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Molecular evaluation of mutations in acute myeloid leukemia patients from Turkey

A single-center study

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Abstract

Certain genetic mutations could have a role in the etiology of acute myeloid leukemia (AML). Hereby, in this study, we primarily aimed to investigate the distribution of genetic mutations in AML patients. We also attempted to analyze the incidence of genetic mutations in AML patients from Turkey.

This retrospective study included a total of 126 patients diagnosed with AML, who had molecular mutation test results or records in their patient files. The patients who were not citizens of the Republic of Turkey were not included in the study.

It was observed that analyses for at least 1 c-kit exon mutation had been carried out on 76 patients, which detected no c-kit mutation among the types of genetic mutations investigated in all of those 76 patients. We found the frequency of FMS-like tyrosine kinase 3-internal tandem duplication mutation as 25%. The prevalence of translocation(15;17) was approximately 11% and the prevalence of translocation(8;21) was % 6.25. In addition, we also showed that the frequency of inversion16 was nearly 3.7%.

Lastly, the possibility of c-kit mutation in AML patients from Turkey might actually be low.

Abbreviations: AML = acute myeloid leukemia, FISH = fluorescence in-situ hybridization, FLT3 = FMS-like tyrosine kinase 3, FLT3–ITD = FMS-like tyrosine kinase 3–internal tandem duplication, FLT3–TKD = FMS-like tyrosine kinase 3–tyrosine kinase domain, inv16 = inversion16, NPM = nucleophosmin, PCR = polymerase chain reaction, t(8;21) = translocation(8;21), t(9;22) = translocation (9;22), t(15;17) = translocation(15;17), WT1 = Wilms' tumor 1.

Keywords: acute miyeloid leukemia, frequency, mutation, Turkey

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request for 2 years. The data that support the findings of this study are available from a third party (The Hospital), but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are only available from the authors upon reasonable request and with permission of the third party.

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1. Introduction

Certain genetic mutations could have a role in the etiology of acute myeloid leukemia (AML). In the routine clinical tests, mutations of nucleophosmin (NPM) gene, FMS-like tyrosine kinase 3 (FLT3) gene, t(8;21) (translocation(8;21)), and inv16 (inversion16) are part of the most frequently investigated genetic abnormalities known to cause AML. A study carried out by Coşkunpınar et al[1] in Turkey to identify the common translocations and the frequency of FLT3 mutations in childhood AML reported that 4% of 50 AML patients had FMS-like tyrosine kinase 3-internal tandem duplication (FLT3–ITD) mutation while 24% had t(8;21) chromosomal aberration. Also, Falini et al[2] reported that 35.2% of patients with AML had cytoplasmic NPM. Furthermore, in another study conducted in non-M3 AML patients by Hou et al,[3] mutation of the Wilms' tumor 1 (WT1) gene was detected in 6.8% of the patients. Moreover, Grimwade et al,[4] on behalf of the National Cancer Research Institute Adult Leukaemia Working Group, reported the frequency of patients who had " translocation(15;17) [t (15;17)] (q22;q21) and variants" as nearly 13% in a study searching the cytogenetic abnormalities among the acute miyeloid leukemia patients. On the other hand, only cytogenetic analysis without FISH (fluorescence in-situ hybridization) and/or PCR (polymerase chain reaction) testing may let the clinician overlook the diagnosis of acute promyelocytic leukemia.

This study primarily aimed to investigate the distribution of genetic mutations in AML patients. It also attempted to analyze the incidence of genetic mutations in AML patients from Turkey.

As our hospital is a referral hospital for hematology and stem cell transplantation, a considerable number of AML patients from across the country present to our clinic. For that reason, although this is a single-center study, the analysis of the data collected here may help to get some idea about the possible mutation frequencies in Turkish AML population.

2. Materials and methods

This retrospective study included a total of 126 patients diagnosed with AML, who had molecular mutation test results or records in their patient files. The patients who had molecular mutations detected by PCR method at the time of diagnosis were included in the study. Mutations detected by methods other than PCR such as FISH were not included in the study. The patient records were retrieved from the archives of the University of Health Sciences, Ankara Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, Leukemia Outpatient Unit. The variables like age, gender and demographic characteristics of the patients were also analyzed. Patients aged 18 and over who were once followed-up or who had a patient file at the hematology outpatient unit were included in the study. The study sample consisted of patients who were citizens of the Republic of Turkey. The patients who did not originate from the people of Turkey were also not included in the study. Nationals of other countries who were born in another country and acquired Turkish citizenship later on were also excluded from the study.

