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The Evaluation of Common Chromosomal Rearrangements and Their Frequencies in Adult Acute Myeloid Leukemia Cases in Malatya Province of Turkey

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ABSTRACT

Recurrent balanced translocations are generally considered as the main parameter for prognosis in acute myeloid leukemia (AML). In recent years, genetic studies have focused on the ascertaintment of molecular aspects of various oncofusion proteins associated with AML, such as t(15;17) PML-RARA, t(8;21) RUNX1-RUNX1T1, t(9;22) BCR-ABL1 and inv (16) CBFB-MYH11. Therefore, we evaluated AML cases with RT-PCR for known specific genetic abnormalities that could lead to more accurate prognosis.

In our study, we retrospectively reviewed the records of 211 cases (59.2% males and 40.8% females). RT-PCR technique was performed to identify t(15;17) PML-RARA, t(8;21) RUNX1-RUNX1T1, t(9;22) BCR-ABL1 and inv (16) CBFB-MYH11.

The most common rearrangement was found to be t (15; 17) (%12.8) followed by t (8; 21) (7.11%), t (9; 22) (7.6%) and inv (16) (1.42%). Also, in two other cases (0.95%) t(15;17) and t(8;21) were seen together. In addition, none of these rearrangement were found in 148 cases (70.14%) with AML.

The presence of chromosomal rearrangements are very important in the diagnosis of AML. Therefore, rapid identification of specific rearrangements during diagnosis is important for prognostic purposes and can help identifying the cause of leukemogenesis and provide new strategies for the treatment of cases. This study is useful for both in Turkey oncologists and transplant centers in other regions will be a reference for the future analyzes and epidemiological data.

Key Words: Acute myeloid leukemia, BCR-ABL1, PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11

Introduction

Acute myeloid leukaemia (AML) is a heterogeneous clonal disorder of haemopoietic progenitor cells and the most common malignant myeloid disorder in adults. The pathophysiology of this disease is being investigated, but it is thought that activation of abnormal genes due to chromosomal translocations and other genetic disorders plays a role in the pathogenesis. Among these, the molecular aspects of several oncofusion proteins associated with subtypes of AML are significant (1).

Acute promyelocytic leukemia (APL) is, a distinctive subgroup representing 5%-15% of AML patients (2). APL is a widespread malignant hematological tumor characterized by the t(15;17) chromosome translocation. Results in the fusion of the retinoic acid receptor alpha (RARA) gene on chromosome 17 and PML gene on chromosome 15, with the expression of a PML-RARA fusion protein (3). There are 3 potential PML-RARA isoforms caused by these translocations. The breakpoint in chromosome 17 is consistently found in intron 2, but alter in chromosome 15. The 3 breakpoints on the PML gene can occur at intron 3 (L-long form), intron 6 (S-short form), and exon 6 (V form). The variation in position of breakpoints within the PML gene produces PML-RARA transcripts of

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different sizes. They are long (bcr-1), variant (bcr-2) and short (bcr-3), respectively (4,5,6,7).

The t(8;21) (RUNX1-RUNX1T1) positive AML cases constitute 5-10% of all AML cases. Results in the fusion of runt-related transcription factor1 (RUNX1) on chromosome 21 and RUNX1T1 (ETO) on chromosome 8. RUNX1, is related in regulating normal hematopoiesis (7,8). According to the FAB (French-American-British) classification, t (8;21) is closely related to AML-M2 subgroup (8).

The inv(16)(p13.1q22) is a subgroup of AML associated with CBFB-MYH11 rearrangement. The inv(16) is seen in approximately 7% of adults with de novo AML (9). Some mouse studies have reported that CBFB/MYH11 rearrangement causes a block in myeloid differentiation, predisposing to leukemia, but additional genetic alterations are required for the development of a leukemic phenotype (10).

The hallmark of chronic myelogenous leukemia (CML) is Philadelphia chromosome (Ph), that is formed by reciprocal translocations between human chromosome 9 and 22, t(9:22) (q34;q11), but it is also found in cases with other acute leukemia (11). The incidence of the Ph in AML is between 0.5% and 3% (12, 13). Philadelphia chromosome positive (Ph+) AML is a rare entity and has been included in the revised World Health Organization (WHO) classification in 2016 as a provisional entity of acute leukemia and myeloid neoplasm. Although the diagnostic criteria and optimal therapy for the Ph+ AML remained unclear because of limited literatüre. (14,15,16).

