Comparison of RT-qPCR and RT-Digital PCR for Detection and Quantification of BCR-ABL1 Transcripts in Chronic Myeloid Leukemia

RT-qPCR ve RT-Dijital PCR Yöntemlerinin Kronik Myeloid Lösemi'de BCR-ABL1 Transkriptlerini Tespit Etme ve Ölçümleme Başarılarının Karşılaştırılması

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ABSTRACT

Aim: Chronic myeloid leukemia (CML) is a hematological malignancy in the group of myeloproliferative neoplasms.Philadelphia chromosome, t(9;22)(q34;q11), results in the BCR/ABL1 fusion gene. The Philadelphia chromosome could be detected in almost all CML cases.RT-qPCR method is still the most commonly used method for monitoring BCR/ABL1 fusion.RT-digital PCR method is an alternative in quantitative measurement of BCR-ABL1 fusion, but there is not enough information in the literature yet. It was planned to evaluate and compare of RT-qPCR and RT-digital PCR for detection and quantification of BCR-ABL1 transcripts in CML.

Materials and Methods: Totaly, 39CML patients were performed.Total RNA was extracted with RNA extraction kit (QIAamp RNA Blood Mini Kit). Qiagene Rotor-Gene-Q system was used for RT-qPCR method and QX200™ Droplet Digital™ PCR (ddPCR™) system was used for RT-digital PCR testing.

Results: There was significant difference between the groups in the BCR-ABL1/ABL comparison of the samples (p=0.017) (Table 1).

Conclusion: Although the significant difference between RT-digital PCR and RT-qPCR in detection and quantification of BCR-ABL1 transcripts in CML, RT-digital PCR is not more sensitive in all samples. Therefore, further research is needed to obtain a clear understanding of the effectiveness of RT-digital PCR.

Key Words: Chronic myeloid leukemia, BCR-ABL1, RT-qPCR, RT-digital PCR

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ÖZET

Amaç: Kronik myeloid lösemi (KML), myeloproliferatifneoplaziler içerisinde sınıflanmış bir hematolojik malignitedir. t(9;22)(q34;q11) translokasyonu ile oluşan Philadelphia kromozomu ile BCR/ABL1 füzyon geni oluşur. Philadelphia kromozomu, neredeyse bütün KML vakalarında tespit edilmektedir. RT-qPCR yöntemi, BCR/ABL1 füzyon geninin kantitatif ölçümünde rutinde en sık kullanılan yöntemdir. RT-dijital PCR yöntemi, alternatif bir yöntem olarak değerlendirilebilir fakat, bu konuda şimdiye kadar literatürde yapılmış yeterli düzeyde çalışma yoktur. Bu çalışmada, RT-qPCR ve RT-dijital PCR yöntemlerinin BCR-ABL1 transkriptlerini tespit etme ve ölçümleme başarılarının kıyaslaması planlandı.

Yöntem: Toplamda 39 KML hastası çalışmaya alındı. RNA izolasyon kiti (QIAamp RNA Blood Mini Kit) ile total RNA izole edildi.RT-qPCR yöntemi için Qiagene Rotor-Gene-Q sistemi,RT-dijital PCR yöntemi içinQX200™ Droplet Digital™ PCR (ddPCR™) sistemi kullanıldı.

Bulgular: Farklı yöntemlerden elde edilen BCR-ABL1/ABL oranları kıyaslandığında iki yöntem arasında anlamlı düzeyde farklılık tespit edildi (p=0.017) (Tablo 1).

Sonuç:KML hastalarındaki BCR-ABL1 transkript düzeyinin ölçümünde, RT-dijital PCR yöntemi RT-qPCR yöntemine göre anlamlı düzeyde daha hassas bulunmuştur, fakat bu üstünlük tüm örneklerde RT-dijital PCR lehine değildir. Bu sebeple, RT-dijital PCR yönteminin etkinliğinin daha iyi anlaşılabilmesi için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Kronik myeloid lösemi, BCR-ABL1, RT-qPCR, RT-digital PCR

