City Country State Ocean Animal
8. What is the minimum number of toothpicks necessary to spell the word "HAT"?	Five Six Seven Eight nine
9. If all Bloobs are Toogs and no Toogs are Goppers.	True False
10. If you rearrange the word "NOPTYH", you would have the name of a/an	City Country State Ocean Animal

Awareness of Code of Medical Ethics in Tomorrow's Doctors in India; A limited study

Hindistan'da Yarının Doktorlarında Tıp Etiği Kurallarının Farkındalığı; Sınırlı Bir Çalışma

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ABSTRACT

Medical Council of India has specified the various unethical acts and misconduct in 'Professional Conduct, Etiquette and Ethics Regulations 2002'. Though medical ethics is not a separate subject, it is being taught as a part of Forensic Medicine in 2nd MBBS. Every medical practitioner should be aware of these codes and should apply them in their daily practice. Medical students are the budding doctors of tomorrow. It is imperative that the medical students are also aware of these codes during their student days so that they can apply them in their future practice. So, the present study attempts to look for the awareness amongst the various years of MBBS students of Kasturba Medical College, Manipal about these codes. In our study it was found that the knowledge of medical ethics was more in 4th year students in comparison to 2nd year and 3rd year students.

Key Words: Awareness, code, medical ethics

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ÖZET

Hindistan Tıp Konseyi 'Mesleki Davranış, Etik Kurallar ve Etik Yönetmelikleri 2002' üzerinde çeşitli etik olmayan davranış ve kötü muameleyi belirtmiştir. Tıp etiği ayrı bir konu olmasa da, ikinci MBBS'de Adli Tıp'ın bir parçası olarak öğretilmektedir. Her hekim bu kurallardan haberdar olmalı ve günlük uygulamalarında kullanmalıdır. Tıp öğrencileri yarının doktorlarıdır. Tıp öğrencilerinin de öğrencilik günlerinde bu kuralların farkında olmaları şarttır, böylece onları gelecekteki uygulamalarına uyarlayabilirler. Bu nedenle, bu çalışma, Kasturba Tıp Fakültesi, Manipal'teki çeşitli yıllardaki MBBS öğrencileri arasındaki bu kurallar hakkındaki farkındalığın araştırılmasını amaçlıyor. Çalışmamızda tıp etiği bilgisinin 4. yıl öğrencilerinde, 2. ve 3. sınıf öğrencilerine göre daha fazla olduğu bulunmuştur.

Anahtar Sözcükler: Farkındalık, etik kurallar, tıbbi etik

Geliş Tarihi: 14.06.2016 **Kabul Tarihi:** 08.12.2016

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INTRODUCTION

Ethics has been defined as "the moral principles that govern a person's behavior or how an activity is conducted" and medical ethics as "the branch of knowledge concerned with moral principles" (1). The application of ethics to medical practice dates back to ancient civilization as even today, all medical graduates must swear symbolic adherence to the Hippocratic oath. Codes of conduct and laws regulating the profession are laid down from time to time (2). There has been growing public awareness regarding the ethical conduct of medical practitioners, and complaints against physicians appear to be escalating. This may reflect an increase in unethical practices by doctors or increasing public awareness of such unethical practices (3). In medicine, professionalism connotes not only knowledge and skills, but also character, especially compassion and ethics (3). It is a commitment to subordinate our self-interest to the interest of patients and it is the foundation of trust upon which our social contract as physicians rests (4).

The recent increase in litigation against doctors is an issue of immediate concern. The reasons for this are social, economic, professional and judicial. Social factors include increasing media awareness about medical facts and fallacies, professional accountability, and rights of patients in terms of information, decision-making and assessing outcomes. Negative publicity in the media about the profession has done further damage (5).

Medical council of India (MCI) has mentioned the code of ethics in its document 'The Indian medical council (professional conduct, etiquette and ethics) regulations 2002'. In the present curriculum medical ethics is taught in 2nd year MBBS as a part of Forensic Medicine and Toxicology.

Every medical student who is a budding doctor of tomorrow should have knowledge of the code of medical ethics which will help him in practicing the art of medicine in an ethical way safeguarding the interest of themselves as well as their patients. With this background the present study was attempted to know the awareness of code of medical ethics in the medical students of Kasturba Medical College, Manipal (A constituent college of Manipal University, Manipal).

METHODS

A cross-sectional study was conducted at Kasturba Medical College, Manipal (a constituent college of Manipal University, Manipal) from July 2015-November 2015. A total of 240 MBBS students were enrolled for the survey with equal distribution of 2nd, 3rd and 4th year (80 in each year) randomly. 'A questionnaire was given to each students containing 10 question based on the chapters on 'unethical acts' and 'misconduct' in the Indian medical council (professional conduct, etiquette and ethics) regulations 2002 which was derived from a study conducted by Arun Babu et al.(6). The students were required to select yes, no or don't know response of all these 10 questions. In addition to the 10 questions, the demographic details of the students like age, sex and year in medical college were gathered as mentioned in the proforma.

Institutional ethics committee clearance was obtained prior to the conduct of the study and participant information sheet (PIS) were given to all of the participants and written informed consent was taken from all the students participating in the study. Data collected was analyzed using SPSS 16 version software to calculate mean and percentage in all the variables.

RESULTS

A group of 240 students (80 each in 2nd, 3rd and 4th year MBBS) participated in the study. Gender distribution of various years is depicted in fig 1, 2 and 3. Five students of 2nd year, 2 students of 3rd and 3 students in 4th year answered all questions correctly. Scenarios and percentage of correct responses for each year is depicted in table no. 1, 2 and 3.

More than 50% correct response was noted to all the questions by 2nd and 4th year MBBS students. More than 50% correct response was noted to all the questions except question no. 3 and 4 by 3rd MBBS students. The overall mean score of all years are depicted in table no. 4.

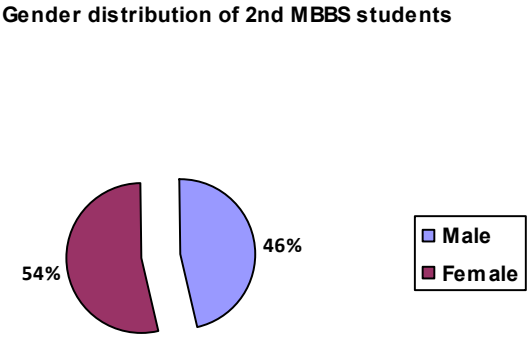

Figure 1. Gender distribution of 2nd MBBS students

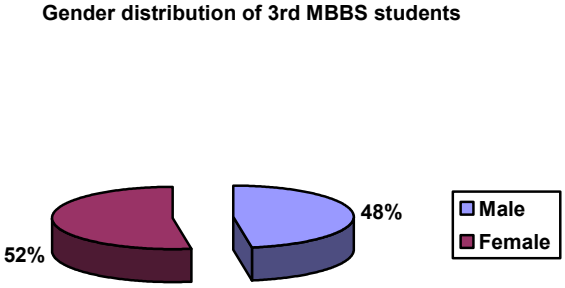

Figure 2. Gender distribution of 3rd MBBS students

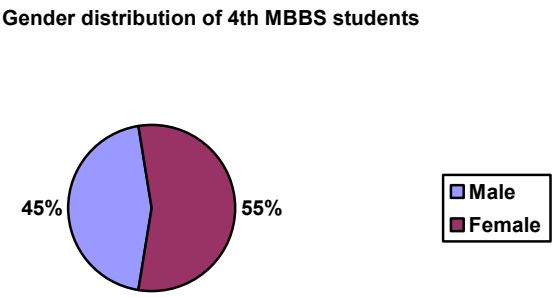

Figure 3. Gender distribution of 4th MBBS students

Table 1. Scenarios and percentage of correct responses of 2nd MBBS students

Sl no.	Questions	Correct response	Percentage of correct responses (n=80)
1.	Printing of one's photograph on the sign board of the consulting room	No	51%
2.	A doctor may disallow the use of his patented discovery even if it benefits a large population	No	60%
3.	A doctor should not own an open pharmacy	Yes	60%
4.	A doctor can receive gifts from medical representatives	No	53%
5.	A doctor can prescribe a drug even if he does not know the exact composition	No	81%
6.	A doctor can conceal signs of domestic abuse in order to prevent marital discord	No	74%
7.	A doctor can perform mercy killing after discussing the matter with the relatives in hopeless cases	No	63%
8.	A doctor should maintain the records of his/her inpatients for 3 years	Yes	78%
9.	A doctor need not display his/ her registration number in the prescription	No	74%
10.	A doctor can disclose secrets of a patient to his/her spouse if they face a serious and identified risk (eg: Retroviral positive patients)	Yes	85%

Table 2. Scenarios and percentage of correct responses of 3rd MBBS students

Sl no.	Questions	Correct responses	Percentage of correct responses (n=80)
1.	Printing of one's photograph on the sign board of the consulting room	No	66%
2.	A doctor may disallow the use of his patented discovery even if it benefits a large population	No	66%
3.	A doctor should not own an open pharmacy	Yes	31%
4.	A doctor can receive gifts from medical representatives	No	45%
5.	A doctor can prescribe a drug even if he does not know the exact composition	No	79%
6.	A doctor can conceal signs of domestic abuse in order to prevent marital discord	No	69%
7.	A doctor can perform mercy killing after discussing the matter with the relatives in hopeless cases	No	55%
8.	A doctor should maintain the records of his/her inpatients for 3 years	Yes	60%
9.	A doctor need not display his/ her registration number in the prescription	No	64%
10.	A doctor can disclose secrets of a patient to his/her spouse if they face a serious and identified risk (eg: Retroviral positive patients)	Yes	74%

Table 3. Scenarios and percentage of correct responses of 4th MBBS students

Sl no.	Questions	Correct responses	Percentage of correct responses (n=80)
1.	Printing of one's photograph on the sign board of the consulting room	No	77%
2.	A doctor may disallow the use of his patented discovery even if it benefits a large population	No	64%
3.	A doctor should not own an open pharmacy	Yes	55%
4.	A doctor can receive gifts from medical representatives	No	56%
5.	A doctor can prescribe a drug even if he does not know the exact composition	No	91%
6.	A doctor can conceal signs of domestic abuse in order to prevent marital discord	No	77%
7.	A doctor can perform mercy killing after discussing the matter with the relatives in hopeless cases	No	55%
8.	A doctor should maintain the records of his/her inpatients for 3 years	Yes	84%
9.	A doctor need not display his/ her registration number in the prescription	No	67%
10.	A doctor can disclose secrets of a patient to his/her spouse if they face a serious and identified risk (eg: Retroviral positive patients)	Yes	71%

Table 4. Mean score of correct response in each year

Year of MBBS Students	Mean Score of correct response (n=80)
2nd	6.67
3rd	6.22
4th	7.11

DISCUSSION

Presently ethics is taught in 2nd year MBBS under the subject Forensic Medicine and Toxicology. Medical Council of India is planning to include ethics teaching in all years (1st, 2nd & final year) in future. The objective of ethics education in medical institutes is to influence upon the student the moral nature of the art and science of medicine and to make aware the learner to ethical issues (7). It is not only medical students who lack awareness of the medical code of ethics, but doctors as well (8). To produce doctors who are aware of the ethical aspects of medicine, medical ethics should be introduced as a distinct and compulsory discipline in the undergraduate curriculum (9).

Many educators believe that ethics has got more to do with the overall moral development of the individual and can be difficult to teach through instruction in the later years of a person's life. Some common arguments against the view that ethics should be imparted are that there are no absolutely right or wrong answers in the domain of ethics. There is a growing cognizance of the need to include medical ethics as a major subject in the regular undergraduate curriculum (10). Arun Babu et al., conducted a medical ethics related study on 118 medical students from Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry. A questionnaire on medical ethics was given to the participants, based on the chapters on 'unethical acts' and 'misconduct' in the Indian Medical Council (Professional Conduct, Etiquette and Ethics) Regulations, 2002 which was also given by us in the present study. Out of 118 students 32 students were in the second MBBS and 43 each in the third and final MBBS. In their study none of the individuals could answer all 10 questions correctly the maximum number of questions answered correctly was eight out of ten. But in the present study 5 students of 2nd year, 2 students of 3rd and 3 students in 4th year answered all questions correctly. In their study the maximum rate of correct responses (more than 50%) was to questions 1, 2, 4, 5, 8 and 9 but in our study more than 50% correct response was noted to all the questions by 2nd and 4th year MBBS students and more than 50% correct response was noted to all the questions except question no. 3 and 4 by 3rd MBBS students. The overall mean score was 6.13 in their study while in our study it was 6.67. The mean scores for second, third and final-year students were 6.19, 5.95 and 6.26 respectively in their study while in present study it was 6.67, 6.22, and 7.11 respectively. As the score was more in our study than theirs which indicates a higher level of awareness of code of medical ethics in our students. Table no 5 shows the comparision between these two studies (6).

In a study conducted to assess the awareness of medical ethics among undergraduates in Midnapur Medical College, West Bengal (11), 52.2% of the students disagreed that it was appropriate for doctors to print their photograph on their signboard, 46.3% disagreed that doctors can "run an open shop for dispensing drugs and appliances prescribed by other doctors". A 64% of them disagreed to the suggestion of euthanasia, the findings of which are comparable to the present work.

In a study conducted to assess the knowledge regarding medical ethics among undergraduates in BM Patil Medical college, Bijapur (12), 61.8% of the students disagreed that it was appropriate for doctors to print their photograph on their signboard, 58.8% disagreed that doctors can "run an open shop for dispensing drugs and appliances prescribed by other doctors", the findings of which are comparable to the present work. However, 25% disagreed to the suggestion of euthanasia, the findings of which are in contrast to the present work.

Table 5. Comparison of our study with Arun Babu et al.

Sl no.	Questions	Percentage of correct responses	
		Present study	Arun Babu et al .
1.	Printing of one's photograph on the sign board of the consulting room	64.6%	82.2%
2.	A doctor may disallow the use of his patented discovery even if it benefits a large population	63.3%	67.8%
3.	A doctor should not own an open pharmacy	48.6%	31.4%
4.	A doctor can receive gifts from medical representatives	51.3%	65.3%
5.	A doctor can prescribe a drug even if he does not know the exact composition	83.6%	94.1%
6.	A doctor can conceal signs of domestic abuse in order to prevent marital discord	73.3%	39.0%
7.	A doctor can perform mercy killing after discussing the matter with the relatives in hopeless cases	57.6%	32.2%
8.	A doctor should maintain the records of his/her inpatients for 3 years	74%	89.8%
9.	A doctor need not display his/ her registration number in the prescription	68.3%	74.6%
10.	A doctor can disclose secrets of a patient to his/her spouse if they face a serious and identified risk (eg: Retroviral positive patients)	76.6%	36.4%

CONCLUSION

In our study it was found that the knowledge of medical ethics was more in 4th year students than in 2nd year and finally it was less in 3rd year students. The reason for this is unknown. In the present study more than 50% correct response was noted to all the questions by 2nd and 4th year MBBS students. Even more than 50% correct response was noted to all the questions except question no. 3 and 4 by 3rd MBBS students. This shows that there is a fair awareness of code of medical ethics in students of our college. Awareness can be increased by coaching ethics as a distinct subject which should be taught as a conventional sector during the formative years of under graduation. Skits, training classes, CME, workshops, conferences and other methods of education can be used to impart the topic commendably.

Reinforcing ethical thinking and judgment in decision making the work skill of multidisciplinary healing proficiency is the need of the hour. Hence enough hours should be given to medical ethics to stress the significance of ethical practice and to make the doctors confident enough to deal with the ethical dilemma by themselves and to adopt those in their day to day medical practice. There should be firm guidelines and penalties to those who unsettle the ethical practice. The regulatory body should be proactive to deal with ethical related matters and to monitor the doctors.

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

REFERENCES

1.Catherine Soanes (editor). The Compact Oxford Reference Dictionary. Oxford University Press; 2001[c16].
2.MCI. Indian Medical Council. Professional Conduct, Etiquette and Ethics Regulations, 2002. Gazette of India. 2002 Apr 6;Part-III-Sec-4.
3.Moser RH. A few thoughts about professionalism (editorial). South Med J. 2000; 93:1132-3.
4. Brennan T, Blank L, Cohen J, Kimball H, Smelser N, Copeland R, et al. Medical professionalism in the new millennium: a physician charter. Ann Intern Med [Internet].2002; 136:243-6.
5. Reddy C. Medical ethics. Indian J Dent Res [Internet]. 2007; 18:47.
6. Arun Babu T, Venkatesh C, Sharmila V . Are tomorrow's doctors aware of the code of medical ethics?. Indian Journal of Medical Ethics. 2013; 10: 192-194.
7. Chattopadhyay S. Teaching ethics in an unethical setting: "doing nothing" is neither good nor right. Indian J Med Ethics. 2009;6:93–6.
8. Brogen AS, Rajkumari B, Laishram J, Joy A. Knowledge and attitudes of doctors on medical ethics in a teaching hospital, Manipur. Indian J Med Ethics. 2009;6:194–7.
9. Gillon R. Medical ethics education. J Med Ethics 1987;13:115–6.
10. Ravindran GD. Medical ethics education in India. Indian J Med Ethics. 2008;5: 18–19.
11. Biswajit Chatterjee, Jhuma Sarkar. Awareness of medical ethics among undergraduates in a West Bengal medical college. Indian Journal of Medical Ethics. 2012; 9: 93-100.
12. M M Angadi, K J Shashank and Arun P Jose. A study to assess knowledge regarding medical ethics among undergraduates in Shri B M Patil Medical College, Bijapur, Karnataka. Int J Pharm Bio Sci .2014; 5: (B) 647 -53.

Proforma
Questionnaire
Sl.No:
Year of MBBS: II/III/IV
Sex: M/F
Age:
Tick the appropriate response after going through the questions.
1. Printing of one's photograph on the sign board of the consulting room is ethical?
Yes/No/Don't Know
2. A doctor may disallow the use of his patented discovery even if it benefits a large population. Is it ethical?
Yes/No/Don't Know
3. A doctor should not own an open pharmacy
Yes/No/Don't Know
4. A doctor can receive gifts from medical representatives
Yes/No/Don't Know
5. A doctor can prescribe a drug even if he does not know the exact composition
Yes/No/Don't Know
6. A doctor can conceal signs of domestic abuse in order to prevent marital discord
Yes/No/Don't Know
7. A doctor can perform mercy killing after discussing the matter with the relatives in hopeless cases
Yes/No/Don't Know
8. A doctor should maintain the records of his/her inpatients for 3 years
Yes/No/Don't Know
9. A doctor need not display his/her registration number in the prescription
Yes/No/Don't Know
10. A doctor can disclose secrets of a patient to his/her spouse if they face a serious and identified risk (eg: retroviral positive patients)
Yes/No/Don't Know

Insulin, Glucagon and Growth Hormone and CIMT in Glucose Intolerance

Glukoz İntoleransında İnsülin, Glukagon, Büyüme Hormonu ve Karotis İntima Media Kalınlığı

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ABSTRACT

Objective: There is an increasing evidence that glucagon and growth hormone (GH)-insulin-like growth factor (IGF) axis may play an important role in glucose metabolism since early stages of glucose intolerance. Carotid intima media thickness is a marker for subclinical atherosclerosis. We aimed to evaluate glucagon, GH and IGF-1 in prediabetic states and their relationship with carotid intima media thickness.
Methods: One hundred subjects underwent a 75 gr oral glucose tolerance test and were divided into 4 groups according to their state of glucose tolerance: (i) normal glucose tolerance (NGT)/Controls (n=21), (ii) impaired glucose tolerance (IGT) (n=35), (iii) impaired fasting glucose (IFG) (n=22), (iv) type 2 diabetes mellitus (n=22). Insulin, glucagon and GH were measured at 0, 60 and 120. minutes of OGTT and their area under the curve (AUC) were calculated. Fasting IGF-1 levels and carotid intima media thickness were determined in all participants.
Results: AUC for Glucagon was significantly higher in subjects with IGT, IFG and type 2 diabetes mellitus compared to NGT subjects. AUC for GH was significantly higher in subjects with IFG compared to subjects with IGT, type 2 diabetes mellitus and NGT. Plasma IGF-1 levels were significantly lower in subjects with abnormal glucose tolerance. CIMT was significantly higher in IFG group and CIMT was found to be negatively correlated with IGF-1 levels in subjects with IFG.
Conclusion: There are pathological alterations of glucagon, GH-IGF-1 and insulin in prediabetic stages. Among these alterations insulin resistance and IGF-1 are associated with CIMT. Further studies needed to investigate the role of treatments targeting insulin sensitivity will have an impact on the association between insulin and early atherogenesis

Key Words: Glucose intolerance, Insulin like Growth Factor-1, carotid intima media thickness, insulin resistance

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ÖZET

Amaç: Glukagonun ve Büyüme Hormonu-İnsülin benzeri büyüme faktörü-1 (IGF-1) aksının glukoz intoleransının erken evrelerinde itibaren glukoz metabolizmasında önemli rollere sahip olduklarını gösteren çalışmalar giderek artmaktadır. Karotis intima media kalınlığı subklinik aterosklerozun önemli bir belirtecidir. Bu çalışmanın amacı prediyabetik hastalarda glukagon, Büyüme hormonu ve IGF-1 düzeyleri ile karotis intima media kalınlığı arasındaki ilişkiyi değerlendirmektir.
Yöntem: Çalışmaya dahil edilen 100 hastaya 75 gr oral glukoz tolerans testi yapıldı ve sonuçlara göre hastalar dört gruba ayrıldı: (i) normal glukoz toleransı (NGT)/kontrol (n=21), (ii) Bozulmuş glukoz toleransı (BGT) (n=35), (iii) Bozulmuş açlık Glukozu (BAG) (n=22), (iv) tip 2 diabetes mellitus (n=22). Test sırasında 0,60 ve 120. Dakikalarda insülin, glukagon ve BH düzeyleri ve 0. dakikada IGF-1 düzeyi ölçüldü. İnsülin, glukagon ve BH için Eğri altında kalan alan hesaplandı. Hastaların karotis intima media kalınlıkları değerlendirildi.
Bulgular: Glukagon için hesaplanan eğri altında kalan alan glukoz intoleransı olan ve diyabetik hastalarda kontrol grubuna göre anlamlı yüksek bulundu. Büyüme hormonu için hesaplanan eğri altında kalan alan bozulmuş açlık glukozu olan hastalarda diğer gruplara göre anlamlı yüksek bulundu. Anormal glukoz toleransı olan hastalarda plazma IGF-1 düzeyleri kontrol grubuna anlamlı düşük bulundu. Karotis İntima media kalınlığı bozulmuş açlık glukozu olan hastalarda diğer gruplara göre anlamlı yüksek sapatndı ve bu hastalarda karotis intima media kalınlığının plazma IGF-1 düzeyleri ile negatif korelasyon gösterdiği görüldü.
Sonuç: Prediyabetik dönemde de glukagon, büyüme hormonu, IGF-1 ve insülin düzeylerinde patolojik değişikilikler olur. Bu değişikliklerden IGF-1 ve insülin direnci karotis intima media kalınlığı ile ilişkilidir. İnsülin direncinin tedavisi ile insülin ve erken aterogenez arasındaki ilişkiye etkisini araştıran ileri çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Glukoz İntoleransı, İnsülin benzeri Büyüme faktörü-1, Karotis intima media kalınlığı, insülin direnci

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INTRODUCTION

Type 2 Diabetes Mellitus (DM) is a heterogeneous syndrome with complex underlying pathophysiology, which involves concomitant impairment of insulin action and insulin secretion (1). There is growing evidence that type 2 diabetes mellitus may not be merely a beta cell dysfunction but also develops as a result of failure of all endocrine pancreas and dysregulation of other associated hormones. Growth hormone (GH), insulin-like growth factor-I (IGF-I) and glucagon may have roles in glucose homeostasis and in pathogenesis of type 2 diabetes mellitus (2,3). It is suggested that alterations of insulin, glucagon and IGF-1 begin in prediabetic stages.

Type 2 DM is associated with increased cardivascular morbidity and mortality(4). Impaired fasting Glucose (IFG) and impaired glucose tolerance (IGT) are also risk factors for cardiovascular events(5,6). Carotid intima media thickness (CMIT), a marker of subclinical atherogenesis and a predictor of cardiovascular events is increased in both diabetes and prediabetic stages. Beta cell dysfunction and insulin resistance is related with increased CMIT however little is known about the relationship between CMIT and glucagon and GH-IGF-1 axis.

In this study, we aimed to evaluate the alterations of insulin, glucagon and GH-IGF-1 axis in prediabetes and the relationship between these alterations and CMIT in prediabetes and DM.

MATERIAL and METHODS

Patient Population

A total of 100 subjects (83 females, 17 males; age range: 35-60 years) were included in the study. Subjects were recruited from outpatient clinics of Ankara University Hospital. All of the subjects were free of cardiovascular, pulmonary, renal, hepatic, or other major organ system disease as determined by history, physical examination, and routine laboratory tests. Patients treated with any drugs that might interfere with glucose metabolism were excluded from the study. The purpose and potential risks involved in the study were carefully explained to all subjects before obtaining their written voluntary consent. The study protocol was reviewed and approved by the Institutional Ethic Committee of the Ankara University. The study was conducted according to the declaration of Helsinki.

The demographic data and the anthropometric measures were obtained from all subjects. Body mass index (BMI) was calculated as weight in kilograms divided by the height in meters squared. Waist and hip circumferences were measured as the subjects standing. Blood pressure was measured twice in a sitting position after 15 minutes rest and the mean of two measurements was considered as the participant's blood pressure. Mean arterial pressure (MAP) was calculated with the use of the following formula: MAP = (2 ×DBP + SBP) / 3.

Measurement of biochemical parameters

All subjects participated in the study underwent 75 gr oral glucose tolerance test (OGTT). Based on the results of OGTT, the subjects were divided into four groups according to American Diabetes Association (ADA) recommendations 7: (i) Normal Glucose Tolerance (NGT) (served as the control group in this study) (n=21), (ii) Impaired Fasting Glucose (IFG) (n=22), (iii) Impaired Glucose Tolerance (IGT) (n=35) and (iv) type 2 diabetes mellitus (n=22). Before the OGTT, a small polyethylene catheter was placed into an antecubital vein and blood samples were collected at 0, 60, and 120 minutes for the measurement of plasma glucose, insulin, glucagon and GH concentrations. Fasting venous blood samples were also obtained for measurement of serum lipids and IGF-1. Samples for analysis of glucagon, GH and IGF-1 were centrifuged immediately and were stored at -20°C for further analysis. Plasma glucose concentration was determined by the glucose hexokinase method (Roche Diagnostics). Plasma glucagon levels were measured by a radioimmunoassay (Linco Research, St Louis, MO). Plasma insulin, GH and IGF-1 levels were measured by double immunoradiometric assay using reagents from Immunotech (Immunotech, Prague, Czech Republic). The lipid parameters (total cholesterol, HDL cholesterol, triglycerides) were measured by standardized methods using autoanalysers. LDL cholesterol levels were calculated according to the formula described by Friedewalds et al 8. Insulin resistance was estimated by using Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), calculated from fasting glucose and insulin concentrations according to following formula: HOMA-IR=(glucose [mg/dl] x insulin[mU/l])/405 9 .

Measurement of Carotid Intima Media Thickness

Carotid Intima Media Thickness (CIMT) was evaluated by a single operator using General Electric Logic 400 Doppler Ultrasound machine and a 12 MHz linear probe. Subjects were examined in the supine position. Each scan of the common carotid artery began just above the clavicle, and the transducer was moved cephalad through the bifurcation and along the internal carotid artery. The near and distant walls of the common carotid artery, the carotid bulb, and the internal carotid artery were scanned for the presence of atherosclerotic plaques. Three segments were identified on each side: 1.0 centimeters (cm) distal to the common carotid artery proximal to the bifurcation, the bifurcation itself and 1.0 cm proximal to the internal carotid artery. At each of the three segments, for distant walls in the left and right carotid arteries, intima-media thickness was defined as the distance between the leading edge of the lumen-intima interface and the leading edge of the media-adventitia interface. Maximum thickness of the wall was calculated at each side. The reported CIMT for each subject is the average of these 10 measurements of distant walls (5 measurements from the right and 5 from the left carotid artery). The investigator (RE) performed all the ultrasonographic examinations blinded to the knowledge of the group the subjects belonged.

Statistical Analysis

All analyses were performed using SPSS software program version 11.5 for windows. Continuous variables are expressed as means ± SD, non-normally distributed variables are expressed as median (ranges). The ANOVA test was applied for normally distributed continuous data and the Kruskal Wallis test was applied to non-normally distributed continuous data. In case of $p<0.05$ between groups, multiple comparison test was used. The Chi Square test was performed for categorical data. The values of the area under the concentration-time curve for insulin, glucagon and GH were calculated by means of trapezoidal rule. For all analyses, a $p<0.05$ value was considered to be statistically significant.

RESULTS

The groups were similar for age, sex, BMI, waist circumference and mean BP (Table 1). There were no significant differences among groups in terms of mean serum levels of Total Cholesterol, LDL Cholesterol, HDL Cholesterol and triglycerides (Table 1). HOMA-IR was significantly higher in subjects with IFG, IGT and type 2 diabetes mellitus compared to control group ($p<0.01$) (Table 1).

The values of area under the concentration-time curve for insulin, glucagon and GH between time 0 and 120 were calculated. Plasma insulin excursion after glucose load in subjects and area under the curve (AUC) values are shown in Figure 1. Area under the curve calculated for insulin was significantly higher in subjects with IFG compared to IGT ($p=0.03$), type 2 diabetes mellitus ($p=0.01$) and control group ($p=0.002$). There was a significant difference between subjects with IGT and NGT ($p=0.03$) while there was no difference between diabetic subjects and NGT ($p=0.515$). Area under the curve calculated for glucagon was significantly higher in subjects with IGT ($p<0.001$), IFG ($p<0.001$) and type 2 diabetes mellitus ($p=0.003$) when compared with NGT subjects. There was no difference between IGT, IFG and diabetic group (Figure 1). Area under curve calculated for GH was significantly higher in subjects with IFG compared to subjects with IGT ($p=0.018$), type 2 diabetes mellitus ($p=0.014$) and NGT ($p=0.004$) (Figure 1).

Table 1 Anthropometric and biochemical characteristics of participants

	IGT (n=35)	IFG (n=22)	T2DM (n=22)	Control (n=21)	p
Age (yrs) †	51.3±6.9	53.9±8.4	53.7±7.2	48.8±7.6	0.065
Sex(F/M)	30/5	19/3	16/6	18/3	0.927
BMI (kg/m²) †	31.0±0.6	33.9±1.9	31.3±1.2	30.5±1.2	0.271
Waist Circumference(cm) †	101.6±10.7	107.6±16.6	102.5±11.1	99.5±15.4	0.215
Mean BP (mmHg) †	99.0±12.0	101.3±13.8	93.0±9.0	94.0±13.3	0.137
Glucose (mg/dl) ‡	93(19)	107(12)	117(12)	91(8)	<0.001[a]
T. Cholesterol (mg/dl) †	202.4±33.0	211.7±48.1	198.7±42.2	193.3±31.4	0.684
LDL (mg/dl) †	121.6±24.9	128.6±40.9	121.1±32.5	116.2±32.5	0.821
HDL (mg/dl) †	45.6±9.8	50.3±9.7	47.7±9.8	50.9±14.3	0.313
Triglycerides (mg/dl) †	179.9±90.8	166.6±74.7	148.7±70.8	130.6±66.4	0.091
IGF-1 (ng/ml) ‡	103(91)	86 (50)	107 (70)	182 (177)	0.020[a]
HOMA-IR‡	3.4(3.1)	4.1(2.7)	3.6(3.5)	2.1(2.0)	0.002[a]
CIMT‡	0.73(0.11)	0.81(0.14)	0.76(0.14)	0.69(0.10)	0.003[b]
Insulin (µU/ml) ‡	14.5 (13.4)	16.1 (12.8)	12.7 (12.4)	9.3 (10.3)	0.065
Glukagon (pg/ml) ‡	74.5 (26.5)	69.0 (19.0)	64.5 (11.5)	52 (12.0)	0.005[a]
GH (ng/ml) ‡	0.13 (0.21)	0.27 (0.28)	0.13 (0.15)	0.10 (0.34)	0.061

† Data are mean±SD
‡ Date are median (interquartil range)
[a]Control vs IGT.IFG and T2DM
[b]Control vs IFG
BP:Blood Pressure. IGT: Impaired Glucose Tolerance. IFG:Impaired Fasting Glucose. T2DM: Type 2 Diabetes Mellitus LDL: Low Density Lipoprotein. HDL: High Density Lipoprotein CRP:C-Reactive Protein. HOMA-IR: Homeostasis Model Assessment for Insulin Resistance CMIT: Carotid Intima Media Thickness

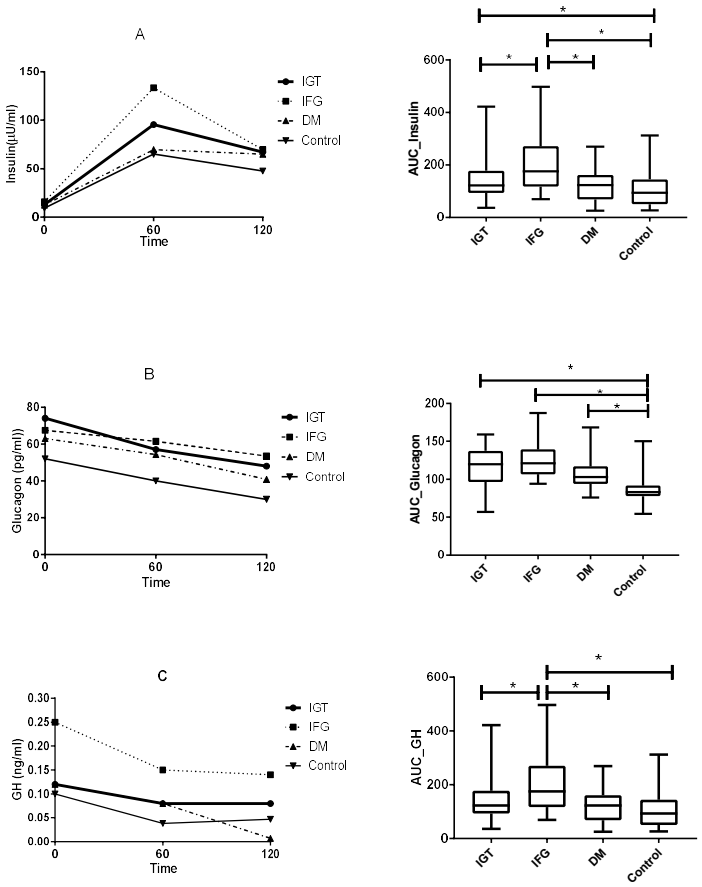

Figure 1. Plasma Insulin (A), Glucagon (B) and Growth Hormone (C) excursion after oral glucose load and area under curve graphics. *p<0,05

Median plasma IGF-1 level was 103 ng/ml (91) for IGT group, 86 ng/ml (50) for IFG group, 107 ng/ml (70) for type 2 diabetes mellitus group, and 182 ng/ml (177) for control group. Plasma IGF-1 levels were significantly lower (p=0.020) in subjects those with abnormal glucose tolerance compared to subjects in control group.

Median CIMT was 0.73 (0.11) mm for IGT group, 0.81 (0.14) mm for IFG group, 0.76 (0.14) mm for type 2 diabetes mellitus group and 0.69 (0.10) mm for control group. While CIMT was significantly higher in subjects with IFG compared to control group (p=0.003), there was no difference in CIMT among subjects with IGT, control and type 2 diabetes mellitus. There was a positive correlation between CMIT and glucose (r=0.304; p=0.005) and HOMA-IR (r=0.308;p=0.005) and a negative correlation between IGF-1 and CMIT (r=-0.330; p=0.003) among all subjects. There was a significant negative correlation between IGF-1 levels and CIMT in subjects with IFG, IGT and DM (p=0.01 r=-0.332). CMIT is not correlated with AUC Insulin (r=0.167;p=0.138); AUC Glucagon (r=0.160;p=0.168) and AUC GH (r=0.124;p=0.265).