Today, these genetic anomalies are being investigated by methods such as classical cytogenetics, fluorescence in situ hybridization, polymerase chain reaction (PCR). Quantitative reverse transcriptase PCR (qRT-PCR) methods are preferred for molecular analysis because of their easy application, lack of radioactivity, no need for electrophoresis and high sensitivity. The aim of our study is to evaluate the results of chromosomal rearrangement (t(15;17) PML-RARA, t(8;21) RUNX1-RUNX1T1, t(9;22) BCR-ABL1 and inv (16) CBFB-MYH11) frequently observed in AML cases by RT-PCR from the Malatya Province in Turkey.

Material and Methods

The detection of chromosomal rearrangement in AML cases was performed by qRT-PCR method with a sensitivity of 10−6. ABL gene amplification was used as a internal control in all applications.

Study Groups: This study has investigated of the presence of [t(15;17) PML-RARA, t(8;21) RUNX1-RUNX1T1, t(9;22) BCR-ABL1 and inv (16) CBFB-MYH11], in cases with newly diagnosed as AML in Inonu University Turgut Ozal Medical Center Department of Hematology. AML was diagnosed and classified according to the French-American-British (FAB) classification. Demographic and clinical features of 211 patients newly diagnosed with AML were evaluated retrospectively. The study group consisted of persons between 19-92 years. The cases are consisting 86 women and 125 men. Each patient is evaluated once.

RNA Extraction: For molecular studies, peripheral blood sample was used. The total RNA isolation was performed using QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany) according to a modification of the manufacturer's protocol. RNA concentration and qualification were measured using the MaestroNano spectrophotometer (Thermo Fisher Scientific).

cDNA Synthesis: cDNA was synthesized from 1 microgram of total RNA. Reverse transcription reaction was done according to the Ipsogen RT synthesis kit protocol (Qiagen, Hilden, Germany).

Real-time Polymerase Chain Reaction: The amplification and analysis were performed using ipsogen PML-RARA (bcr1, bcr2 and bcr3), RUNX1-RUNX1T1, CBFB-MYH11 and BCR-ABL1 MBCR IS-MMR KIT and BCR-ABL1 mbcr kit protocol (Qiagen, Hilden, Germany) on the instrument Rotor Gene Q as per the manufacturer's instructions.

Statistical Analysis: The normality of the data was evaluated by the Shapiro–Wilk test and Kolmogorov-Simirnov test due to the sample size. Since, the data were not normally distributed, median, minimum and maximum values were used as descriptive statistics for quantitative data. For group comparisons Kruskal-Wallis test was used. Qualitative data were summarized by count and percentage, Pearson chi-square test was used for comparisons. In all analyses, significance level was considered to be 0.05.

Results

The study group consisted of 211 cases, where 59.2% were males and 40.8% females. Table 1 shows the distribution of t(15;17), t(8;21), t(9;22) and inv (16) positivity rates by sex. The cases

clinical and hematological characteristics are summarized in Table 2. AML cases carrying any of these rearrangement [t(15; 17), t(8; 21), t(9;22) and inv (16)] had a median age of 56 years (min-max, 21-88). Median age of those without rearrangement was 58 years (min-max, 19-92).

The most common rearrangenment t(15;17) was found in 27 cases (12.8%). As a result of molecular analysis found that long form or bcr1 occurs by 48.2% (13 cases), variant form or bcr2 by 11.1% (3 cases), and the short form or bcr3 by 40.7% (11 cases). t(8; 21) and t(9; 22) rearrangenment were detected in 15 (7.11%) and 16 (7.6%) cases, respectively. The inv (16) anomaly seen in 3 cases (1.42%). Also, in two other cases t(15;17) and t(8;21) were seen together. In addition, none of these rearrangenments were found in 148 cases with AML (70.14%).

Next, we evaluated the relationship between these rearrangements and hematological parameters (WBC, RBC, Hb and PLT) in cases with AML. Summary of hematological characteristics of all AML cases are reported in Table 2.

Discussion

The molecular genetic is testing play an important role for the diagnosis, risk stratification, planning of the effective therapeutic strategies, and disease monitoring in hematological malignancies (17).

AML is a clonal heterogeneous hematopoietic progenitor cell disease which is more common in adults than children. t(15;17) PML-RARA, t(8;21) RUNX1-RUNX1T1, t(9;22) BCR-ABL1 are and inv (16) CBFB-MYH11, is among the most common genetic defects in AML cases. Diagnosis of genetic defects are may help to recognize cause of leukemogenesis and provide new strategies for treatment of cases (1).

