The Importance of Test Variability in Acute Myeloblastic Leukemia: A Case Report

Akut Miyeloblastik Lösemi Tanısında Test Çeşitliğinin Önemi: Bir Olgu Sunumu

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ABSTRACT

Acute myeloid leukemia (AML), phenotypically and genotypically is a quite heterogeneous disease. More than 100 cytogenetic aberrations and gene mutations that are specific to this disease are defined (1). A patient who applied to hematology polyclinic of our hospital with various complaints was diagnosed with AML. In genetic analyses, t(8;21) and FLT3-ITD were found to be positive. Additionally, in chromosome analysis trisomy 8 was observed. After remission was ensured in the patient, allogenic stem cell transplantation was carried out. By conducting t(8;21), FLT3 mutation and trisomy 8 analyses on the patient at regular intervals, prior information was gathered about the relapse and minimal residual disease.

Key Words: Minimal ResiduelDisease,acute myeloid leukemia, chimerism, trisomy 8, FLT3 mutation, t(8;21)

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ÖZET

Akut myeloid lösemi (AML), fenotipik ve genotipik olarak oldukça heterojen olan bir hastalıktır. Bu hastalığa özgü 100'den fazla sitogenetik aberasyon ve gen mutasyonu tanımlanmıştır (1). Hastanemiz hematoloji polikliniğine çeşitli şikayetlerle başvuran hastaya AML tanısı konulmuştur. Bunu takiben yapılan genetik analizlerde t(8;21) ve FLT3-ITD pozitif bulunmuştur. Ayrıca kromozom analizinde trizomi 8 gözlenmiştir. Hastada remisyon sağlandıktan sonra allojenik kök hücre transplantasyonu yapılmıştır. Düzenli aralıklarla hastanın kimerizm, t(8;21), FLT3 mutasyon ve trizomi 8 analizleri yapılarak relaps ve minimal rezidüel hastalık hakkında önceden bilgi sağlamak amaçlanmıştır

Anahtar Sözcükler: Minimal rezidüel hastalık, Akut Myeloid Lösemi, Kimerizm, Trizomi 8, FLT3 mutasyonu, t(8;21).

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INTRODUCTION

In almost all of leukemias lots of different chromosomal anomalies are notified. While the importance of some parts of these anomalies in disease pathology was investigated, it has been observed that some parts of these anomalies have a prognostic significance. In acute myeloid leukemia, a set of chromosomal anomalies are also reported; and it is indicated that these anomalies are related to prognosis. From chromosomal anomalies, t(8;21), t(15;17) and inv(16) are accepted as good prognostic factors (2,3), whereas, other chromosomal anomalies such as -5, -7, -5q, -7q, trisomy 8, apparent hyperdiploidy, and t(6;9) are accepted as poor prognostic values. Thus, an arrangement of a treatment plan based on these prognostic values is proposed (2). Elucidation of the pathogenesis of cytogenetically normal AMLs has been a distinct research subject. Nowadays, in all leukemia guidelines it is suggested that an examination of fms-related tyrosine kinase 3 (FLT-3) and Nucleophosmin 1 (NPM) mutations be made in all cytogenetically normal AML patients (4,5). Also, the fact that information can be obtained about minimal residual disease through a detection of chromosomal anomalies, increases the importance of determining these anomalies at diagnosis. In this case report, we will present a case which had trisomy 8, t(8;21), and FLT3 mutations.