DISCUSSION

In this study, subjects with both impaired glucose tolerance, impaired fasting glucose and type 2 diabetes mellitus were relatively hyperglucagonemic compared to normal glucose tolerant subjects. Plasma glucagon concentrations are inappropriately elevated in diabetic individuals, and α-cell suppression by hyperglycaemia is blunted (10). Although dysregulated glucagon secretion and elevated glucagon levels are known to be associated with high blood glucose in type 2 diabetes mellitus(11), these conditions are usually considered a consequence instead of a cause of diabetes, and it remains controversial whether glucagon, plays an active role in diabetes development (12). Our data suggest that combined decreased insulin secretion and increased insulin resistance may not be sufficient to induce hyperglycaemia without dysregulated glucagon secretion, and thus reveal a previously underappreciated role of the regulation of glucagon secretion in the development of diabetes. Increased glucagon secretion may also promote diabetes development at prediabetic stages such as impaired glucose tolerance and impaired fasting glucose. There was no significant correlation between AUC glucagon and CIMT in our study. There is little known about the relationship between glucagon and atherosclerosis in diabetic patients. However studies suggest that Glucagon like peptide-1 analogs which suppresses glucagon secretion have cardioprotective role independent of their glucose lowering effects(13). Further studies needed in this era.

In our study, we showed a negative correlation between serum IGF-1 levels and CIMT and positive correlation between HOMA-IR and CIMT among all participants. Observational studies investigating associations between IGF1 and its binding proteins (IGFBPs) with CIMT have yielded inconsistent results. Both increased and decreased levels of IGF-1 was found to be associated with thickening of carotid intima media (14-18). Previous analysis by Martin et al. revealed no association between IGF-1 and IMT(19). There are IGF-1 receptors on human endothelial cells and IGF-1 is potent mitogen and antiapoptotic factor for vascular smooth muscles(20). In early stages of atherosclerosis decrease of IGF-1 levels might be beneficial but in advanced stages IGF-1 has an important role on plaque stability(21). This dual role of IGF-1 on atherosclerosis may explain the negative correlation between CIMT and IGF-1 in our study. All participants in our study were obese. Obesity is characterized with low IGF-1 levels(22). This might be another explanation of low IGF-1 levels of our patients.

One important result of our study was positive correlation between HOMA-IR and CIMT. This is an agreement with a recent study that has shown that patients with higher CIMT had lower insulin sensitivity(23). Roussel et al. showed that insulin secretion is associated with early atherosclerosis in non diabetic individuals(24). Insulin receptors are expressed both on endothelial cells and vascular smooth muscle. Insulin signaling pathways in vascular endothelium stimulates vasodilatator NO and also stimulates vasoconstrictor ET-1(25). Inappropriate insulin secretion and action may triggers an imbalance between these signaling pathways in the endothelium and may cause subclinical atherosclerosis.

Plasma IGF-1 levels were significantly lower in subjects those with abnormal glucose tolerance compared to subjects with NGT. Our data is consistent with a previous study by Sesti et al. who found a significant positive correlation between insulin sensitivity and endogenous IGF-I concentration among patients with varying degrees of glucose intolerance(26). There is considerable evidence suggesting that IGF-1 has insulin-like effects on peripheral uptake of glucose and fatty acids(27). Obesity, an insulin resistance state and a strong risk factor for type 2 DM, was associated with altered IGF-1 levels. Taken together with our present data suggests that IGF-1 may have role in pathogenesis of type 2 DM.

Area under curve calculated for GH was significantly higher in subjects with IFG compared to subjects with IGT, type 2 diabetes mellitus and NGT. We also found that the patients with IGT had the lowest IGF-1 levels compared to other groups. This may be the result of inadequate negative feedback of low plasma IGF-I concentrations at the level of the hypothalamus and/or pituitary, thus resulting in growth hormone (GH) hypersecretion and a decrease in insulin sensitivity.

Our study has some limitations. We only measured total IGF-1 and not the free fraction, which is the form that interacts with IGF-1 receptor and is responsible for the peripheral effect. Relatively small sample size and cross sectional design are also the limitations of the study.

As a conclusion, there are pathological alterations of glucagon, GH-IGF-1 and insulin in prediabetic stages. Among these alterations insulin resistance and IGF-1 are associated with CIMT. Further studies needed to investigate the role of treatments targeting insulin sensitivity will have an impact on this association between insulin and early atherogenesis.

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Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1.Del Prato S, Marchetti P. Beta- and alpha-cell dysfunction in type 2 diabetes. Horm Metab Res 2004;36:775-81.

2.DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am 2004;88:787-835, ix.

3.Holt RI, Simpson HL, Sonksen PH. The role of the growth hormone-insulin-like growth factor axis in glucose homeostasis. Diabet Med 2003;20:3-15.

4.Diabetes mellitus: a major risk factor for cardiovascular disease. A joint editorial statement by the American Diabetes Association; The National Heart, Lung, and Blood Institute; The Juvenile Diabetes Foundation International; The National Institute of Diabetes and Digestive and Kidney Diseases; and The American Heart Association. Circulation 1999;100:1132-3.

5.Karbek B, Cakal E, Cakir E, et al. Cardiovascular risk factors, carotid artery intima media thickness, and HSCRP levels in patients with impaired glucose metabolism. Minerva Endocrinol 2013;38:297-304.

6.Shin JY, Lee HR, Lee DC. Increased arterial stiffness in healthy subjects with high-normal glucose levels and in subjects with pre-diabetes. Cardiovasc Diabetol 2011;10:30.

7.American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care 2013;36 Suppl 1:S67-74.

8.Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry 1972;18:499-502.

9.Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

10. Spellman CW. Pathophysiology of type 2 diabetes: targeting islet cell dysfunction. J Am Osteopath Assoc 2010;110:S2-7.

11. Virally M, Blickle JF, Girard J, Halimi S, Simon D, Guillausseau PJ. Type 2 diabetes mellitus: epidemiology, pathophysiology, unmet needs and therapeutical perspectives. Diabetes Metab 2007;33:231-44.

12. Dunning BE, Gerich JE. The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. Endocr Rev 2007;28:253-83.

13. Sarraju A, Kim SH, Knowles JW. Cardiometabolic Effects of Glucagon-Like Peptide-1 Agonists. Curr Atheroscler Rep 2016;18:7.

14. Colao A, Spiezia S, Di Somma C, et al. Circulating insulin-like growth factor-I levels are correlated with the atherosclerotic profile in healthy subjects independently of age. J Endocrinol Invest 2005;28:440-8.

15. Laughlin GA, Barrett-Connor E, Criqui MH, Kritz-Silverstein D. The prospective association of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-1 levels with all cause and cardiovascular disease mortality in older adults: the Rancho Bernardo Study. The Journal of clinical endocrinology and metabolism 2004;89:114-20.

16. Denti L, Annoni V, Cattadori E, et al. Insulin-like growth factor 1 as a predictor of ischemic stroke outcome in the elderly. The American journal of medicine 2004;117:312-7.

17. Spilcke-Liss E, Friedrich N, Dorr M, et al. Serum Insulin-like Growth Factor-I and its Binding Protein 3 in their Relation to Intima Media Thickness: Results of the Study of Health in Pomerania (SHIP). Clinical endocrinology 2011.

18. Sirbu A, Nicolae H, Martin S, et al. IGF-1 and Insulin Resistance Are Major Determinants of Common Carotid Artery Thickness in Morbidly Obese Young Patients. Angiology 2016;67:259-65.

19. Martin RM, Gunnell D, Whitley E, et al. Associations of insulin-like growth factor (IGF)-I, IGF-II, IGF binding protein (IGFBP)-2 and IGFBP-3 with ultrasound measures of atherosclerosis and plaque stability in an older adult population. The Journal of clinical endocrinology and metabolism 2008;93:1331-8.

20. Arnqvist HJ, Bornfeldt KE, Chen Y, Lindstrom T. The insulin-like growth factor system in vascular smooth muscle: interaction with insulin and growth factors. Metabolism 1995;44:58-66.

21. Wang J, Razuvaev A, Folkersen L, et al. The expression of IGFs and IGF binding proteins in human carotid atherosclerosis, and the possible role of IGF binding protein-1 in the regulation of smooth muscle cell proliferation. Atherosclerosis 2012;220:102-9.

22. Berryman DE, Glad CA, List EO, Johannsson G. The GH/IGF-1 axis in obesity: pathophysiology and therapeutic considerations. Nat Rev Endocrinol 2013;9:346-56.

23. Andreozzi F, Gastaldelli A, Mannino GC, et al. Increased carotid intima-media thickness in the physiologic range is associated with impaired postprandial glucose metabolism, insulin resistance and beta cell dysfunction. Atherosclerosis 2013;229:277-81.

24. Roussel R, Natali A, Balkau B, et al. Beta-cell function is associated with carotid intima-media thickness independently of insulin resistance in healthy individuals. J Hypertens 2016;34:685-91.

25. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006;113:1888-904.

26. Sesti G, Sciacqua A, Cardellini M, et al. Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. Diabetes Care 2005;28:120-5.

27. Rajpathak SN, Gunter MJ, Wylie-Rosett J, et al. The role of insulin-like growth factor-I and its binding proteins in glucose homeostasis and type 2 diabetes. Diabetes Metab Res Rev 2009;25:3-12.

Effects of Picroside II on Myocardial Ischemia-Reperfusion Injury in Streptozotocin-Induced Diabetic Rats

Streptozosin ile Diyabet Oluşturulan Ratlarda Pikrozid II'nin Miyokard İskemi Reperfüzyon Hasarı Üzerine Etkisi

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ABSTRACT

Objective: Diabates mellitus, is a chronic metabolic disorder accompanied by an increase in oxidative stress. Ischemia-reperfusion injury is a cascade of events initiated by tissue ischemia. The cellular damage produced by reperfusion leads to an active inflammatory response. This study was performed to investigate the effect of picroside II on myocardial ischemia-reperfusion injury in rats with streptozotocin-induced diabetes.
Methods: Animals were equally (n:6) divided for five groups as follows; Control (C), diabetes [D], diabetes+picroside II [DP], diabetes+I/R [DIR], and diabetes+I/R+ picroside II [DIRP]. In DIR group, a left anterior descending artery branch was occluded for 60 minutes, the reperfused for 120 minutes. In DIRP group, picroside II was administrated via 10 mg/kg intraperitoneal route 30 minutes before ligating the left anterior descending artery. At the end of the study, myocardial tissues were taken for total oxidant status and total antioxidant status level determinations.
Results: Total oxidant status levels were significantly higher in DIR group, when compared with C, DP, and DIRP groups (*p*:0.001, *p:0.*019, and *p*:0.031, respectively). Total antioxidant status levels were significantly higher in DIR group, when compared with C, DP, and DIRP groups (*p*:0.006, *p*:0.024, and *p:0.*007, respectively).
Conclusion: These results indicate that administration of picroside II may have protective effects against I/R injury.

Key Words: Ischemia-reperfusion, total oxidant status, total antioxidant status, picroside II, myocardial tissue, rat

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ÖZET

Amaç: Diyabetes mellitus, oksidatif stres artışının eşlik ettiği kronik metabolik bir hastalıktır. İskemi-reperfüzyon (İ/R) hasarı doku iskemisi tarafından başlatılan olayların bir kaskadıdır. Reperfüzyon sonucunda oluşan hücre hasarı inflamatuar yanıtı aktive eder. Bu çalışma, Streptozosin kaynaklı diyabeti olan ratlarda miyokard İ/R hasarı üzerine Pikrozid II'nin etkisini araştırmak amacıyla yapıldı.
Yöntem: Hayvanlar, beş gruba (n=6) olacak şekilde ayrıldı; Kontrol grubu (K), Diyabet grubu [D], Diyabet + Pikrozid II grubu [DP], Diyabet + İ/R grubu [DİR] ve Diyabet + İ/R + Pikrozid II grubu [DİRP]. DİR grubunda, sol ön inen arter dalı iskemi amaçlı 60 dakika süre ile kapatılmış ardından reperfüzyon için akım sağlanarak 120 dakika beklenmiştir. DİRP grubuna sol ön inen arter dalı kapatılmadan 30 dakika önce Pikrozid II, 10 mg/kg olmak üzere intraperitoneal olarak verildi. Çalışmanın sonunda, miyokard doku örnekleri total oksidan durum ve total antioksidan durum seviyesinin ölçülmesi için alındı.
Bulgular: Toplam oksidan durum seviyeleri DİR grubunda, diğer gruplarla karşılaştırıldığında (K, DP, DİRP) anlamlı yüksek bulunmuştur (sırasıyla p:0.001, p:0.019, ve p:0.031). Total antioksidan durum seviyeleri DİR grubunda, diğer gruplarla karşılaştırıldığında (K, DP, DİRP) anlamlı olarak yüksek bulunmuştur (sırasıyla p:0.006, p:0.024, ve p:0.007).
Sonuç: Bu bulgular Pikrozid II'nin İ/R hasarına karşı koruyucu etkiye sahip olabileceğini göstermektedir.

Anahtar Sözcükler: İskemi-reperfüzyon, total oksidan seviye, total antioksidan seviye, pikrozid II, miyokard dokusu, rat

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INTRODUCTION

Ischemic heart disease is a leading cause of morbidity and mortality worldwide (1). Oxygen-derived free radicals are important agents of tissue injury during ischemia and reperfusion (2).

In diabetic patients and diabetic rats studies have shown that, oxygen free radicals and lipid peroxidation are significantly increased, and oxidative stress is an important agent of the etiology and progression of diabetes (3).

Picrorhiza scrophulariiflora belongs to the plant family, Scrophulariaceae. The roots of this plant are beneficial to health and often used in traditional Chinese medicine to treat a number of conditions, including dyspepsia, chronic diarrhea, and upper respiratory ailments (4). Numerous published studies have shown that picroside II has a wide range of pharmacological effects, including, antioxidant (5-7)**,** anticarcinogenic (8), and immune modulating activities (9).

The aim of the present study was to examine the potential protective effects of picroside II on myocardial ischemia-reperfusion (I/R) in a diabetic rat model, using biochemical aspects.

MATERIALS and METHODS

Experimental Groups

A total of 30 adult Wistar-albino rats, weighing between 210 to 300 g were used in this study. The present study was approved by the Gazi University Institutional Local Animal Care and Use Committee. All animals received humane care, in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and the Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institutes of Health (NIH publication no. 85–23, revised in 1985). Rats were housed in cages at an average temperature of 22°C in a light-dark cycle-controlled environment with free access to food and tap water.

Study Design

Animals were equally (n:6) divided for five groups as follows; Control (C), diabetes [D], diabetes+ picroside II [DP], diabetes+I/R [DIR], and diabetes+I/R+ picroside II [DIRP]). The rats were kept alive for four weeks after the streptozotocin injection to allow the development of chronic diabetes before they were exposed to I/R. Picroside II (i.p) (Sigma Aldrich Co. Ltd. [CAS No: 39012-20-9, purity greater than 98%, molecular formula: $C_{23}H_{28}O_{13}$]) was administered via 10 mg/kg 30 minutes before ligating the left anterior descending artery to the DIRP group. A small plastic snare was threaded through the ligature and placed in contact with the heart. The artery could then be occluded by applying tension to the ligature for 60 minutes, then reperfusion was achieved by releasing the tension for 120 minutes. However, after the above procedure, the coronary artery was not occluded or reperfused in the C, DC, or DP rats. At the end of the reperfusion period, all rats were sacrified under anesthesia, and myocardium was taken for biochemical analyses.

Diabetes was performed with streptozotocin (Sigma Chemical, St. Louis, MO, USA) by giving a single doses of 55 mg/kg intraperitoneally (i.p). 72 hours after the injection the blood glucose levels were measured. If the blood glucose levels exceed 250 mg/dL, then we said the rats become diabetic. 100 mg/kg (i.p) of ketamine were administered to the rats for anesthesia. The trachea was cannulated for artificial respiration. The chest was shaved, and each animal was fixed in a supine position on the operating table. The chest was opened by a left thoracotomy, followed by sectioning the fourth and fifth ribs about 2 mm to the left of the sternum. Positive-pressure artificial respiration was started immediately with room air, using a volume of 1.5 mL/100 g body weight, at a rate of 60 strokes/min. Sodium heparin (500 IU/kg) was administered through the peripheral vein from the tail. The heart was exteriorized with gentle pressure on the right side of the rib cage after the pericardium was incised. An 8/0 silk suture attached to a 10 mm micropoint reverse-cutting needle was quickly placed under the left main coronary artery. The heart was then carefully replaced in the chest and the animal was allowed to recover for 20 minutes.

Biochemical Examination

The heart tissue was collected into a sterile microcentrifuge tube and kept at -80°C until being analyzed for total antioxidant/oxidant status and oxidative stress index. The sample was removed from the microcentrifuge tube and dissolution without allowing tissue left quickly weighed 80 to100 mg using a No. 22 surgical scalpel. These tissue pieces were crushed in liquid nitrogen in a porcelain bowl. The powdered tissue was transferred to the homogenization tube, and for every gram of tissue, the dilution of 1/10 140 mM KCl solution was added. Maintaining homogenization in the homogenization tube, a glass beaker full of snow was used to avoid raising the temperature, and the homogenization rocess was complete in two minutes at 50 rpm in a speed homogenizer. After homogenization, the microcentrifuge tubes were covered with Parafilm and then centrifuged for 10 minutes at 3,000 rpm. After centrifugation, the supernatant was put into another microcentrifuge tube for measurement of total oxidant status (TOS) and total antioxidant status (TAS).

Measurement of myocardial tissue TOS

Myocardial tissue TOS levels were determined using a commercially available kit, developed by Erel (10) (REL Assay Diagnostics, Mega Tip, Gaziantep, Turkey). In this method, the oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ions. Glycerol molecules, which are abundantly present in the reaction medium, enhance the oxidation reaction. The ferric ions produces a colored complex with Xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed as μmol H_2O_2 equivalent/L. Hydrogen peroxide and other derivatives of peroxides, produced physiologically in organisms and occurring in higher concentrations under some pathologic conditions, diffuse into plasma. The level of total peroxide was measured and expressed as TOS in this study.

Measurement of myocardial tissue TAS

Myocardial tissue TAS levels were determined using a commercially available kit developed by Erel (REL assay diagnostics, Mega Tip, Gaziantep, Turkey) (11). In this method, hydroxyl radical, which is the most potent radical, is produced via a Fenton reaction. In the classical Fenton reaction, the hydroxyl radical is produced by mixing a ferrous ion solution and a hydrogen peroxide solution. In the most recently developed assay by Erel, the same reaction is used. In the assay, a ferrous ion solution, which is present in Reagent 1, is mixed with hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals, such as brown-colored dianisidinyl radical cation produced by the hydroxyl radical, are also potent radicals. In this assay, we measured the antioxidative effect of the sample against the potent free radical reactions initiated by the hydroxyl radical. The assay has excellent precision values, lower than 3%. The results are expressed as mmol Trolox equivalents.

Statistical Analyses

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 20.0 program was used for statistical analyses. The Kolmogorov-Smirnov test was used for the comparisons to determine the distribution of all variable groups. We assessed the variations in TOS and TAS levels by using the Kruskal-Wallis test. The Bonferroni-adjusted Mann-Whitney U test was used after the Kruskal-Wallis test to determine which group differs from the others. Results were expressed as mean ± standard deviation (mean ± SD). P values less than 0.05 were considered as statistically significant.

RESULTS

There was a statistically significant difference between the groups when they were compared among themselves by means of TOS levels in myocardial tissue (*p*: 0.026). TOS levels were significantly higher in DIR group when compared with C, DP, and DIRP groups (*p*: 0.001, *p*: 0.019, and *p*: 0.031, respectively). In addition, the DC groups TOS enzyme activity was significantly higher than the C groups (*p*: 0.023) (Figure 1).

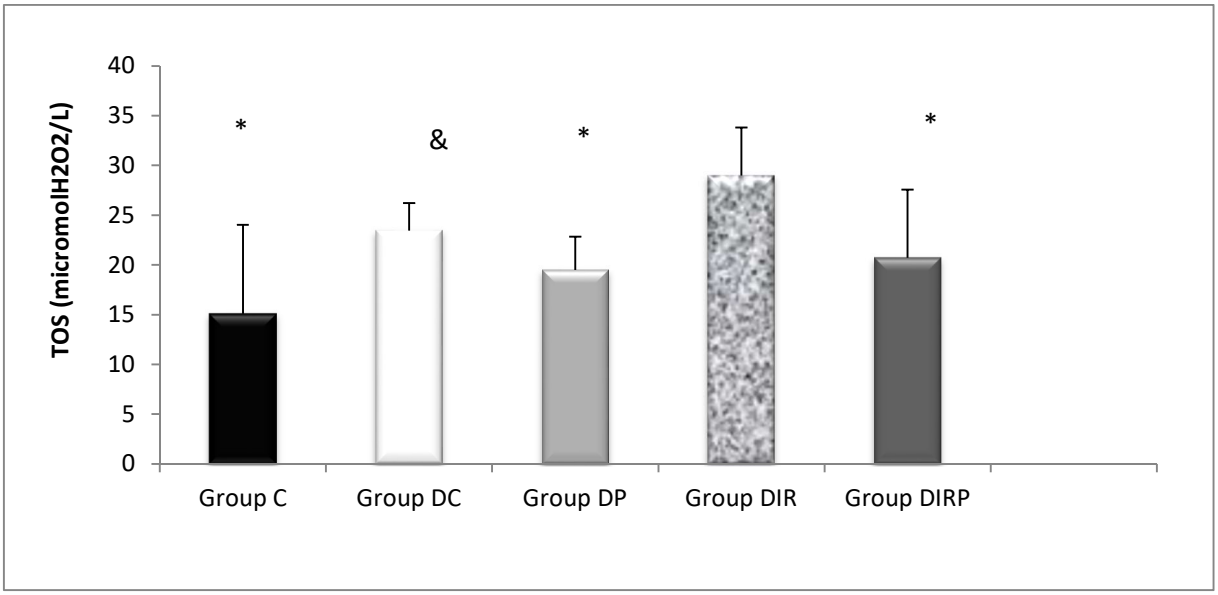

Figure 1. Myocardial tissue total oxidant status (TOS) level [mean ± SD)]
* $p < .05$ compared to Group DIR and & $p < .05$ compared to Group C

A statistically significant difference was found among the groups when they were compared among themselves for TAS levels in myocardial tissue (*p*: 0.012). TAS levels were significantly higher in DIR group when compared with C, DP, and DIRP groups (*p*: 0.006, *p*: 0.024, and *p*: 0.007, respectively). In addition, TAS enzyme activity of DC groups was significantly higher than the C groups activity (*p*: 0.032) (Figure 2). TAS and TOS levels were shown in Table 1.

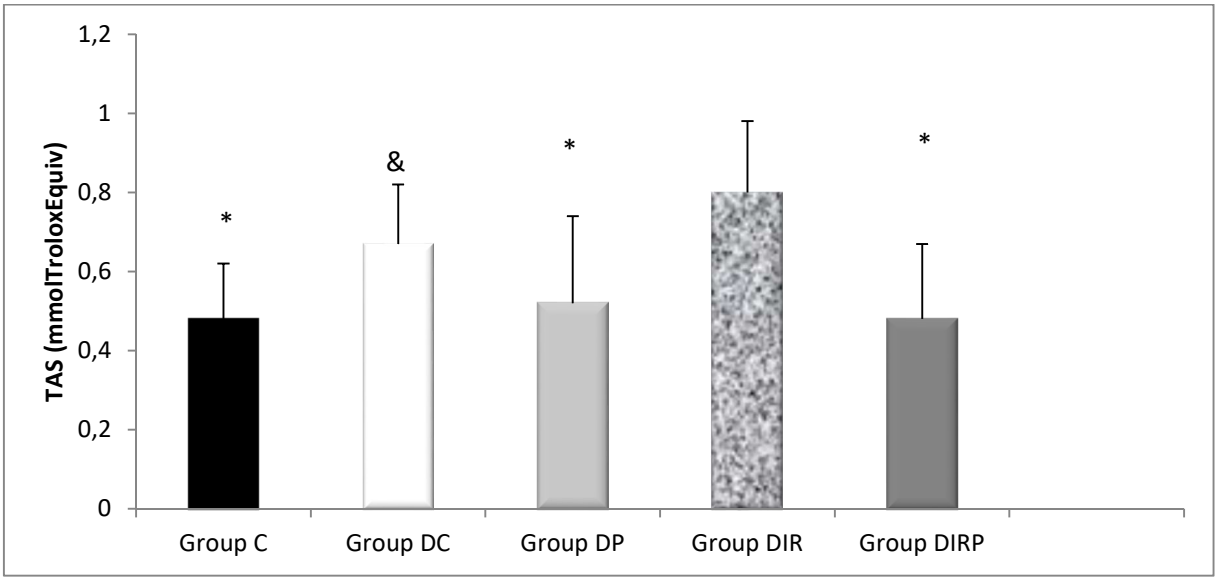

Figure 2. Myocardial tissue total antioxidant status (TAS) level [mean ± SD)]
* $p < .05$ compared to Group DIR and & $p < .05$ compared to Group C

Table 1. Total oxidant status (TOS) and total antioxidant status (TAS) levels of the study groups. [Mean ± SD)]

	Group C (*n* = 6)	**Group DC (*n* = 6)**	**Group DP (*n* = 6)**	**Group DIR (*n* = 6)**	**Group DIRP (*n* = 6)**	***P*****
TOS (µmol H_2O_2 /L**)**	15.08 ± 8.95*	23.4 5 ± 2.76&	19.48 ± 3.37*	28.98 ± 4.83	20.72 ± 6.86*	0.026
TAS (mmol TroloxEquiv)	0.48 ± 0.14*	0.67 ± 0.15&	0.52 ± 0.22*	0.80 ± 0.18	0.48 ± 0.19*	0.012

*P***: $p < .05$ is considered to be significant using Kruskal-Wallis test.
* $p < .05$ compared to the Group DIR and &$p < .05$ compared to Group C

DISCUSSION

Jennings and colleagues (12), were decribe the I/R injury in 1960. At that time the study of reperfusion injury has become significant to various studies had been done on the cerebrovascular, hepatic, renal, and cardiovascular systems.

Reactive oxygen species (ROS) generation, intracellular calcium overload, adenosine triphosphate depletion, myocardial apoptosis, and endothelial dysfunction are all considered the end results of an I/R cascade (13, 14). The disclosure of these mechanisms of several drugs has yielded encouraging results in animals and a few have been tested in humans; however, none of these modalities has been widely accepted (15, 16).

In this study, we examined the effect of picroside II on I/R injury in myocardial streptozotocin-induced diabetic rats and in the control group, with the relationship between oxidant and antioxidant effects of picroside II. The study reflects total antioxidant protection against the attacks of free radicals in the organism (TAS) and the total value of oxidative stress (TOS) markers used.

Restoration of the blood supply to the ischemic tissue results in ROS generation. Excessive ROS production causes lipid peroxidation in cell membranes and oxidative damage to DNA and proteins (17). A number of agents, such as levosimendan and dexmedetomidine, have been proposed as useful against I/R-induced myocardial injury (18, 19).

Picroside II, an iridoid glycoside, has been demonstrated to have multiple pharmacologic actions, including decreasing oxidative stress, inhibiting apoptosis, and downregulating the expression of related inflammatory factors (20, 21). Studies have also observed the kidney and myocardial protective effect of picrocide II by decreasing oxidative stress and downregulating the expression of related inflammatory factors (22, 23).

In two publications (24, 25), a reversible and dose-related inhibition of oxygen production (by neutrophils) was demonstrated in animal models of ischemic myocardial damage in the presence of iodine. In addition, iodine has proven to significantly decrease malondialdehyde (MDA) in animal models of abdominal aortic I/R (26), induce liver peroxidation (27), and reduce hydrogen peroxide-induced pathological glaucomatous changes in cultured cells (28).

Proinflammatory cytokines, such as tumor necrosis factor-alpha and interleukin-1 beta, and adhesion molecule, such as the intercellular adhesion molecule-1 (ICAM-1 mRNA), levels were reduced with picroside II (22). In another study of the effect of picroside II on I/R damaged models, MDA in serum decreased and superoxide dismutase with glutathione peroxidase increased (23, 29).

In this study, we used a novel measurement method to evaluate the extent of oxidative stress in rat myocardium after I/R. This provides a useful method for the rapid evaluation of TAS and TOS, valuable parameters in conditions involving oxidative stress. TOS indicates the total oxidative products in tissue. Oxidative products such as ROS, reactive nitrogen species, hydrochloric acid, MDA, and lipid peroxides constitute TOS (6). In our study, TOS levels significantly increased after myocardial I/R. We also found that I/R+picroside II significantly reduced TOS levels. TAS levels significantly increased in Group DIR. Our findings are consistent with previous papers reporting the antioxidant effects of picroside II on animal models of organ injury induced by myocardial I/R (22, 23). The mechanism of protective effect of picroside II against myocardial injury cannot be explained only by its antioxidative effect, since I/R injury is a complex process. We have hypothesized that to some extent it may also have an antioxidative effect on myocardial injury. Our findings need to be supported by further studies evaluating different oxidative parameters.

CONCLUSION

Biochemical findings of this study demonstrate that, administration of picroside II may have protective effects, against myocardial injury induced by left anterior descending artery I/R injury and encourage us to investigate this agent in different dosage strategies with alternate administration protocols. Further studies evaluating histological and other biochemical parameters are required to confirm our findings and to elucidate the exact mechanisms of action before clinical use.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006; 3: e442.
2. Hensley K, Robinson KA, Gabbita SP, et al. Reactive oxygen species, cell signaling, and cell injury. Free Radic Biol Med 2000; 15;28: 1456-62.
3. Pitkanen OM, Martin JM, Hallman M, et al. Free radical activity during development of insulin dependent diabetes mellitus in the rat. Life Science 1992; 50: 335-9.
4. Bhat WW, Dhar N, Razdan S, et al. Molecular characterization of UGT94F2 and UGT86C4, two glycosyltransferases from Picrorhiza kurrooa: comparative structural insight and evaluation of substrate recognition. PLoS ONE 2013; 8 e73804.
5. Sood H, Chauhan RS. Biosynthesis and accumulation of a medicinal compound, Picroside-I, in cultures of *Picrorhiza kurroa* Royle ex Benth. Plant Cell Tiss Organ Cult 2010; 100: 113–7.
6. Ansari RA, Aswal BS, Chander R, et al. Hepatoprotective activity of kutkin—the iridoid glycoside mixture of Picrorhiza kurroa. Indian Journal of Medical Research. 1988; 87: 401–4.
7. Banerjee D, Maity B, Nag SK, et al. Healing potential of *Picrorhiza kurroa* (Scrofulariaceae) rhizomes against indomethacin-induced gastric ulceration: a mechanistic exploration. BMC Complement Altern Med 2008; 31;8:3.
8. Rajkumar V, Guha G, Kumar RA. Antioxidant and anti-neoplastic activities of Picrorhiza kurroa extracts. Food Chem Toxicol 2011; 49: 363–9.
9. Sud A, Chauhan RS, Tandon C. Identification of imperative enzymes by differential protein expression in *Picrorhiza kurroa* under metabolite accumulating and non-accumulating conditions. Protein Pept Lett 2013; 20: 826–5.
10. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38: 1103-11.
11. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004; 37: 277-85.
12. Jennings RB, Sommers HM, Smyth GA, Flack HA, Linn H. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. Arch Pathol 1960; 70: 68–78.
13. Mozaffari MS, Liu JY, Abebe W, Baban B. Mechanisms of load dependency of myocardial ischemia reperfusion injury. Am J Cardiovasc Dis 2013;3: 180–96.
14. Turer AT, Hill JA. Pathogenesis of myocardial ischemia-reperfusion injury and rationale for therapy. Am J Cardiol 2010; 106:360–8.
15. Cohen MV, Yang XM, Downey JM. The pH hypothesis of postconditioning: Staccato reperfusion reintroduces oxygen and perpetuates myocardial acidosis. Circulation 2007;115: 1895–903.
16. Ji Y, Pang QF, Xu G, Wang L, Wang JK, Zeng YM. Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia-reperfusion injury. Eur. J.Pharmacol 2008; 587:1–7.
17. Oyar EO, Kiris I, Gulmen S, et al. The protective effect of adrenomedullin on renal injury, in a model of abdominal aorta cross-clamping. Thorac Cardiovasc Surg 2012; 60:5-10.
18. Kiraz HA, Poyraz F, Kip G, et al. The effect of levosimendan on myocardial ischaemia reperfusion injury in streptozotocin induced diabetic rats. Libyan J Med 2015 Dec 7;10:29269. doi: 10.3402/ljm.v10.29269. eCollection 2015.
19. Arslan M, Poyraz F, Kiraz HA, et al. The effect of dexmedetomidine on myocardial ischaemia reperfusion injury in streptozotocin induced diabetic rats. Anaesth Pain & Intensive Care 2015; 19: 444-51.
20. Chang Z. Role of toll-like receptors in regulatory functions of T and B cells. Chinese Science Bulletin 2008; 53(8): 1121–7.
21. Liu G, Zhang L, Zhao Y. Modulation of immune responses through direct activation of Toll-like receptors to T cells. Clin Exp Immunol 2010;160: 168–75.
22. Wang L, Liu XH, Chen H, Chen ZY, Weng XD, Qiu T, Liu L. Picroside II protects rat kidney against ischemia/reperfusion-induced oxidative stress and inflammation by the TLR4/NF-κB pathway. Exp Ther Med 2015; 9: 1253-8.
23. Wu N, Li W, Shu W, et al. Protective effect of picroside II on myocardial ischemia reperfusion injury in rats. Drug Des Devel Ther 2014 May 14; 8:545-54
24. Fantone JC, Kinnes DA. Prostaglandin E1 and prostaglandin I2 modulation of superoxide production by human neutrophils. Biochem Biophys Res Commun 1983; 113: 506-12.
25. Simpson PJ, Mickelson J, Fantone JC, et al. Iloprost inhibits neutrophil function in vitro and in vivo and limits experimental infarct size in canine heart. Circ Res 1987; 60: 666-73.
26. Kiris I, Tekin I, Yilmaz N, et al. Iloprost downregulates expression of adhesion molecules and reduces renal injury induced by abdominal aortic ischemia-reperfusion. Ann Vasc Surg 2009; 23: 212-23.
27. Bursch W, Taper HS, Somer MP, et al. Histochemical and biochemical studies on the effect of the prostacyclin derivative Iloprost on CCl4-induced lipid peroxidation in rat liver and its significance for hepatoprotection. Hepatology 1989;9: 830-8.
28. Yu AL, Fuchshofer R, Kampik A, et al. Effects of oxidative stress in trabecular meshwork cells are reduced by prostaglandin analogues. Invest Ophthalmol Vis Sci 2008; 49: 4872-80.
29. Gao H, Zhou YW. Anti-lipid peroxidation and protection of liver mitochondria against injuries by picroside II. World J Gastroenterol 2005; 11(24): 3671-4.

The Species Composition of Malaria Mosquitoes in the Kharkov Region (Ukraine): Natural Factors of Malaria Spread

Ukrayna'daki Kharkov Bölgesi'ndeki Sıtma Sivrisineklerinin Tür Bileşimi: Sıtma Yayılmasının Doğal Faktörleri

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ABSTRACT

Objective: Article describes the species composition of malaria mosquitoes dominating in the Kharkov region, Ukraine, season of their possible effective infection, as well as antimalarial precautions taken.
Methods: When collecting the material, the conventional methods of evaluation of abundance of mosquitoes were used. Collection of larvae and pupae was carried out with standard butterfly net or photo tray with subsequent recalculation per m^2.
Results: On the territory of the region were studied 30 species of bloodsucking mosquitoes of such three genera as *Anopheles, Culex, Aedes* were found.
Conclusion: Facts demonstrate the favorable environmental conditions for malaria spread such as; increase in the number of vectors, increase in precipitation, long temperature period of transmission of infection.

Key Words: Malaria, *Anopheles* genus, epidemiology, evaluation of abundance, hydrotechnical measures

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ÖZET

Amaç: Bu makale, Ukrayna'nın, Kharkov bölgesinde egemen olan sıtma sivrisineklerinin, muhtemel etkili enfeksiyon mevsimindeki tür bileşiminin yanı sıra alınan antimalaryal önlemlerini açıklamaktadır.
Yöntem: Materyali toplarken, sivrisineklerin mikarının değerlendirilmesi için geleneksel yöntemler kullanılmıştır. Larvaların ve pupaların toplanması standart kelebek ağı veya fotoğraf tepsisi ile yapıldı ve m^2 başına sonraki yeniden hesaplamalar yapıldı.
Bulgular: Bölgenin topraklarında *Anofel, Kuleks, Aedes* gibi 30 tür içerisinden üç cins kan emen sivrisinek bulundu.
Sonuç: Durum; sıtma yayılımı için olumlu çevresel koşulları göstermektedir. Bunlar arasında, vektör sayısındaki artış, yağış artışı, enfeksiyonun aktarımında uzun sıcaklık periyodu sayılabilir.

Anahtar Sözcükler: Sıtma, *Anofel* türü, epidemiyoloji, miktarın değerlendirilmesi, hidroteknik önlemler

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INTRODUCTION

Bloodsucking mosquitoes (*Diptera, Culicidae*) are numerous annoying bloodsuckers and vectors of pathogens of dangerous diseases of humans and animals-parasitic invasions, viral and bacterial infections. Change in abundance and the species composition of bloodsucking mosquitoes have a significant impact on the course of the epizootic process, and therefore, on the epidemiological safety of the area, the health status of the population and domestic animals. Many authors have investigated the fauna of blood-sucking mosquitoes, but today the issues of ecology and the epidemiological significance remain relevant all over the world.