The aim of this study is to retrospectively determine the t(15;17), t (8;21), t(9;22) and inv (16) in 211 adults AML. The median age of AML cases carrying any of these rearrangements [t(15;17), t(8;21), t(9;22) and inv (16)] was 56 years. The majority was of newly diagnosed cases of AML have a mean age of more than 55 years (18,19). AML is rarely diagnosed before the age of 40 years (18). However, Abuhelwa and Kamaneh were determined their median age as 36 and 44, in their study, respectively (20,21). Among the reasons for this discrepancy include differences in case sample sizes, differences in inclusion and exclusion criteria, and geographic distribution of nonrandom and ethnic differences.

AML in adults has a slight male predominance in most countries (22, 23). In our study, AML is more common in men.

In our study, the most common rearrangement among cases with AML was t(15;17) and determined 12.8% with 27 cases. In two different studies in Lebanon, t(15;17) fusion rates were determined as 7.6% and 25.0%, respectively (24,25). The frequency of this fusion is reported to be 11% in the study of Enjeti et al. (26). The frequency of the potential PML-RARA isoforms (bcr1, bcr2 and bcr3) caused by t(15;17) in our study were 48.2%, 11.1% and 40.7% respectively. In several studies were from the USA and Europe, which was approximately 50-55% for PML(L) RARA (bcr1), 8-20% for PML(V) RARA (bcr2) and 27-49% for PML(S) RARA (bcr3) (27). In a study by Chatterjee et al from India PML(L) RARA (bcr1) isoform was found to be the predominant isoform (42.85%) followed by PML(S) RARA isoform (38.09%) (5).

The lower frequency is of inv (16) (1.42%) in our case group is not unique in the literature. Lower frequencies were ranging between 1 and 2% have also been declared in Singapore and Denmark (26,28). In our study determined t(8;21) fusion at 7.11%.

According to the sources that we were can reach, the rearrangement of t(8;21) in AML cases is reported to be 3.3% in Denmark and 12.4% in Tunisia in the highest frequency (28,29).

In our study, Ph+ was determined as 7.6%. The incidence Ph+ of de novo AML ranges from 0.5% to 3% (11,12,13,30). As a retrospective article of medical records, this study suffers a few limitations. The relatively small sample size, unavoidable because of the low of cases with Ph+ AML, limits firm statistical results regarding long-term conclusion.

Chromosomal abnormalities accompanying t(15;17) are reported in 26%-39% of APL cases (31,32,33). We also identified expression of t(8;21) in addition to t(15;17) in two cases (0.95%) with APL diagnosis in our study. Marileila Varella-garcia et al. and Uz et al. also found similar results in their studies (34,35). Co-expression of t(15;17) and t(8;21) is seldomly seen in APL patients (36). The role of recurrent cytogenetic/molecular translocations other than t(15;17) in APL is still unclear (35).

Early detection of chromosomal rearrangements observed in childhood and adulthood is critical for both the clinician and the patient. It was a valuable method to confirm the diagnosis, guide the treatment for molecular remission and follow up minimal residual diseases. However,

Table 1. t(15;17), t(8;21), t(9;22) and inv(16) positivity rates by sex

sex	Positive n(%)	Negative n(%)	Total n(%)	p value
Female	28 (32.6)	58 (67.4)	86 (100.0)	
Male	35 (28.0)	90 (72.0)	125(100.0)	0.477
Total	63 (29.9)	148 (70.1)	211(100.0)	

Table 2. Clinical characteristics stratified by t(15;17), t(8;21), t(9;22) and inv(16) status in all cases

	t(15;17)	t(8;21)	inv (16)	t(9;22)	t(15;17) and t(8;21)	No rearrangement	p-value
n	27	15	3	16	2	18	
Age (Years)	49 (22-79)	66 (27-86)	74 (22-88)	55 (21-74)	50 (45-55)	58 (19-92)	0.363
WBC (109/L)	6 (0.1-88.1)	9.6 (0.4-45.2)	3.6 (0.7-38.1)	1.9 (0.1-38.1)	20.25 (5.7-34.8)	5.42 (0.1-258.4)	0.057
RBC (1012/L)	3.39 (0.6-4.7)	3.02 (2.1-5.46)	2.99 (2.9-3.24)	3.13 (2.1-5.04)	3.76 (2.9-4.6)	3.12 (1.4-6.03)	0.827
Hb (g/dL)	10.5 (2.2-14.8)	8.9 (6.7-17)	8.9 (8.9-12)	9.1 (6.5-15.8)	11.95 (8.8-15.1)	9.4 (3.2-18.3)	0.365
PLT (109/L)	36 (5-208)	86 (5-381)	19 (17-167)	34 (8-399)	51 (17-85)	54 (5-380)	0.555

comprehensive studies were with higher numbers of cases with more detailed translocation analysis are recommended.

Conflicts of interest: The authors have no conflicts of interest to declare.

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