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Mutations at exons 11 and 17 of c-kit Gene in Patients of Acute Myeloid Leukaemia

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Abstract

Objective: To determine the frequency of mutations at exons 11 and 17 of c-kit gene in patients of Acute Myeloid Leukaemia.
Methodology: It was descriptive study and conducted in departments of Hematology and Human Genetics and Molecular Biology, University of Health Sciences, Lahore. Total 80 newly diagnosed patients of AML from different hospitals of Lahore were included in the study The study included both peripheral blood and bone marrow aspirate slides of 80 newly diagnosed patients of AML. Each sample was amplified for exons 11 and 17 of c-kit gene using specific primers by direct DNA sequencing.
Results: Among 80 patients, 67.5% (n = 54) were males and 32.5% (n = 26) were females with a median age of 38 years (range 12-85 years). Our DNA sequencing results indicated that none of the patient had been observed to have mutation in both exons of c-kit gene. Only a polymorphism was identified in exon 11. So, it is concluded that our screened AML population was negative for these two exons of c-kit gene.
Conclusion: The study concluded that our selected AML patients were negative for these two exons but SNP was found in exon 11 of c-kit gene.
Keywords: Tyrosine Kinase class III (RTK), AML (Acute Myeloid Leukaemia), SNP (Single Nucleotide Polymorphism)

Introduction

Acute myeloid leukemia (AML) is a clonal haemopoietic malignancy, characterized by decreased production of normal hematopoietic cells by inhibiting cellular differentiation. This leads to proliferation of blasts that interferes with normal haemopoietic process resulting in decreased cell lines i.e. cytopenias.[1] Blasts or abnormal cells move to blood from bone marrow and sometimes spread to other areas of body like spleen, liver, lymph nodes and central nervous system (CNS). These abnormal cells interfere with normal hematopoietic process and organs function.[2]

The median age of incidence for AML, which is the most prevalent kind of leukemia in adults, is 68 years. [3] It can develop in patients with another hematological disorder, as a result of prior chemotherapy treatment with alkylating drugs, or as a result of radiation exposure. However, the majority of the time, the disease develops in previously healthy individuals as a new cancer. Because of chromosomal translocations like t(15:17) and t(8:21) and gene mutations like NPM1, FLT3-ITD, IDH, and c-kit, the disease progression of AML involves the uncontrolled generation and differentiation of myeloid stem cells clone. [4]

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The c-kit gene is proto-oncogene that encodes c-kit receptor (CD117). It is expressed by early hematopoietic progenitor cells, bone marrow stem cells and CD34+ cells. It plays a pivotal role in their proliferation, differentiation and survival. [5] C-kit gene comprise of 21 exons and their size ranges from 100bp to 300bp. [6] Different types of mutations in c-kit gene have been linked with specific types of malignancy. Regarding leukemia, c-kit has been studied in 78% of AML, 80% of CML in blast phase, 2% of ALL patients and no expression has been studied in CLL. [7] Duplication and insertion mutations and point mutations that activate the c-kit receptor are the most common types of mutations in AML. [8] The c-kit gene exons 2, 8, 9, 10, 11, 13, 14 and 17 have been the most thoroughly studied mutations in AML patients. [9]

Many studies on AML in Pakistan have been carried out and mutations like NPM1 and FLT3-ITD and cytogenetic abnormalities like t(8;21), t(15;17) and inv16 have been identified. These mutations and cytogenetic abnormalities are routinely analyzed on patients' blood and bone marrow samples by different techniques. But still a lot of work has to be needed for the better prognosis of patients. C-kit gene mutation is also related to poor prognosis of AML and identification of this mutation is recommended for risk stratification.

No study has yet reported the incidence of these mutations in Pakistani population. According to literature review, mutations at exon 11 and 17 of c-kit are more prevalent in India. As, Pakistan and India are in the same Asian sub-continent and both countries have same indigenous populations, these two exons of c-kit gene are selected for this study. The present study was planned to look for the mutations at exons 11 and 17

of c-kit gene in patients of acute myeloid leukemia in order to provide baseline information for better treatment strategy.

Methodology

It was descriptive study and conducted in departments of Hematology and Human Genetics and Molecular Biology, University of Health Sciences, Lahore. Total 80 newly diagnosed patients of AML from different hospitals of Lahore were included in the study. After taking informed consent from each patient, 3ml of whole blood was obtained using aseptic measures. The blood was collected in EDTA vacutainer and was stored at – 20°C until DNA extraction. Blood counts were done using Sysmex XT-1800i and peripheral blood smears were made and stained with Giemsa to look for morphological details. Patients with myeloblasts on a peripheral smear were considered for bone marrow aspiration. The aspirate was used to make slides, which were stained with Giemsa and then cytochemically stained for confirmation of the diagnosis. DNA was extracted from both peripheral blood and unstained bone marrow aspirate slides (10)using the standard phenol chloroform method.