In the longitudinal study conducted in Sekong Province in the southern region of Laos was studied the prevalence of malaria mosquitoes in three malaria endemic villages. Of the 16 species of mosquitoes of *Anopheles* genus being under study *An. dirus A, An. maculatus sl.* and *An. jeyporiensis* appeared to be infected with sporozoites was obserevd high correlation between the sporozoite index and humidity of the malaria epidemiological season. It was also noted the ambivalence of mosquitoes in the choice of food since about 50% of insects with equal probability attack humans both indoors and outdoors (1). According to the studies conducted in the malaria endemic areas of such villages as Lenia, Kuala Lipis, Pahang in Malaysia the population of *Anopheles maculatus Theobald* mosquitoes was 31.0%. High frequency level of attacks of *Anopheles maculatus Theobald* on humans was recorded in December, and in January the activity of attacks decreased. Larval stage of mosquitoes was taken by a rapid flow of the river, thereby causing a reduction in their number. Of the five dominant species of *Culicidae* family only *An. annulitarsis* had a significant positive correlation of abundance with monthly precipitation. Activity of attacks of *An. maculatus* was observed from 10:00 to 11:00 p.m. (2). According to the studies conducted by the Center for Control of Diseases in Papua New Guinea when catching mosquitoes of *Anopheles* genus using baits and insect light traps *Anopheles koliensis Owen* was caught most often, then *An. punctulatus Dönitz, An. karwari (James), An. farauti Laveran (sensu lato), An. longirostris Brug* and *An. bancroftii Giles* followed. Percentage of mosquitoes affected by sporozoites of *Plasmodium falciparum Welch* and *P. vivax* in light traps was much higher than in mosquitoes caught by the "on oneself" method. It implies the attractiveness of light traps for older mosquitoes. *An. punctulatus* and *An. Farauti* were more often affected by sporozoites (3). In Western and East Georgia three related species of malarial mosquitoes of the *An. maculipennis* complex – *An. maculipennis Meigen*, 1818; *An. melanoon Hacket*, 1934; *An. sacharovi Favre*, 1903 were identified (East Georgia) (4). Entomological observations of malaria-transmitting mosquitoes were conducted in the area of the Korean demilitarized zone (Paju, Gyeonggi Province) from April to October 1999 where there were cases of malaria. Trapping of mosquitoes of the adult stage was performed using the dark field and light traps with ultraviolet radiation in five and two stations, respectively. Weekly capture of the larval stages was also conducted in five rice fields located close to the stations of trapping of adult species. Malarial index appeared to be higher in mosquitoes of 11 species selected throughout the study period in 1999, and it was 47-48% of the total number of species caught in dwelling houses and cattle barns. In all five observation stations mosquitoes of *Anopheles* genus were caught in ultraviolet light traps from May, and they were the most numerous in the stations located near ponds and rice fields. Percentage of the population density of the larval and adult stage constantly grew since June and reached its maximum value in the second decade of July (112 females/trap/night). Cross correlation showed a significant relationship between the number of adult females of *Anopheles* genus and the number of larvae collected on the same day, previous day, as well as 3 and 7 days ago (5).

According to the data of the European Center for Control and Prevention of Diseases *Anopheles labranchiae* species is of special interest of researchers. It is the endophilic species and a harmful bloodsucker involved in transmission of malaria in Spain, Portugal, France, Corsica in recent years, and in 2011 – in Italy. *Anopheles labranchiae* was and is the most important link in spreading malaria (Becker et al., 2010). It was suggested that *An. labranchiae* was involved in transmission of malaria in Spain, Portugal, France and Italy (6). Historically, there is the evidence of natural infection with *An. labranchiae* by exotic strains of *malaria plasmodium*, and recently the studies have shown that these data are confirmed in the laboratory. In general, *An. labranchiae* is considered to be non-effective for transmission of exotic strains (7). Recently, it was reported that *An. labranchiae* was a vector of an imported case of tertian malaria (*pl. vivax*) in Corsica in 1970-ies (8). Difficulties of the population control and the prevalence of *An. labranchiae* are explained by a high fertility of this species (9).

The European Center for Control and Prevention of Diseases also reports that *Anopheles sacharovi* was distributed in the coastal areas of Italy, Sardinia, Corsica, Croatia, the former Yugoslav Republic of Macedonia, Albania, Bulgaria, Romania, in the southern regions of the former USSR, Turkey, Lebanon, Israel, Jordan, Syria, Iraq, Iran (10, 11, 12, 13). Preliminary results of the recent research in Moldova suggest that there is a significant part of the population of this species on the south of the country. For the first time this species was registered in Greece in 1928, and later a numerous population was found in all coastal areas (14). Previously *Anopheles sacharovi* was registered in abundance in Armenia, but disappeared in 1965. The recent studies indicate re-colonization of some regions of Armenia (15). There are also records of occurrence of this species in Cyprus since 2009. *Anopheles sacharovi* is an important factor in spreading malaria (10). Historically, this is a known vector of malaria in Armenia (15), and it was confirmed not only in Turkey, Syria, Northern Iraq and Iran (12), but in Greece as well (14). In Turkey, it remains the main vector of malaria (16). It was also suggested that *Anopheles sacharovi* was involved in transmission of malaria in Corsica, France, the Balkans, Italy, Romania and Greece (6). It was the main vector in the North-Eastern coast of Italy (15). In 2011 *Anopheles sacharovi* was the vector of 42 cases of tertian malaria in Greece (17, 18, 19). Presumably, malaria transmission was by *An. sacharovi* because of migrants from countries endemic for malaria. This is the most common species of malarial mosquitoes in Greece (17, 20, 21). Taking into consideration the high epidemiological importance, as well as frequent complaints of people on the bites of troublesome bloodsuckers we considered it appropriate to analyze the modern status of the population of *Anopheles* genus mosquitoes within the territory of the Kharkov region (Ukraine).

MATERIALS and METHODS

Analysis of the entomological and meteorological situation in Ukraine was conducted in the Kharkov region according to the data of the Ukrainian Center of Control and Monitoring of Diseases at the Ministry of Health of Ukraine, as well as the Kharkov Regional Laboratory Center.

Collection of the material (adult and larval) was carried out on the territory of natural and artificial reservoirs the Kharkov region in the period of 2013 – 2014.

When collecting the material the conventional methods of evaluation of abundance of mosquitoes were used. Collection of larvae and pupae was carried out with standard butterfly net (net diameter 20 cm, bag depth 25 cm, handle length 1 m) or photo tray (area 13 × 18) with subsequent recalculation per m^2. Unit of account is carrying out a net in the water for one meter. During examination net was submerged in the water to half of the rim and quickly carried out on the surface of one meter. Content of the net was rinsed in the tub and out of it were caught larvae and pupae of different stages, counting the number of both. Accounting of winged mosquito population was carried out 1 hour before sunset, at sunset or 1 hour after sunset. Considering that various species attack man at different times, were carried out three accounting for one hour, and the average number of mosquitoes of all species on one account. Mosquitoes were caught by the method "on the body" for 15 minutes on a naked forearm with a test tube-killing bottle (22).

RESULTS

On the territory of the region were studied 30 species of bloodsucking mosquitoes of such three genera as *Anopheles, Culex, Aedes* were found. Determination of species belonging of mosquitoes was carried out at the larval and imaginal stages using determinants (22, 23). Epidemiological role of each species is determined by a number of conditions. Dangerous vectors can be only the species occurring in large amount with a significant percentage of the population feeding on the human blood, having a rather long season of activity and a sufficient number of females surviving to the age of the possible maturation of sporozoites in their bodies (24).

Depending on the situation each species of *Anopheles* meets these conditions to varying degree. The same *Anopheles* species can be a dangerous vector in one area and be for nothing in another locality. There are species that are dangerous vectors under a wide range of conditions, while others transmit malaria only in exceptional cases or do not have the epidemiological value at all (24).

In Ukraine the most important vectors are *Anopheles maculipennis, An. m. messeae, An. m. atroparvus, An. claviger* (25) (Table 1).

List of the dominant species of mosquitoes (*Diptera*, *Culicidae*) of the Kharkov region includes: *Anopheles* genus – *An.maculipennis, An.messeae; Aedes Meigen* genus – *Ae.cataphylla, Ae.leucomelas, Ae. dorsalis, Ae.excrucians, Ae.vexans; Culex* genus – *C. pip.pipiens, C.pip.molestus.*

Table 1. Relative abundance of Anopheles mosquitoes Kharkov region

	List of species		*Relative abundance
Anopheles mosquitoes	1	*Anopheles maculipennis*	10.2 on a sq.m.
	2	*An.m.messeae*	10.8 on a sq.m.
	3	*An.claviger*	7.4 on a sq.m.
	4	*An.atroparvus*	6.8 on a sq.m.

All mosquito species registered on the territory of the Kharkov region are susceptible to the species of human malaria parasites currently known. Moreover, dominant species in urban landscapes are *An. maculipennis* and *An. messeae.* These species possess all the qualities that are necessary to be considered a dangerous vector of malaria. They are well infected with three main species of human malaria parasites (24).

On the territory studied under the conditions of urban landscapes the gonoactive females occurred within 3.5–4 months, and the larval stages in ponds – approximately within 4.5 months. Maximum number of species was observed in mid-July. Due to the high number and activity of the attack in the summer, as well as proximity of breeding places to human settlements such species as *An. maculipennis, An. messeae* are of the greatest epidemiological risk (25).

DISCUSSION

As previously noted, in order to correctly identify malaria control the knowledge concerning the species composition of *Anopheles* that are prevalent in the given area, epidemiological values of each species, subspecies and biology of major vectors is required. Control measures against a vector must be based on the knowledge of its biology and the seasonal changes. Rational duration of antimalarial precautions is determined primarily by seasonal phenomena in the life of malaria mosquitoes. When planning and implementing the measures for prevention of malaria in Ukraine the scientifically proven terms for conducting such measures based on the long-term study of phenology of malaria mosquitoes are used. Best evaluation criterion of anti-mosquito measures is the age composition of female malaria vectors. Malaria transmission occurs by *Anopheles* mosquitoes in the presence of favorable temperatures for the maturation of malarial parasites in the body of a mosquito (25).

According to the data of the Kharkov regional hydrometeorological center the end of spring in 2013 was hot. Temperature reached +30°C. Summer of 2013 was moderate with sufficient rainfall. Daytime temperatures, as a rule, did not exceed the level of +35°C. July was the hottest month, the average temperature of the month was +25 °C. Maximum temperature observed in August 7 (+37 °C). Autumn was warm and rainy, maximum temperature reached up to +25°C. All facts mentioned above demonstrate improvement of environmental conditions in the possible transmission of imported cases of malaria (26).

In order to monitor the possible occurrence of indigenous cases of malaria and transmission of imported, it is critical to track the beginning and end of the season of effective infection of mosquitoes with malaria plasmodium not only in the Kharkov region, but throughout Ukraine. Season of effective infection of malaria mosquitoes by a causative agent of tertian malaria – *P. vivax* in 2013 began in 18 regions of Ukraine before May 10, earlier (before March 18) – in the Chernivtsi region; later – in the Ivano-Frankivsk region (May 31, 2013). End of the season of effective infection of mosquitoes in 13 regions of the country was in the I-II decade of August; in 6 regions – in the III decade of August, in 4 regions – in September; and in the Sumy region, the Chernihiv region and Kyiv – before August (August 7, 2013); later – in the Odesa region (October 7, 2013). The longest season of effective infection of mosquitoes by a causative agent of tertian malaria was observed in the Odesa region (163 days) (25).

Season of a possible infection of persons with a causative agent of vivax malaria began in the II-III decades of May in 15 regions and in the I-II decades of June in 7 regions; prior the specified period this season began in the Chernivtsi region – before April 15; later – in the Ivano-Frankivsk region – before July 28. End of the season was in October in 19 regions and in September in 3 regions; in the Cherkasy region – in September 10, 2013; in the Odesa region – in November 25, 2013. The longest season of the possible transmission of malaria was recorded in the Odesa region – 191 days (25).

Epidemiological situation of malaria is complicated by deterioration of the entomological monitoring of vectors – bloodsucking mosquitoes. In 2012 specialists of entomological groups of sanitary-epidemiological institutions conducted supervision over the sanitary condition of water reservoirs, fish breeding ponds, ornamental ponds, basements, and other areas. Antimalarial hydrotechnical measures, monitoring in determining the species and age composition of malaria mosquitoes were conducted. After reforming the sanitary and epidemiological service in December 2012 the Parasitological and Entomological Sections were significantly reduced.

In the Kharkov region during 2012 compared to 2011 the average number of the larval stages of *Anopheles* genus mosquitoes increased by 3.9% (25).

In 2013 on the territory of the Kharkov region 5082.6 ha of anophelogenous areas of water reservoirs with the average seasonal index of abundance of larvae of *Anopheles* genus mosquitoes – 8.7 were registered. In 2013, as well as in previous years, to control the number of bloodsucking mosquitoes environmental (hydrotechnical) measures and chemical (insecticidal) agents were used, but in much smaller amounts (25). But it should be remembered that resistance to insecticides can be a challenge all over the world. For instance, the study on resistance to insecticides to reduce the number of mosquitoes of *Anopheles* genus was conducted in southern Turkey in Adana, Adiyaman, Antalya, Aydin and Mugla. These areas where malaria is endemic are diverse with relation to the use of insecticides, geographical features, social infrastructure, development of agriculture and peculiarities of tourism. All these factors can influence on the mosquito resistance to insecticides. Dominant species *An. sacharovi, An. superpictus* were exposed to different insecticides – DDT, dieldrin, malathion, pirimiphos methyl. Resistance to dieldrin was detected in *An. sacharovi* in 1970; resistance to karbofos was first registered in 1974. Cross-resistance was observed for a wide range of organophosphorous and carbamate insecticides although previously they had never been used for treating dwelling houses and premises. Aim of the study was to compare the baseline information on susceptibility of *Anopheles* genus mosquitoes under experimental conditions with the results obtained on the samples in nature in order to assess the current trends in resistance of mosquitoes to insecticides on the southern coast of Turkey (27).

Operational area of treatment against adult malaria mosquitoes in Ukraine in the premises was reduced by 20% of the amount of work of 2012 and was the lowest in the last 5 years, and against the larvae of malaria mosquitoes – by 2.5 times (25).

Antimalarial hydrotechnical measures were conducted in all sectors to the fullest extent from the work leading to elimination of the mosquito breeding to the work creating unfavorable conditions for larval development (25).

CONCLUSION

Abovementioned facts demonstrate the favorable environmental conditions for malaria spread: increase in the number of vectors, increase in precipitation, long temperature period of transmission of infection.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Vythilingam I, Phetsouvanh R, Keokenchanh K, Yengmala V, Vanisaveth V, Phompida S et al. The prevalence of Anopheles (Diptera: Culicidae) mosquitoes in Sekong Province, Lao PDR in relation to malaria transmission. Trop Med Int Health. 2003; 8:525-35.
2. Ali WN, Ahmad R, Nor ZM, Ismail Z, Lim LH. Population dynamics of adult mosquitoes (Diptera: Culicidae) in malaria endemic villages of Kuala Lipis, Pahang, Malaysia. Southeast Asian J Trop Med Public Health. 2011; 42:259-67.
3. Hii JL, Smith T, Mai A, Ibam E, Alpers MP. Comparison between anopheline mosquitoes (Diptera: Culicidae) caught using different methods in a malaria endemic area of Papua New Guinea. Bull Entomol Res. 2000; 90:211-9.
4. Bezzhonova OV, Babuadze GA, Gordeev MI, Zvantsov AB, Ezhov MN, Imnadze P et al. Malaria mosquitoes of the Anopheles maculipennis (Diptera, Culicidae) complex in Georgia Med Parazitol (Mosk). 2008; 3:32-6.

5. Shin EH, Lee WJ, Lee HI, Lee DK, Klein TA. Seasonal population density and daily survival of anopheline mosquitoes (Diptera: Culicidae) in a malaria endemic area, Republic of Korea. J Vector Ecol. 2005; 30:33-40.
6. Alten B, Kempen H, Fontenille D. Malaria in southern Europe: resurgence from the past. In Takken W, Knols BGJ, editors. Emerging pests and vector-borne diseases in Europe. Wageningen (the Netherlands): Wageningen Academic Publishers; 2007. p. 35-58.
7. Toty C, Barre H, Le Goff G, Larget-Thiery I, Rahola N, Couret D et al. Malaria risk in Corsica, former hot spot of malaria in France. Malar J. 2010; 9:231.
8. Baldari M, Tamburro A, Sabatinelli G, Romi R, Severini C, Cuccagna G et al. Malaria in Maremma, Italy. Lancet. 1998; 351:1246-7.
9. Boccolini D, Toma L, Di Luca M, Severini F, Cocchi M, Bella A et al. Impact of environmental changes and human-related factors on the potential malaria vector, *Anopheles labranchiae* (Diptera: Culicidae), in Maremma, Central Italy. J. Med Entomol. 2012; 49:833-42.
10. Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C et al. Mosquitoes and their control. Second ed. Berlin: Springer-Verlag Berlin Heidelberg; 2010; 577.
11. Schaffner F, Angel G, Geoffroy B, Hervy JP, Rhaiem A, J. B. The mosquitoes of Europe (CD ROM). Montpellier, France: IRD Edition and EID Méditerranée; 2001.
12. Yurttas H, Alten B. Geographic differentiation of life table attributes among *Anopheles sacharovi* (Diptera: Culicidae) populations in Turkey. J Vector Ecol. 2006; 31:275-84.
13. Merdic E. Checklist of mosquitoes (Diptera: Culicidae) of Croatia. European Mosquito Bulletin. 2004; 17:8-13.
14. Patsoula E, Samanidou-Voyadjoglou A, Spanakos G, Kremastinou J, Nasioulas G, Vakalis NC. Molecular characterization of the *Anopheles maculipennis complex* during surveillance for the 2004 Olympic Games in Athens. Med Vet Entomol. 2007; 21:36-43.
15. Romi R, Boccolini D, Hovanesyan I, Grigoryan G, Di Luca M, Sabatinell G. *Anopheles sacharovi* (Diptera: Culicidae): a reemerging malaria vector in the Ararat Valley of Armenia. J Med Entomol. 2002; 39:446-50.
16. Alten B, Caglar SS, Simsek FM, Kaynas S. Effect of insecticide-treated bednets for malaria control in Southeast Anatolia. Turkey. J Vector Ecol. 2003; 28:97-107.
17. Danis K, Baka A, Lenglet A, Van Bortel W, Terzaki I, Tseroni M et al. Autochthonous Plasmodium vivax malaria in Greece, 2011. Euro Surveill. 2011; 16:19993.
18. European Centre for Disease Prevention and Control. Meeting Report: Consultation on Plasmodium vivax transmission risk in Europe, Stockholm 17-18 January 2012. Stockholm: ECDC, 2012.
19. Hellenic Center for Disease Prevention and Control. Epiemiological Surveillance Report, Malaria in Greece, 2013. Athens; 2013.
20. Vakali A, Patsoula E, Spanakos G, Danis K, Vassalou E, Tegos N, et al. Malaria in Greece, 1975 to 2010. Euro Surveill. 2012; 17:20322.
21. Kousoulis AA, Chatzigeorgiou KS, Danis K, Tsoucalas G, Vakalis N, Bonovas S et al. Malaria in Laconia, Greece, then and now: a 2500-year-old pattern. Int J Infect Dis. 2013; 17:e8-e11.
22. Prudkina NS. Krovososuschie dvukryilyie nasekomyie. Fauna, biologiya, ekologiya, mediko-veterinarnoe znachenie. Uchebnoe posobie: H. Kollegium; 2011 (in Russian).
23. Sheremet VP. Krovosisni komarI Ukrayini. Navchalniy posIbnik dlya studentIv bIologIchnogo fakultetu. K.: “KiYivskiy unIversitet”; 1998 (in Russian).
24. Zvantsov AB, Ezhov MN, Artemev MM. Perenoschiki malyarii (Diptera, Culicidae, Anopheles) sodruzhestva nezavisimyih gosudarstv (SNG). Kopengagen: Vsemirnaya organizatsiya zdravoohraneniya. Evropeyskoe regionalnoe byuro. Programma «Obratim vspyat malyariyu»; 2003 (in Russian).
25. Statistical data of the Ukrainian Center of Control and Monitoring of Diseases at the Ministry of Health of Ukraine (http://ucdc.gov.ua/en/).
26. https://www.timeanddate.com/weather/ukraine/kharkiv/historic.
27. Kasap H, Kasap M, Alptekin D, Herath PRJ. Insecticide resistance in Anopheles sacharovi Favre in southern Turkey. Bull World Health Organ. 2000; 78:687-92 (in Russian).

Thrombophilic Status of Extracted Fetal Tissues of Spontaneously Aborted Embroys

Spontan Abortus Embriyolarından Ekstrakte Edilen Fetal Dokuların Trombofilik Durumu

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ABSTRACT

Objective: The reports about Factor V (FV) Leiden, Factor II (FII) G20210A and Methylenetetrahydrofolate reductase (MTHFR) C677T gene mutations of parents and fetal viability are frequently encountered in the literature, despite the fetal side of thrombophilia is scant. To clarify the three common thrombophilic gene mutations of the spontaneously aborted embryos, an accurate algorithm was followed to extract the fetal tissues and then the mutations were searched.
Material and Methods:70 spontaneous abortion materials were included to the study and all were karyotyped. Cytogenetically abnormals were excluded from the study. To extract the fetal tissues, amplifications of sex determination region of chromosome-Y (SRY) gene and genotypings were performed, respectively. Extracted fetal tissues of spontaneous aborted embroys and parents were screened for the thrombophilic gene mutations via electronic microarray.
Results: After excluding chromosomally abnormal and maternally contaminated ones totally ten fetal tissues were screened for the FII G20210A, FV Leiden and MTHFR C677T gene mutations, and two carry F II G20210A and F Leiden heterozygote mutations, and six carry heterozygote forms of MTHFR C677T.
Conclusion: The present study performed on the limited number of abortion materials, has a value for distinguishing the fetal tissues before analyzing the three common mutation of thrombophilic genes which make the results are very substantial.

Key Words: Fetal thrombophilia , Spontaneous abortion, SRY gene

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ÖZET

Amaç: Ebeveynlerdeki Faktör V (FV) Leiden, Faktör II (FII) G20210A ve Metilen Tetrahidrofolat redüktaz (MTHFR) C677T gen mutasyonları ve fetal tutunma ile ilgili bilgiler literatürde sıklıkla karşılaşılmasına rağmen, trombofilinin fetal yönü üzerine yapılan çalışmalar oldukça sınırlıdır. Bu üç sıklıkla rastlanılan gen mutasyonunu spontan abort embriyolarında netleştirmek için fetal dokuları ekstrakte edebilmek maksadıyla doğru bir algoritma takip edildi ve mutasyon taraması gerçekleştirildi.
Yöntem: Toplam 70 spontan abortus materyali çalışmaya dâhil edildi ve hepsinin karyotip analizi yapıldı. Sitogenetik olarak anormal olanlar çalışmadan çıkartıldı. Fetal dokuları ekstrakte edebilmek için Y kromozomu üzerindeki SRY gen bölgesi amplifikasyonu ve genotiplendirme işlemleri ayrı ayrı yapıldı. Spontan abort embriyolarının ekstrakte edilen fetal dokuları elektronik mikroarray kullanılarak trombofilik gen mutasyonları açısından tarandı.
Bulgular: Kromozom anomalisi ve maternal hücre kontaminasyonu tespit edilenler çalışma grubundan çıkartıldıktan sonra 10 fetal dokuda FV Leiden, FII G20210A ve MTHFR C677T gen mutasyonlarının taraması gerçekleştirildi, iki tanesinin FII G20210A ve FV Leiden heterozigot mutasyon taşıdığı ve altı tanesinin de MTHFR C677T heterozigot mutasyon taşıdığı tespit edildi.
Sonuç: Sınırlı abort materyalinde yapılan bu çalışmada ilgili üç trombofilik gen mutasyonunun analizinden önce fetal dokuların ayırt edilmesi elde edilen sonuçları değerli kılmaktadır.

Anahtar Sözcükler: Fetal trombofili, spontan abort, SRY geni

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INTRODUCTION

The majority of the spontaneous abortions occur during the first trimester and over 50% of these are detected chromosomally abnormal (1,2). Thrombophilia can predispose an individual to thromboembolism and this condition could have been a significant role of the production of the spontaneous abortions (3). Although several studies are available concerning the relationship between thrombophilic pattern of the parents and abortions, thrombophilia mutations of the extracted fetal tissues of spontaneously aborted embroys are very limited.

Factor V (FV) Leiden, Factor II (FII) G20210A and Methylenetetrahydrofolate reductase (MTHFR) C677T gene alterations are the common mutations of the thrombophilia. Detecting these mutations on the abortion materials have difficulties because of the maternal decidual cell contamination (MCC) of the pregnancy loss tissues and additional techniques are necessary to extract the fetal tissues (4-6). In this present study after excluding abnormally karyotyped ones, cytogenetically normal females materials were searched to detect the very tiny component of chromosome Y by using amplifications of sex determination region of chromosome-Y (SRY) gene. Then if negative, genotyping procedures were used to ensure the origin of the materials. The details of the procedures were presented in our previous paper publicated on 2010 (7).

The study give us the results of the electronic microarray screening of the FV Leiden, FII G20210A and MTHFR C677T gene mutations of the spontaneously aborted materials XY karyotyped ones and additionally XX karyotyped ones which were confirmed via molecular genetic techniques.

METHODS

Tissue culture and chromosome analysis

A total of 70 spontaneous abortions which occurred between the fifth and twentieth weeks of pregnancies (singleton gestations) were referred for cytogenetic evaluation. Written informed consent forms were obtained from all participants and the study was approved by the ethics committee of Gazi University. Materials were transferred immediately to the laboratory in a sterile culture medium. After separating the chorionic villi in small pieces in sterile condition, long-term tissue cultures were set up using a slightly modified procedure of Verma and Babu (8). Karyotyping was performed by using Giemsa-trypsin banding (GTG) and five metaphase spreads were analyzed; fifteen metaphase spreads were counted for each one of the specimens which were cultivated for a period shorter than two weeks from two separate primer cell cultures (9). Some parts of the materials were stored at -20°C for DNA isolations.

DNA isolation

The abortion materials which were stored in -20°C for molecular studies were placed in 1000 µl of lysis buffer, 50 µl SDS and 20 µl Proteinase K (20 mg/ml) for overnight at 37°C. A 750 µl of ammonium acetate was added and agitated 20 times. Following incubation for 10 min at room temperature centrifugation at 3500 rpm for 15 min was done. The supernatant was separated to a new tube and absolute alcohol was added. DNA was taken to the tubes that contain 50 µl TE. After dissolving the DNA at room temperature for one day, it was stored at -20°. Genomic DNAs were isolated from paternal and maternal peripheral venous blood cells by using proteinase K digestion using salt extraction method according to the slightly modified procedure of Miller et al (10).

SRY Amplification and Genotyping

The primers of SRY gene and G6PDH gene as an internal control were used in PCR reaction. DNAs were amplified in three step cycles: denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, extension at 72°C for 30 sec. After 35 cycles, the DNAs were given a final extension step at 72°C for 5 min. By using high-polymorphic microsatellite DNA markers, chorionic villi DNAs and maternal and paternal DNAs were evaluated. Totally four different DNA markers including chromosome 10, 15 and X (D15S999, D10S1714, DXS987 and DXS1058) were used. The information about sequences and amplification conditions were obtained from Genome Data Base. Amplified PCR products were visualized on a 2% agarose gel by staining ethidium bromide. These products were separated in 167 bp, 193 bp, 206-244 bp and 273-285 bp respectively by 6% denatured polyacrylamide gel electrophoresis. The DNAs of the fetuses and their related parents were loaded to electronic microarray for mutation screening. Electronic microarray is a reliable method that is based on hybridization between molecules on the surface of streptavidin-coated chip and DNA molecules that are labelled with biotin. Finally the analyzes are done according to the signals.

RESULTS

Of the 70 spontaneous abortion materials, chromosomal abnormalities were identified in 26 (37.1%); 3 of them were structural (11.5%), 23 of them were numerical (88.5%) aberrations. The cytogenetic analyses of the 6 abortion materials were revealed as male karyotypes and screened directly for the FII G20210A, FV Leiden and MTHFR C677T gene mutations. SRY gene amplifications via PCR were performed on the 38 XX karyotyped abortion materials and a part of SRY gene was observed on the 16 of them (42.1% of the materials were maternally contaminated) as shown in figure 1. The genotypings were performed on the remaining twenty-two materials and only four of them were evaluated as chorionic villi (81.8% of the materials were maternally contaminated). Finally the MCC (-) four chorionic villi materials were screened for the mutations and the results are shown on table 1. No homozygous mutant abortion materials were detected in terms of FV Leiden, MTHFR C677T and FII G20210A. The strongest association between thrombophilic patterns and spontaneously aborted materials were observed in MTHFR C677T. Six of ten materials were detected heterozygous carrier for this gene and the results are shown on table 2. Combined thrombophilia was found in 2 of them; one of them was both MTHFR C677T and FII G20210A, the other is both MTHFR C677T and FV Leiden respectively.

Table 1. The results of the abort materials screened for maternal cell contamination, Factor II G20210A, Factor V Leiden, and MTHFR C677T mutations. G: gravida P: para A: abortion L: living het: heterozygote MI: maternally inherited PI: paternally inherited

No	Maternal age	Gestation weeks	Obstetric history	Karyotype	MCC	Factor II G20210A	Factor V Leiden	MTHFR C677T
1	29	8,3	G2P0A2L0	46,XY	(-)	wt	het/MI	wt
2	33	7,3	G1P0A1L0	46,XY	(-)	wt	wt	het/MI
3	26	7,5	G3P1A2L1	46,XY	(-)	wt	wt	het/MI
4	32	10,2	G3P0A3L0	46,XY	(-)	wt	wt	wt
5	38	15	G2P0A2L0	46,XY	(-)	wt	wt	wt
6	28	6,5	G1P0A1L0	46,XY	(-)	wt	wt	het/MI
7	36	8,1	G2P0A2L0	46,XX	(-)	het./MI	wt	wt
8	35	6,3	G5P2A3L2	46,XX	(-)	het./PI	wt	het/PI
9	32	8	G3P1A2L1	46,XX	(-)	wt	het/MI	het/MI or PI
10	27	8,5	G1P0A1L0	46,XX	(-)	wt	wt	het/MI or PI
11	35	6,10	G2P0A2L0	46,XX	SRY (+)	UD	UD	UD
12	30	7,6	G2P1A1L1	46,XX	SRY (+)	UD	UD	UD
13	25	7,6	G2P0A2L0	46,XX	SRY (+)	UD	UD	UD
14	29	7,4	G6P0A6L0	46,XX	SRY (+)	UD	UD	UD
15	27	7,3	G2P0A2L0	46,XX	SRY (+)	UD	UD	UD
16	37	6	G2P1A1L1	46,XX	SRY (+)	UD	UD	UD
17	29	6	G1P0A1L0	46,XX	SRY (+)	UD	UD	UD
18	29	8,4	G1P0A1L0	46,XX	SRY (+)	UD	UD	UD
19	38	8,2	G1P0A1L0	46,XX	SRY (+)	UD	UD	UD
20	22	9,5	G2P0A2L0	46,XX	SRY (+)	UD	UD	UD
21	36	9	G1P0A1L0	46,XX	SRY (+)	UD	UD	UD
22	34	6	G3P1A1L1 (D&C:1)	46,XX	SRY (+)	UD	UD	UD
23	30	6,6	G1P0A1L0	46,XX	SRY (+)	UD	UD	UD
24	23	7	G1P0A1L0	46,XX	SRY (+)	UD	UD	UD
25	25	8	G2P0A2L0	46,XX	SRY (+)	UD	UD	UD
26	37	9,1	G4P1A4L1	46,XX	SRY (+)	UD	UD	UD
27	36	7	G2P1A1L1	46,XX	(+)/ matDNA	UD	UD	UD
28	29	12,6	G3P0A3L0	46,XX	(+)/ matDNA	UD	UD	UD
29	29	10,4	G1P0A1L0	46,XX	(+)/ matDNA	UD	UD	UD
30	37	8,1	G3P2A1L1	46,XX	(+)/ matDNA	UD	UD	UD
31	31	6,3	G3P1A2L1	46,XX	(+)/ matDNA	UD	UD	UD
32	37	5,6	G4P2A1L2 (D&C:1)	46,XX	(+)/ matDNA	UD	UD	UD
33	41	10,2	G4P0A4L0	46,XX	(+)/ matDNA	UD	UD	UD
34	29	6,5	G1P0A1L0	46,XX	(+)/ matDNA	UD	UD	UD
35	33	6,3	G3P1A2L0	46,XX	(+)/ matDNA	UD	UD	UD
36	29	9,5	G2P1A1L1	46,XX	(+)/ matDNA	UD	UD	UD
37	31	8,4	G1P0A1L0	46,XX	(+)/ matDNA	UD	UD	UD
38	43	6,2	G1P0A1L0	46,XX	(+)/ matDNA	UD	UD	UD
39	32	6	G2P0A2L0	46,XX	(+)/ matDNA	UD	UD	UD
40	41	5,6	G5P0A5L0	46,XX	(+)/ matDNA	UD	UD	UD
41	30	8	G2P0A2L0	46,XX	(+)/ matDNA	UD	UD	UD
42	29	7	G1P0A1L0	46,XX	(+)/ matDNA	UD	UD	UD
43	26	6,5	G1P0A1L0	46,XX	(+)/ matDNA	UD	UD	UD
44	27	10,6	G3P0A3L0	46,XX	(+)/ matDNA	UD	UD	UD

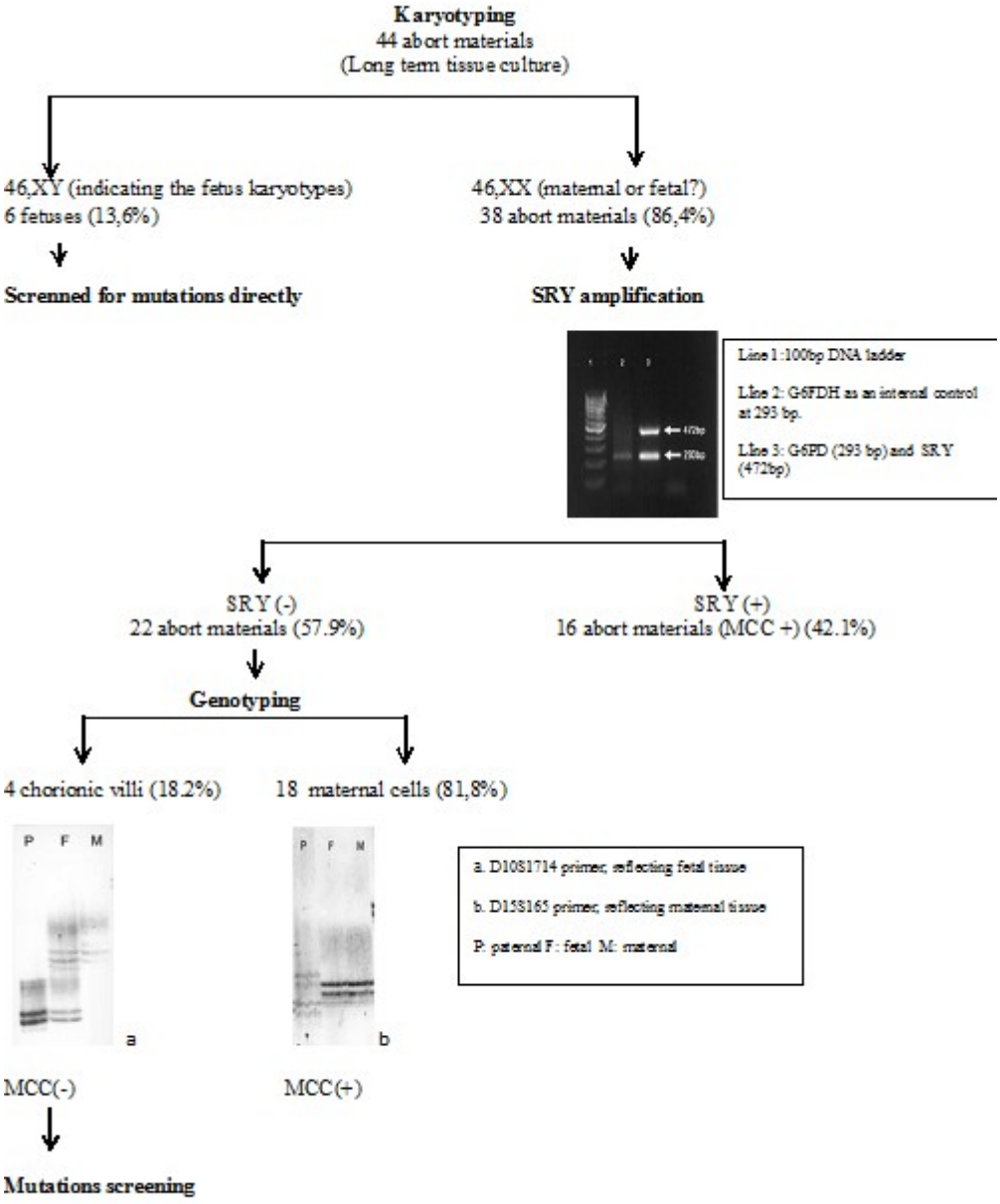

Figure 1. The algorithm followed for excluding maternal cell contamination of the 46, XX karyotyped abortion materials. MCC: Maternal cell contamination

Table 2. The genotypes and allelle frequencies of FII G20210G, FII G20210A, FII A20210A, FV G1691G, FV G1691A, FV A1691A, MTHFR C677C, MTHFR C677T, MTHFR T677T in aborted materials and their parents in the current limited results.