CASE REPORT

In November, a 32 year-old, male patient applied to our polyclinic with weakness and fever complaints. In physical examination of the patient no abnormal finding was detected except for paleness and the coarsening of the breathing sound. Myeloblasts were observed in the peripheral smear of the patient and he also had leucocytosis in his hemogram. With AML pre-diagnosis, t(9;22), t(8;21), t(15;17), inv(16), FLT3 mutation and NPM1 mutation tests were requested from our department. t(8;21) was found to be positive with 8,86 ratio. FLT3-ITD was also found to be positive (Figure 1,2). In addition to that in cytogenetic analysis of bone marrow, trisomy 8 was observed. After the patient received a treatment, in January 2013 cytogenetic analysis and Fluorescence In Situ Hybridization (FISH) were both normal karyotype; t(8;21) and FLT3-ITD were found as negative. One month later, in the analysis of t(8;21), a low positivity (0,000307) was detected. Whereas FLT3-ITD was observed to be negative. Cytogenetic analysis and FISH were both normal. In March, 2013, from an appropriate donor, allogenic hematopoietic stem cell transplantation was carried out on the patient. In analyses, which were performed 30 days after the transplantation, cytogenetic analysis and FISH were both normal, and t(8;21) and FLT3-ITD were negative (Figure 3,4).

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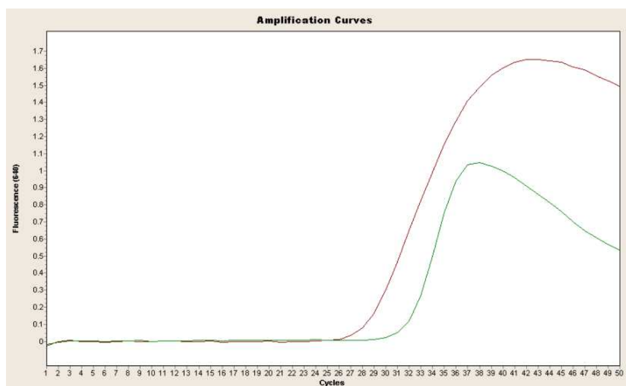

Figure 1: RT PCR for t(8;21) at diagnosis. Red curve is internal control gene's curve, gren curve is t(8;21) AML1-ETO fusion gene's curve

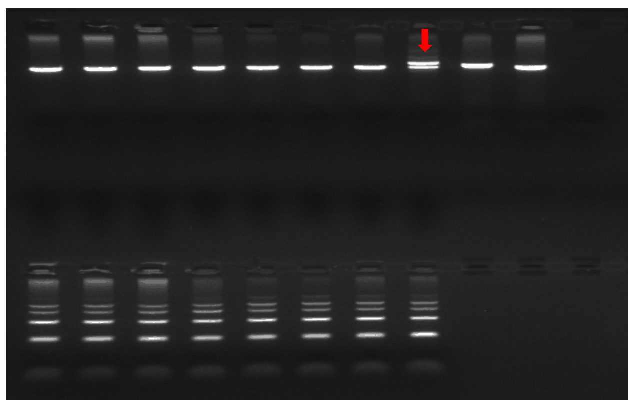

Figure 2: Gel elecrophoresis of FLT3-ITD mutation at diagnosis

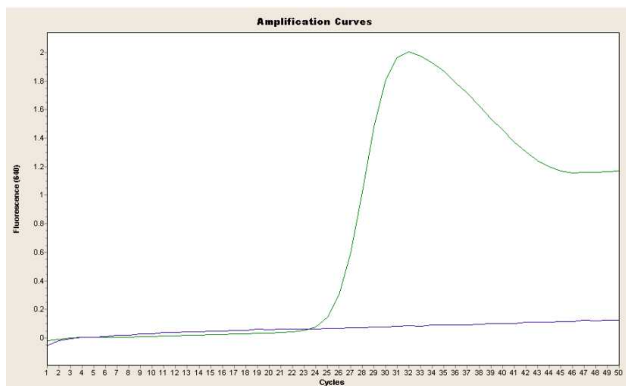

Figure 3: RT PCR for t(8;21) after bone marrowtransplantationRedcurve is internalcontrolgene'scurve, greencurve is t(8;21) AML1-ETO fusion gene'scurve