After quantification of DNA, polymerase chain reaction (PCR) was carried out using forward and reverse primer of each exon. The primer sequences utilized in this study were previously published in peer-reviewed journals. The UCSC genome browser was used to validate these primers (www.genome.ucsc.edu). A 2% agarose gel was used to evaluate the PCR results. In 120 mL of 1XTBE buffer, 2.4 gm agarose was dissolved. After amplification of exons 11 and 17, bands of 257bp and 220bp were seen. To eliminate unincorporated dNTPs and residual oligonucleotides from the PCR result, an ethanol purification procedure was applied. It was carried out in order to acquire good sequencing results. Sequencing analysis was carried out on a commercial basis. Chromas light software v2.01 was used to examine the sequencing data and outcomes.

Statistical analysis was done using SPSS version 25. Qualitative variables like gender, patient clinical presentation, disease sub-classification and presence or absence of mutations were reported in form of frequencies and percentages. Quantitative variables like patient age, CBC parameters, LDH and blasts counts and duration of illness were reported in form of mean and standard deviation. Normal distribution of the data was checked by applying the Shapiro Wilk test and the *p* value>0.05 was considered as significant. Mean ± SD was reported for normal distributed parameters and the median (IQR) was reported for not normal distributed parameters. Mann Whitney U was applied on not normally distributed parameters while t-test was applied on normally distributed parameters and the *p* value ≤0.05 considered as significant and vice versa.

Results

Newly diagnosed 80 AML patients were included in the study. Out of 80 patients, 54 (67.5%) were males and 26 (32.5%) were females, the age of patients ranges from 12-85 years with a median of 38.0 (24) years. These patients were further categorized, in accordance with FAB classification. AML-M2 being the commonest. All 80 patients of AML were analyzed for disease associated mutations but none of them was found to have mutation in the exon 11 and 17 of c-kit gene. However, two polymorphisms, an intronic variant upstream of the coding sequence and a base pair deletion in the coding region of exon 11 was found in six patients (Figure 1). In one patient, a heterozygous deletion (c.1752delT) in exon 11 was identified. The c.1752delT is known as rs869225486 in the SNP data base (Figure 2).

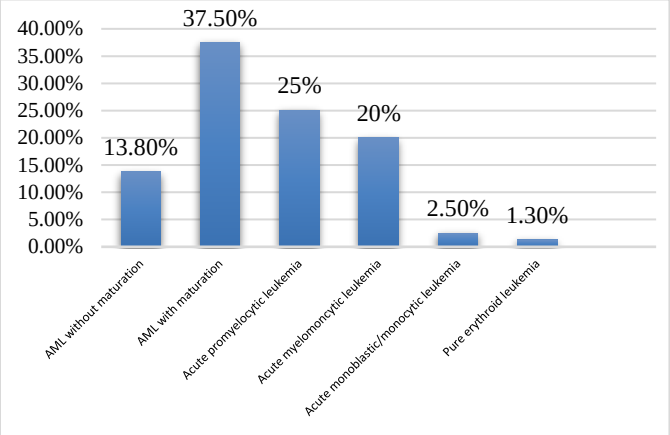

Figure 1. Bar chart showing FAB classification of selected AML patients, which showed that AML with maturation was the most common category.

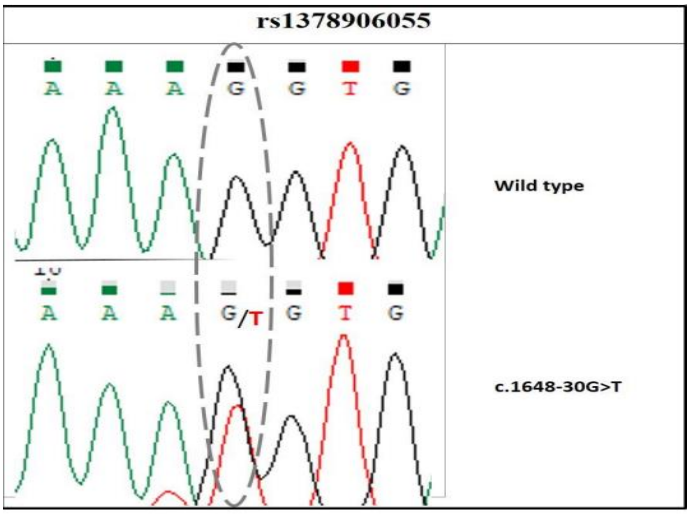

Figure 2. SNP in exon 11 intronic region.