In cases where the reports of genetic analysis results were not available in the patient files, the physician's notes regarding the genetic results were also used in the study. Patients whose genetic results could not be obtained during the study time or who had contradicting results in the file record system were also excluded from the study. We determined the prevalence of certain types of mutations, namely WT1, FLT3, t(8;21), t(15;17), inv16, t(9;22) (translocation(9;22)), NPM and c-kit mutations, which have been routinely investigated in AML patients for a long time in the clinical practice of our hospital. The rates of positivity and negativity status of the tested mutations were calculated in percentages.

The genetic test results obtained after induction chemotherapy and those tested after chemotherapy in our hospital once the patient had received the therapy in another health center previously were not used in the study. Patients who were started chemotherapy in another center and lacked prechemotherapy PCR test results in the patient file were also not included in the study. Besides, the study excluded patients with mutation data before treatment/at the time of diagnosis if such data were not obtained through PCR method or suspected to have been tested by the FISH method. All results recorded as FLT3–TKD (FMS-like tyrosine kinase 3–tyrosine kinase domain) or as FLT3-D835 were classified as FLT3–TKD and analyzed under this classification.

The genetic results reported to have been obtained by the FISH method at the time of diagnosis were not used in the study. The distribution of recorded genetic results of patients was determined and their frequency distribution was analyzed. AML patients with a history of solid organ cancer prior to the diagnosis of AML were excluded, as well as those developing AML after myelodysplastic syndrome, myeloproliferative neoplasm, Fanconi anemia or lymphomas. Patients who had diagnosis of mixed phenotypic and/or biphenotypic acute leukemia along with AML in their patient file records were also not included in the study.

The study sample consisted of 126 patients who were followed up in the hematology outpatient unit or who admitted to hematology outpatient unit until 24 April 2019. The patient age was calculated by including the year in which patient was living to the number of completed years since birth. For some patients, no age calculation was performed, instead the age in the patient file recorded at the time of diagnosis was used in the data processing. Patients with a file in the archive of the leukemia outpatient unit during the data collection time were included in the study. Ethical approval for this study was obtained from the local ethics committee at the hospital mentioned in the first affiliation in the title page. (Decision No: 2019-04/252, Decision Date: 24.04.2019.

3. Results

One patient was diagnosed with simultaneous rectal adenocarcinoma and AML at the age of 66. This male patient had WT1 gene mutation, but not FLT3–ITD/D835, t(8;21), t(15;17), inv16 or NPM gene mutations.

It was observed that analyses for at least 1 c-kit exon mutation had been carried out on 76 patients, which detected no c-kit mutation among the types of genetic mutations investigated in all of those 76 patients. However, not all types of c-kit exon mutations known to be common in AML patients had been studied thoroughly in all of these 76 patients. Furthermore, at least 1 PCR analysis had been performed to evaluate t(9;22) translocation status in 102 patients, but none of these 102 patients tested positive for t(9;22) gene mutation. Lastly, t(8;21), NPM gene mutation, t(15;17) and inversion 16 frequencies are shown in Table 1.

Of the AML patients included in the study, 92 patients had been tested for WT1 mutation, and 87 (≈94.56%) of these patients had WT1 mutation. No threshold had been determined for the WT1 mutation, and all results reported to be positive even at low levels were accepted as WT1 positive.

The retrospective review of medical records performed to determine FLT3–ITD mutation positivity revealed that 27 patients were positive for FLT3–ITD and 81 were negative (Table 1). It was observed that 6 patients had been identified as FLT3 negative, but the exact mutation type or types had not been recorded. Only 1 patient was recorded as FLT3 positive, but again the exact type or types of mutation were not recorded. Therefore, these 7 patients were excluded from FLT3–ITD and FLT3–TKD analyses. Only 27 patients known to be FLT3–ITD positive and 81 patients known to be FLT3–ITD negative were included in the analysis for the frequency of FLT3–ITD mutation. In order to evaluate the frequency of FLT3–TKD mutation, only 10 patients known to be FLT3–TKD positive and 94 patients known to have been recorded as FLT3–TKD negative were taken into account.