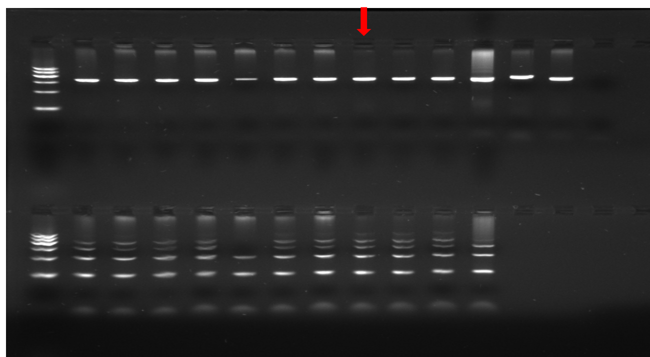

Figure 4: Gel elecrophoresis of FLT3-ITD mutationafter bone marrowtransplantation

Chimerism ratios for peripheric blood and bone marrow were observed to be 99,41% and 99,91% respectively (Figure 5). In the 60th day after the transplantation, chimerism ratios for peripheric blood and bone marrow were observed to be 99,4% and 98,7%, respectively. In the 90th day after the transplantation, cytogenetic analysis and FISH were both normal. t(8;21) and FLT3-ITD were found to be negative; and chimerism ratios for peripheric blood and bone marrow were recorded as 99,8% and 98,4%, respectively. In the 120th day after that transplantation, again cytogenetic analysis and FISH were both normal. t(8;21) and FLT3-ITD analyses were negative; and chimerism ratio for peripheric blood was observed to be 98,3%. By testing the patient at regular intervals, the patient was followed-up and protected against minimal residual disease.

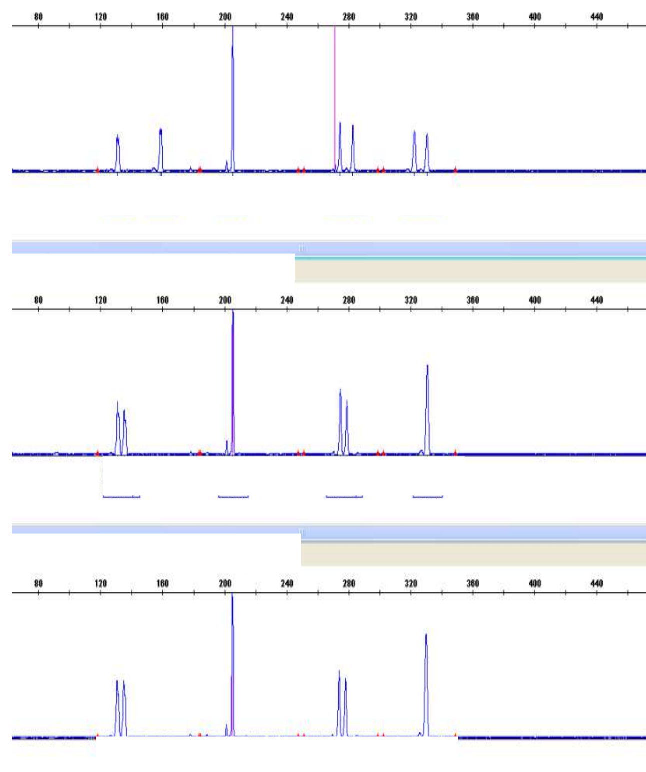

Figure 5: Chimerism Analysis: First line patient's pretransplantation, second line donor's and the third line patients posttransplantation STR markers

DISCUSSION

The aim in the treatment of hematological malignancies is the reduction of tumor cell load as much as possible (6). Even though there is no clinical finding about leukemia, the presence of cells which escape from treatment and then cause a relapse, is described as minimal residual disease (MRD) (7). In recent years, for the detection of minimal residual disease much research is done. In many hematologic malignancies such as acute leukemia, chronic myeloid leukemia, non-hodgkin lymphoma, multiple myeloma, lymphoproliferative diseases, it was demonstrated that there is a prognostic value of MRD (6,7). Since the detection of MRD at a right time produces opportunities like the classification of the disease, grouping of the diseases based on risk factors, application of goal-oriented therapy protocols, and the induction of an effective treatment on time, the importance of such a detection gradually increases in routine studies (1).