	Fetus (n/%)	Mother (n/%)	Father (n/%)
FII G20210G	8/80	9/90	9/90
FII G20210A	2/20	1/10	1/10
FII A20210A	-/0	-/0	-/0
FV G1691G	8/80	8/80	10/100
FV G1691A	2/20	2/20	-
FV A1691A	-/0	-/0	-/0
MTHFR C677C	4/40	1/10	3/30
MTHFR C677T	6/60	3/30	3/30
MTHFR T677T	-/0	2/20	-/0

DISCUSSION

Venous thromboembolism and pre-eclampsia are the most frequent pregnancy complications. Heritable prothrombotic factors lead to an increased risk of thromboembolism as hypercoagulable state within the fetal circulation could lead to fetal stem vessel thrombosis, placental infarction in the distribution of fetal vessels and spontaneous abortion. By that way these factors play a substantial role in the pathogenesis of spontaneous abortions. Various studies in the recent years have been examined the incidence of specific thrombophilic gene mutations in women with unexplained spontaneous abortion. Some of these studies have been demonstrated an association between thrombophilic gene mutations and abortions (11-13) whereas others have shown the lack of any association (14-16).

It was hypothesized that when the fetus itself has an inherited risk of thrombosis, pregnancy is also more prone to placental infarction at the maternal–fetal interface resulting in an increased risk for intrauterine death but most of the studies have focused on genetic contribution of parents either than the fetuses (17). It is reliable to study with the late pregnancy losses as umbilical cord blood and neonate cord blood collected during the delivery which are the specimens that belong to the fetus. The prevalence of FV Leiden and FII G20210A allele in the umbilical cords of 139 cases (intrauterine exitus) with a gestational age of more than 16 weeks were analyzed.

Fetuses born from an uncomplicated pregnancies were used as control group and the incidence of thrombosis was found higher in the study group, suggesting an important role of abnormal coagulation in placentation (18).

Placental samples of eighty-six patients with pregnancy complicated in the third trimester by idiopathic intrauterine fetal death (IUFD) and 100 healthy pregnant controls were screened for MTHFR C677T, FV Leiden and FII G20210A mutations. It was determined that carrier status of mutant MTHFR C677T must be considered a risk factor for intrauterine fetal demise. As most of the studies have focused their attention on maternal biologic samples to search for the genetic contribution of the mother, they highlight the importance of analyzing the mother–fetus–father triad DNA to screen the thrombophilic mutations in the evaluation of the risk of IUFD (19). Supporting this data in our study six of ten abortion materials were found to be carrying mutant allele of MTHFR C677T in a heterozygote manner.

The umbilical cords from 75 patients with preeclampsia and 92 cords as control group were screened for inherited thrombophilic gene mutations; FV Leiden, MTHFR C677T, and FII G20210A as thrombotic vascular disease may predispose patients to the development of preeclampsia (20). No significant differences between patients with severe preeclampsia and controls in terms of maternal FV Leiden mutation, MTHFR C677T mutation or FII G20210A mutation were determined.

When studying with the early fetal losses, there can be misdiagnosis because of the probability of MCC without the verification of the origins of the materials. Pauer et al., screened FV Leiden mutations on the 139 abortion materials (with the mean gestational age of twelve weeks) and maternal materials (17). It was figured out that there was a tendency towards the pregnancy loss if both fetus and mother carry the same mutation. Based on no information about the verification of the origin of the materials on this report and the others, the results could not be considered completely accurate (12,17,21,22). In our study after confirmation of the fetal tissues in terms of MCC, trombophilic status of the fetal tissues were searched and FV Leiden mutation was detected in heterozygous manner in two of ten materials and they both inherited maternally.

As far as we met in the literature there are only two reports addressing the examination of the maternal tissue contamination by using hyper-polymorphic short tandem repeats. In Zeeteberg et al., study the embryonic tissues were analyzed with microsatellite markers and their haplotypes were compared with the corresponding pattern of their parents (23). Eighty fetal tissue samples from spontaneous abortions that occurred between sixth and twentieth weeks and 125 DNA samples from healthy blood donors as control group were analyzed for MTHFR C677T and A1298C polymorphisms. They found significantly higher frequencies in abortion materials indicating that the MTHFR polymorphisms may have a major impact on fetal survival. Yalcintepe et al., investigated the possible role of multiple inherited thrombophilic gene variations in women with unexplained spontaneous abortions (24). For this aim, they genotyped the F V Leiden, F II G20210A, MTHFR C677T, PAI-1 4G/5G, ACE I/D, eNOS E298D and Apo E E2/E3/E4 mutations in spontaneously aborted fetal materials, and their mothers. 22 abortion materials and their mothers, 22 control subjects with have at least two healthy birth were studied. According to their results combined thrombophilic gene variations may be associated with increased risk for spontaneous abortions. Departure from our study, karyotyping of the fetal tissues was not performed and the chromosome constitution of the fetal tissues were obscure in these two study (23,24).

In the present study it was aimed to study hereditary thrombophilic gene mutations on unmixed spontaneous abortion specimens. For this purpose, efficient algorithms were constructed to exclude the maternal decidual cells. To our knowledge this is the first study that the thrombophilic gene mutations are screened in abort materials after an accurate algorithm. It is find out that MTHFR C677T mutations of the fetus could be more effectual in the formation of abortion. Despite the low number of materials screening for mutation, the parameters are very substantial as they almost reflect the pregnancy loss tissues. As a result a conservative algorithm should be performed to analyze the unmixed materials of the abortions otherwise the results may not be accurate and direct the scientists improperly. There is a need for larger studies to explore the effect of thrombophilic factors in family members for pregnancy losses.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Choi TY, Lee HM, Park WK, Jeong SY, Moon HS. Spontaneous abortion and recurrent miscarriage: A comparison of cytogenetic diagnosis in 250 cases. Obstet Gynecol Sci 2014;57:518-25.
2. Nagaishi M, Yamamoto T, Iinuma K, Shimomura K, Berend SA, Knops J. Chromosome abnormalities identified in 347 spontaneous abortions collected in Japan. J Obstet Gynaecol Res. 2004;30:237-41.
3. Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N. Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. Thromb Haemost 1999;82:6-9.
4. Jarret KL, Michaelis RC, Phelan MC, Vincent VA, Best RG. Microsatellite analysis reveals a high incidence of maternal cell contamination in 46, XX products of conception consisting of villi or a combination of villi and membranous material. Am J Obstet Gynecol 2001;185:198-203.
5. Nikitina TV, Lebedev IN, Sukhenova NN, Sazhenova EA, Nazarenko SA. Maternal cell contamination of cultures of spontaneous abortion fibroblasts: Importance for cytogenetic analysis of embryonic lethality. Genetika 2004;40:800-9.
6. Karaoguz MY, Nas T, Konac E, Ince D, Pala E, Menevse S. Is cytogenetic diagnosis of 46, XX karyotype spontaneous abortion specimens erroneous? Fluorescence in situ hybridization as a confirmatory technique. J Obstet Gynaecol Res 2005;31:508-13.
7. Karaoguz MY, Percin EF, Pala E, Biri AA, Korucuoglu U. SRY gene amplifications and genotypings revealed the occurrence of the hidden maternal decidual cells in 46, XX karyotyped spontaneous abortions. Genet Couns. 2010;21:9-17.
8. Verma RS, Babu A. Tissue culture techniques and chromosome preparation. In: Human Chromosomes Principles and Techniques. 2nd edition. New York, McGraw-Hill. 1995;6-71.
9. Seabright M. A rapid banding technique for human chromosomes. Lancet ii. 1971;971-2.
10. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
11. Hubacek JA, Rynekrova J, Kasparova D, Adamkova V, Holmes MV, Fait T. Association of MTHFR genetic variants C677T and A1298C on predisposition to spontaneous abortion in Slavonic population. Clin Chim Acta 2015;440:104-7.
12. Callejón G, Mayor-Olea A, Jiménez AJ, Gaitán MJ, Palomares AR, Martínez F, Ruiz M, Reyes-Engel A. Genotypes of the C677T and A1298C polymorphisms of the MTHFR gene as a cause of human spontaneous embryo loss. Hum Reprod 2007;22:3249-54.
13. Glueck CJ, Pranikoff J, Aregawi D, Haque M, Zhu B, Tracy T, Wang P. The factor V Leiden mutation, high factor VIII, and high plasminogen activator inhibitor activity: etiologies for sporadic miscarriage. Metabolism 2005;54:1345-9.
14. Korteweg FJ, Folkeringa N, Brouwer JL, Erwich JJ, Holm JP, Van Der Meer J, Veeger NJ. Fetal loss in women with hereditary thrombophilic defects and concomitance of other thrombophilic defects: a retrospective family study. BJOG 2012;119:422-30.
15. Rai R, Shlebak A, Cohen A, Backos M, Holmes Z, Marriott K, Regan L. Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage. Hum Reprod 2001;6:961-5.
16. Bae J, Shin SJ, Cha SH, Choi DH, Lee S, Kim NK. Prevalent genotypes of methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) in spontaneously aborted embryos. Fertil Steril 2007;87:351-5
17. Pauer HU, Voigt-Tschirschwitz T, Hinney B, Burfeind P, Wolf C, Emons G, Neesen J. Analyzes of three common thrombophilic gene mutations in German women with recurrent abortions. Acta. Obstet. Gynecol. Scand., 2003,82:942-7.
18. Dizon-Townson DS, Meline L, Nelson LM, Varner M, Ward K. Fetal carriers of the factor V Leiden mutation are prone to miscarriage and placental infarction. Available from: URL: Am J Obstet Gynecol 1997;177:402-5.
19. Dekker JW, Lind J, Bloemenkamp KW, Quint WG, Kuijpers JC, van Doorn LJ, de Groot CJ. Inherited risk of thrombosis of the fetus and intrauterine fetal death. Eur J Obstet Gynecol Reprod Biol 2004;117:45-8.
20. Tranquilli AL, Saccucci F, Giannubilo SR, Cecati M, Nocchi L, Lorenzi S, Emanuelli M. Unexplained fetal loss: the fetal side of thrombophilia. Fertil Steril 2010;94:378-80.
21. Isotalo PA, Wells GA, Donnelly JG. Neonatal and fetal methylenetetrahydrofolate reductase genetic polymorphisms: an examination of C677T and A1298C mutations. Am J Hum Genet 2000;67:986-90.
22. Bae J, Shin SJ, Cha SH, Choi DH, Lee S, Kim NK. Prevalent genotypes of methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) in spontaneously aborted embryos. Fertil Steril 2007;87:351-5.
23. Zetterberg H, Regland B, Palmér M, Ricksten A, Palmqvist L, Rymo L, Arvanitis DA, Spandidos DA, Blennow K. Increased frequency of combined methylenetetrahydrofolate reductase C677T and A1298C mutated alleles in spontaneously aborted embryos. Eur J Hum Genet 2002;10:113-8.
24. Yalcintepe S, Ozdemir O, Haciveliogl SO, Akurut C, Koc E., Uludag A, Cosar E, Silan F. Multiple Inherited Thrombophilic Gene Polymorphisms in Spontaneous Abortions in Turkish Population. Int J Mol Cell Med 2015;4:120-7.

Are Peripheral Blood Derived Inflammation Markers Prognostic Indicators in Breast Cancer?

Periferik Kanda Ölçülen İnflamasyon Belirteçlerinin Meme Kanserli Hastalarda Prognostik Önemi var mı?

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ABSTRACT

Objective: Peripheral blood derived inflammation based scores are proposed as prognostic markers in solid tumors especially in gastrointestinal system. We aimed to investigate the association between neutrophyl/lymphocyte ratio (NLR, platelet/lymphocyte ratio (PLR) and breast cancer in means of prognostic forecast.
Methods: Of 190 patients diagnosed and operated for breast cancer. 160 patients with available pretreatment blood count were included. Ultrasound, mammography, and pathology results were also recorded.
Results: The median age was 50 years at the time of diagnosis (28-90 years). Family history was positive in 11 patients. There were 139 patients with invasive ductal carcinoma, 15 patients with invasive lobuler carcinoma. There was no association with histopathological type of the tumor and peripheral blood derived inflammation markers. The difference in PLR between T1 and T4 tumors was statistically significant. Also both NLR and PLR were significant when N0 patients compared with N1 patients ($p<0.05$). Neither estrogen nor progesterone receptor status was shown to have an association with NLR and PLR. PLR was found to be statistically significant in different pathological grade groups.
Conclusion: Both NLR and PLR were found to be correlated with advanced stage breast cancer so with prognosis. In order to use these markers in clinical practice, more clinical trials with large number of patients and long follow up period are needed.

Key Words: Breast cancer, neutrophyl/lymphocyte ratio, platelet/lymphocyte ratio

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ÖZET

Amaç: Periferik kanda saptanan inflamasyon belirteçleri gastrointestinal sistem tümörleri öncelikli olmak üzere birçok solid tümörde prognostik belirteç olarak kullanılmaktadır. Biz de bu çalışmada nötrofil/lenfosit oranı ve trombosit/ lenfosit oranları ile meme kanseri arasında prognoz belirleme bakımından ilişki olup olmadığını araştırdık.
Yöntem: Meme kanseri nedeniyle ameliyat edilen 190 hastadan preoperatif kan tetkikleri sonucuna ulaşılan 160 hasta çalışmaya dâhil edildi. Ultrasonografi, mamografi ve patoloji sonuçları da kaydedildi.
Bulgular: Hastaların yaşı 28 ile 90 arasında değişmekte olup tanı anındaki median yaş 50 idi. Aile öyküsü 11 hastada vardı. 139 hasta invaziv duktal karsinom tanısı alırken, 15 hasta invaziv lobuler karsinom tanısı almıştı. Kanserin histopatolojik tipi ile inflamasyon belirteçleri arasında istatistiksel olarak anlamlı ilişki saptanmadı. Tümör boyutları açısından bakıldığında T1 ve T4 tümörler arasında trombosit/lenfosit oranı açısından anlamlı fark saptandı. N0 ile N1 hastalar karşılaştırıldığında ise hem nötrofil/lenfosit oranı hem de trombosit /lenfosit oranı açısından fark istatistiksel olarak anlamlıydı ($p<0,05$). Östrojen ve progesteron reseptörlerinin negatif veya pozitif olmasının ise ne nötrofil/lenfosit oranı ne de trombosit/ lenfosit oranı üzerinde etkisi olmadığı görüldü. Tümör *Grade*'leri açısından karşılaştırma yapıldığında da trombosit/lenfosit oranında istatistiksel olarak anlamlı fark saptandı.
Sonuç: Hem nötrofil/lenfosit oranı hem de trombosit/lenfosit oranı'nın ileri evre meme kanseri ve dolayısıyla prognoz beklentisi ile ilişkili olduğu görülmüştür. Klinikte kullanıma girebilmesi için bu belirteçlerle ilgili daha geniş vaka serileri ve uzun takip sürelerini içeren meta analizlere ihtiyaç vardır.

Anahtar Sözcükler: Meme kanseri, nötrofil/ lenfosit oranı, trombosit/ lenfosit oranı

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INTRODUCTION

Cancer progression and prognosis are affected by the host's inflammatory response in the tumor microenvironment (1). As components of systemic inflammatory response; lymphocytes, neutrophils and platelets are increasingly being recognized to have an important role in carcinogenesis and progression of tumor (2). Peripheral blood derived inflammation based scores are proposed as prognostic markers in solid tumors commonly in gastrointestinal system especially in colorectal cancer (3). However the role of these biomarkers in breast cancer prognosis is less well known (4). We aimed to investigate the association between pre-treatment neutrophyl/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and breast cancer in means of prognosis forecast.

METHODS

Between 2012 and 2014, 190 patients diagnosed and operated for breast cancer by the same surgical team. 160 patients with available data were included in the study. Demographic data of the patients, family history and risk factors for breast cancer, physical examination, laboratory findings, ultrasound, mammography and pathology results including tumor characteristics were all recorded. In order to ascertain that the blood count results were pre-treatment values, the initial treatment dates of the patients were checked. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count and PLR was defined as the absolute platelet count divided by the absolute lymphocyte count.

After the operations, the patients were followed up in the breast unit by the same surgical team. The follow-up time was calculated from the time of diagnosis to the end of the study period. If any, date and cause of the death was recorded. Statistically categorical variables were compared by using x^2 - test and continuous variables were expressed in "median" and compared by using Kruskal-Wallis test.

RESULTS

The median age was 50 y at time of diagnosis (28 y-90 y). Family history was positive in 11 patients (7%). There were 139 patients with invasive ductal carcinoma, 15 patients with invasive lobular carcinoma, 4 patients with ductal-lobular carcinoma and 2 patients with medullary carcinoma. There was no statistically significant association between histopathological type of the tumor and peripheral blood derived inflammation markers (Table 1).

The difference in PLR between T1 and T4 tumors was statistically significant ($p<0.05$). Also both NLR and PLR were significant when N0 patients compared with N1 (Table 2). Neither estrogen nor progesterone receptor status was shown to have an association with NLR and PLR. PLR was found to be statistically significant in different pathological grade groups (Table 3).

The patients were followed up every 3 months for the first two years and then every 6 months up to 5 years. Those who were lost to follow-up were excluded. Any unexpected event related with breast cancer was documented. No death from the progression of the disease was noted.

Table 1: RDW. PDW. NLR and PLR values according to the histopathological type

Pathology	RDW	PDW	NLR	PLR
Invasive ductal ca (86%)	14.41±1.77	13.51±2.37	2.43±1.43	149.94±61.34
Invasive lobular ca (9%)	14.51±1.63	14.02±3.25	2.38±0.93	141.29±62.55
Invasive ductal +lobular ca (3%)	15.22±0.78	14.52±2.3	2.53±0.94	136.43±48.56
Medullary ca (2%)	13.4±0.28	13.65±1.2	2.16±0.45	95.67±36.30
p value	NS	NS	NS	NS

RDW: red cell distribution witdh. PDW: platelet cell distribution width

Table 2: RDW. PDW. NLR and PLR values according to the TNM's

	n	%	RDW	PDW	NLR	PLR
T						
T1	43	27	14.06 ± 1.34	13.29 ± 2.75	2.19 ± 1.01	135.68 ± 50.75
T2	92	57.5	14.4 ± 1.62	13.82 ± 2.42	2.46 ± 1.39	147.01 ± 50.66
T3	13	8	14.86 ± 2.8	13.23 ± 1.66	2.21 ± 0.97	138.69 ± 61.96
T4	12	7.5	15.47 ± 2.11	31.21 ± 2.2	3.31 ± 2.29	211.15 ± 114.81
P			a	NS	NS	a
N						
N0	66	41	14.31 ± 1.53	13.93 ± 2.62	2.2 ± 0.9	131.98 ± 43.87
N1	82	51	14.54 ± 1.9	13.23 ± 2.25	2.67 ± 1.67	160.03 ± 67.62
N2	10	6	13.88 ± 0.72	14.13 ± 2.24	2.22 ± 0.86	141.21 ± 57.64
N3	2	2	16.3 ± 4.1	14.45 ± 5.02	1.05 ± 0.27	222.59 ± 153.24
P			NS	NS	b	b
M						
0	146	91	14.36 ± 1.68	13.65 ± 2.47	2.37 ± 1.26	144.35 ± 59.1
1	14	9	15.15 ± 2.18	12.94 ± 2.1	3.08 ± 2.2	187.02 ± 67.75
P			NS	NS	NS	0.038

a: T1& T4; p=0.04 for RDW. p=0.04 for PLR
b: N0 & N1. p= 0.029 for NLR. p=0.003 for PLR

Table 3: RDW. PDW. NLR and PLR values acoording to the grade and receptor status

	n	%	RDW	PDW	NLR	PLR
GRADE						
1	14	12	14.57 ± 2.02	14.71 ± 1.62	2.49 ± 2.17	141.5 ± 73.83
2	77	65	14.49 ± 1.79	13.33 ± 2.4	2.24 ± 0.87	138.99 ± 46.47
3	27	23	14.46 ± 1.74	13.32 ± 2.35	2.62 ± 1.2	177.44 ± 77.04
P			NS	c	NS	c
ER						
Negatif	33	79	14.55 ± 1.61	13.89 ± 2.91	2.33 ± 1.1	168.01 ± 74.03
Pozitif	127	21	14.4 ± 1.77	13.51 ± 2.32	2.45 ± 1.43	143.09 ± 56.34
p			NS	NS	NS	NS
PR						
Negatif	36	22.5	14.26 ± 1.5	13.73 ± 2.59	2.28 ± 1.05	158.45 ± 74.26
Pozitif	124	77.5	14.48 ± 1.8	13.54 ± 2.41	2.47 ± 1.45	145.18 ± 56.56
p			NS	NS	NS	NS
HER reseptörü						
Negatif	99	62	14.45 ± 1.72	13.48 ± 2.41	2.6 ± 1.55	154.19 ± 60.13
Pozitif	61	38	14.39 ± 1.77	13.75 ± 2.51	2.15 ± 0.95	138.07 ± 61.31
p			NS	NS	0.028	NS

c: Grade 1 & 2. p=0.013 for PDW; Grade 1 & 3. p=0.03 for PDW; Grade 2 & 3; p=0.02 for PLR

DISCUSSION

It is now widely recognized that outcomes in patients with cancer are not determined by tumor characteristics alone but patient related factors are also playing key roles (5). Cancer associated inflammation is a key determinant of disease progression and survival in most cancers (6). The inflammatory response involves systemic alterations triggered by cytokines and chemokines like an increase in neutrophyl count or platelet count. In addition red cell distribution width (RDW) and mean platelet volume are routine and easy to measure inflammatory markers (7). A recent meta-analysis of 40559 patients with solid tumors found that an NLR greater than 4.00 was associated with a substantial increase in risk for all cause mortality (8). Although PLR is not seen to be associated with either disease-free survival or over-all survival in women with breast cancer (9), in our study both NLR and PLR were found to be correlated with advanced stage breast cancer so with prognosis.

Previous studies had found that the impact of NLR and PLR on breast cancer prognosis varies according to the cancer subtypes (10) but we did not find a significant effect. In order to use these readily available biomarkers in clinical settings and call them predictive prognostic indicators more clinical trials with large number of patients and long follow-up periods are needed.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Hanahan D. Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144: 646-74.

2. DeNardo DG. Coussens LM. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Res. 2007; 9: 212.

3. Li MX. Liu XM. Zhang XF. et al. Prognostic role of neutrophil-to-lymphocyte ratio in colorectal cancer: a systematic review and meta-analysis. Int J Cancer. 2014; 134: 2403-13.

4. Azab B. Bhatt VR. Phookan J. et al. Usefulness of the neutrophil-to-lymphocyte ratio in predicting short- and long-term mortality in breast cancer patients. Ann Surg Oncol. 2012; 19: 217-24.

5. Guthrie GJ. Charles KA. Roxburgh CS. et al. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. Crit Rev Oncol Hematol. 2013; 88: 218-30.

6.Hanahan D. Weinberg RA. The hallmarks of cancer. Cell. 2000; 100: 57-70.

7. Balta S. Demirkol S. Kucuk U. Sarlak H. Kurt O. Arslan Z. Neutrophil to lymphocyte ratio may predict mortality in breast cancer patients. J Breast Cancer. 2013; 16: 354-5.

8.Templeton AJ. McNamara MG. Šeruga B. et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014; 106: dju124.

9.Cihan YB. Arslan A. Cetindag MF. Mutlu H. Lack of prognostic value of blood parameters in patients receiving adjuvant radiotherapy for breast cancer. Asian Pac J Cancer Prev. 2014;15: 4225-31.

10. Noh H. Eomm M. Han A. Usefulness of pretreatment neutrophil to lymphocyte ratio in predicting disease-specific survival in breast cancer patients. J Breast Cancer. 2013; 16: 55-9.

Rektumda Polipoid-Malign Görünümlü Lezyonla Başvuran Soliter Rektal Ülser Olgusu

A Case of Solitary Rectal Ulcer Presenting with Polipoid-Malignant Appearance in Rectum

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ÖZET

Soliter rektal ülser sendromu (SRÜS), sıklıkla genç-erişkin yaş grubunda görülen etiyolojisi tam olarak bilinmeyen kronik bir hastalıktır. Tanı semptomlar, endoskopik bulgular ve histopatolojik incelemelerin kombinasyonu ile mümkündür. Bu yazıda maligniteyi taklit eden soliter rektal ülser olgusu sunulmuştur**.**

Anahtar Sözcükler: Soliter rektal ülser, meselazin

Geliş Tarihi: 06.07.2015 **Kabul Tarihi:** 04.10.2016

ABSTRACT

Solitary rectal ulcer syndrome (SRUS) is a rare pathology which is usually seen the young-adult group with unknown etiology. Diagnosis has been made possible by a combination of symptoms, endoscopic findings and histopathological examinations. In this article, we report a case with solitary rectal ulcer mimicking malignancy.

Key Words: Soliter rectal ulcer, meselasin

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GİRİŞ

Soliter rektal ülser sendromu (SRÜS), sıklıkla genç-erişkin yaş grubunda görülen etiyolojisi tam olarak bilinmeyen kronik bir hastalıktır. Tanı semptomlar, endoskopik bulgular ve histopatolojik incelemelerin kombinasyonu ile mümkündür. Bu yazıda maligniteyi taklit eden soliter rektal ülser olgusu sunulmuştur.

OLGU SUNUMU

Onsekiz yaşında erkek hasta konstipasyon ve defekasyon sonrası tam boşalamama hissi ile gastroenteroloji polikliniğine başvurdu. Hastanın öyküsünde yaklaşık 2 yıldır dışkılama güçlüğü olduğu öğrenildi. Yapılan rektosigmoidoskopik incelemede rektum ön duvarda 5. cm de lümende yaklaşık 3 cm çapında ülsero-vejetan görünümde polipoid lezyon izlendi (Resim 1). Malign görünümü andıran lezyondan örneklemeler yapıldı. Bunun üzerine dışkılama esnasında parmak ile dışkılama (dijital) kullanımının olup olmadığı tekrar sorgulandı ve dijital kullanımı olduğu öğrenildi. Histopatolojik incelemede kronik inflamasyon bulguları, lamina propriada mononükleer hücre infiltrasyonu ile uyumlu olup malignite yönünden bulgu saptanamadı olarak rapor edildi.

Yapılan anaorektal manometrik çalışmalar sonucunda rektoanal dissinerji ile uyumlu bulgular saptandı. Hastada bu bulgular ile soliter rektal ülser ön planda düşünüldü. Hastaya biofeed back yapılarak günlük uygulaması öğretildi.

Dijital kullanmaması konusunda uyarıldı ve mesalazin lavman 1 gram 3 aylık tedavi verildi. Üç ay sonraki rektal incelemede lezyonunda makroskopik olarak büyük oranda gerilediği sadece bir alanda üzerinde yüzeyel ülsere alan ve kenar mukozasının hiperemik olduğu tipik SRÜ görünümü izlendi(Resim 2). Biofeed back sonrası defekasyonla ilgili şikayetlerinin de azaldığı öğrenildi.

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TARTIŞMA

Soliter rektal ülser sendromu (SRÜS) kronik bir hastalıktır özellikle genç erişkinlerin hastalığıdır. Kadın cinsiyette daha sıktır (1). Başlangıç semptomları kanlı mukuslu dışkılama, konstipasyon, tenesmus, anorektal ağrı ve defekasyon sonrası tam boşalmama hissi olarak sayılabilir. Klinik semptomlar karakteristik değildir. Bu sebeple SRÜS kolonun diğer malign veya benign hastalıklarıyla karışabilir. Bizim hastamızda öncelikli tanımız (detaylı sorgulamadan önce) malignite idi. Ancak biyopsi sonuçlarıyla maligniteyi dışladık. Alınan doku örneklerinin histopatolojik incelemesinde fibromüsküler hiperplazi, lamina propriada düz kas hücresi ve kollajen infiltrasyonu, muskülaris mukozada kalınlaşma ve kript yapılarında bozulmaların olması ve lamina propriada görülen kollajen infiltrasyonu SRÜS için tipik bir bulgular olup (2), bizim olgumuzda da benzer şekilde rapor edilmiştir.

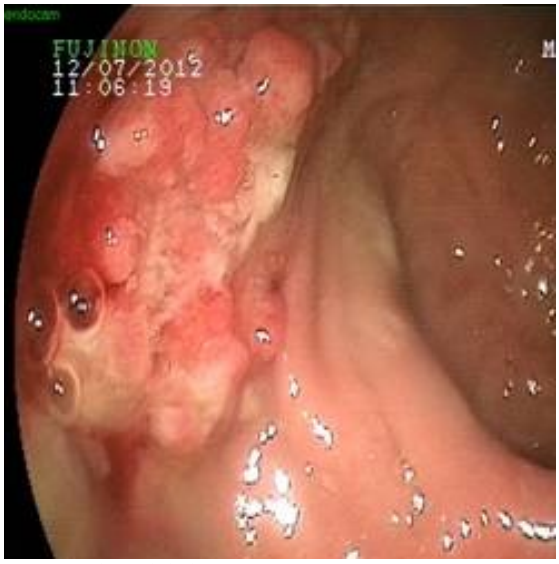
Lezyon etrafı eritemli, tek bir ülser, multipl ülserler veya polipoid lezyonlar şeklinde de görülebilir. Bir seride ülserasyon prevelansı %57, polipoid lezyonlar %25, hiperemik mukozal yama şeklinde görünüm %18 olarak bildirilmiştir (3).

Sıklıkla rektum anterior duvarda (vakaların %68-95) ve anal verge'den itibaren 6-10 cm de görülmektedir (1-4). SRÜS etyopatogenezi tam olarak ortaya konulamamıştır. Ancak bazı olası mekanizmalar düşünülmektedir. Etkilenen hastalarda gizli ya da açık rektal prolapsus olduğu, pelvik taban kaslarının uyumsuz şekilde kasıldığı ve mukozadaki kan akımının azaldığı yapılan çalışmalarla gösterilmiştir (5,6).

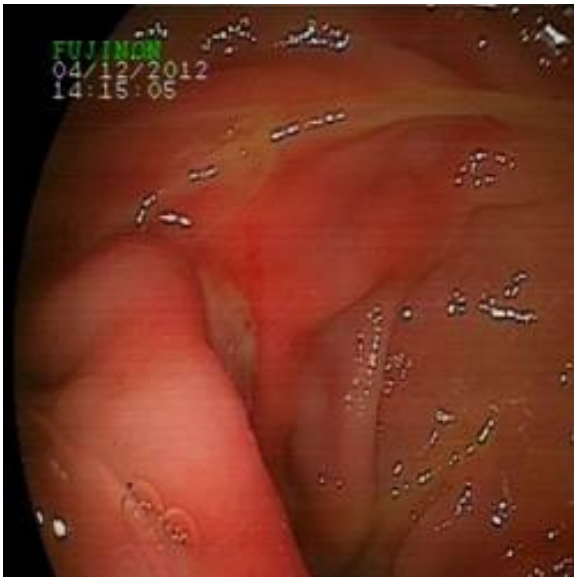
Rektum mukozasında travmaya bağlı ülser geliştirebilecek diğer bir neden de şiddetli konstipasyonu olan hastalarda rektal içeriği parmak yardımıyla boşaltmaya çalışmaktır (2,7).

Tekrarlayan girişimler sırasında mukozal bütünlük zedelenmektedir. Ancak anal kanala parmak mesafesinden daha uzak ülserler için bu mekanizmanın geçerli olduğunu düşünmek zordur. Yapılan kontrollü çalışmalarda defekasyon sırasında koordinasyon bozukluğu olması ve uzun süreli zorlanma ile şiddetli ıkınmaların SRÜS için anahtar rol oynadığı bildirilmiştir (8). Bizim olgumuzda gözlenebilir bir rektal prolapsus yoktu. Ancak öyküsünden kronik konstipasyonunun olduğu ve detaylı sorgulamalarda parmak kullanım öyküsü olduğu öğrenilmişti.

SRÜS'te tedavi kişiye özel ve eğer saptanabiliyorsa nedene yönelik olmalıdır. Diyete lif eklenmesi ve laksatif kullanımınına yanıt alınamazsa medikal tedavi yöntemleri denenebilir. Ancak bu yaklaşımlara yanıtsız olan hastalara defekografi yapılmadır. Defekografi normal sınırlarda saptanırsa alternatif tedavi yöntemleri denenebilir. Defekografide pelvik taban kaslarında uyumsuz kasılmalar saptanırsa pelvik taban disfonksiyonu düşünülür. Pelvik taban disfonksiyonu olan hastalarda diğer tedavi yöntemlerinden fayda görmeyenlere ve özellikle konstipe olanlara biofeed back uygulaması denenebilir. Bu tedavinin amacı defekasyon sırasında pelvik taban kaslarını ve anal sfinkteri gevşeterek rektoanal koordinasyonu sağlamak ve normal defekasyon dinamiğini gerçekleştirmektir (9,10). Davranışsal tedaviler ile semptomlarda belirgin düzelmelerin sağlandığı rapor edilmiştir (11,12).



Resim 1: Rektumda kitle



Resim 2: Tedavi sonrası lezyon

SONUÇ

Malign görünümlü rektal tümörlerde ayrıca tanıda soliter rektal ülser ayırıcı tanıda mutlaka düşünülmelidir. SRÜ olduğu düşünülen olgularda digital kullanımı tekrar sorgulanmalıdır. Konstipasyonla seyreden olgulara anoreaktal manometrik çalışma yapılmalı ve biofeed back uygulanmalıdır.

Çıkar Çatışması

Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

KAYNAKLAR

1. Tjandra JT, Fazio VW, Church JM, Lavery IC, Oakley JR,Milsom JW. Clinical conundrum of solitary rectal ulcer. DisColon Rectum 1992; 35: 227-34.
2. Remes-Troche JM, Rao S SC. Anorectal motor disorders. Best Practice & Research Clinical Gastroenterology 2007;21:733-48.
3. Martin CJ, Parks TG, Biggart JD. Solitary rectal ulcer syndrome in Northern Ireland, 1971-1980. Br J Surg 1981; 68: 744-7.
4. Levine DS. "Solitary" rectal ulcer syndrome. Gastroenterology 1987; 92: 243
5. Felt-Bersma RJ & Cuesta A. Rectal prolapse, rectal intussusception, rectocele, and solitary rectal ulcer syndrome. Gastroenterol Clin North Am 2001;30:199-222.
6. Jarrett MED, Vaizey CJ, Emmanuel AV et al. Behavioral therapy (biofeedback) for solitary rectal ulcer syndrome improves symptoms, mucosalblood flow. Gut 2004;53:368-70.
7. Sharara AI, Azar C, Amr SS et al. Solitary rectal ulcer syndrome: endoscopic spectrum and review of the literature. Gastrointest Endosc 2005;62:755-62.
8. Morio O, Meurette G, Desfourneaux V et al. Anorectal physiology in solitary ulcer syndrome: a casematched series. Dis Colon Rectum 2005;48:1917-22.
9.Rao SS, Ozturk R, De Ocampo S et al. Pathophysiology and role of bi-ofeedback therapy in solitary rectal ulcer syndrome. Am J Gastroente- rol 2006;101:613-8
10. Bishop PR & Nowicki MJ. Nonsurgical therapy for solitary rectal ulcer syndrome. Curr Treat Options Gastroenterol 2002;5:212-23.