4. Discussion

The study by Østergaard et al[5] that compared 133 newly diagnosed AML patients with healthy controls showed that expression levels of WT1 was higher than normal in 118 (≈89%) of AML patients. In our study, WT1 positivity was frequent among patients, which could be primarily explained by the fact that no cut-off value was determined for WT1 positivity. Another reason was that in ≈27% (n = 34) of the patients, no records about the WT1 mutation status could be found. Due to the

Table 1

Frequency of genetic abnormalities in patients with known mutation status.

	Positive, n	Negative, n	Total, n
NPM gene mutation	39 (≈ 33.33%)	78	117
t(8;21)	7 (6.25%)	105	112
t(15;17)	12 (≈ 11%)	97	109
Inversion 16	4 (≈ 3.7%)	104	108
FLT3–ITD mutation	27 (25%)	81	108
FLT3–TKD mutation	10 (≈ 9.6%)	94	104

retrospective design of our study, there were a number of patients with unknown WT1 mutation status with the possibility of significantly changing the outcome of the frequency of WT1 mutation. In our study, we accepted all patients as WT1 positive, including those reported to be positive for WT1 gene mutations, regardless of the low number of WT1 copies.

Patel et al[6] reported the frequency of KIT mutation as 6% in their study performed by analysis of the samples from newly diagnosed AML patients included in the Eastern Cooperative Oncology Group E1900 clinical trial. In our study, however, there was no patient with any known c-kit mutations. One reason for this could be the absence of any record in the significant number of patient files regarding the c-kit testing. Thus, any conclusion in that regard would be misleading as all types of c-kit exon mutations known to be common in AML patients had not been routinely performed in every patient in previous examinations. Future studies with larger samples may yield more precise data by testing each patient for all types of c-kit mutations commonly seen in AML.

In a retrospective analysis carried out on 776 AML patients who were entered on AML treatment protocols of the Eastern Cooperative Oncology Group (ECOG) and had karyotypes available, it was reported that 7 (≈0.9%) of the patients had the Philadelphia (Ph) chromosome.[7] However, in our retrospective clinical study with patients from Turkey who developed de novo AML, we did not detect any t(9;22) positivity. It should be noted here that our study excluded patients with prior history of myeloproliferative neoplasms, such as chronic myeloid leukemia, also known as chronic myelogenous leukemia. Besides, our relatively small sample size might have played a role in the lack of t(9;22) positive patients. In our study, only 102 patients had been tested for t(9;22) by PCR, so further studies with larger samples will provide better insight into this matter.

In their study conducted in Iran, Rezaei et al[8] reported the frequency of FLT3–ITD mutation as about 25.9% in cytogenetically normal-acute myeloid leukemia patients. Similarly, we found the frequency of FLT3–ITD mutation as 25% in analyses performed without taking cytogenetic properties into account. If the 6 patients "who had been identified as FLT3 negative, but the exact mutation type or types had not been recorded" were included in the group negative for FLT3–ITD mutation, then the frequency of FLT3–ITD mutation in our study would be ≈23.68% (27/114). In addition, since our study was retrospective in design, FLT3–ITD allelic ratios could not be evaluated. In a study by Sakaguchi et al,[9] the frequency of FLT3–TKD mutation was reported to be approximately 5% in Japanese AML patients. In their study, FLT3–ITD mutation frequency was higher than FLT3–TKD mutation frequency.[9] In our study, however, the frequency of FLT3–TKD mutation was lower than that of FLT3–ITD mutation, which could be explained by limited number of patients with a file in the archive of leukemia outpatient unit during retrospective data collection. Therefore, we need future research with prospective design to prevent bias and gather more conclusive evidence in this field.

In conclusion, the possibility of c-kit mutation in AML patients from Turkey might actually be low. However, more comparative studies should be conducted with larger samples by thoroughly testing each patient for all types of known exon mutations.

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