MRD can be studied with several methods such as immunophenotyping by using flow cytometry (FC), or the demonstration of chromosomal aberrations in molecular level or detection of clone-specific immunoglobulin (Ig) and TCR gene rearrangements, via polymerase chain reaction (PCR). Observation of specific fusion genes in AML with PCR comes into prominence as compared to other methods. Aberrations like t(8;21), t(15;17), inv(16) and the follow-up of FLT3 gene mutations along with duplications can be used for this purpose (8).

One of the chromosomal defects that is most commonly observed in AML is t(8;21). While this defect is seen in adult AML at a ratio of 12-15%, it is seen in pediatric AML at a ratio of 30%.

In this defect, AML1 gene from chromosome 21 comes next to the ETO gene which is located on the chromosome 8, and this generates a fusion gene. After a chemotherapy and hematopoietic stem cell (HSC) transplantation, even though patients remain in remission for a long time with RT-PCR techniques, the presence of this fusion gene can still be demonstrated. It is considered that positive reactions arise from monocytes, B cells, stem cells which are in silent position. For this reason, its value in MRD follow-up is controversial. However, in RQ-PCR studies, the observation of progressive increases in the fusion gene can be an indicator of a forthcoming relapse (3).

Apart from chromosome anomalies, there are lots of gene anomalies that accompany hematological malignancies. FLT3 gene mutations which are observed in AML cases, and which are indicators of a poor prognosis along with very high expressions of Wilms tumor gene might be given as examples. In FLT3 gene FLT3-Internal Tandem Duplication (FLT3-ITD) mutations which demonstrate themselves with random repeats in various numbers can be regarded as an example for these, as well.

Allogenic hematopoietic stem cell transplantation (alloHSCT) is the most effective approach which promises cure in AML. Transplantation is not suggested for cytogenetically good subtypes (AML; t(8;21), inv(16), t(15;17)) in their first complete remission (1.CR). If a relapse occurs, transplantation is suggested in their second complete remission (2.CR). For AML with a standard or high risk, alloHSCT is suggested in the first CR (2). Thus, at the moment of initial diagnosis to be able to execute all tests on the patient is critically important. In our patient, in the case that only translocation parameters were studied, the patient was seemed to be having a good prognostic marker. However, with the detection of trisomy 8 by cytogenetic examination, it was realized that the patient actually had a medium risk marker. The detection of FLT3-ITD mutation in this patient increases the risk.

In chimerism studies following HSCT, by examining the equilibrium between donor and recipient cells it is investigated that whether the engraftment occurred or not, along with whether hematopoietic cells of the recipient are present or not (9). In studies that are performed after HSCT even though the absence of MRD is accepted as a good prognostic factor, interpretation of the presence of MRD is not that much easy. Despite the presence of MRD, some patients can remain in the remission state regardless of the amount of MRD that is found. Also there are studies which demonstrate the presence of MRD in months before the relapse in KML (1). In our patient, our follow-up parameter for minimal residual disease with RT-PCR was t(8;21). FLT3-ITD was our follow-up parameter with conventional PCR. Additionally in our patient, the chimerism follow-up was done in capillary electrophoresis by the analysis of short tandem repeats (STR). From the aspect of MRD follow-up, concordant results were obtained from both conventional PCR and RT-PCR and also from the capillary electrophoresis. From these three methods, the one which has the highest sensitivity is RT-PCR, then in the second place comes capillary electrophoresis, and the third is the conventional PCR.

CONCLUSION

From the point of laboratory testing due to the difference in the sensitivities of the methods, using several methods for the follow-up of minimal residual disease might be required. In this case that we presented, we attempted to emphasize the importance of examining all chromosomal anomalies along with known gene mutations in newly diagnosed AML patients at different stages of the treatment involving diagnosis, follow-up and the prognosis.

Conflict of Interest

No conflict of interest was declared by the authors.

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