Acute Coronary Syndrome due to Diclofenac-Induced Anaphylaxis: Type 1 Kounis Syndrome

Diklofenak'ın Tetiklediği Anafilaksi Nedenli Akut Koroner Sendrom: Tip 1 Kounis Sendromu

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ABSTRACT

Kounis syndrome is a potentially life-threatening medical emergency with both acute coronary syndrome and a severe allergic reaction which can occur even in patients with angiographically normal coronary arteries. In this report, we present a patient who developed acute coronary syndrome (type 1 Kounis syndrome) after diclofenac sodium intake.

Key Words: Kounis syndrome, acute coronary syndrome, diclofenac, anaphylaxis.

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ÖZET

Kounis sendromu, anjiyografik olarak normal koroner arterleri olan hastalarda meydana gelen ve potansiyel olarak yaşamı tehdit eden şiddetli alerjik bir reaksiyon ile akut koroner sendrom birlikteliğinin olduğu acil bir durumdur. Bu olguda, diklofenak sodyum alımı sonrasında akut koroner sendrom (tip 1 Kounis sendromu) gelişen bir olgu sunulmuştur.

Anahtar Sözcükler: Kounis sendromu, akut koroner sendrom, diklofenak, anafilaksi.

Geliş Tarihi: 19.09.2015 **Kabul Tarihi:** 02.11.2016

INTRODUCTION

Kounis syndrome is a potentially life-threatening medical emergency with both acute coronary syndrome and a severe allergic reaction which can occur even in patients with angiographically normal coronary arteries. This complication should be diagnosed early and proper treatment should be initiated quickly. There are several causes underlying this syndrome including some drugs, latex, foods, as well as various conditions and environmental exposures (1).

In this report, we present a patient who developed acute coronary syndrome (type 1 Kounis syndrome) after diclofenac sodium intake.

CASE REPORT

A non-steroidal antiinflammatory drug diclofenac sodium was initiated by orthopedics clinic to 51 year-old male patient for his knee pain. After 45 minutes of ingestion of 100 mg diclofenac sodium lacrimation in his eyes, widespread pruritus in his body and severe chest pain had started and he was presented to our emergency department. On admission physical examination was normal except widespread erythematous rashes. His vital signs were stable including blood pressure 105/66 mmHg and heart rate 96 bpm. In his medical history there was not a history of any allergic disease and except being an ex-smoker he was devoid of any cardiovascular risk factor.

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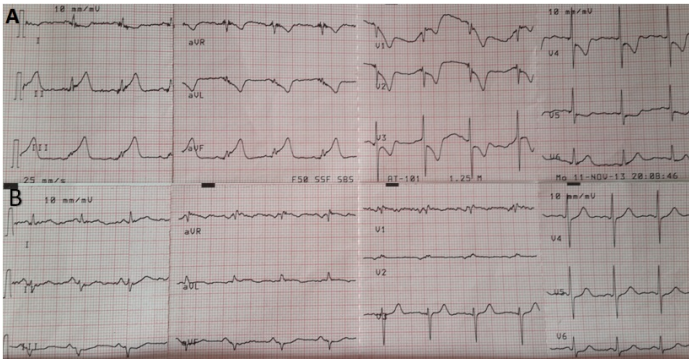

Figure 1.(A) ECG recording showing approximately 2-3 mm ST-elevation in inferior derivations, reciprocal ST- segment depression up to 3 mm and invers T wave in entire precordial leads **(B)** Resolution of ST-segment elevations after antihistaminic and corticosteroid slow intravenous injection therapy

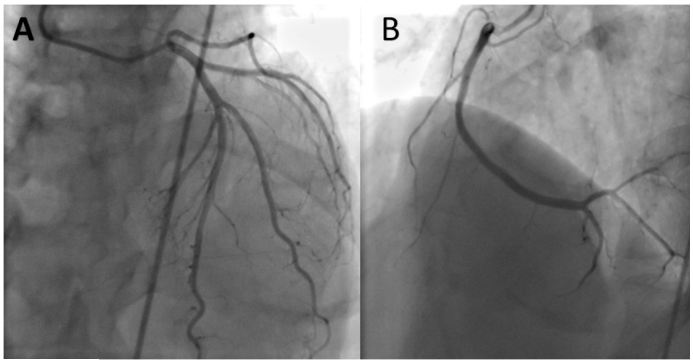

Figure 2. (A) Selective left coronary angiogram demonstrating normal left coronary system (**B)** Selective right coronary angiogram demonstrating normal right coronary artery

Because of chest pain an ECG was performed and it revealed 2-3 mm ST segment elevation in DII-DIII and aVF leads and reciprocal changes in other leads (Figure 1A). Because of presence of systemic allergic reaction pheniramine (antihistaminic) and dexamethasone (corticosteroid) treatments were initiated. Beside this because of the suspect of acute coronary syndrome enoxaparine and acetylsalicylic acid were administered. After initial treatment symptoms of patient was regressed and chest pain was resolved. Repeated ECG showed completely normal findings (Figure 1B). The cardiac enzyme panel was within normal limits [troponin I (<0.005 ng/ml), CK-MB (2.25 ng/ml) and CK (131.8 u/l)]. The echocardiography was within normal limits and the coronary angiogram showed normal coronary arteries without any obstruction (Figure 2).

DISCUSSION

In this report we presented a patient who developed acute coronary syndrome after oral administration of 100 mg diclefenac sodium.

In our patient, according to the development of allergic and cardiac symptoms after drug administration, dramatic respsonse to antihistaminic and corticosteroid treatment and according to the absence of coronary artery stenosis we thought that diclofenac was the triggering factor of an allergic reaction for development of this clinical picture.

In 1991 Kounis and Zavras described the syndrome of allergic angina and allergic myocardial infarction, currently known as Kounis syndrome (2). This allergic reaction is known to be caused by inflammatory mediators such as histamine, chemokines and cytokines. There are several causes underlying this syndrome including some drugs, latex, foods, as well as various conditions and environmental exposures (1). Nonsteroidal anti-inflammatory drugs are frequently used in daily clinical practice and they are the second most commonly seen class of medications causing anaphylaxis (3). Because NSAIDs are among the most widely used drugs, their possible side effects should be known by all physicians. The systemic anaphylactic reaction caused by inflammatory mediators released during the activation process should be controlled early in the management of these patients. A previous case report emphasized that a 74-year-old woman had felt chest pain and her ECG recording showed ST-elevation in inferior derivations after intravenous administration of diclefenac at emergency department (4). However, this patient had undergone successful coronary angioplasty with implantation of bare-metal stents two months ago (type 2 Kounis syndrome). In our case we established a rapid diagnosis and the appropriate antiallergic treatment was started accordingly. The coronary angiogram showed normal coronary arteries without any obstruction. The result was excellent with the full recovery of our patient.

CONCLUSION

Kounis syndrome should be considered in patients without any risk factor for coronary artery disease when they develop acute coronary syndrome (especially inferior myocardial ST segment elevation) after any drug intake. These patients should be treated with steroids, antihistamines, acetylsalicylic acid and enoxaparine before transfer to the coronary angiography laboratory (5).

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Fassio F, Almerigogna F. Kounis syndrome (allergic acute coronary syndrome): different views in allergologic and cardiologic literature. Intern Emerg Med 2012;7:489-95

2. Kounis NG, Zavras GM. Histamine-induced coronary artery spasm: the concept of allergic angina. Br J Clin Pract 1991;45:121-8.

3. Brown AF, McKinnon D, Chu K. Emergency department anaphylaxis: a review of 142 patients in a single year. J Allergy Clin Immunol 2001;108:861-6.

4. Cakar MA, Gündüz H, Kocayiğit I, Binak DF, Vatan MB, Tamer A. Acute coronary syndrome due to diclofenac potassium induced anaphylaxis: two Kounis syndrome variants in the same patient. Anadolu Kardiyol Derg 2011;11:88-9.

5. Cevik C, Nugent K, Shome GP, Kounis NG. Treatment of Kounis syndrome. Int J Cardiol 2010;143:223-6.

Gebelikte Kolon Kanseri Metastazına Bağlı Krukenberg Tümörü: Olgu Sunumu

Krukenberg Tumor Metastasized from Colon Cancer in Pregnancy: A Case Report

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ÖZET

Krukenberg tümörü, gastrointestinal kanserin overlere metastazı olarak tanımlanmaktadır, prognozu oldukça kötüdür ve beş yıllık yaşam süresi ortalama %12-23.4 dir. Krukenberg tümörünün primer kaynağı sıklıkla gastrik kanserdir, buna rağmen kolon, apendiks, meme, akciğer ve pankreas kanserlerinin de overe metastazları gösterilmiştir. Krukenberg tümörü sıklıkla hayatın beşinci dekadında ve ortalama 45 yaşında görülmektedir. Gebelik sırasında tanısı oldukça nadirdir. Olguların %80 'i bilateraldir. Burada akut pelvik ağrı ile başvuran kolon karsinomuna sekonder Krukenberg tümörlü 17 hafta ile uyumlu gebeliği bulunan bir olguyu sunmaktayız.

Anahtar Sözcükler: Krukenberg tümörü, gebelik, kolon karsinomu

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ABSTRACT

Krukenberg tumor refers to gastrointestinal cancer metastatic to the ovaries, and has an extremely poor prognosis, with a five-year survival rate ranging from 12% to 23.4%. Gastric cancer has been reported as the most frequent primary source of Krukenberg tumor; however, tumors of the colon, appendix, breast, lung, and pancreas have also been reported to metastasize into the ovaries. Krukenberg tumors are usually seen in the fifth decade of life, with an average age of 45 years and cases diagnosed during pregnancy are thus extremely rare. They are bilateral in 80% of the cases. Here, we report a case of a Krukenberg tumor secondary to colon carcinoma in a pregnant woman with acute pelvic pain. The prenatal diagnosis was made at 17 weeks' gestation.

Key Words: Krukenberg tumor, pregnancy, colon carcinoma.

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GİRİŞ

Krukenberg Tümörü; gastrointestinal sistem kanserinin tek taraflı veya iki taraflı overe metastaz yapmasını ifade eder. Beş yıllık yaşam süresi %12 ile %23.5 arasında değişmektedir. Solid tümörlerdendir ve %80 iki taraflıdır(1). Krukenberg tümörü genellikle yaşamın beşinci dekadında görülmektedir, prognozu kötüdür (2,3) ve letal olarak seyretmektedir(4). Klinik olarak değişkenlik göstermekle birlikte genellikle pelvik ağrı, adneksiyal kitle, bulantı-kusma, kilo kaybı ve maternal, çok nadir olarak ta fetal virilizasyon (5) ile birlikte görülmektedir. Gebelikte nadir görülen Krukenberg tümörünün diğer pelvik ağrı ve adneksiyal kitlelerden ayırıcı tanısının erken dönemde konulması prognoz açısından oldukça önemlidir. Genç ve arkadaşları, erken teşhisi takiben hamilelik sırasında yapılan ameliyat ve kemoterapinin bu hastalarda olumlu sonuca neden olabileceğini öne sürmüşlerdir (4). Buna rağmen gebelikteki Krukenberg tümörü için tedavi stratejisi net değildir. Bu yazıda, 27 yaşında akut pelvik ağrı ile kliniğimize başvuran ve kolon adenokarsinomu metastazına bağlı oluşan Krukenberg tümörü tanısı konulan gebe olgu sunulmaktadır.

OLGU SUNUMU

Yirmi yedi yaşında, primigravid, 17 haftalık gebeliği bulunan hasta kliniğimize akut pelvik ağrı şikayeti ile başvurdu. Yapılan ultrasonografide, gestasyonel hafta ile uyumlu tek canlı gebelik ve sağ adneksiyal bölgede overden kaynaklanan yaklaşık 140×130 mm çapında semisolid kitle ve batın içinde yaygın asit gözlendi (Şekil 1). Hastanın karnı oldukça gergin ve abdominal muayenede yaygın hassasiyet mevcut idi, ovaryan torsiyondan şüphelenilerek acil laparatomi yapıldı. Hasta ve eşi bilgilendirilerek onam formu alındı. Laparatomi esnasında, batında yaygın asit, sağ overde yaklaşık olarak 160×140 mm nekrotik tümörden kaynaklanan overyan torsiyon ve omentum üzerinde metastazlar gözlendi. Batın sitolojisi alındı, hastaya tek taraflı ooferektomi ve omentektomi yapıldı. Over ve omentumdan alınan örneklerin histopatolojik inceleme sonucu muhtemel primeri gastrointestinal karsinom kaynaklı adenokarsinom metastazı olarak rapor edildi (Şekil 2a,b). Sitokeratin 20 (+++) ve karsino embriyonik antijen (CEA) (+++) ile immünohistokimyasal olarak pozitif boyandı (Şekil 2c). Asit mayinin sitolojik incelmesinde malign hücrelere rastlanmadı.

Bu olgu 14. FMF Dünya Kongresi'nde bildiri olarak sunulmuştur.

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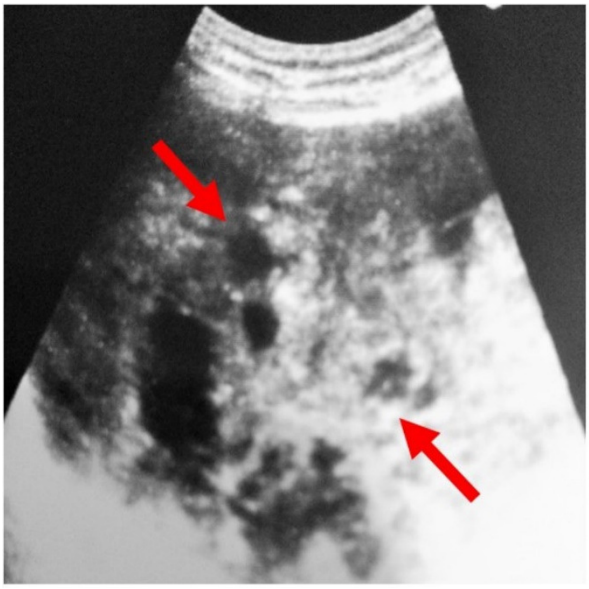


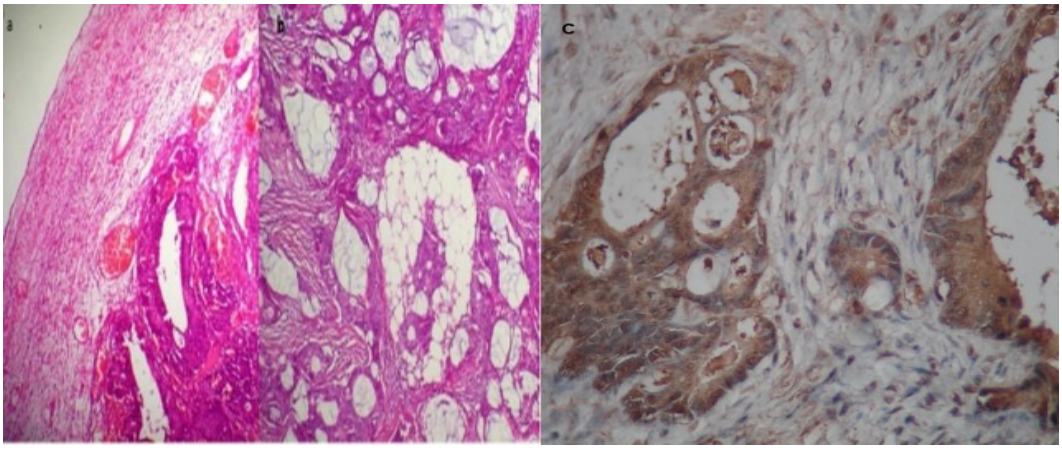
Şekil 1: Transabdominal ultrasonografide sağ adneksiyal bölgede yaklaşık 140×130 mm çapında semisolid kitle

Hastanın üst gastrointestinal sistem endoskopisi normaldi fakat kolonoskopi de inen kolondan kaynaklanan lümenin üçte ikisini kaplayan kitle gözlendi. Kitleden yapılan biyopsilerin histopatolojik inceleme sonucu primer kolon adenokarsinomu olarak rapor edildi. Kötü maternal prognoz nedeni ve hastanın istemi ile gebelik sonlandırıldı. Hasta Krukenberg tümörü tanısı aldıktan bir ay sonra exitus oldu.

TARTIŞMA

Krukenberg tümörü genellikle hayatın beşinci dekadında görülmektedir. Erken tanı ve tedavi, prognozun kötü olmasından dolayı oldukça önemlidir. Gebelikte erken dönemde görülen bulantı-kusma gibi gastrointestinal semptomlar ve fetal büyüme ile eş zamanlı abdominal büyüme altta yatan gastrointestinal ve ürogenital sitemden kaynaklanan tümöral durumları taklit edip maskeleyebilir. Gebelikteki bulantı-kusmanın, kilo kaybının ve pelvik ağrı gibi bulguların gebelik haftası ilerledikçe devam etmesi ve şiddetlenmesi mutlaka gastrointestinal sistem ile ilgili diğer patolojilerden ayırıcı tanısının yapılması gerektiğini göstermektedir.

Krukenberg tümörü kaynaklandığı gastrointestinal sistemdeki orjinine göre farklı klinik tablolar gösterebilir; mideden kaynaklı olanlar daha sık bulantı-kusma, kilo kaybı, iştahsızlık gibi semptomlar gösterebilirken, gastrointestinal sistemin diğer bölgelerinden kaynaklananlar (kolon, ince bağırsak, duedonum vb.) bağırsak obstrüksiyonu, adneksiyal kitle, gaitada kanama, abdomino-pelvik ağrı, bulantı-kusma, iştahsızlık ve kilo kaybı ile kendini gösterebilir (6). Fakat bu semptomların hepsi gebelikte, gebeliğin non-spesifik bulguları ile karışıp maskelenebilir. Literatürde gebeliğin ovaryan kanserin yayılmasında veya büyümesine olumsuz etki ettiğini gösteren herhangi bir bulgu yoktur. Fakat gebelikte tümör torsiyonu veya rüptürüne bağlı akut kliniğinin görülme olasılığı artmaktadır. Burada sunulan olgu 17. gebelik haftasında akut pelvik ağrı ile başvurmuş, semptomların çok aşikar olması nedeni ile yapılan ultrasonografide maternal batında yaygın asit ve sağ ovaryan lojda semisolid kitle saptanması üzerine, hastaya over torsiyonu ön tanısı ile acil olarak laparatomi yapılmıştır. Ooferektomi materyalinin ve omentumdaki yaygın kitlelerden alınan örneklerin incelenmesi sonrası adenokarsinom tanısı konulmuştur.



Şekil 2a,b: Overde adenokarsinom metastazı, histopatolojik görünüm
Şekil 2c: Sitokeratin 20 (+++) ve karsino embriyonik antijen (CEA) (+++) ile immünohistokimyasal olarak pozitif boyanma

Krukenberg tümörü tanısı alan bazı vakalarda primer tümör bulunamadan hastalar kaybedilebilir. Krukenberg tümörü %6-22 üst gastrointestinal sistemden, %15-32 de alt gastrointestinal kaynaklanmaktadır (7,8). Bizim hastamızda yapılan endoskopi de hiçbir bulgu saptanmamış, kolonoskopide inen kolondan kaynaklanan lümenin üçte ikisini dolduran kitle saptanmıştır. Yapılan multipl biyopsiler sonrasında primer kolon adenokarsinomu tanısı konulmuştur.

Erken tanı ve tedavi yönetiminin hastanın yaşam süresi açısından önemli olmasına rağmen hastanın yaşı, gastrointestinal sistemdeki primer tümörün yeri, eş zamanlı gebelik olması ve primer orjinini saptanmadan önce overe metastaz yapması prognozun kötü olduğunu göstermektedir (9,10). Krukenberg tümöründe mümkün olan en kısa süre içerisinde kombine sitoredüktif cerrahi ve kemoterapi uygulanması ve olumsuz sonuçları en aza indirmek için, gebe olan ve olmayan hastalarda tedavi yaklaşımında fark olmaması gerektiği ifade edilmektedir (4,10).

SONUÇ

Burada terminal dönem kolon adenokarsinomuna bağlı Krukenberg tümörü tanısı alan ve 17 haftalık canlı gebeliği bulunan bir olgu sunulmuştur. Radikal cerrahi tedavi yapılamayan hasta Krukenberg tümör tanısı aldıktan bir ay sonra exitus olmuştur.

Çıkar Çatışması
Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

KAYNAKLAR

1.Ritter DB, McGill FM, Greston WM. Krukenberg tumor part II: Identification during pregnancy. Female Patient. 1999;24:19
2.Sun HD, Hsiao SM, Chen YJ, Wen KC, Li YT, Wang PH. Advanced endocervical adenocarcinoma metastatic to the ovary presenting as primary ovarian cancer. Taiwan J Obstet Gynecol 2015;55:201e3
3.Jiang R, Tang J, Cheng X, Zang RY. Surgical treatment for patients with different origins of Krukenberg tumors: outcomes and prognostic factors. Eur J Surg Oncol 2009;35:92e7.
4.Genç M, Genç B, Solak A, Gür E, Sezgin C. Bilateral Krukenberg tumor in a 16- week pregnant woman. Eur J Gynaecol Oncol 2014;35:95e6.
5.Papakonstantinou E, Liapis A, Kairi-Vassilatou E, Iavazzo C, Kleanthis CK, Kondi-Pafiti A. Virilizing ovarian Krukenberg tumor in a 27-year-old pregnant woman. A case report and literature review. Eur J Gynaecol Oncol. 2011;32:331-3.
6.Goff BA, Mandel LS, Drescher CW, et al. Development of an ovarian cancer symptom index: possibilities for earlier detection. Cancer 2007; 109:221.
7.de Waal YR, Thomas CM, Oei AL, et al. Secondary ovarian malignancies: frequency, origin, and characteristics. Int J Gynecol Cancer 2009; 19:1160.
8.Kondi-Pafiti A, Kairi-Vasilatou E, Iavazzo C, et al. Metastatic neoplasms of the ovaries: a clinicopathological study of 97 cases. Arch Gynecol Obstet 2011; 284:1283.
9.Tamussino K, Scholl W, Reich O, Winter R. Gastric carcinoma presenting as a Krukenberg tumor in the 24th week of gestation. Eur J Obstet Gynecol Reprod Biol 1995; 62: 251-252,
10. Glisić A, Atanacković J. Krukenberg tumor in pregnancy. The lethal outcome. Pat Oncol Res 2006;12:108-10.

Komplikasyonlarla Seyreden Bir Boğmaca Olgusu Nedeniyle "Koza Stratejisi"

A Case of Whooping Cough with Complications: The Importance of "Cocooning" Immunization

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ÖZET

Boğmaca hastalığı, üst üste gelen inatçı ve spazmodik öksürük nöbetleri ile karakterize, *Bordetella pertussis* ile oluşan çocukluk çağının akut solunum yolu enfeksiyonudur. Yaygın ve yüksek oranlardaki aşılamaya rağmen, ergenler ve genç erişkinlerde hastalık sıklığı yıllar içinde artış göstermektedir. Süt çocukluğu ve yenidoğan döneminde hastalığın kaynağını temasta oldukları erişkin ve ergenler oluşturmaktadır. Bu dönemde hastalıktan korunmanın önemli yöntemi "koza stratejisi" ile aşılamadır. Burada elli günlük boğmaca tanısı ile izlenirken, ağır sekonder pnömöni ve nöbet komplikasyonu görülen bir süt çocuğu sunulmaktadır. Bu olgu ile aşısız ya da inkomplet aşılı süt çocuklarıyla yakın temasta olan ve *B. Pertussis* açısından ana kaynak olarak gösterilen ergen ve erişkinlerin, aşılamasına ve hastalığın önlenmesine vurgu yapılması amaçlanmıştır.

Anahtar Sözcükler: *Bordetella pertussis*, koza stratejisi, komplikasyon, nöbet, pnömöni

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ABSTRACT

Whooping cough caused by *Bordetella pertussis* is a common acute respiratory infection that is characterized by paroxysmal spasmodic cough in childhood. Despite to the high vaccination rates, the incidence of the disease has been increasing in adolescents and in also adults. This population is standing as the common source of the infection for newborns and infants. At this stage, the primary way to get protected from the disease is the "cocooning" immunization. Herein we report a fifty-days-old infant who was diagnosed as whooping cough and then complicated as severe secondary pneumonia and also seizure during the period of the disease. By the way, it is aimed to point out the importance of the immunization of adolescents and adults who are in contact with newborns and infants who are at risk for the disease.

Key Words: *Bordetella pertussis*, cocooning immunization, complications, seizure, pneumonia

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GİRİŞ

Boğmaca hastalığı, üst üste gelen inatçı ve spazmodik öksürük nöbetleri ile karakterize çocukluk çağının akut solunum yolu enfeksiyonudur(1). Hastalığın etkeni *Bordetella pertussis* gram negatif, aerobik, sporsuz ve hareketsiz bir kokobasildir (2).Yaygın ve yüksek hızlardaki aşılamaya rağmen, ergenler ve genç erişkinlerde hastalık sıklığı yıllar içinde artış göstermektedir (3). Süt çocukluğu ve yenidoğan döneminde hastalığın mortalitesi ve morbiditesi en yüksektir (3). Yenidoğanları, aşısız ya da eksik aşılı süt çocuklarını hastalıktan korunmanın önemli yöntemi, yakın temasta olan ergen ve erişkinlerin aşılanmasının önerildiği "koza stratejisi" ile aşılamadır. Bu amaçla, özellikle annelerin gebelik döneminde ya da hemen doğumdan sonra aşılanması önem taşımaktadır. Ayrıca bebekle yakın temasta bulunan ve bebeğe bakım veren sağlık personelinin aşılanması gereklidir.

Burada boğmaca tanısı ile izlenirken, ağır sekonder pnömöni ve nöbet komplikasyonu görülen bir süt çocuğu, koza stratejisinin öneminin tekrar hatırlatılması amacıyla sunulmuştur.

OLGU SUNUMU

Elli günlük erkek hasta, öksürük, morarma ve öksürüğün ardından katılma şeklinde nöbet geçirme nedeniyle başvurdu. Hastanın 10 gün önce başlayan ve şiddeti giderek artan, peşpeşe 15 kez tekrarlayan öksürüğü mevcuttu. Ateş ve burun akıntısı yoktu. Özgeçmiş ve soygeçmişinde özellik olmayan hastanın ailede yaklaşık 3 haftadır öksüren 15 yaşında ablası vardı. Fizik muayenede; tipik iç çekmenin eşlik ettiği arka arkaya 20 kez morarma ve kızarma ile birlikte olan öksürük nöbeti izlendi. Laboratuvar incelemesinde; Hb 12 gr/dl, MCV 99,9 fl, MCH 34 pg, BK 63.760 / mm^3, PLT 468.000 / mm^3, CRP <3.45 mg/dl, kan biyokimya değerleri normal idi. Akciğer grafisinde yaygın interstisiyel infiltrasyon (Resim1A) izlendi. Tipik öksürük nedeniyle alınan boğmaca kültüründe; *Bordetella Pertussis* üremesi saptandı. Hasta sefotaksim (150 mg/kg/gün), ampisilin (200 mg/kg/gün) ve klaritromisin (30 mg/kg/gün) tedavileri ile izleme alındı. Tedavinin 3. gününde solunum sıkıntısı başlayan hastanın solunum sayısı 70/dk, her iki hemitoraksta subkostal retraksiyonlar, dinlemekle sağ akciğerde havalanma azlığı tespit edildi. BK 78.000/mm^3, CRP 156 mg/dl bulundu.

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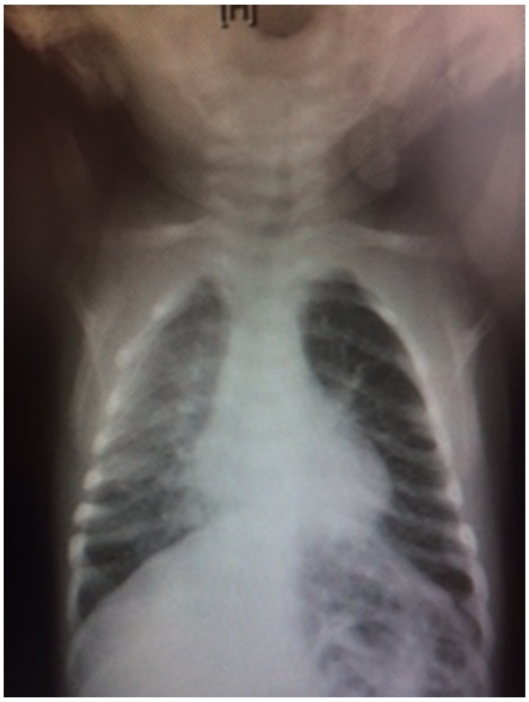
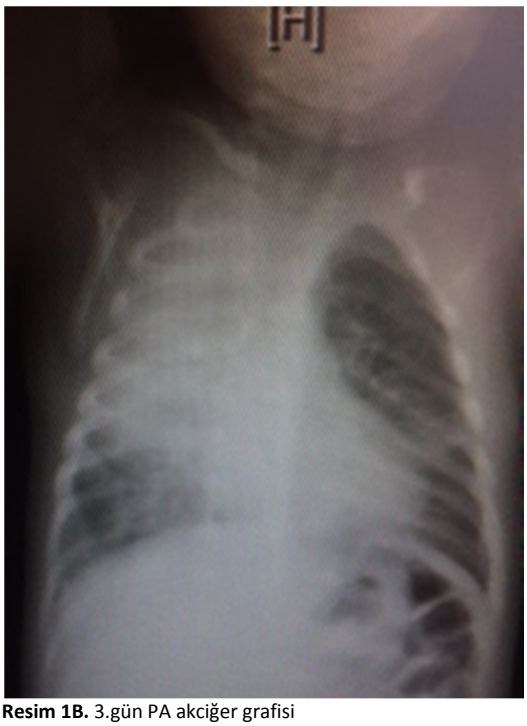
Periferik yaymasında, %65 lenfosit, %30 nötrofil, %5 monosit; kan gazı incelemesinde; pH: 7,45, PO2: 99 mmHg, PCO2: 40 mmHg, HCO3:25 mmol/L, BE: 2,5, O_2 satürasyonu: %99,5 bulundu. Kan kültüründe üreme saptanmadı. Akciğer grafisinde sağ akciğer üst ve orta lobda konsolidasyon saptandı (Resim 1B). Tedavi, meropenem (120 mg/kg/gün) olarak değiştirildi. Bu tedavi süresince iki kez öksürük ataklarını takiben boş bakma ile birlikte, kol ve bacaklarında klonik atımlar gözlendi. Hipoksiye bağlı nöbet geçirdiği düşünülen hastanın, kan elektrolit düzeyleri, lomber ponksiyon incelemesi, transfontanel ultrasonografi ve elektroensefalografi (EEG) değerlendirmesi normal olarak saptandı. Akut dönemde hastaya fenobarbital (3 mg/kg/gün) tedavisi başlanıldı. Primer immün yetmezlikler yönünden yapılan tetkiklerde (serum immünglobülin düzeyleri, lenfosit alt grupları ve CH50 ve kompleman düzeyleri) yaşına uygun normal aralıklarda bulundu. Takipte 21 gün meropenem ve 14 gün klaritromisin tedavisi sonrası bulguları düzelen ve nöbeti olmayan hasta, yaklaşık 40 günlük izlem sonunda şifa ile taburcu edildi. Hastanın İl Sağlık Müdürlüğüne bildirimi yapıldı. Taburculuk sonrası takiplerinde nörolojik muayeneleri normal saptandı. Bir ay sonra yapılan beyin magnetik rezonans görüntülemesi ve 3 ay sonra yapılan kontrol EEG'leri normal saptanması üzerine ve akut semptomatik nöbet olarak kabul edilmesi nedeniyle fenobarbital tedavisi, 6 ay içinde azaltılarak kesildi.



Resim 1A. Başvuru PA akciğer grafisi



Resim 1B. 3.gün PA akciğer grafisi

TARTIŞMA

Aşılamanın düzenli ve yaygın biçimde yapılıyor olmasına ve aşılama ile olgu sayısında %99 oranında azalma sağlanmasına rağmen; tüm dünyada her yıl yaklaşık 190.000 civarında boğmaca olgusu görülmektedir ve 89.000 ölümle sonlanmaktadır (2,4,12). Gelişmiş ülkelerde, aşı ile korunabilir hastalıkları arasında sıklığı hala yüksek kalan tek enfeksiyon boğmaca hastalığıdır (2,12). Bebekler, aktif aşılamanın başladığı ikinci aya kadar annelerinden geçen antikorların hızlı düşüşü ile hastalığa karşı en duyarlı olan yaş grubudur (5). İki ayın altındaki bebeklerde yıllık insidans 150/100.000'dir (2) Bir yaşın altındaki bebekler boğmaca hastalığı nedeniyle olan mortalitenin %40'ını oluştururlar (2).

Hastalık tipik olarak üst solunum yolu enfeksiyonu bulguları ile başlar. Progresif öksürük, iç çekme ve apne epizodları olabildiği gibi, bebeklerde klinik değişken olabilir (1,2).Hastalığın başlıca komplikasyonları; apne, pnömöni gibi ikincil enfeksiyonlar ve şiddetli öksürüğe bağlı fiziksel sekellerdir (3). Hipoksemi ile birlikte nöbetler sık görülür (2). Ateş, devam eden takipne ve ataklar arasında solunum güçlüğü, sekonder pnömöni için işaretler olup en sık *Staphylococcus aureus* ve *Streptococcus pneumoniae* etkendir (2,4). Hastamız solunum yetmezliği bulgusu olmadan, ağır sekonder pnömöni ve hipoksiye bağlı tekrarlayan nöbet komplikasyonları yaşamıştır. Bu komplikasyonların fatalitesi ve morbiditesi yüksek olup, aşı ile önlenebilir bir enfeksiyon hastalığı için son derece ağırdır.

Boğmaca hastalığı ile ilişkili komplikasyonların en fazla altıncı ayın altında görülüyor olması, aşılaması tamamlanmamış çocukların koruma stratejileri üzerinde çalışılmaya neden olmuştur (12). Bu amaçla belirlenen evrensel iki strateji, anneden bebeğe doğrudan pasif antikor transferini sağlayan hamilelik süresince annelerin aşılanması ve koza stratejisidir. Gebelerin aşılanmasında optimum aşılama zamanının üçüncü trimester, tercihen 28 ile 38 hafta arası olduğu belirtilmektedir (12). Koza stratejisi, aşılaması eksik olan bebek ile yakın temas eden ve enfeksiyon kaynağı olabilecek bireylerin, ergenlerin, erişkinlerin ve sağlık personelinin aşılanmasını amaçlamakta ve böylece bebeklerin indirekt olarak korunmalarını sağlamaktadır (2,3,12).

Prospektif ve serolojik çalışmalar, boğmacanın özellikle ergen ve erişkin yaş grubunda ayırıcı tanıda akla nadiren gelmesi ve zor tanı alması nedeniyle tanımlanamadığını ve tahmini yıllık olgunun yılda 600.000 olduğunu göstermektedir (5-8,11). Bu grupta aşı etkinliğinin zamanla azalmış olması, bu bireyleri enfeksiyon için duyarlı hale getirmektedir. Ergen ve erişkinlerde boğmaca enfeksiyonu uzun süreli öksürük, öksürük sonrası kusma gibi tipik bulgular olmaksızın atipik veya asemptomatik seyreder. İki haftadan uzun süren öksürük yakınması ile başvuran ergen ve erişkin hastalarda yapılan çalışmalarda boğmaca infeksiyonu % 13-52 gibi yüksek oranda saptandığı bildirilmektedir (3). Ülkemizde yapılan bir çalışmada, iki haftadan uzun öksüren 0-16 yaş grubundaki olguların % 16,9'unda boğmaca saptanmıştır (6). Ülkemizde yakın zamanda yapılan bir başka çalışmada, uzamış öksürüklerde boğmaca seroprevelansı ergenlerde %12 olarak raporlanmıştır (7). Hastamızın henüz boğmaca aşısının yapılmamış olması ve evde ablasının benzer şikâyetlerinin olması boğmaca gelişmesine neden olmuştur. Ülkemizde boğmaca aşılaması, 1968 yılında difteri-boğmaca-tetanoz (DTP) şeklinde, hayatın 2., 3. ve 4. aylarında ve 16-24. aylarında rapel olarak uygulanmaya başlamıştır (8). İlk uygulama yıllarında %20-30'larda olan aşılama hızları, 2014 yılı Sağlık Bakanlığı Sağlık İstatistikleri verilerine göre % 97'lere ulaşmıştır (9). Buna paralel olarak, boğmaca olgu sayısı ve insidansında önemli düşüş olmuştur. Ancak, insidanstaki düşüşe rağmen, boğmaca ülkemizde hâlâ tüm yaş gruplarını özellikle ergen, erişkin ve bebekleri etkileyen bir enfeksiyon hastalığı olmaya ve siklik patern göstermeye devam etmekte, 3-5 yıl aralıklarla salgınlar yaparak morbidite ve mortaliteye yol açmaktadır (10).Aşılama hızlarının yüksek olmasına rağmen, boğmaca olgularının görülme nedeni, tam aşılı çocuklarda da aşının koruyuculuğunun belli bir süre sonra azalmasıdır. Yaş gruplarına göre yapılan seroprevelans çalışmalarında; tam aşılı çocukların ilköğretime başladıkları dönemde antikor düzeylerinin düştüğü ve boğmaca sıklığının bu yaşlarda arttığı gösterilmiştir (11).