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INTRODUCTION

Chronic myeloid leukemia (CML) is a hematological malignancy in the group of myeloproliferative neoplasms (MPN), which is caused by abnormal proliferation of pluripotent stem cells. The prevalence is approximately 1-2/100000 cases in adults, and CML accounts for about 15% of newly diagnosed leukemia cases (1). CML is more common in males than in females and is diagnosed between 50-60 years of age. Although the etiology of CML is not fully understood, exposure to ionizing radiation, especially at the early stages of life, may increase the risk (2). CML is the first malignancy associated with a chromosomal abnormality (3). In 1960, Nowel and Hungerford discovered that chromosome 22 was shorter in patients with CML. This short chromosome 22 was called Philedelphia chromosome. Reciprocal translocation between chromosomes 9 and 22 were discovered, 13 years later. Breakpoints are defined on t(9; 22)(q34; q11) (4). This reciprocal translocation results in the BCR/ABL1 fusion gene. This fusion occurs at 5' end of the BCR gene and 3' end of the c-ABL proto-oncogene. This newly formed fusion gene produces a protein called p210 weighing 210 (kDa). The Philadelphia chromosome could be detected in almost all CML cases (5, 6). If the breakpoints are different, different proteins are also produced. p190 protein is detected in 5% of cases; e14a3, e13a3, e1a3, e19a3, e6a2, e8a2 and e18a2 fusion proteins are seen less than 1%. p210 protein has an increased tyrosine kinase activity. Increased tyrosine kinase activity causes the release of growth factors and proliferation of abnormal cell clones (7-10).

CML is usually diagnosed by complet blood count (CBC) in the chronic phase. Leukocytosis, anemia and thrombocytosis are common findings. Bone marrow examination shows hypercellular bone marrow (11). Genetic tests have an importance in the diagnosis and follow-up of CML. The presence of the Philedelphia chromosome in the cytogenetic analysis from the bone marrow sample is diagnostic (12). Molecular cytogenetic method (fluorescence in situ hybridization = FISH) is particularly important when sufficient metaphase cannot be obtained from conventional cytogenetic analysis (13).

Molecular genetic tests are especially important in the follow-up of CML patients, response to treatment, relapse monitoring and also providing prognostic information. A real-time quantitative polymerase chain reaction method (RT-qPCR) is used to measure the amount of BCR-ABL1 fusion as a molecular test (14).This method measures the ratio of BCR/ABL1 fusion transcripts to the number of ABL1 gene transcripts. It is known that the amount of BCR-ABL1 fusion transcript measured by molecular methods is correlated with the number of residual leukemic cells. RT-qPCR method can detect one leukemic cell in 100,000 normal transcripts. According to the molecular result, the molecular response status of the disease is determined during treatment. BCR-ABL1/control gene ratio <0.1% (≥3 log reduction) was defined as the major molecular response (MMR), and BCR-ABL1=0 as the full molecular response (CMR) (15).

RT-qPCR method is still the most commonly used method for monitoring molecular response in CML. Residual cell monitoring is actually crucial for early diagnosis of minimal residual disease (MRD). One fusion cell can be detected in 100000 normal copies by RT-qPCR method. Although highly sensitive quantitative results are obtained, more sensitive methods are needed for MRD follow-up, which provide to detect the relapse status of the disease much earlier. RT-digital PCR method have been used in gene expression studies, and detection of hot-spot mutations and single nucleotide changes (CNV), in recent years (16). Although there are studies comparing the success of RT-digital PCR method in quantitative measurement of BCR-ABL1 fusion, there is not enough information in the literature yet (17, 18).

In this study, it was planned to evaluate and compare of RT-qPCR and RT-digital PCR for detection and quantification of BCR-ABL1 transcripts in CML.

MATERIALS and METHODS

Patients and samples

Totaly, 39CML patients were performed at the University of Health Sciences, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Medical Genetics Clinic.Ethical committee of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital approved the study (2019-07/326). Written informed consent was obtained from all patients before testing for the use of their RNA samples for research purposes.Samples were obtained at the same time for RT-qPCR and RT-digital PCR. BCR-ABL1/ABL value was compared with statistical analysis.

RT-qPCR testing

Total RNA from EDTA-anticoagulated peripheral blood was extracted with an RNA extraction kit (QIAamp RNA Blood Mini Kit). RNA was reverse transcribed with an Ipsogen® RT Kit. cDNA was stored at -20°C. BCR-ABL1 cDNA was performed on Qiagene Rotor-Gene-Q with TaqMan probes, according to manufacturer's instructions. Each Ipsogen BCR-ABL1 Mbcr Kit provides four standard dilutions for ABL and five standard dilutions for Mbcr. Use of the Ipsogen BCR-ABL1 Mbcr kits enables detection and quantification of BCR-ABL1 and ABL transcripts. The reaction was initiated according to the optimized protocols defined by the manufacturer.

RT-digital PCR testing

The QX200™ Droplet Digital™ PCR (ddPCR™) system was used for RT-digital PCR testing. Mastermix was prepared according to manufacturer's instructions. QX200™ Droplet Generator hadthree wells per sample. Mastermix was added to the first well and oil was added to the second well for droplet formation. The droplet generator was placed in the wells on the device.The other well was filled with oil. Two minutes later the droplets were ready. Droplets were transferred to PCR plate by pipette.After the PCR, the droplets in the plate were transferred to QX200™ Droplet Reader.