Sağlık Bakanlığı verilerine göre, 1986 yılında boğmaca olgu sayısı 1048, yıllık insidans 2.03/100,000 iken 2005 yılına gelindiğinde olgu sayısının 272'e ve insidansın 0.38/100,000'e düştüğü görülmektedir (8). Ülkemizde bildirim yetersizliği yanında tanı zorlukları, boğmacanın ön tanıda akla gelmemesi, atipik veya asemptomatik olguların sık görülmesi gibi nedenlerle gerçek insidansın saptanması güçtür (10). Ülkemizde yapılan çalışmalarda boğmaca insidansının bildirilenden yüksek olduğunu göstermektedir (5,8, 10). Doğurganlık çağındaki kadınların yarısında, ilk doz boğmaca aşısı yapılana kadar bebeği koruyacak antikor titresinin olmadığı gösterilmiştir. Bu çalışmaların ışığında; adölesan, erişkinler ve gebe kadınlara aselüler boğmaca aşısı uygulanması önerilmiştir(5). Boğmaca enfeksiyonunun artık sadece bir pediatrik enfeksiyon olmadığının fark edilmesi gerekmektedir.

Sonuç olarak, dünyanın diğer ülkelerinde olduğu gibi, ülkemizde de ergen ve erişkinlerde boğmaca enfeksiyonunda belirgin artış vardır (5-6, 8). Enfekte ergen ve erişkinler, aşılanması yapılmamış ya da henüz tamamlanmamış bebekler için önemli kaynak oluştururlar. Bebekleri korumak ve boğmaca enfeksiyon sıklığını azaltmak için; ülkemizde mevcut bebek aşılamasının güçlenerek devamı, tüm ilköğretim birinci sınıflara beşinci doz aşı uygulamasının düzenli sürdürülmesi yanında, ergen aşılamasının önemi unutulmamalıdır. Aşılaması tam olan ergen ve erişkinlere Tdap olarak, tek doz pekiştirme önerilmelidir. Ayrıca, bebekleri koruma için "koza stratejisi" içinde, annelerin gebelikte aşılanması veya doğum sonrası yenidoğanla sıkı temasta olan aile bireylerinin, kardeş, bakıcı, büyükanne – büyükbaba gibi her bireyin ve bebeklere bakım hizmeti veren sağlık personelinin aşılanması planlanmalıdır.

Çıkar Çatışması
Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

KAYNAKLAR

1- Somer A. Boğmaca: Epidemiyoloji ve Klinik. ANKEM Derg 2011; 25(Ek2): 218-23.
2- Long SS. Pertussis (Bordetella pertussis and Bordetella parapertussis). In:Behrman RE, Kliegman RM, Jenson HB, Stanton BF (eds). Nelson Textbook of Pediatrics. 20th ed.Philadelphia PA: Elsevier, 2016: 1377- 82.
3- Cherry JD. Epidemiology of pertussis. Pediatr Infect Dis J 2006; 25: 361-2.
4- Crowcroft NS, Stein C, Duclos P, Birmingham M. How best to estimate the global burden of pertussis? Lancet Infect Dis 2003; 3: 413-7.
5- Esen B, Coplu N, Kurtoglu D, Gozalan A, Akin L. Prevalence of high antibody titers of pertussis in Turkey: reflection of circulating microorganism and a threat to infants, J Clin Lab Anal 2007;21: 154-61.
6- Yıldırım I, Ceyhan M, Kalaycı O ve ark. Frequency of pertussis in children with prolonged cough. Scand J Infect Dis 2008;40:314-9.
7- Karagul A, Ogunc D, Midilli K, Ongut G, Baysan BO, Donmez L, et al. Epidemiology of pertussis in adolescents and adults in Turkey. Epidemiol Infect 2015; 143: 2613-8.
8- Dilli D, Bostanci I, Dallar Y, Buzgan T, Irmak H, Torunoglu MA. Recent findings on pertussis epidemiology in Turkey, Eur J Clin Microbiol Infect Dis 2008;27:335-41.
9- T.C. Sağlık Bakanlığı Sağlık İstatistikleri Yıllığı 2014, Hastalıkların Önlenmesi ve Sağlığın Korunması, DTaB aşılama oranları. In: T.C. Sağlık Bakanlığı Sağlık İstatistikleri Yıllığı, ANKARA, 2015. S: 59.
10- Kurugöl Z. Boğmaca aşısı ve sorunlar. ANKEM Derg 2011;25(Ek 2):212-7.
11- Cevik M, Beyazova U, Aral AL, Duyan Camurdan A, Ozkan S, Sahin F, Aybay C. Seroprevalence of IgG antibodies against Bordetella pertussis in healthy individuals aged 4-24 years in Turkey. Clin Microbiol Infect 2008;14:388-90.
12- www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/passive/pertussis/en/
13- Forsyth K, Plotkin S, Tan T, Wirsingvon König CH. Strategies to decrease pertussis transmission to infants. Pediatrics 2015;135:e1475-82.

Life Threatening Bradycardia Requiring Temporary Transvenous Pacemaker in Spinal Cord Injury

Spinal Kord Hasarında Geçici Kalp Pili Gerektiren Yaşamı Tehdit Edici Bradikardi

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ABSTRACT

Spinal cord injury (SCI) is a real health problem and cardiovascular disorders are the most common cause of mortality in SCI. Bradycardia in patients with spinal cord injury is related with autonomic instability and may be resistant to pharmacologic therapy. In this study, we report a case of life threatening bradycardia requiring temporary transvenous pace maker implantation in spinal cord injury. Placement of a temporary transvenous pace maker can be life saving in acute phase in spinal cord injury and all patients with spinal cord injury regardless of the level and severity should be closely monitored .

Key Words: Spinal injury, bradycardia, cardiac pacemaker

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ÖZET

Spinal kord hasarı (SCI) gerçek bir sağlık sorunudur ve spinal kord hasarında kardiovasküler hastalıklar en sık mortalite nedenidir. Spinal kord hasarlı hastalarda görülen bradikardi otonomik instabilite ile ilişkilidir ve farmakolojik terapiye dirençli olabilir. Bu yazımızda, spinal kord hasarında geçici kalp pili takılmasını gerektiren yaşamı tehdit edici bradikardi vakasını bildirdik. Spinal kord hasarının akut fazında geçici kalp pili takılması yaşam kurtarıcı olabilir ve tüm hastalar spinal kord hasarının seviyesi ve ciddiyetinden bağımsız olarak yakından takip edilmelidir.

Anahtar Sözcükler: Spinal hasar, bradikardi, kalp pili

Geliş Tarihi: 26.07.2016 **Kabul Tarihi:** 10.10.2016

INTRODUCTION

Spinal cord injury (SCI) constitutes a devastating traumatic injury associated with autonomic dysregulation and secondary hemodynamic instability related to bradycardia and loss of vascular tone(1). Morbidity and mortality associated with SCI is primarily attributable autonomic instability and it is associated cardiovascular hemodynamic effects. Bradycardia and hypotension due to high vagal tone as well as tachyarrhythmias are common and account for approximately 30% of deaths in SCI. Specific complication dependent on the period of time after trauma like spinal shock and autonomic dysreflexia are also reviewed. Spinal shock occurs during the acute phase following SCI and is a transitory suspension of function and reflexes below the level of the injury. Neurogenic shock as part of spinal shock consists of severe bradycardia and hypotension. Autonomic dysreflexia appears during the chronic phase, after spinal shock resolution, and it is a life-threatening syndrome of massive imbalanced reflex sympathetic discharge occurring in patients with SCI above the splanchnic sympathetic outflow (T5-T6). Besides all this, additional cardiac complications, such as cardiac deconditioning and coronary heart disease may also ocur(2,3).

We present a case with cervical vertebral trauma and symptomatic bradycardia requiring transvenous pace maker implantation.

CASE REPORT

A 89 year old male was found unconscious at home and brought to our University Hospital. Past medical history was unenlightening. His blood pressure was 70/30 mm Hg with a heart rate of 34 beats per minute(bpm). ECG revealed third-degree AV block (fig.1A) and measured mass CK-MB level was 6,18 ng/ml (0 - 4,94 ng/ml); high sensitive troponin T level was 13,75 pg/ml (0 - 14 pg/ml) . There was no significant changes in serial cardiac enzymes.İnitial neurological examination revealed that he was tetraplegic. He subsequently developed respiratory failure requiring mechanical ventilation. Due to severe hemodynamic collapse; a transvenous pacemaker was placed and the patient was admitted to the intensive care unit. His blood pressure increased to 100/60 mm Hg and bedside transthoracic echocardiography revealed normal ejection fraction without segmental wall motion abnormalities. Magnetic resonance imaging of the cervical vertebra revealed a C4-C5 fracture and dislocation with spinal cord compression (fig. 2). After a consultation with the neurosurgical department of our hospital about the patient, a cervical traction was performed immediately. After cervical traction his intrinsic heart rate increased to 50 bmp (fig.1B) and the patient underwent surgical stabilization. He did not require any more pacing support over the next 7 days and a pace maker was discontinued.

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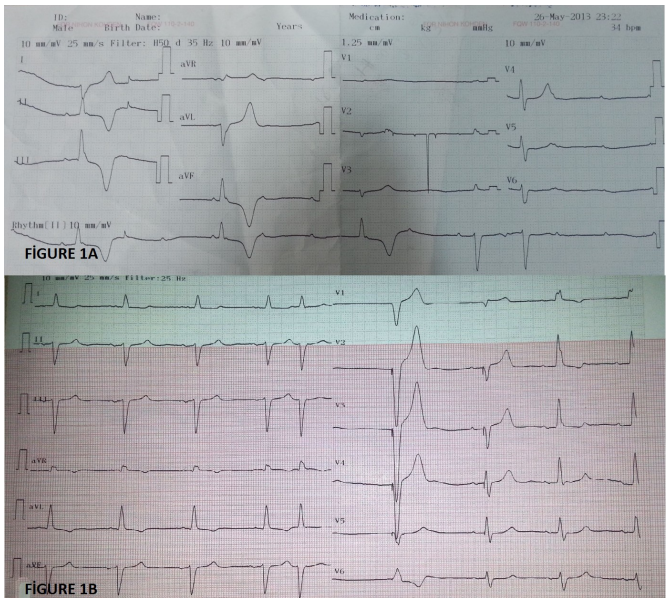

Figure (1A) Initial ECG at admission (third-degree AV block) **(1B)** ECG after cervical traction

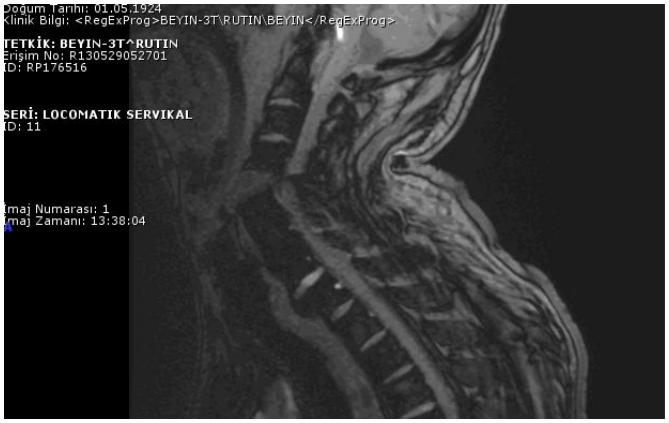

Figure 2. Magnetic resonance imaging of the cervical vertebra

DISCUSSION

Spinal cord injury (SCI) happens for a wide variety of reasons . Injuries due to trauma are the most common. Cardiovascular complications are main cause of death in patients with SCI and bradycardia is a common complication in almost all cases(3).

Primary mechanism of bradyarhytmias appears as a result of post injury imbalance in the autonomic nervous system and the injured cardiac sympathetic nerve located in the cervical spinal cord(4). The most cardiac manifestations reported included premature atrial and ventricular contractions and heart block of varying degrees. Precipating factors such as tracheal suction or hypoxemia should be avoided in spinal cord injured patients(5). Normally tracheal suctioning results in increased heart rate because of sympathetic stimulation from the mechanical irritation. Due to anatomic sympathetic denervation, bradycardia and asystole result from predominance of the vasovagal reflexes(6).

All patients with spinal cord injury regardless of the level and severity should be closely monitored. Administration of atropine, epinephrine, aminophyline could be used in treatment modalities(7). Atropine can partially and transiently treat bradycardia because of sympathetic deficiency and should be considered as first line treatment (8). Medical neccesity of pace maker implantation must be viewed in the context of the overall management of the particular patient. In the setting of bradycardia leading to hemodynamic collapse, temporary transvenous pacing is indicated. Silbert et al., reported a case report in which the patient was treated with a temporary transvenous pacemaker to avoid extreme bradycardia, asystole and syncope attacks(9). Permenant pace maker implantation is advocated for patients with high cervical spinal cord injuries and refractory or reccurrent bradyarrhythmias. Moerman et al. ,stated that early replacement of a cardiac pacemaker was beneficial in patients with high spinal cord injury and could help early stabilization in these patients(10).

Syncope caused by heart block (Stokes - Adam attacks) and carotid sinus syncope may lead to traumatic injuries especially in elderly patients and should be considered in differential diagnosis. In our case report; predominance of the parasympathetic nervous system due to anatomic sympathetic denervation results in severe bradycardia and hypotension. The patient's heart rate increased dramatically after cervical traction and therefore a third degree block was considered to be related to spinal cord injury.

CONCLUSION

Bradycardia and asystolic cardiac arrest are potentially preventable complications in SCI. Patients with spinal cord injury regardless of the level and severity should be closely monitored in intensive care unit and need a proactive stance with low threshold for pacemaker implantation; temporary if the cord injury is reversible. Bridging treatment to pacemaker implantation with use of beta agonists, atropine and aminophyline can temporarily relieve bradycardia and hypotension allowing time for more definitive management with a pacemaker. Awareness of this life threatening complication would decrease the likelihood of unexpected death in these patients.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. McKinley W, Garstang SV, Wieting JM, Talavera F, Foye PM, Allen KL, et al. Cardiovascular concerns in spinal cord injury. eMedicine Specialties/Physical Medicine and Rehabilitation/Spinal Cord Injury. 2006.

2. Claydon VE, Steeves JD, Krassioukov AV. Orthostatic hypertension following spinal cord injury: understanding clinical pathophysiology. Spinal Cord.2006; 44: 341-51.

3. Devivo MJ, Krause JS, Lammertse DP. Recent trends in mortality and causes of death among persons with spinal cord injury. Arch Phys Med Rehabil. 1999; 80: 1411-9.

4. Singh A, Garg R, Weachter R. Cardiac bradyarrhythmias in a patient with cervical spine injury. Am Heart Hosp J. 2010; 8 :63-5.

5. Peyrol M, Sbragia P, Trigano JA, Paganelli F. Dorsal spinal cord injury as a cause of recurrent asystole requiring permanent cardiac pacing. Europace . 2012;14 :146-7.

6. A G Abd , N M Braun .Management of life-threatening bradycardia in spinal cord injury. Chest.1989; 95:701-2.

7. Schulz-Stübner S. The use of small-dose theophylline for the treatment of bradycardia in patients with spinal cord injury.Anesth Analg. 2005 ;101 :1809–11.

8. Partida E, Mironets E, Hou S, Tom VJ. Cardiovascular dysfunction following spinal cord injury. Neural Regen Res. 2016 ; 11 :189-94.

9. Silbert PL, Davis JM. Late asystole in high cervical spinal cord injury: case report. Paraplegia. 1990 ; 28 :137-40.

10. Moerman JR, Christie B 3rd, Sykes LN, Vogel RL, Nolan TL, Ashley DW. Early cardiac pacemaker placement for life-threatening bradycardia in traumatic spinal cord injury. J Trauma. 2011 ;70 :1485-8.

Pilomatricoma of the Retroauricular Area

Retroaurikuler Bölgede Pilomatrikoma

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ABSTRACT

Pilomatricoma is a benign skin tumor of hair matrix cells. The head and neck is the most commonly affected site of the body. This article describes 41 year-old man who referred to our clinic with an asymptomatic, slow-growing mass on the right retroauricular, suboccipital region. The tumor was excised and histopathologically diagnosed as pilomatricoma. Because of its frequency and predilection of head and neck region, otolaryngologists should be familiar of this tumor.

Key Words: Pilomatricoma, skin neoplasm, calcifying epitelioma.

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ÖZET

Pilomatrikoma, kıl folliküllerinin matriks hücrelerinden köken alan benign deri tümörleridir. Bu tümörün en sık görüldüğü yerleşim yeri baş ve boyun bölgesidir. Bu yazıda, 41 yaşında sağ retroauriküler, suboccipital bölgede semptomsuz, yavaş büyüyen kitle ile başvuran bir hasta tarif edilmektedir. Bu tümör çıkarılarak histopatolojik incelemeye gönderilmiş ve pilomatrikoma tanısı almıştır. Baş ve boyun bölgesinde sık görülme eğilimi nedeniyle kulak burun boğaz hekimlerinin bu tümörün klinik sunumunun bilincinde olması ve baş boyun kitlelerinin ayırıcı tanısında bu tümörleri akla getirmesi önerilmektedir.

Anahtar Sözcükler: Pilomatrikoma, deri tümörü, kalsifiye epitelyoma

Geliş Tarihi: 05.09.2016 **Kabul Tarihi:** 28.09.2016

INTRODUCTION

Pilomatricoma, also described as calcifying epitelioma of Malherbe is a skin neoplasm, which originates from hair matrix cells (1-3). This tumor is benign and the neck is the one of the most commonly affected sites of the body. This tumor is actually not uncommon neoplasia that pathologist confirmed this diagnosis for one of every 500 specimens (4). Despite the frequency of pilomatricoma, it is rarely come to mind as prediagnosis by clinicians (5). Its varying presentations may be a cause of misdiagnosis. Thus, it should be important to be awere of this tumor. This article describes a case of pilomatricoma in the retro-auricular, sub-occipital region and discuss this neoplasia.

CASE REPORT

A 41 year-old male patient referred to our clinic with a a right retro-auricular, sub-occipital mass. The tumor has grown in 8 months. No pain, discharge or discoloration occured in this area. There were no other masses or lymphadenopathy or significant medical history of the patient. The superficial, solid, mobile mass measured aproximately 3 cm x 1,5 cm in the right retro-auricular, sub-occipital region (Figure 1). The other parts of ENT examination and systemic examination was normal. Superficial ultrasound examination revealed a 3 cmx 1,5 cm subcutaneous cystic lesion with containing solid areas. Excision surgery was performed under general anesthesia.

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Skin incision was made in the sub-occipital region and then by proceeding with blunt dissection of the subcutaneous tissue, the mass was achieved. A firm calcified mass, which is non-adherent to the surrounding tissues was excised (Figure 2). The pathologist explained the diagnosis as pilomatricoma (Figure 3).

Figure 1: Photograph of a mass in the retroauricular, suboccipital region.

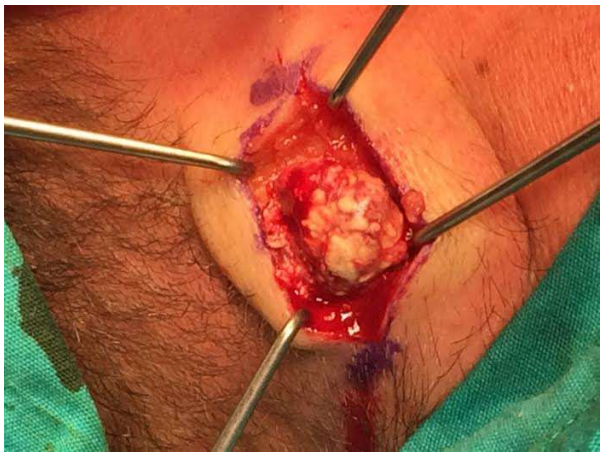

Figure 2: Intraoperative photograph of a firm, calcified mass

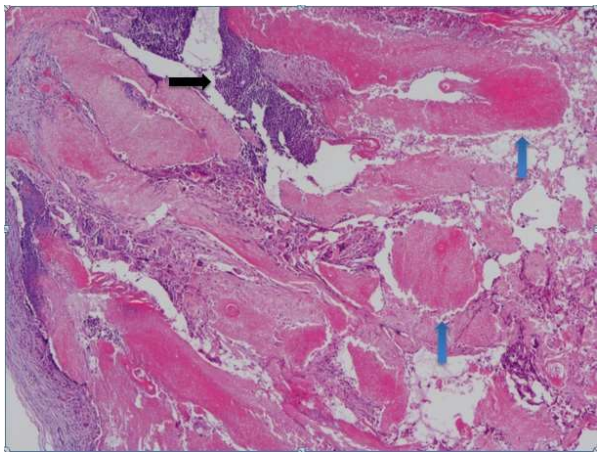

Figure 3: Photomicrograph of pilomatricoma showing the characteristic basaloid (black arrow) and (blue arrow) shadow cells. Among them, multinucleated giant cells with chronic inflammatory infiltrates are also seen (H&E, ×40)

DISCUSSION

The formation mechanism of pilomatricoma, which is the tumor of hair matrix cell, had explained by the failure of the hair follicle cycle that cause the breakdown in the differentiation of pilar keratinocytes into mature hair follicules. (6,7). Furthermore, the association of pilomatricoma with the genes for myotonic dystrophy, polyoma virüs have been demostrated (8,9). β-catenin gene mutations have also been shown to be associated with pilomatricoma (10).

Clinically, pilomatricoma presents as a superficial, firm, freely mobile, cystic or solid mass. Discoloration as reddish-blue or ulceration of the skin may be seen. These tumors generally are painless, occasionally they present with pain and tenderness (5, 6, 11,12). Pilomatricoma commonly occurs at 5-15 years and at 50-65 years (13) and it has female predominance (5). Although these tumors are solitary lesions, multiple lesions reported in patients with myotonic dystrophy, Turner syndrome, Gardner syndrome, Steinert disease (5, 14). Pilomatricoma can be usually seen in haired parts of the body. Thus, they occur most frequently in the cervicofasial region and the limbs are the second common area. In the head and neck region, because of the high density of hair follicles, the cheek, neck, peri-auricular, and peri-orbital are the common areas fo this tumor. (6,11,15). To avoid misdiagnosis, benign and malignant tumors such as sebaceous and epidermoid cysts, basal cells and scuamous cell carsinoma, vascular lesion, lymph node, lipoma, branchial remnants, preauricular sinuses, chondroma and parotid region tumors should be considered before intervention to the mass (5,12,16,17).

For diagnostic investigation, ultrasonography can be performed for the determination of the position and the calsification of the mass, especially for young patients (6). Computed tomography or magnetic resonance imaging are mainly requested for certain cases such as preauricular and extensive tumors (5, 12). As a part of diagnostic investigation fine needle aspiration has been described (18).

Histopathologically, pilomatricoma is markedly limited nodules with a fibrous capsule. The islands of well-organized malpighian cells are the most frequent microscopic features. The tumor contains basaloid cells with nucleus settled in the peripheral of the island and eozinofilic shadow cells (ghost cells) without nucleus settled in the center (5,6,12). Calsification in the ghost cell region is common feature that ranges from 69% to 85% (18). Malign forms have atypical, undifferentiated cells, a large epithelial cell component and may infiltrate blood vessels and fibrous capsule (12,20).

The target of treatment is the total removal of the tumor (5). This tumor has low recurrence and malignant transformation rate (6). Malignant transformation may occur with advancing ages in males (20). Also death due to the malignant metastatic pilomatricoma was reported (21).

CONCLUSION

Pilomatricoma is one of the most common benign skin neoplasm. Otolaryngologists should consider this tumor in a patient who presented with mobile, superficial, slowly progressive mass in the head and neck region. Cilical presentation is usually suggestive for the diagnosis. Radiologic assesment is important in certain cases. Complete resection of the mass is essential for the treatment. Recurrence and malignant transformation rates are low.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Malherbe A, Chenantais J. Note sur l'epitheliome calcifie des glandes sebacees, Prog. Med. 1880; 8: 826–37.

2. Forbis R, Helwig EB. Pilomatrixoma (calcifying epithelioma), Arch. Dematol. 1961; 83: 606–7.

3. Arnold HL. Pilomatricoma, Arch. Dermatol. 1977; 113.

4. Marrogi, A. J., Wick, M. R., and Dehner, L. P. Pilomatrical neoplasms in children and young adults.Am. J.Dermatopathol. 1992; 14: 87.

5. Lan MY, Lan MC, Ho CY, Li WY, Lin CZ. Pilomatricoma of the head and neck: a retrospective review of 179 cases.Arch Otolaryngol Head Neck Surg 2003; 129: 1327–30.

6. Ashkan Pirouzmanesh, B.S., John F. Reinisch, M.D., Ignacio Gonzalez-Gomez, M.D.,Ebonie M. Smith, B.A., and John G. Meara, M.D., D.M.D. Pilomatrixoma: A Review of 346 Cases.Plast Reconst Surg. 2003;112: 1784-9.

7. Goufman LTDB, Murrell CDRGL, Watkins CDRDV. Quiz case 2: diagnosis: pilomatricoma (calcifying epitelioma of Malherbe). Arch Otolaryngol Head Neck Surg. 2001: 127: 218-20.

8. Harper, P. S. Calcifying epithelioma of Malherbe: Association with myotonic muscular dystrophy.Arch. Dermatol. 1972; 106: 52.

9. Hashimoto, K., Magré, L. A., and Lever, W. F. Electron microscopic identification of viral particles in calcifying epithelioma induced by polyoma virus. J. Natl. Cancer Inst. 1967; 39: 977.

10. Kajino, Y., Yamaguchi, A., Hashimoto, N., Matsuura, A., Sato, N., and Kikuchi, K. -Catenin gene mutation in human hair follicle-related tumors. Pathol. Int. 2001; 51: 543.

11. Kwon D, Grekov K, Krishnan M, Dyleski R. Characteristic of pilomatrixoma in children : A review of 137 patients. International Journal of Pediatric Otorhinolaryngology 2014; 78: 1337–41.

12. Duflo S, Nicollas R, Roman S, Magalon G, Triglia JM. Pilomatrixoma of the head and neck in children: a study of 38 cases and a review of the literature, Arch. Otol. 1998; 124: 1239–42.

13. Gupta V, Marwah N, Jain P, Dua S, Gupta S, Sen R. Diagnostic pitfalls of pilomatricoma on fine needle aspiration cytology, Iran J. Dermatol. 2012; 15: 59–61.

14. Trufant J, Kurz W, Frankel A, Muthusamy V, McKinnon W, Greenblatt M, Lazar A, Cook D, Bosenberg MJ. Familial multiple pilomatrixomas as a presentation of attenuated adenomatosis polyposis coli. J. Cutan. Pathol. 2012; 39: 440-3.

15. Schwarz Y, Pitaro J, Waissbluth S, Daniel SJ. Review of head and neck pilomatrixoma. Int Ped Otorhinolaryngol. 2016; 39:573.

16. O'Connor N, Patel M, Umar T, Marcpherson DW, Ethunandan M. Head and neck pilomatricoma: an analysis of 201 cases. British Journal of Oral and Maxillofacial Surgery 2011; 49: 354–8.

17. Aydın S, Bilmez ZE, Erdoğdu S, Altintoprak N, Kayıpmaz Ş. Complicated Giant Pilomatrixoma of the Parotid Region. J Maxillofac Oral Surg. 2016;15111-5.

18. Wang J, Cobb CJ, Martin SE, Venegas R, Wu N, Greaves TS. Pilomatrixoma: clinicopathologic study of 51 cases with emphasis on cytologic features.Diagn Cytopathol 2002; 27: 167–72.

19. Boyd AS, Martin RW III. Pilomatricoma (calcified epitelioma of Malherbe) with secondary ossification. Arch Otolaryngol Head Neck Surg. 1992; 118: 212-5.

20. Hardisson D, Linares MD, Cuevas-Santos J, Contreras F. Pilomatrix carcinoma: a clinicopathologic study of six cases and review of the literature.Am J Dermatopathol 2001; 23: 394–401.

21. Walker DM, Dowthwaite S, Cronin D, Molden-Hauer T, McMonagle B. Metastatic pilomatrix carcinoma: Not so rare after all? A case report and review of the literature. Ear Nose Throat J. 2016;95:117-20.

Variation of Bilateral Multiheaded Sternocleidomastoid Muscle

Bilateral Çok Başlı Musculus Sternocleidomastoideus Varyasyonu

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ABSTRACT

The sternocleidomastoid muscle is important anatomically and clinically because of its relationship with many neurovascular structures in the neck. The muscle descends obliquely across the side of the neck and divides it into anterior and posterior triangles. In this case report, the additional clavicular head of bilateral sternocleidomastoid muscle was shown. During routine dissections of the neck for anatomy education in the Department of Anatomy Laboratory in the Faculty of Medicine at the Gazi University, additional clavicular heads of sternocleidomastoid muscle were observed bilaterally. The additional clavicular heads originated from the superior surface of the middle third of the clavicle. Furthermore, on the left side of the neck, muscle fibers originating from sternal head of sternocleidomastoid muscle and blending with clavicular head of sternocleidomastoid muscle were seen. Both of the minor supraclavicular triangles were narrower than normal. Unusual clinical cases in medical area makes diagnosis and treatment more difficult. Knowledge of anatomical variations is very important for avoiding of this difficulty.

Key Words: Additional head, anatomical variation, sternocleidomastoid, muscle variation, additional clavicular head

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ÖZET

Boyunda birçok nörovasküler yapılarla ilişkisi nedeniyle, musculus sternocleidomastoideus (SCM) anatomik ve klinik açıdan önemlidir. Kas, boynun yan tarafı boyunca oblik olarak iner ve boynu ön ve arka üçgenlere böler. Bu vakada, bilateral musculus sternocleidomastoideus'un ek klavikular başı gösterildi. Gazi Üniversitesi Tıp Fakültesi Anatomi Anabilim Dalı Laboratuvarı'nda, anatomi eğitimi için yapılan rutin boyun diseksiyonu sırasında, bilateral olarak musculus sternocleidomastoideus'un ek klavikular başı gözlendi. Ek klavikular başlar clavicula'nın üçte bir orta kısmının üst yüzünden orijin almakta idi. Ayrıca, boynun sol tarafında, SCM'nin sternal başından kaynaklanan ve SCM'nin klavikular başıyla karışan kas lifleri görüldü. Her iki trigonum supraclaviculare minor normalden daha dar gözlendi. Medikal alanda, nadir klinik olaylarda tanı ve tedavi zor hale gelir. Bu zorluğu önlemek için anatomik varyasyonların bilinmesi çok önemlidir.

Anahtar Sözcükler: Ek baş, anatomik varyasyon, sternocleidomastoid, kas varyasyonu, ek klavikular baş

Geliş Tarihi: 21.09.2016 **Kabul Tarihi:** 29.09.2016

INTRODUCTION

Because of its relationship with many neurovascular structures in the neck, the sternocleidomastoid muscle (SCM) is important surgical landmark. The muscle descends obliquely across the side of the neck and divides it into anterior and posterior triangles(1-3).

The SCM is attached inferiorly by two heads. The medial head, which is rounded and tendinous, originates from the upper part of the anterior surface of the manubrium sterni. It is called the sternal head and ascends posterolaterally. The lateral head, which is variable in width and contains muscular and fibrous elements, originates from the superior surface of the medial third of the clavicle. It is called the clavicular head and ascends almost vertically. Near their origins, the two heads are separated by a triangular interval which corresponds to a surface depression and is known as the lesser supraclavicular fossa.

The SCM is inserted into the lateral surface of the mastoid process from its apex to its superior border by a strong tendon and into the lateral half of the superior nuchal line by a thin aponeurosis. While the sternal fibers are more oblique and superficial and extend to the occiput, the clavicular fibers reach mainly to the mastoid process(4).

The upper part of the SCM is supplied by branches of the occipital and posterior auricular arteries, middle part of the SCM is supplied by the superior thyroid artery and the lower part of the SCM is supplied by the suprascapular artery. SCM is innervated by the spinal part of the accessory nerve. Besides, branches of the ventral rami of the second, third, and sometimes fourth cervical spinal nerves innervate the muscle. Although it was believed that these cervical rami were only proprioceptive, clinical evidence suggests that some of their fibers are motor(4).Acting alone, the SCM flexes the neck laterally and rotates the face to the other side. When the muscles of the two sides contract together, they flex the head and neck forcibly (1, 5).

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CASE REPORT

During routine dissections of the neck for anatomy education in the Department of Anatomy Laboratory in the Faculty of Medicine at the Gazi University, variations of SCM were found bilaterally in a 66-year-old male cadaver. The incision was performed on each side of the neck in accordance with traces of SCM. Skin, superficial fascia, platysma and deep fascia were removed and the muscles of the neck were exposed. During the dissections, two clavicular heads in addition to a sternal head were observed on the right, and similarly a sternal head and two additional clavicular heads were observed on the left. However, on the left some groups of fibers on the left of the sternal piece were splitting and were first connecting to the clavicular in the medial(Figure 1A and 1B).This cluster of muscle fibers was initially perceived as a head. All the heads on the left and right ended normally as a single joint body extending.

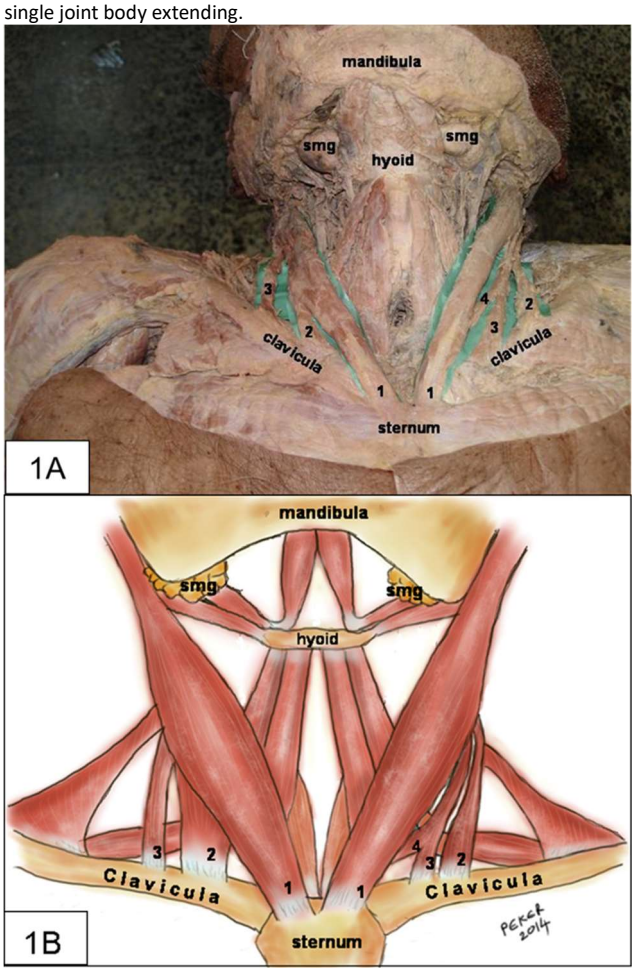

Figure 1A and 1B: General view of variation of sternocleidomastoid muscle. *smg* submandibular gland, *1* sternal head of sternocleidomastoid muscle, *2* clavicular head of sternocleidomastoid muscle, *3* accessory clavicular head of sternocleidomastoid muscle, *4* accessory clavicular head of sternocleidomastoid muscle between sternal head and accessory clavicular head

After these processes, additional clavicular heads of SCM were observed bilaterally. The additional clavicular heads originated from the superior surface of the middle one third of the clavicle. It was discovered that the right additional clavicular head was 8 cm laterally of the outer edge of the sternal head and the left one 7 cm laterally of the outer edge of the sternal head. The lengths of both SCM were measured as 25 cm. Besides, it was determined that right additional clavicular head blended with the other fibers of SCM 17 cm superiorly of the sternum and the left additional clavicular head blended with the other fibers of SCM 15 cm superiorly of the sternum.

Respectively, the lengths of right and left additional clavicular heads were measured as 11 cm and 12 cm. The measurements were performed with a mechanical caliper (BTS-12003, China). Measurement precision is 0.02 mm. Both of the lesser supraclavicular triangles were seen to be narrower than normal and it was shown that the insertion of the SCM was normal bilaterally.