Statistical analysis

The data were evaluated in the SPSS statistical package program (Version 15.0). According to the normal distribution of parameters, concordance was examinedwith visual (histogram and probability graphs) and analytical methods (Kolmogorov-Simirnov / Shapiro-Wilk tests). Because measurements of BCR1/ABL do not show normal distribution,results were compared using Wilcoxon test. Mean value, standard deviation and median-minimum-maximum values were given as descriptive statistics, $p < 0.05$ was considered statistically significant.

RESULTS

The amount of BCR/ABL1 fusion transcripts in both RT-qPCR and RT-digital PCR method were compared to 39 patients who were referred to our clinic and followed up with the diagnosis of CML. There was significant difference between the groups in the BCR-ABL1/ABL comparison of the samples ($p=0.017$) (Table 1).

Table 1: Detected BCR/ABL and ABL copy numbers by RT-qPCR and RT-digital PCR.

	RT-qPCR		RT-digital PCR	
Patient (P)	**BCR/ABL**	**ABL**	**BCR/ABL**	**ABL**
P1	0	105161	0	81000
P2	0	115620	0	72000
P3	0	102885	0	54000
P4	12	62200	31	95670
P5	3	80877	8	88670
P6	3	102885	3	56000
P7	22	92223	11	48000
P8	2	47489	4	58160
P9	6	24995	11	38000
P10	4	66906	5	60000
P11	1	37330	14	53000
P12	4	29132	10	64000
P13	10	46123	10	50000
P14	12	42879	10	57000
P15	2	183059	5	117000
P16	7	31796	11	36000
P17	124	28920	119	34000
P18	0	83271	1	76000
P19	14	65458	5	41000
P20	0	123465	2	68000
P21	0	18569	1	12000
P22	2	74464	4	86000
P23	1	61437	1	49000
P24	2	91547	1	63000
P25	1	91500	1	80000
P26	2	61437	2	47000
P27	2	21565	9	26000
P28	2	25585	2	35000
P29	2	37318	3	64000
P30	0	73411	2	38000
P31	1	33776	1	44000
P32	1	41525	4	42000
P33	2	19106	6	24000
P34	1	27085	2	50000
P35	1	34018	0	28000
P36	48	76616	86	88000

DISCUSSION

BCR-ABL1 follow-up is vital in monitoring treatment response, determining resistance development, and reorganizing treatment protocol in CML patients (19).The sensitivity of the follow-up is a necessity in detecting MRD and taking early measures.Genetic diseases diagnostic centers are currently using conventional cytogenetic, FISH and RT-qPCR technologies for routine follow-up of CML patients.Many studies have been conducted worldwide to find an effective method for MRD detection.In this context, RT-digital PCR method, which has become more widespread in recent years, is considered to be an alternative in the measurement of BCR-ABL1 copy number.

According to the results of the study, both the RT-qPCR method and the RT-digital PCR method were able to detect CML patients with the number of copies on the logarithm of the odds (LOD) value, but there was a difference between the two methods in terms of sensitivity (Table 1).The LOD value shows a higher sensitivity from 1 log to 2 log in a previous study(18).According to another previous study, RT-digital PCR has an comparable performance to RT-qPCR over a 4 log dynamic range for the quantification of BCR-ABL1(17).In this study, there were no differences in logarithmic levels detected in previous studies.On the basis of the differences between the results, the differences in device and kit systems and the characteristics of the study groups can be shown.CML patients included in this study were randomly selected for this study. Future studies can be used to group patients according to the stage of the disease and in which stage the sensitivity increases especially.In this study, RT-digital PCR was found to be more sensitive in low copy numbers.The studies to be conducted in a larger sample of patients with low copy numbers may provide statistically more satisfactory information.

It is thought that simultaneous RT-digital PCR to the sample of the patient, who is considered to be cured, will provide significant information on this subject. In this way, the patient who is considered to be cured with qPCR might be detected with low number of BCR1/ABL copies over the LOD and the treatment may be prolonged.Relapsealso could be detected with this more sensitive method.Early detection of relapse is an important factor that increases treatment success.

Although the significant difference between RT-digital PCR and RT-qPCR in detection and quantification of BCR-ABL1 transcripts in CML, RT-digital PCR is not more sensitive in all samples. Therefore, further research is needed to obtain a clear understanding of the effectiveness of RT-digital PCR.

Conflict of interest

No conflict of interest was declared by the authors.

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