DISCUSSION

Unusual clinical cases in medical area makes diagnosis and treatment more difficult. Knowledge of anatomical variations is very important for avoiding of this difficulty (6). In order to understand anatomical variations, knowledge of human embryology is very valuable. The SCM and trapezius develop from a common premuscle mass from the last two occipital and upper cervical myotomes. This common mass splits and separates at 9 mm stage and these two divisions grow independently along the upper limb bud. The mass forming SCM becomes fixed first to the clavicle and later to the sternum, occipital bone and mastoid process at 14 mm stage (7). Abnormality of the separation of common mass may be cause of variations of SCM.

Although several variations of origin of the SCM are common, variations of its insertion are very rare(1, 3). Different variations related to additional heads of the SCM have been reported by various authors. Cherian and Nayak (1) reported unilateral additional clavicular head of the SCM on the left side of the neck of 65-year-old male cadaver. Similarly, during the gross dissection of the neck region of a 65-year-old male cadaver, Rani et al. (7) found an additional clavicular head on the left side.

During the routine anatomy dissection, Mehta et al. (3) detected a bipartite clavicular head of the SCM on the left side of the neck of a male cadaver, whereas the sternal head of SCM was normal. Aparna (8) reported an additional clavicular head of SCM on the left side, during the routine student dissection of the head and neck region of a 50-year-old male cadaver.

Ramesh et al. (5) and Amorim et al. (6) observed an additional clavicular head of the SCM bilaterally on a male cadaver as in our case. Boaro and Fragoso Neto (9) reported the presence of three clavicular heads, while the sternal head was normal on a nine-month-old infant. Natsis et al.(10) noticed bilateral supernumerary heads of the SCM that had an additional sternal head and three additional clavicular heads, making six heads in total with normal heads of SCM. In addition to these variations, similar to our case, they revealed that the lesser supraclavicular triangle was very narrow. During routine dissection of the neck of a male cadaver for undergraduate students, Kaur et al.(2) also observed that the right SCM was formed by six heads, two sternal and four clavicular, whereas the left SCM was without any variation.

The SCM is a landmark for physicians such as orthopedic surgeons, neurosurgeons and especially anesthesiologists who intervene in the lesser supraclavicular fossa during instillation of a central venous line. The presence of additional clavicular heads can misdirect them, and consequently the catheter may puncture the pleura and possibly lead to pneumothorax. While trying to access the vital elements that are located in the lesser supraclavicular fossa, variations of SCM may cause some complications. For example, the spinal accessory nerve can be damaged and cannulation of the internal jugular vein can be unsuccessful. So, in order to prevent complications in this area, surgeons and anesthetists should be aware of anatomical variations of SCM (8, 10).

The knowledge of anatomical variations of SCM is also useful for plastic surgeons. The SCM can be used in several ways during surgery. The additional head of the SCM may not have any functional advantage on the movement of the neck, but it may be used in various reconstructions of the head and neck region. Plastic surgeons can use this additional head for muscle graft surgeries. It may be utilized as a myocutaneous flap in reconstructing the oral floor, as a suture line to protect the carotid arteries, or, along with a portion of the clavicle, to reconstruct the mandible (1, 3).

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

REFERENCES

1. Cherian SJ, Nayak S. A rare case of unilateral third head of sternocleidomastoid muscle. Int J Morphol2008; 26:99-101.
2. Kaur D, Jain M, Shukla L. Six heads of origin of sternocleidomastoid muscle: a rare case. Internet Journal of Medical Update 2013;8: 62-4.
3. Mehta V, Arora J, Kumar A, Nayar AK, Ioh HK, Gupta V et al. Bipartite clavicular attachment of the sternocleidomastoid muscle: a case report. Anat Cell Biol2012;45: 66-9.
4. Standring S. Gray's Anatomy. 39th ed. London: Elsevier Churchill Livingstone; 2005.
5. Ramesh RT, Vishnumaya G, Prakashchandra SK, Suresh R.Variation in the origin of sternocleidomastoid muscle. A case report. Int J Morphol2007;25: 621-3.
6. AmorimAA, Lins CCSA, Cardoso APS, Damascena CG.Variation in clavicular origin of sternocleidomastoid muscle. Int J Morphol 2010;28:97-8.
7. Rani A, Srivastava AK, Rani A, Chopra J. Third head of sternocleidomastoid muscle. International Journal of Anatomical Variations 2011;4: 204-6.
8. Aparna G. Additional head of sternocleidomastoid muscle with 3rd supraclavicular fossa. Int J PharmBioSci2013;4: 586-9.
9. Boaro SN, FragosoNeto RA. Topographic variation of the sternocleidomastoid muscle in a just been born children. Int J Morphol 2003;21:261-4.
10. Natsis K, Asouchidou I, Vasileiou M, Papathanasiou E, Noussios G, Paraskevas G. A rare case of bilateral supernumerary heads of sternocleidomastoid muscle and its clinical impact. Folia Morphol2009; 68: 52-4.

Y-Type Urethral Duplication Presented with Perianal Fistula in an Infant

İnfantta Perianal Fistül Şeklinde Bulgu Veren Y-Tipi Üretral Duplikasyon

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ABSTRACT

Urethral duplications are rare lower urinary tract anomalies. Here we present an unusual presentation of urethral duplication in which, completely patent accessory urethra was coursing laterally on perianal region, near the anus, and presented as perianal fistula. A 2-month-old boy presented with discharge from the perianal region. On physical examination a fistula was observed on perianal region, in the 1 o'clock position and only 1 cm near the anus. Fistulography showed an opening to the prostatic urethra. The opening to the urethra was seen left superior part of the verumontanum on cystourethroscopy by giving methylene blue from the fistula. The accessory urethra was dissected from the skin to the prostatic part of the orthotopic urethra and excised completely with anterior sagittal approach. His postoperative course was uneventful. In unusual form of urethral duplications of Effmann Type IIA2, as in our case, orthotopic urethra is normal and ventral urethra opens to the perineum. It should be kept in mind that especially laterally placed ones can be misdiagnosed as anal fistula.

Key Words: Urethra, congenital abnormalities, urethral duplication, fistula, infant

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ÖZET

Üretral duplikasyon, alt üriner sistemin nadir anomalilerindendir. Olgumuz, duplike üretranın anüsün çok yakınında ve orta hattın lateraline açılan ve anal fistülle karışan nadir bir üretral duplikasyon şekli olması nedeniyle sunulmuştur. 2 aylık erkek hasta perianal bölgeden akıntı şikayetiyle başvurdu. Fizik muayenede perianal bölgede, anüse 1 cm mesafede, saat 1 hizasında fistül saptandı. Fistülografide fistülden verilen opak maddenin prostatik üretraya açıldığı görüldü. Sistoüretroskopide, fistülden verilen metilen mavisinin prostatik üretrada, verumontanumun hemen üst kısmında, sol yandan geldiği görüldü. Anterior sagital yöntemle, aksesuar üretra ciltten ayrılıp, ortotopik üretranın prostatik kısmına kadar serbestleştirilip, total olarak eksize edildi. Hastanın postop takiplerinde sorun olmadı. Olgumuzda olduğu gibi üretral duplikasyonun nadir bir formu olan Effmann tip IIA2 üretral duplikasyonda, ortotopik üretra tamamen normalken, ventral üretra perineye açılır. Özellikle orta hattın dışına açılan üretral duplikasyonların yanlışlıkla anal fistülle karışabileceği unutulmamalıdır.

Anahtar Sözcükler: Üretra, konjenital anomaliler, üretral duplikasyon, fistül, infant

Geliş Tarihi: 26.09.2016 **Kabul Tarihi:** 25.10.2016

INTRODUCTION

Duplication of urethra is a rare lower urinary tract anomaly and is more common in males (1,2). Multiple anatomical variants were described with different clinical manifestations (3). We report here an unusual presentation of urethral duplication in which, completely patent accessory urethra was coursing laterally on perineal region, near the anus, and presenting as an anal fistula.

CASE REPORT

A 2-month-old boy presented with discharge from the perianal region. There was no story of perianal abscess, no instrumentations and no other traumas in his past medical history. On physical examination, a fistula was observed on perianal region, at the 1 o'clock position and only 1 cm near the anus. Other physical findings and abdominal ultrasonography were normal. Informed consent was obtained from the patient's relatives prior to the procedure.

This case was presented at 12th Balkan Congress of Radiology in October 16-19, 2014; İstanbul, Turkey.

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The fistulography with water-soluble contrast material showed that the fistula tract was coursing anteriorly from the perineum to the posterior prostatic urethra (Figure 1). He was diagnosed as urethral duplication and cystourethroscopy was made first to evaluate the orthotopic urethra.

The orthotopic urethra, the verumontanum and the bladder were normal. During cystourethroscopy methylene blue was injected into the fistula on the perianal region and the flow was seen in the left part of prostatic urethra just near the verumontanum (Figure 2). The therapeutic decision was excision of the posterior ventral tract communicating between the urethra and the perineum. A ureteral stent was inserted into the meatus of accessory urethra on the perineum (Figure 3). Anterior sagittal incision was made between the anus and scrotum. The accessory urethra was dissected from the skin to the prostatic part of the orthotopic urethra and was excised totally (Figure 4). His postoperative course was uneventful.

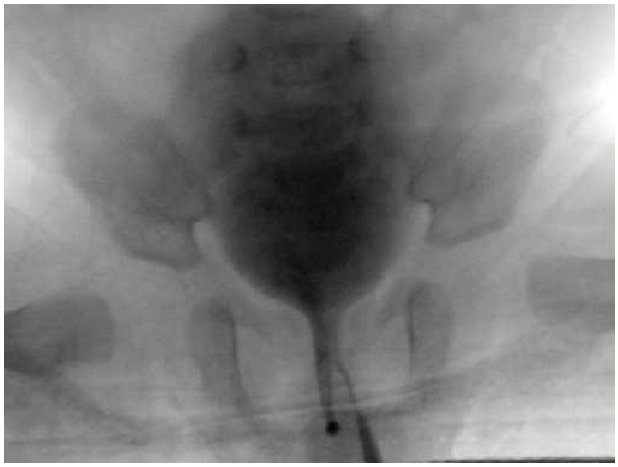

Figure 1. Contrast material outlining the accessory urethral tract from the left perianal region to the prostatic urethra.

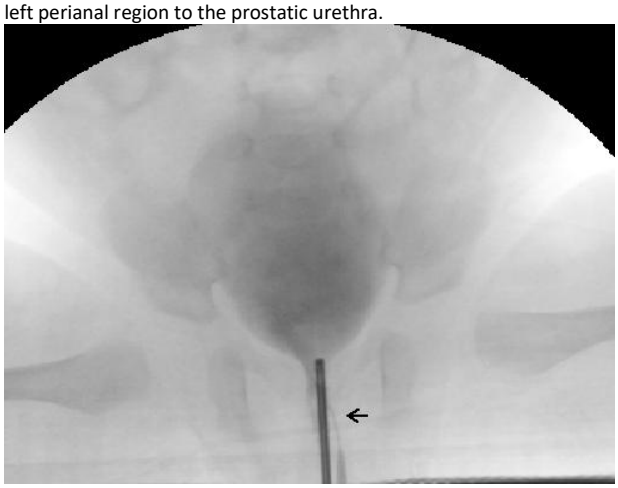

Figure 2. Communication of the duplicated urethra with the prostatic urethra is demonstrated by injection of contrast material through the perianal orifice during the cysto-urethroscopy procedure (cysto-urethroscope is in the native urethra).

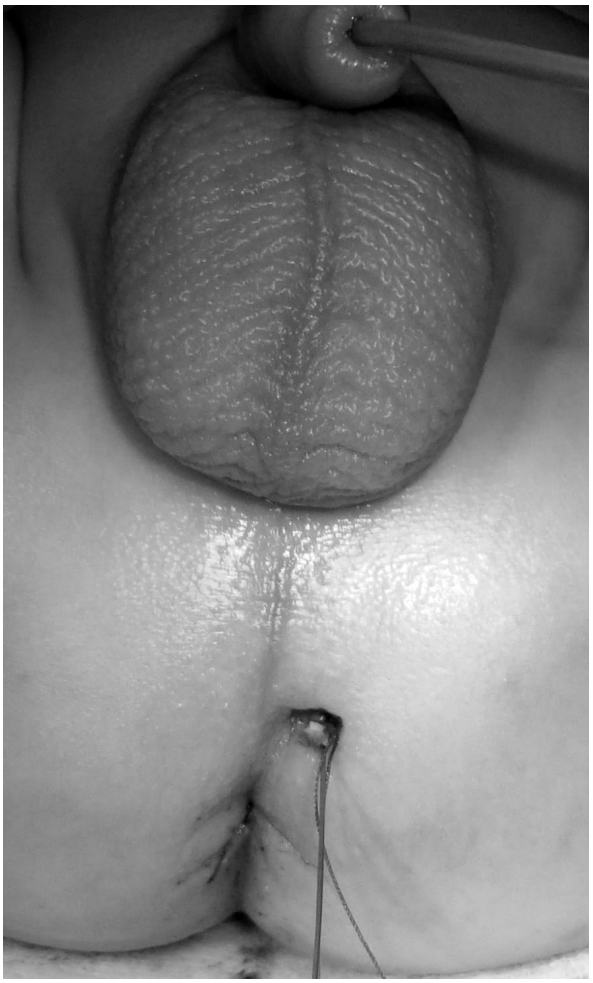

Figure 3. The accessory urethral tract is dissected by the aid of traction sutures and a stent inserted in the duplicated accessory urethra while a Foley catheter is in the native urethra.

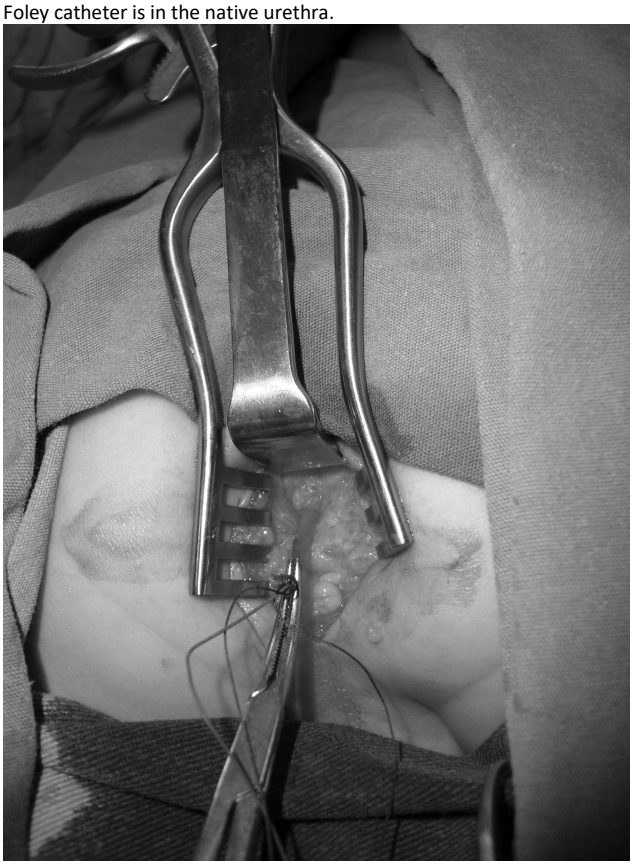

Figure 4. Dissection of the accessory urethra through a midline perineal incision is in progress.

DISCUSSION

Urethral duplications may be sagittal or coronal. Most of them occur in the sagittal plane with two channels running one above the other in the sagittal plane. Effman et al, classified urethral duplications into three types and elaborated them. In type I, there is partial duplication of the urethra. Type II is the complete duplication of urethra and can be classified in two subtypes. There are two meatus in Type IIA. In Type IIB there is two urethral channels arise from bladder or posterior urethra and they unite at the distal part and has one meatus. Type III urethral duplication comprises complete duplication of the urethra and bladder (1).

Y-Type duplication is a special form of Type IIA2 urethral duplications. In this type of duplication both two urethras originate from a common bladder neck or posterior urethra and the ventral accessory urethra opens onto perineum independently. Possible mechanism of embryologic development of Y-Type duplication is the fistula formation in the dorsal margin of urogenital sinus due to possible vascular accident. On the other side impaired growth of the dorsoinferior wall of urogenital sinus and faulty closure of the urorectal membrane are the other suspicious mechanisms for the development of Y-Type duplication (4,5).

Patients may present with different clinical manifestations and on different ages. Clinical symptoms may be very variable such as double urinary stream, urinary incontinence, recurrent urinary tract infections, epididymitis, perineal abscess and outflow obstructions. Diagnosis of Y-Type urethral duplication is made with voiding cystourethrography, retrograde urethrography and fistulography. Cystourethroscopy should be performed to confirm the radiographic findings. It is important to determine which urethra is the more functioning one, especially in Y-Type duplications. The normal urethra has larger calibration, a well developed sphincter mechanism and a normal verumontanum (6,7).

There are two variants of Y-Type duplications:

(i)Stenotic orthotopic: Orthotopic dorsal urethra is stenotic, more functional accessory ventral urethra opens into the perineum or anal channel.

(ii)Unusual form: There is hypoplastic ventral urethra and dorsal urethra is the normal channel. Because in all duplications except this very rare form, the ventral urethra is the more functioning urethra (1,6,7).

The excision of accessory perineal urethra is the surgical treatment of unusual forms, and it is enough alone. Y-Type duplications with stenotic orthotopic urethra, due to the perineal urethra has got verumontanum and sphincter mechanisms, and require more complex surgical interventions (2,4,5,6,7).

CONCLUSION

It should be kept in mind, especially the duplications that open laterally, outside the midline, such as in our case may be misdiagnosed as an anal fistula. Cystourethroscopy should be performed to confirm the radiographic findings and to determine the functioning urethra, especially for Y-Type urethral duplications, then the surgical management should be planned.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Onofre LS, Gomes AL, Leão JQ, Leão FG, Cruz TM, Carnevale J. Urethral duplication--a wide spectrum of anomalies. J Pediatr Urol 2013; 9: 1064-71.

2. Dayanc M, Irkilata HC, Kibar Y, Bozkurt Y, Basal S, Xhafa A. Y-type urethral duplication presented with perineal fistula in a boy. Ger Med Sci 2010; 8: 1-4.

3. AbouZeid AA, Safoury HS, Mohammad SA, El-Naggar O, Zaki AM, Hassan TA, *et al*. The double urethra: revisiting the surgical classification. Ther Adv Urol 2015; 7: 76-84.

4. Sánchez MM, Vellibre RM, Castelo JL, Arias MP, Sarmiento RC, Costa AR. A new case of male Y-type urethral duplication and review of literature. J Pediatr Surg 2006; 41: e69-71.

5. AbouZeid AA, Mohammad SA, Radwan NA, Safoury HS, El-Naggar O, Hay SA. Y-Type Urethral Duplication: A True Variant of the Anomaly or a Misnomer? Eur J Pediatr Surg 2016; 26: 245-51.

6. Salle JL, Sibai H, Rosenstein D, Brzezinski AE, Corcos J. Urethral duplication in the male: review of 16 cases. J Urol 2000; 163: 1936-40.

7. Wagner JR, Carr MC, Bauer SB, Colodny AH, Retik AB, Hendren WH. Congenital posterior urethral perineal fistulae: a unique form of urethral duplication. Urology 1996; 48: 277-80.

Lipoma Bağlı Akut Gelişen Posterior İnterosseos Sinir Sendromu: Olgu Sunumu

Lipoma Causing Acute Posterior Intreosseous Nerve Syndrome: Case Report

Bahar Say

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ÖZET

Posterior interosseos sinir sendromu radial sinirin derin dalının, dirsek ekleminin hemen distalinde gelişen tuzak nöropatisidir ve nadir görülür. Kliniğinde el parmaklarında ekstansiyon kaybı olur. En sık nedeni radius kemiğinin tramvatik kırık ve çıkıkları olup diğer nedenler ise ön kolda yer kaplayan oluşumlar (ganglion, tümöral oluşumlar, romatoid artritde dirsekte gelişen sinovial hipertrofi, kırık sonrası kallus oluşumu, kas hipertrofisi, lipom) olabilir. Lipomlar ön kol yerleşimli olduğunda parmak ekstensör kaslarında yavaş seyirli bir güçsüzlükle sonuçlanan posterior interosseos sinir basısı yapabilir. Bu yazıda, nadir görülen, akut posterior interosseos sinir sendromu gelişen 68 yaşında bir bayan hasta sunulmuş olup, etkilenen bölgede MRG ile lipom saptanmıştır. Tanı ve lokalizasyonda elektronöromiyografinin önemi belirtilmiştir.

Anahtar Sözcükler: Posterior interosseos sinir sendromu, lipom, ENMG (Elektronöromiyografi).

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ABSTRACT

Posterior interosseous nerve syndrome is an entrapment neuropathy of the deep terminal branch of the radial nerve which develops right at the distal of the elbow joint and it is unusual. In the clinic version there is an extension loss of fingers. It's most widespread reason being traumatic fractures-dislocations of the radius bone, the other reasons can bulky formations (synovial hypertrophy that develops at the elbow in rheumatoid arthritis, callus formation after fractures, and muscle hypertrophy, lipoma) located in forearm. Lipomas when occurring in the proximal forearm they can compress the posterior interosseous resulting in an insidious onset of weakness of digital extensor muscles. In this case report is about an unusual case of a 68-year-old-woman with an acute posterior interosseous nerve syndrome and lipoma has been detected in the affected area by MRI. We also emphasize the importance of electroneuromyography (ENMG) in diagnosis and localization.

Key Words: Posterior intreosseous nerve syndrome, lipoma, ENMG (Electroneuromyography).

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GİRİŞ

Radial sinir lateral epikondil düzeyinde yüzeyel ve derin (posterior interosseos sinir) olmak üzere iki dala ayrılır. Yüzeyel dal saf duyu dalı olup, posterior interosseos sinir ise saf motor daldır. Posterior interosseos sinir ön kolda supinatör kas, ekstensör carpi radialis ve ekstensör digitorum kaslarını innerve eder. Kliniğinde el parmaklarında ekstansiyon paralizisi gözlenir. Sinir en sık travmatik radius kemiği kırık ve çıkıklarında etkilenirken kompresyonu, en sık içinden geçtiği supinatör kas düzeyinde olup, yer kaplayan oluşumlar, lipoma gibi benign yumuşak doku tümörleri, ganglionlar ve sinovia hipertrofisine (romatoid artrit) bağlıdır. Kliniğinde düşük elden çok düşük parmak bulgusu saptanırken kompresyon nedenli nöropatilerde klinik yavaş gelişmekte ve progressif bir seyir izlemektedir.

Bu yazıda akut gelişen posterior interosseos sinir sendromu olan bir hasta sunulmuş olup tanıda ve lokalizasyon belirlemede elektronöromiyografinin önemi de belirtilmiştir.

OLGU SUNUMU

Altmış sekiz yaşında kadın hasta sol el parmaklarında güçsüzlük şikâyeti ile başvurdu. Bir yıl önce sabah uyandığında, aniden, sol el parmaklarında güçsüzlük fark etmiş. Sol elinin parmaklarını yukarı doğru kaldıramıyormuş. Bu nedenle başvurduğu merkezlerde antienflamatuar tedavi verilmiş, ancak fayda görmemiş. Özgeçmişinde, hipertansiyon ve diyabet mevcuttu. Nörolojik muayene de sol el bilek metakarpofarengeal eklem ve interfarengeal eklemlerinde belirgin bir ekstansiyon kaybı (0/5) ve sol elde hafif radial deviasyon mevcuttu.

Hastanın şikâyeti ve muayene bulguları doğrultusunda posterior interosseos sinir sendromu ön tanısı ile ENMG yapıldı. ENMG çalışmasında üst ve alt ekstremitelerde duyu iletimlerinde hızlarda yavaşlama saptandı. Alt ekstremitelerde motor iletimlerde amplitüd ve hızlarda azalma ve F dalga latanslarında uzama saptandı.

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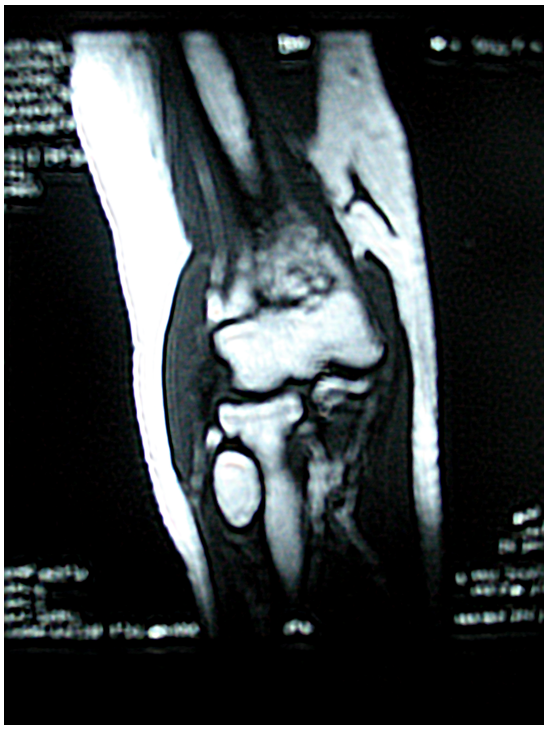
En dikkat çekici bulgu ise, sol üst ekstremitede süperfisial radial sinir duyu ve sol radial sinir motor iletimleri normal iken sol posterior interosseos sinir motor iletiminde BKAP amplitüdünde düşme ve ileti hızında yavaşlama saptanmasıydı. Buna karşılık sağ posterior interosseos sinir motor ileti çalışması normaldi. İğne çalışmasında solda ekstensör indicis proprius ve ekstensör digitorum kaslarında akut denervasyon bulguları saptanmış olup brachioradial ve triceps kası normal bulundu.

Bu bulgular doğrultusunda, ENMG yaygın periferik sensorimotor nöropati zemininde sol posterior interosseos sinirin brachioradialis kası distalinde, ekstensör digitorum innervasyonu öncesindeki parsiyel nöropatisi ile uyumlu olarak yorumlandı ve lezyon yeri belirtilerek sol dirsek ve ön kol MRG istendi.

MR görüntülemesinde radius proksimali düzeyinde, kemiği çevreleyen, supinator kas ile kemik arasında yaklaşık 30x14x23 mm boyutlarında hafif lobule konturlu, tüm sekanslarda yağ ile izointens baskılamanın gözlendiği lipom ile uyumlu olabileceği düşünülen kitle mevcuttu (Resim 1). Kitle total olarak eksize edildi. Patoloji tarafından lipom olarak değerlendirildi. Hastanın postoperatif muayenesinde sol el metakarpofarengeal ve interfarengeal eklem ekstansiyonunda hafif düzelme mevcuttu.



Resim 1: Sol dirsek ve ön kol MRG

TARTIŞMA

Posterior interosseos sinirin ön kolda lipom, fibrom, arteriovenöz malformasyon, bursa, ganglion, romatoid artrit veya osteoartritteki sinovial kist gibi nedenlerle nöropatisi görülebilir (1). Kliniğinde el parmaklarında ekstansiyon paralizisi görülür. Tanı, klinik ve muayene ile konur, elektrofizyolojik çalışma ile de doğrulanır. Elektrofizyolojik çalışmalar nontramvatik, palpabl olmayan yumuşak doku tümörlerine bağlı vakalarda tanıda oldukça kullanışlıdır (2).

Lipomlar sık gözlenen benign yumuşak doku tümörleridir ve genellikle subkutan yerleşimli, asemptomatik, nadiren de derin yerleşimli olabilirler. Lipom radius başına komşu ve derin yerleşimli olduğunda interosseos sinir nöropatisine yol açabilmektedir (3). Literatürde lipomun neden olduğu posterior interosseos sinir nöropatisi sınırlı sayıdadır. Bu olgularda parmak ekstansiyonunundaki kayıp tipik olarak yavaş olup, genellikle de progressif bir seyir izlemiştir (4). Bununla birlikte, yalnızca Bugnicort ve arkadaşlarının sunduğu vakada lipoma bağlı posterior interosseos sinir nöropatisi akut olarak gözlenmiş ve bu nedenle tanıda öncelikle akut serebrovasküler hastalık düşünülerek bu yönde tedavi uygulanmıştır. Ancak olgunun iki ay sonraki muayenesinde ön kol posteriorunda motor güçsüzlükte artış ve kas atrofisinin saptanması üzerine tuzak nöropati düşünülüp ENMG ile posterior interosseos sinir nöropatisi tanısı konulmuştur (5).

Bu yazıda sunulan olguda lipoma bağlı akut posterior interosseos sinir sendromu gözlenmiştir. Akut gelişen klinik, vasküler kompresyon sonucunda, iskemik nedenli olabilir. Bu durumu hastanın diyabeti de kolaylaştırmış olabilir.

Olgunun ENMG, görüntüleme ve patoloji sonucu doğrultusunda nöropati tanısı ve lipom birlikteliği doğrulanmıştır. Nöropati tanısı ve lezyon lokalizasyonunda ENMG'nin önemi büyük olmuştur.

Çıkar Çatışması
Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

KAYNAKLAR

1. Salama H, Kumar P, Bastawrous S. Posterior interosseous nerve palsy caused by parosteal lipoma: A case report. Case Rep Med. 2010;2010
2. Valer A, Carrera L, Ramirez G. Myxoma causing paralysis of the posterior interosseous nerve. Acta Orthop Belg. 1993;59:423-5
3. Allagui M, Maghrebi S, Touati B, Koubas M, Hadhri R, Hamdi MF, Abid A. Posterior interosseous nerve syndrome due to intramuscular lipoma. EurOrthop Traumatol.2014;5:75-9.
4. Murphy A, Williams J. Posterior interosseous nerve palsy caused by lipoma: A case report. Can J Plast Surg. 2009 Winter,17:e42-4
5. Bugnicourt JM, Peiter J, Merle PE, Le Gars D. Acute peripheral nerve compression by a lipoma mimicking stroke. Clin Neurol Neurosurgery. 2009;111:395-6.

Sitoredüktif Cerrahi ve HİPEK'te Anestezi ve Perioperatif Bakım

Anesthesia and Perioperative Care in the Cytoreductive Surgery and HIPEC

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ÖZET

Hipertermik intraperitoniyal kemoterapi ve sitoredüktif cerrahi seçilmiş peritoniyal karsinomatozisli olgularda popülaritesi gittikçe artan, etkili multimodal tedavi seçeneklerindendir. Sitoredüktif cerrahi, cerrahi alanın büyük olduğu, kan ve sıvı kaybının fazla olduğu, sıvı şiftlerinin görüldüğü, koagülasyon değişikliklerinin meydana geldiği hipertermik intraperitoniyal kemoterapi öncesi uygulanan fazdır ve süre olarak da uzundur. Hipertermik intraperitoniyal kemoterapifazı ise önemli hematolojik, hemodinamik ve metabolik değişikliklerin görüldüğü fazdır. Bu cerrahi prosedür anestezistler içinde oldukça zor bir süreçtir. Anestezistin görevi her fazda meydana gelen patofizyolojik değişiklikleri bilmek ve buna göre perioperatif dönemde önlem almaktır. Bu hastalar intraoperatif ve postoperatif yakın izlem gerektirir. Primer hastalığın yanı sıra cerrahinin büyüklüğü, anestezik yönetim, verilen sıvı miktarı ve kan transfüzyonu sonuçları etkileyebilir. Bu konuda ileri çalışmalara ihtiyaç vardır. Gittikçe artan sıklıkla yapılmasına rağmen anestezi uygulaması ile ilgili oluşmuş bir görüş birliği bulunmamaktadır. Bu yazıda sitoredüktif cerrahi ve hipertermik intraperitoniyal kemoterapide perioperatif anestezik yaklaşım anlatılmaktadır.

Anahtar Sözcükler: Sitoredüktif cerrahi, hipertermik intraperitoniyal kemoterapi, anestezi

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ABSTRACT

Cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy is a major surgical procedure that is being used increasingly frequently in therapeutic option for selected patients with peritoneal surface malignancies. This is more blood and fluid shift and coagulation changes could be seen in cytoreductive surgery phase. It is applied before hyperthermic intraperitoneal chemotherapy phase and process is longer than hyperthermic intraperitoneal chemotherapy phase. Significant hematologic, hemodynamic and metabolic changes have been seen is in hyperthermic intraperitoneal chemotherapy phase. This surgical procedure is quite a difficult process of anesthetists. The task of anesthesiologist is to know during pathophysiological changes occurring in each phase and accordingly take measures in perioperative. These patients require close monitoring of intraoperative and postoperative period. Primary disease as well as the size of the surgical area, anesthetic administration and the amount of fluid and blood transfusion may affect the outcome. There is a need for further work on this issue. There is no consensus gradually formed on anesthesia despite the increasing frequency. In this article, perioperative anesthetic management are described in cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy.

Key Words: Cytoreductive surgery, hyperthermic intraperitoneal chemotherapy, anesthesia.

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GİRİŞ

Hipertermik intraperitoniyal kemoterapi (HİPEK) ve sitoredüktif cerrahi (SRC) son 20 yılda seçilmiş peritoniyal karsinomatozisli olgularda popülaritesi gittikçe artan, etkili multimodal tedavi seçeneklerindendir**.** Bu kompleks tedavinin uygulanması sırasında oluşan metabolik ve fizyolojik değişikliklerden dolayı anestezistler perioperatif dönemde gelişebilecek komplikasyonlar için oldukça dikkatli olmak zorundadırlar. Hastaların seçimi ve tümörün titizlikle çıkarılması en iyi klinik sonuçları elde etmek için zorunludur. Gittikçe artan sıklıkla yapılmasına rağmen anestezi uygulaması ile ilgili oluşmuş bir görüş birliği bulunmamaktadır. Bu yazıda SRC ve HİPEK'te perioperatif anestezik yaklaşım anlatılmaktadır.

Sitoredüktif cerrahi ve HİPEK ilk olarak Sugarbaker tarafından 1995 yılında tanımlanmıştır. Bu teknik ile kolorektal, gastrik, ovaryan ve peritoniyal mezotelyoma gibi farklı kanserlerden kaynaklanan peritoniyal karsinomatozisli olgularda survi ve hayat kalitesi artar. 2008 yılında yapılan 6. peritoniyal yüzey kanserleri çalıştayında SRC ve HİPEK peritoniyal karsinomatozisli olgularda deneyimli merkezlerde standart tedavi şeklini almıştır. Morbidite % 12 ile % 67.6 arasında değişirken, mortalite %0 ile %9 arasındadır (1,2). SRC intraabdominal makroskopik tüm tümörlerin çıkarıldığı pariyetal ve visseral peritonektomi prosedürüdür.

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Sadece omentektomiden, gastrointestinal trakt, pankreas, dalak, safra kesesi, uterus, overler, karaciğerin bir parçasının ve 2.5 mm'den büyük lenf nodlarının alındığı geniş abdominal rezeksiyonu içerir.

SRC cerrahi alanın büyük olduğu, kan ve sıvı kaybının fazla olduğu, sıvı şiftlerinin görüldüğü, koagülasyon değişikliklerinin meydana geldiği HİPEK öncesi uygulanan fazdır ve süre olarakta uzundur. Hipertermik intraperitoniyal kemoterapi fazı ise önemli hematolojik, hemodinamik ve metabolik değişikliklerin görüldüğü fazdır. HİPEK tedavisi ile skar dokusu, adezyon ve anastomoz bölgelerinde malign hücrelerin yayılımını önlemek hedeflenir. HİPEK sırasında kullanılan kemoterapötik ajanlar tümör dokusunda maksimum konsantrasyon ile etki ederken normal dokuya etkileri minimaldir; peritoniyal sıvıdan plazmaya doğru geçiş ve peritoneal klirensleri yavaştır. HİPEK sırasında 4 adet dren ve ısı probu batın içine yerleştirilir. Bu drenlerden bir tanesi batın içine ısıtılmış perfüzatı ve kemoterapötik ajanı verirken, diğer üç tanesi ise bu perfüzatı dışarı drene eder. Perfüzat batın içinde roller pompa aracılığıyla 60-90/dk döndürülür ve sıcaklık 41-42 °C'ye kadar çıkarılır. Ardından kemoterapötik ajan eklenir. HİPEK sırasında taşıyıcı solüsyonların tipi ve miktarı konusunda dikkatli olunmalıdır. Taşıyıcı solüsyon olarak genellikle %5 dekstroz ya da izotonik salin tercih edilir. HİPEK teknik olarak açık ve kapalı olarak uygulanabilir. Çoğu merkez ısı kaybının az olması, dokuya daha iyi penetrasyon sağlaması ve çevre kontaminasyonunun daha az olması nedeniyle kapalı tekniği tercih etmektedir. Açık teknikte ise kemoterapötik ajan daha homojen dağılırken çevre kontaminasyonu daha fazla olmaktadır. HİPEK sırasında sıcaklığının artırılması ile kemoterapotik ajanın tümörosidal etkinliği artırılmakta, hücreler sistemik kemoterapi uygulanmasıyla karşılaştırıldığında 20 kat daha fazla kemoterapötiğe maruz kalmaktadır. Kolon kanserlerinde sistemik kemoterapi ile ortalama 16-24 aylık survi bildirilirken; HİPEK'te 5 yıllık yaşam şansı yaklaşık % 30-45 oranındadır (3,4). Tümörün tipine göre farklı kemoterapötik ajanlar uygulanmaktadır. Yan etkilerine ek olarak uygulama sırasında alerjik reaksiyonlar, bulantı, kusma ya da flushing meydana gelebilir.

Hipertermik intraperitoniyal kemoterapi sırasında kolon kanserlerinde; Oksaliplatin+5 Flurourasil, over kanserlerinde; Sisplatin+Doksorubisin, kolorektal kanserlerde Mitomisin C kullanılmaktadır. Mitomisin C nefrotoksisite, pulmonertoksisite yaparken, Sisplatin; periferal nöropati, miyelotoksiste, nefrotoksisite, Oksaliplatin; nörotoksisite yapabilmektedir. SRC ve HİPEK uygulamasında başarılı sonuçlar için hasta seçimi önemlidir. Kolorektal kaynaklı peritoniyal karsinomatozis tanısı konan hastalardan 3 ve daha az karaciğer metastazı olan, biliyer obstrüksiyonu olmayan ve sistemik kemoterapiye iyi yanıt vermiş olanlar, SRC ve HİPEK tedavisi için en uygun adaylardır (5) . SRC ve HİPEK tedavisi aktif kardiyak şikayeti olmayan yada medikal tedavi ile stabil olan, ekstra-abdominal hastalığı olmayan, yaygın karaciğer metastazı olmayan, retroperitoneal yayılımı olmayan, 70 yaş altı ve peritoniyal lezyonu tam ya da tama yakın rezeke edilebilecek hastalara yapılmalıdır (5,6).

PREOPERATİF HAZIRLIK

Hastalar operasyon öncesi ayrıntılı bir fizik muayeneden geçirilmelidir. Rutin preoperatif testlerin yanısıra preoperatif kemoterapötik ajanların özellikle doksorubisinin ve bleomisinin kardiyak ve solunum fonksiyonlarını etkilemelerinden dolayı hastaların kardiyovasküler ve solunum sistemi rezervleri değerlendirilmelidir. Preoperatif renal değerlendirmede üre, kreatinin ve glomerüler filtrasyon hızının hesaplanması hastaların intraoperatif ve postoperatif böbrek hasarı açısından önemlidir. İntraoperatif kan kaybının fazla olabileceği ve oluşabilecek koagülasyon değişiklikleri ihtimaline karşın yeterli kan ve kan ürünleri hazırlanmalıdır. Genel anesteziye ilave olarak cerrahiye stres yanıtı azaltması, intraoperatif opioid tüketimini azaltması, postoperatif ventilasyon gereksinimini azaltması, yoğun bakımda ventilatör gereksinimini azaltması ve postoperatif ağrı tedavisi nedeniyle torakal epidural kateter uygulanabilir. Ancak, koagülasyon sisteminde oluşabilecek değişikler nedeniyle epidural hematom gelişme riski ve hastaların immünsüpresif olmalarından dolayı epidural apse oluşumu gözönünde bulundurulmalıdır. Bu yüzden bu hastalarda kanama ile ilgili anamnezin yanı sıra kullandığı ilaçlar da gözden geçirilmelidir. Epidural kateter takılması ise deneyimli bir anestezist tarafından uygulanmalıdır. Tüm hastalar major laparotomilerde rutin uygulanan şekilde entübe edilip ventile edilir. Postoperatif dönemde bu hastaların mekanik ventilatör desteğine ihtiyaç duyabileceği unutulmamalıdır. İntraoperatif sıvı yönetiminde kullanılmak üzere geniş damar yolu açılmalıdır. Santral venöz kateterizasyon sıvı idamesinin sağlanmasında önem arz eder. Arteriyal kateterizasyon hemodinamik monitorizasyonda kullanılabilir. Kardiyak outputu ölçmek ve sıvı yönetiminde kullanmak için ise pulse kontur analiz, transözofagiyal ekokardiyografi, özofagiyal dopler ya da pulmoner arter kateteri tercih edilebilir.

İNTRAOPERATİF DÖNEM

Volüm durumu (sıvı, protein, kan), koagülasyon değişiklikleri, ısı (hipotermi, hipertermi), kemoterapinin etkileri ve kardiyovasküler stabilite intraoperatif olarak dikkatle izlenmelidir.

SIVI YÖNETİMİ

İntraoperatif dönemde SRC fazında çok ciddi kan ve sıvı kayıpları olmaktadır. Bu kayıplara bağlı olarak hipovolemi, uzamış cerrahiye bağlı hipotermi ve ciddi kanamaya bağlı koagülopatiler bu fazda majör problemlerdir. Bu dönemde amaç HİPEK fazında meydana gelen önemli patofizyolojik değişiklikler öncesi normovolemik durumun kristaloid, kolloid ya da kan ve kan ürünleri kullanarak sağlanması ve idame ettirilmesidir. Operasyon sırasında normovolemiyi sağlamak için 6-12 ml/kg/saat sıvı replasmanı gerekebilir. Bazı hastalarda ise masif kan transfüzyonu gerekebilir. SRC fazında normovolemiyi sağlamak, HİPEK öncesi kardiyovasküler stabiliteyi ve elektrolit imbalansını düzeltmek, HİPEK fazında ise ortalama arteriyal basıncın bazale göre % ± 20 sinin sağlanması ve yeterli idrar çıkışını sağlamak esas olmalıdır. Saatlik 0.5-1 ml/kg idrar çıkışı, santral venöz basınç (CVP)'nin 6-8 cm/H_20 civarında tutulması yeterlidir. İdrar çıkışı kontrol edilerek furosemid (ortalama 25mg) yapılabilir. Böbrek fonksiyonlarını korumak için düşük doz dopamin kullanılması tavsiye edilmez (7). Son on yılda çeşitli çalışmalar göstermiştir ki düşük doz dopamin başlanmasının böbrek fonksiyonlarını iyileştirmesi üzerine etkisi yoktur (8,9). Renal fonksiyonların idame ettirilmesi ve renal zedelenmenin önlenmesi perioperatif sonuçları olumlu etkiler. HİPEK sırasında nefrotoksik kemoterapötiklerin kullanımı, abdominal hipertansiyon ve abdominal sıvı geçişine bağlı intravasküler volüm açığı nedeniyle %1.3-%5.7 hastada akut böbrek hasarı görülmektedir (10). Böbrek hasarı genellikle multi-faktöriyeldir. Böbrek yetmezliği için majör risk faktörleri; hipovolemi, hipotansiyon, majör cerrahi, nefrotoksik ilaçlar, kan transfüzyonu, sisplatin kullanımı ve sistemik inflamasyondur. Abdominal cerrahilerde sıvı yönetiminde kristaloid ya da kolloid sıvı seçimi tartışmalıdır (11). Kolloidler damariçi onkotik basıncı sağlamak için iyi bir alternatif olabilir. Üçüncü jenerasyon hidroksietil starch solüsyonları (%6 HES 130/0.4) bu amaçla kullanılabilir. Aşırı kristaloid kullanmı intestinal ödeme ve mortalitede artışa neden olmaktadır (12,13). Kajdi ve arkadaşları (14) kristaloid kullanımının renal fonksiyonlar üzerine negatif etkisinin olmadığını göstermişlerdir. Buna karşılık HES kullanımının ise renal fonksiyonları önemli ölçüde negatif etkilediğini bildirmişlerdir (14). Ayrıca debulking aşamasında ve asit drenajı esnasında ciddi protein kayıpları olabilmektedir. Bu protein kaybını karşılayabilmek için human albümin ya da taze donmuş plazma replasmanı gerekebilir. Sıvı tedavisi sırasında aşırı sıvı yüklenmesi endotelyal glikokaliksin zarar görmesine neden olabilmektedir (15). Bu yüzden anestezistler dengeli bir sıvı tedavisi ile hipovolemi ve hipervoleminin etkilerinden hastalarını koruyup, bölgesel ve sistemik perfüzyonun sağlanması ve idame ettirilmesine özen göstermelidirler. Bu hastalarda liberal ya da restriktif sıvı rejimi uygulamalarının sonucu nasıl etkilediği bilinmemektedir ve bu konuda ileri çalışmalara ihtiyaç vardır.

HEMODİNAMİK ve METABOLİK DURUM

Hipertermik intraperitoniyal kemoterapi fazı hipermetabolik bir süreçtir. Kardiyovasküler ve metabolik çok sayıda parametrede önemli değişiklikler meydana gelir. Bu fazda kalp hızı ve kardiyak output artarken, sıcaklığın artması ile birlikte sistemik vasküler rezistansta düşme meydana gelir. Kardiyak outputtaki artış, pulmoner arter kateteri ya da transözofagiyal ekokardiyografi ile gösterilebilir (16,17). Vücudun sıcaklığa ilk tepkisi periferik vazodilatasyondur. Azalan periferik vasküler rezistansa karşılık kardiyak outputu sürdürebilmek için kalp hızı artar (7,16). Swanz Ganz kateteri, transözofagiyal eko ya da pulse kontur analiz gibi invazif monitorizasyonlar rutin olarak bu hastalarda önerilmemektedir. Ancak, kalbin her atımında kardiyak outputu ölçebilen, nabız şekli analiz yöntemleriyle ekstravasküler akciğer volümü ya da nonkardiyojenik akciğer ödemi hakkında bilgi sahibi olunabilir. Arteriyal kateterizasyon, CVP ve idrar sondası gibi standart monitörizasyon tekniklerinin yanı sıra, sıvı durumu hakkında bilgi edinmek için kalibrasyon gerektirmeyen arteryal dalga şekline göre kardiyak output ölçebilen yeni teknolojiler ile özofagiyal eko ya da doppler gibi daha az invazif yöntemlerden yararlanılabilir (7,17). CVP kardiyak önyükün ve volüm durumunun zayıf bir göstergesidir. İntraabdominal olarak uygulanan solüsyon batın içindeki basıncı artırır. Artan intraabdominal basınç diyaframı yukarı doğru iter, fonksiyonel rezidüel kapasite azalır, havayolu basıncı ve CVP artar (17). HİPEK sırasında ortalama CVP yaklaşık 3-4 mmHg artar (18). Batın içindeki basınç değişiklikleri kardiyak outputu etkiler. Splenik damar direncinin artması, abdominal kan miktarının azalması venöz dönüşü azaltır (19,20). Sistemik ve bölgesel perfüzyonun idame ettirilmesinde kan volümü önemli rol oynar (7).

Anestezist için böbrek yetmezliğinin önlenmesi ve sıvı dengesinin sağlanması için kardiyak outputtaki değişiklikleri bilmek önemlidir. Hipotansiyon gelişirse vazoaktif ilaçlar intraoperatif ve postoperatif dönemde kullanılabilir. Tüm hastalarda kombine metabolik ve respiratuar asidoz gelişebilir. Metabolik asidoz çoğu kez HİPEK fazı öncesi başlar ve HİPEK boyunca devam eder. Bikarbonat seviyesinde düşme, laktat miktarında artma gözlemlenir. Karbondioksit üretimi artarken, sistemik oksijen tüketimi de artar. Schmidt ve arkadaşlarının çalışmalarında ortalama oksijen ekstraksiyon oranı (PaO_2/FiO_2) SRC sırasında 60.7 iken, HİPEK 'le birlikte bu oranın 50.9'a düştüğü ve HİPEK sonlandırıldıktan sonra ise en düşük 41.6 olduğu tespit edilmiştir (18). HİPEK sırasında SRC dönemi ile karşılaştırıldığında intraabdominal basınç artışına bağlı olarak hava yolu basıncı artarken, paralel olarak end-tidal karbondioksit ($ETCO_2$)'de artmıştır. İntraoperatif dönemde kemoterapi ve sıcaklığın etkisiyle birlikte çeşitli elektrolit bozuklukları gelişebilir. Taşıyıcı solüsyon olarak %5 dekstroz kullanıldığı zaman insülin tedavisi gerektirecek kadar hiperglisemi gelişebilir. Dilüsyonel hiponatreminin serebral ödeme neden olarak mortalite ve morbiditeyi artırdığı gösterilmiştir (21). Ayrıca hemodinamik durumda kemoterapötik ajanların indüklediği kardiyak yan etkilerde göz ardı edilmemelidir. Literatürde sisplatinin intraperitoniyal uygulanması sonrası amiodarona dirençli ventriküler taşikardi gelişen bir olgu sunumu da bulunmaktadır (22).

ISI DENGESİNİN SAĞLANMASI

Sitoredüktif cerrahi ve HİPEK sırasında vücut sıcaklığında önemli değişiklikler meydana gelmektedir. Bu yüzden tüm hastalara rutin ısı monitorizasyonu uygulanmalıdır. Özofagiyal, rektal, vezikal ya da timpanik ısı probu tercih edilebilir ve sürekli ısı monitorizasyonu sağlanmış olur. SRC sırasında önemli miktarda kan kaybı olmaktadır. Hemodinamik olarak stabilite sağlamak amacıyla sıvı ve kan transfüzyonu uygulanmaktadır. Ancak cerrahi alanın oldukça büyük olması, verilen sıvıların ısıtılmaması ve ısı kaybı nedeniyle SRC fazı sırasında hipotermi beklenebilmektedir. Bu aşamada sıvıların ısıtılarak verilmesi, hastanın blanketler yardımı ile ısıtılması ile ısı kaybı azaltılabilir. Isı dengesinin sağlanması ile hipoterminin koagülasyon ve hemostaz mekanizmalarına etkileri de önlenmiş olur (23). Ayrıca antiinflamatuar süreç, metabolik denge ve nörolojik durum da hipoterminin zararlı etkilerinden korunmuş olur.

Hipertermik intraperitoniyal kemoterapi süresince intraperitoniyal uygun sıcaklığı elde etmek için uygulanan hiperterminin süresi ve sıcaklık tartışmalıdır. Optimal sıcaklıkta farklı uygulamalar mevcuttur. Literatürde 40 ile 45°C arasında değişen protokoller bulunmaktadır. HİPEK sırasında sıklıkla kullanılan ilaçlar 50°C'ye kadar kimyasal stabilitelerini korumaktadır. Sitotoksik ajanların hipertermi ile birlikte tümörosidal aktiviteleri 39°C ve üstünde artmaktadır. HİPEK sırasında ısıtılmış perfüzatın intrabdominal kaviteye verilmesiyle birlikte ortalama vücut ısısında da artış olmaktadır. Uygulamayla birlikte hastalarda vücut ısısı 40.5°C'ye kadar çıkmaktadır (17,18). Ancak; ısı klinik olarak kabul edilebilir sınırlarda kalmaktadır (24). Kamal ve arkadaşlarının çalışmalarında ise HİPEK fazında aktif soğutma uygulanmasına rağmen hastalarının %18'inde vücut sıcaklığı 39°C ve üstüne çıkmıştır (25). Hipertermi aşamasında oksijen tüketiminde artma, kalp hızında ve $ETCO_2$ miktarında artma, metabolik asidoz ve arteriyal laktat miktarında artma gibi metabolik değişiklikler görülür. Bu yüzden uzun süreli hipertermi uygulamalarında vücudun ısısını azaltmak için soğutma sistemleri kullanmak gerekir. Ayrıca anestezistin HİPEK boyunca ventilasyon parametrelerinde değişiklik yaparak metabolik dengeye etki etmesi de gerekebilir. Ne yazık ki hayvanlarda veya insanlarda hipertermi süresinin artması konusunda sistematik bir çalışma yoktur. Uzun süreli hipertermi ve anestezik ajanlarla etkileşimleriyle ilgili ileri çalışmalara ihtiyaç vardır.

Bilindiği üzere ısı nöromüsküler ilaçların etki sürelerini etkiler. Karaciğerdeki aktif transport işlemi ısı bağımlıdır ve hipotermi ile bu işlem inhibe olur. Veküronyumun etki süresi bu yüzden hipotermide uzar (26). Atrakuryumunda 30°C'de kardiyopulmoner bypass sırasında kas gevşemesini sağlamak için gerekli olan dozun %35 oranında azaldığı tespit edilmiştir (27). Bu azalmanın nedeni multifaktoriyeldir. Hofmann eliminasyonu ısı ve pH bağımlı bir süreçtir. Ayrıca ısı ile birlikte karaciğer ve böbrekten ekstraksiyonu da etkilenmektedir (27). Hipertermi sırasında ise ve küronyumun etki süresi ile ısı arasında doğrusal bir ilişki bulunmazken; atrakuryumda doğrusal bir ilişki bulunmuştur. Atrakuryumun hipertermi ile birlikte etki süresinde kısalma meydana gelmiştir(28). Adachi ve ark., yaptığı çalışmada ise hipertermik dönemde veküronyum potensinde azalma tespit edilmiştir. HİPEK sırasında kas gevşeticilerin efektif kullanımı açısından nöromusküler monitorizasyonun uygulanması önerilmektedir (29). Kas gevşeticiler ve hipertermi ile ilgili ileri çalışmalara ihtiyaç vardır.

KOAGÜLASYON

Sitoredüktif cerrahi gibi majör cerrahilerde önemli miktarda kan kaybı olmaktadır. Bu hastaların yaklaşık yarısına intraoperatif dönemde, hastaların üçte birine ise postoperatif dönemde kan ve taze donmuş plazma replasmanı gerekmektedir (18). Literatürde operasyon süresinin 9 saatten uzun olması, preoperatif INR değerinin 1.2 den yüksek olması ve preoperatif hemoglobin değerinin 12.5 gr/dl den daha az olması, intraoperatif kan transfüzyonu için önemli risk faktörleri olarak belirtilmiştir (30).

Kanser cerrahisinde kan transfüzyonu artmış morbidite ve mortalite ile birliktedir. Bu yüzden cerrahi kanamanın kontrolü ve koagülasyon bozukluklarının erken tanınması ve tedavisi önemlidir (31).Banka kanı ile karşılaştırıldığında daha fizyolojik pH da, daha fazla 2,3 difosfogliserat içeren, potasyum miktarının daha az olduğu, eritrosit morfolojisinin daha düzgün ve yaşam süresinin daha uzun olduğu, ışınlanarak kanser hücrelerinden uzaklaştırılarak yapılan otolog kan transfüzyonu kan kaybının replasmanında alternatif olabilir (32,33). Kan kaybı sadece cerrahi nedenlerle değildir. Kanamaya da yatkınlık artmıştır. Bu yatkınlığın nedeni tam olarak anlaşılamamıştır. Bu yatkınlıkta geniş sıvı şiftlerinin ve döngüsünün olması, protein kaybının olması, tümör yükü ve hipertermik kemoterapi uygulaması sorumlu olabilir. Artmış olan koagülasyon bozukluğunu saptamada laboratuvar testleri yardımcı olabilir. INR de uzama, antitrombin III ve fibrinojen seviyelerinde azalma,aktive parsiyel tromboplastin seviyelerinde artma ve trombosit sayısında azalma tespit edilebilir. Ek olarak bazı koagülasyon faktörlerinin azalması örneğin faktör 13, standart laboratuar testleri ile tespit edilemeyebilir (18). Bu yüzden tromboelastometri kullanımı yararlı olabilir.

POSTOPERATİF YÖNETİM

Postoperatif dönemde tüm hastalar hemodinamik ve solunum parametreleri, asit-baz dengesi, ve organ fonksiyonları stabilize edilinceye dek postoperatif yoğun bakım ünitelerinde takip edilmelidir. Hastaların yoğun bakımda kalma süreleri değişkendir. Bu sürede hastanın yaşı, komorbid hastalık varlığı, verilen sıvı ve kan miktarı, cerrahinin büyüklüğü etkilidir. Hasta genç ve komorbid bir hastalığı yoksa bu süre kısadır. Çeşitli çalışmalarda ortalama kalış süresi 4.4 (2-10) gün olarak belirtilmiştir (25). Hastaların çoğu diyafragmatik yaralanma, unstabil hemodinami ya da ventilasyon problemlerinden dolayı operasyon bitiminde ekstübe edilemez ve postoperatif mekanik ventiasyon takibi gerekebilir. Bu hastalarda genel durum stabilize edildikten sonra erken dönemde ekstübasyon düşünülmelidir. Arakelian ve arkadaşları ekstübasyon sonrası CPAP uygulanmasının yararlı olabileceğini bildirmişlerdir (34). Solunum sistemi komplikasyonlarını önlemek için antibiyotik profilaksisi, erken dönemde fizyoterapi, yeterli ağrı kontrolü sağlanmalıdır. Atelektazi, plevral efüzyon, pulmoner ödem, pnömoni ve pnömototoraks gibi torasik komplikasyonlar bazı literatürlerde %86 gibi yüksek oranlarda bildirilmiştir (25).

Postoperatif ilk 72 saat içinde yüksek sıvı kaybı halen devam etmektedir ve yakın takip gerektirir (3,35,36). Sıvı kaybı çoğunlukla nazogastrik ve drenlerden olmaktadır. Literatürde postoperatif 6. güne kadar nazogastrikten günlük yaklaşık 1000 cc gelen vaka serileri bulunmaktadır (25). Hemodinamik değişiklikler, CVP, serum elektrolitleri, idrar çıkışı ile sıvı tedavisi düzenlenmelidir. Kan ve kan ürünleri transfüzyonuna ise hemoglobin düzeyi, hematokrit miktarı ve koagülasyon parametreleri takip edilerek karar verilmelidir. Onkolojik cerrahide kan transfüzyonu artmış morbidite ve mortalite ile birliktedir (31,37). Kajdi ve arkadaşlarının çalışmaları ise artmış transfüzyon ihtiyacı ile majör komplikasyon riskinin arttığını destekler niteliktedir (14). Kanama miktarının ve sıvı şiftinin fazla olmasından ötürü kristaloid, kolloid ya da kan ürünleri ile efektif dolaşım volümü sağlanmaya çalışılır. Gerekli ise inotropik ajanlar, vazopressörler de kullanılabilir (14). Protein kaybından dolayı albumin seviyelerinde düşme beklenebilir ve replasman gerekebilir (38). Hastaların büyük çoğunluğunda postoperatif 1. ve 2.günlerde vücut sıcaklığının 37.7 ile 37.9 °C arasında seyrettiği ve sonrasında normalize olduğu gözlemlenmiştir (25). Baratti ve arkadaşları ise sepsis bulguları olmamasına rağmen hastaların bazılarında postoperatif 10 gün boyunca sıcaklığın 38 °C'ye kadar yükseldiğini rapor etmişlerdir(39).

Hipertermik intraperitoniyal kemoterapi sırasında farklı kemoterapötik ajanlar kullanılmaktadır. Kullanılan ajanlara göre postoperatif dönemde kemik iliği depresyonu, kalp, karaciğer ve böbrekte toksisite gözlenebilir (40,41). Doksorubisine bağlı miyokardiyal depresyon, bleomisine bağlı pulmoner fibrozis, sisplatine bağlı ise böbrek yetmezliği gözlenebilir. İntraoperatif koagülasyon faktörlerinin dilüsyonu ve eksilmesi ile birlikte INR'de postoperatif dönemde uzama ve taze donmuş plazma verilmesi gerekebilir.

Ayrıca karaciğer fonksiyon testlerinde bozukluk ta postoperatif dönemde görülebilir. Hastanemizde alınan 36 hastanın kayıtları incelendiğinde hastaların preoperatif ve postoperatif Htc, Hb ve platelet değerlerinde istatistiksel olarak anlamlı bir fark vardı . Postoperatif tüm hastalarda beyaz küre değerlerinde ise yine preoperatif ve postoperatif 1. günde fark gözlemledik. Hastalarımızın tümünde kreatin değerlerinde farklılık gözlemlemedik. Bunu kullanılan sisplatin dozunun uygun aralıkta olmasına ve yeterli sıvı dengesi sağlanmasına bağlamaktayız. Bu hastalarda intraoperatif dönemden başlayarak yeterli hidrasyonun ve idrar çıkışının sağlanması, kaybedilen kan ürünlerinin yerine konulması ve düzenli laboratuvar takibinin yapılmasının gelişebilecek komplikasyonları önlemede etkili olabileceğini düşünmekteyiz.

Bu hastalarda postoperatif ağrı ve yönetimi de önem arz eder. Torakal epidural kateter takılması ile lokal anestetik ve opioid infüzyonu ile etkin analjezi sağlanabilir. Ayrıca, erken ekstübasyon, mobilizasyon, postoperatif pulmoner komplikasyonların azaltılması ve kronik ağrı sendromu gelişimi de azaltılmış olur. İntravenöz opioid kullanımının azalması ile barsak atonisi ve solunum depresyonu riski gelişmesi önlenebilir(42,43). Ancak gelişebilecek olan trombositopeni ve koagülasyon faktörlerinin eksikliğine bağlı spinal hematom riski akılda tutulmalıdır(44). Kajdi ve arkadaşlarının bir hastasında ise dekompresyon cerrahisi gerektirecek epidural kitle tespit edilmiştir (14). Biz de hastalarımızın sadece 9 tanesine torakal epidural kateter taktık ve herhangi bir komplikasyon gözlemlemedik.

SONUÇ

Hipertermik intraperitoniyal kemoterapi ile SRC seçilmiş hastalarda standart tedavi haline gelen majör hemodinamik ve metabolik değişikliklerin olduğu yüksek riskli bir cerrahi prosedürdür. Bu genişletilmiş cerrahi anestezistler içinde oldukça zor bir süreçtir. Hastalarda ciddi sıvı şiftleri, kan ve protein kaybı, hipermetabolik durum ve artmış intraabdominal basınç mevcuttur. Anestezistin görevi her fazda meydana gelen patofizyolojik değişiklikleri bilmek ve buna göre önlem almaktır. SRC fazında geniş sıvı şiftleri olduğundan dolayı normovolemi sağlamak ve idame ettirmek, HİPEK fazında ise artmış metabolik aktiviteyle ve gelişen koagülasyon değişiklikleri ile ilgili önlem almak esastır. Sürekli intraoperatif ve postoperatif yakın izlem gerektirir. Primer hastalığın yanı sıra cerrahinin büyüklüğü, anestezik yönetim, verilen sıvı miktarı, kan transfüzyonu sonuçları etkileyebilir. Bu konuda ileri çalışmalara ihtiyaç vardır.

Çıkar Çatışması
Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

KAYNAKLAR

1. Elias D, Honore C, Ciuchendea R, Elias D, Honoré C, Ciuchendéa R,,.et al. Peritoneal pseudomyxoma: results of a systematic policy of complete cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Br J Surg 2008;95:1164 –71.
2. Witkamp AJ, de Bree E, Kaag MM, van Slooten GW, van Coevorden F, et al. Extensive surgical cytoreduction and intraoperative hyperthermic intraperitoneal chemotherapy in patients with pseudomyxoma peritonei. Br J Surg 2001;88:458–63.
3. Elias D, Lefevre JH, Chevalier J, Brouquet A, Marchal F, Classe JM, et al. Complete cytoreductive surgery plus intraperitoneal chemohyperthermia with oxaliplatin for peritoneal carcinomatosis of colorectal origin. J Clin Oncol 2009; 27:681–5.
4. Elias D, Gilly F, Boutitie F, Quenet F, Bereder JM, Mansvelt B, et al. Peritoneal colorectal carcinomatosis treated with surgery and perioperative intraperitoneal chemotherapy: retrospective analysis of 523 patients from a multicentric French study. J Clin Oncol 2010;28:63–8.
5. Esquivel J, Sticca R, Sugarbaker P, Levine E, Yan TD, Alexander R, et al; Society of Surgical Oncology Annual Meeting. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in the management of peritoneal surface malignancies of colonic origin: a consensus statement. Society of Surgical Oncology. Ann Surg Oncol 2007;14:128-33.
6. Fleisher LA, Beckman JA, Brown KA, Calkins H, Chaikof E, Fleischmann KE, et al.ACC/AHA 2007 guidelines on perioperative cardiovascular evaluation and care for noncardiac surgery: executive summary—a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Anesth Analg 2008;106:685-12.
7. Cafiero T, Di Iorio C, Di Minno RM, Sivolella G, Confuorto G. Noninvasive cardiac monitoring by aortic blood flow determination in patients undergoing hyperthermic intraperitoneal intraoperative chemotherapy. Minerva Anestesiol 2006; 72:207–15.
8. Kellum JA, Decker JM. Use of dopamine in acute renal failure: a meta-analysis. Crit Care Med 2001; 29:1526–31.
9. Karthik S, Lisbon A. Low-dose dopamine in the intensive care unit. Semin Dialysis 2006; 19:465–71.
10. Kusamura S, Baratti D, Younan R, Laterza B, Oliva GD, Costanzo P, et al. Impact of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy on systemic toxicity. Ann Surg Oncol 2007;14:2550-8
11. Chappell D, Jacob M, Hofmann-Kiefer K, Conzen P, Rehm M. A rational approach to perioperative fluid management. Anesthesiology 2008; 109:723–40.
12. Lowell JA, Schifferdecker C, Driscoll DF, Benotti PN, Bistrian BR. Postoperative fluid overload: not a benign problem. Crit Care Med 1990; 18:728–33.
13. Payen D, de Pont AC, Sakr Y, Spies C, Reinhart K, Vincent JL . A positive fluid balance is associated with a worse outcome in patients with acute renal failure. Crit Care 2008;12:R74.
14. Kajdi ME, Beck-Schimmer B, Held U, Kofmehl R, Lehmann K, Ganter MT. Anaesthesia in patients undergoing cytoreductive surgery with hyperthermic intraperitoneal chemotherapy: retrospective analysis of a single centre three-year experience. World J Surg Oncol 2014 May 1;12:136
15. Rehm M, Zahler S, Lötsch M, Welsch U, Conzen P, Jacob M, et al. Endothelial glycocalyx as an additional barrier determining extravasation of 6% hydroxyethyl starch or 5% albümin solutions in the coronary vascular bed. Anesthesiology 2004; 100:1211–23.
16. Shime N, Lee M, Hatanaka T. Cardiovascular changes during continuous hyperthermic peritoneal perfusion. Anesth Analg 1994; 78:938–42.
17. Esquivel J, Angulo F, Bland RK, Stephens AD, Sugarbaker PH. Hemodynamic and cardiac function parameters during heated intraoperative intraperitoneal chemotherapy using the open 'coliseum technique'. Ann Surg Oncol 2000; 7:296–300.
18. Schmidt C, Creutzenberg M, Piso P, Hobbhahn J, Bucher M. Peri-operative anaesthetic management of cytoreductive surgery with hyperthermic intraperitoneal chemotherapy. Anaesthesia 2008;63:389-95.
19. Bickel A, Arzomanov T, Ivry S, Zveibl F, Eitan A. Reversal of adverse hemodynamic effects of pneumoperitoneum by pressure equilibration. Arch Surg 2004; 139:1320–5.
20. Mertens zur Borg IR, Lim A, Verbrugge SJ, IJzermans JN, Klein J. Effect of intraabdominal pressure elevation and positioning on hemodynamic responses during carbon dioxide pneumoperitoneum for laparoscopic donor nephrectomy:a prospective controlled clinical study. Surg Endosc 2004; 18:919–23.
21. Rueth NM, Murray SE, Huddleston SJ, Abbott AM, Greeno EW, Kristein MN, et al. Severe electrolyte disturbances after hyperthermic intraperitoneal chemotherapy: oxaliplatin versus mitomycin C. Ann Surg Oncol 2011;18:174-80.
22. Thix CA, Königsrainer I, Kind R, Wied P, Schroeder TH. Ventricular tachycardia during hyperthermic intraperitoneal chemotherapy. Anaesthesia 2009; 64:1134–6.
23. Michelson AD, MacGregor H, Barnard MR, Kestin AS, Rohrer MJ, Valeri CR. Reversible inhibition of human platelet activation by hypothermia in vivo and in vitro. Thromb Haemost 1994; 71:633–40.
24. Kanakoudis F, Petrou A, Michaloudis D, Chortaria G, Konstantinidou A. Anaesthesia for intra peritoneal perfusion of hyperthermic chemotherapy. Haemodynamic changes, oxygen consumption and delivery. Anaesthesia 1996;51:1033-6.
25. Kamal JM, Elshaikh S M, Nabil D, Mohamad MA. The perioperative course and anesthetic challenge for cytoreductive surgery with hyperthermic intraperitoneal chemotherapy. Egyptian J Anaesthesia 2013;29:311-8.
26. Caldwell JE, Heier T, Wright PMC, Lin S, McCarthy G, Szenohradszky J, et al.Temperature-dependent pharmacokinetics and pharmacodynamics of vecuronium. Anesthesiology 2000;92:84–93.
27. Flynn PJ, Hughes R, Walton B, Jothilingam S. Use of atracurium infusions for general surgical procedures including cardiac surgery with induced hypothermia. Br J Anaesth 1983;55:135–8S.
28. Jonker G, Hoogenboom LJ, van Ramshorst B, Bruins P. Atracurium during induced hyperthermia. J Anesth 2009;23(3):442-4.
29. Adachi T, Shinomura T, Nomura R.Duration of vecuronium induced neuromuscular blockade is shortened during hyperthermic intraoperative intraperitoneal chemotherapy. Br. J. Anaesth 2003;91:160-1
30. Saxena A, Yan TD, Chua TC, Fransi Sal, Almohaimeed K, Ahmed S, et al. Factors for massive blood transfusion in cytoreductive surgery: a multivariate analysis of 243 procedures. Ann Surg Oncol 2009;16:2195–203.
31. Theusinger OM, Spahn DR, Ganter MT. Transfusion in trauma: why and how should we change our current practice? Curr Opin Anaesthesiol 2009,22:305–12.

32. Hansen E, Knuechel R, Altmeppen J, Taeger K. Blood irradiation forintraoperative autotransfusion in cancer surgery: demonstration of efficientelimination of contaminating tumor cells. Transfusion 1999; 39:608–15.

33. Hansen E, Bechmann V, Altmeppen J. Intraoperative blood salvage with irradiation of blood in cancer surgery: answers to current queries. Anasthesiol Intensivmed Notfallmed Schmerzther 2002; 37:740–4.

34. Arakelian E, Gunningberg L, Larsson J, Norlén K, Mahteme H. Factors influencing early postoperative recovery after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Eur J Surg Oncol 2011; 37:897–03.

35. Yan TD, Deraco M, Baratti D, Kusamura S, Elias D, Glehen O et al. Cytoreductive surgery and hyperthermicintraperitoneal chemotherapy for malignant peritoneal mesothelioma:multiinstitutional experience. J Clin Oncol 2009; 27:6237–42.

36. Verwaal VJ, Bruin S, Boot H, van Slooten G, van Tinteren H. 8-year follow-up of randomized trial:cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy in patients with peritoneal carcinomatosis of colorectalcancer. Ann Surg Oncol 2008; 15:2426–32.

37. Dixon E, Datta I, Sutherland FR, Vauthey JN. Blood loss in surgical oncology: neglected quality indicator? J Surg Oncol 2009, 99:508–12.

38. Cooksley TJ, Haji-Michael P. Postoperative critical care management of patients undergoing cytoreductive surgery and Heated Intraperitoneal Chemotherapy (HIPEC). World J Surg Oncol 2011; 9:169.

39. Baratti D, Kusamura S, Laterza B, Balestra M, Deraco M. Early and long-term postoperative management following cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. World J Gastrointest Oncol 2010;2(1):36–3.

40. Miao N, Pingpank JF, Alexander HR, Royal R, Steinberg SM, Quezado MM, et al. Cytoreductive surgery and continuous hyperthermic peritoneal perfusion in patients with mesothelioma and peritoneal carcinomatosis: hemodynamic, metabolic, and anesthetic considerations. Ann Surg Oncol 2009;16(2):334–44.

41. Glehen O, Osinsky D, Cotte E, Kwiatkowski F, Freyer G, Isaac S, et al. Intraperitoneal chemohyperthermia using a closed abdominal procedure and cytoreductive surgery for the treatment of peritoneal carcinomatosis: morbidity and mortality analysis of 216 consecutive procedures. Ann Surg Oncol 2003;10:863–9.

42. Zielmann S, Grote R. The effects of long-term sedation on intestinal function.Anaesthesist 1995; 44 (Suppl 3):549–58.

43. Blumenthal S, Min K, Nadig M, Borgeat A. Double epidural catheter with ropivacaineversus intravenous morphine: a comparison for postoperative analgesia after scoliosis correction surgery. Anesthsiology 2005; 102:175–80.

44. Wulf H. Epidural anaesthesia and spinal haematoma. Can J Anaesth 1996;43:1260–71.