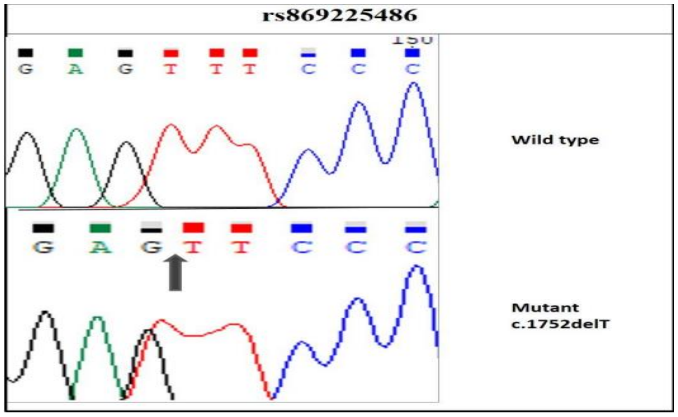

Figure 3. Electropherogram showing c.1752delT

were reported. Activating c-kit mutations found in leukemia patients cause bad prognosis of AML.[11] Despite advancements in treatment of leukemia globally, the prognosis of AML is still bad in Pakistan. Therefore, c-kit gene was selected to assess the prognosis.

In our study, the male to female ratio came out to be 1.8:1. Another study on Pakistani population also showed the similar trend of male predominance towards AML and the male to female ratio was 1.5:1.[12] This finding was consistent with of that European population which showed highest incidence of AML in males (3.7/100,000) as compared to females (1.88/100,000). The highest incidence in males was may be due to some physiological and genetic factors. [13]

Table II: Comparison of age with other hematological and biochemical parameters

Parameters	Age (Years)	Mean ± SD	Median (IQR)	Shapiro Wilk Test (*P*-Value)	*P*-value
Hb (g/dl)	<40	7.67 ± 1.93	8.20 (2.85)	0.191	0.796[a]
	>40	7.76 ± 1.57	7.9 (1.3)	0.600	
WBC (x10^6/µL)	<40	57.85 ± 71.22	40.0 (54.0)	0.000	0.946[b]
	>40	44.91 ± 41.81	40.30 (57.2)	0.000	
Platelets (x10^6/µL)	<40	58.89 ± 32.65	50.0 (25)	0.000	0.541[b]
	>40	58.54 ± 24.08	53.0 (22.0)	0.000	
BM Blast (n)	<40	69.51 ± 17.3	72 (27)	0.20	0.205[a]
	>40	65.5 ± 14.5	66 (20)	0.444	
LDH (U/L)	<40	814.96 ± 399.16	764.0 (413.5)	0.000	0.488[b]
	>40	754.17 ± 307.8	720 (390.0)	0.097	
Duration of Illness (weeks)	<40	28.55 ± 12.6	28.0 (21.0)	0.423	**0.012**[a]
	>40	21.11 ± 12.93	20.0 (21.0)	0.332	

a = t-test, b = Man-Whitney U test, *p* value ≤ 0.05 = Significant

Table II: The Shapiro wilk test was applied and the *p* value > 0.05 showing normal distribution.

Parameters	Mean ± SD	Median (IQR)	Shapiro Wilk Test (*P*-Value)	Data Distribution
Age (Years)	39.87 ± 16.6	38.0 (24)	0.012	Not-Normal
Hb (g/dl)	7.71 ± 1.8	7.90 (2.47)	0.141	Normal
WBC (x10^6/µL)	52.19 ± 60.2	40.15 (54.83)	0.000	Not-Normal
Platelets (x10^6/µL)	58.74 ± 29.0	50.00 (23.75)	0.000	Not-Normal
BM Blast (n)	67.75 ± 16.3	68.00 (25)	0.016	Not-Normal
LDH (IU/L)	788.36 ± 361.2	742.50 (407)	0.000	Not-Normal
Duration of Illness (weeks)	6.57 ± 3.6	6.00 (5.38)	0.013	Not-Normal

We further classified the patients into two age groups: 40 years and >40 years, to compare age with other hematological and biochemical characteristics. We did not observe any significant difference between the mean Hb values, median WBC and platelet count, blasts percentages and LDH levels. Regarding duration of illness, the patients with <40 years of age had significantly high mean duration as compared to those with >40 years of age (p = 0.012) (Table II & Table III).

Discussion

The c-kit gene receptor tyrosine kinase, involved in intracellular signaling and the mutated form of c-kit plays an important role in hematological malignancies. Total 21 exons of c-kit gene

The median age of presentation of 80 AML patients was 38.0 (24) years. An Indian study also showed the median age of 40 years.[14] This lower median age as compared to other developed nations was, perhaps due to different genetic makeup and late referring of patients to the tertiary care hospitals.

AML is actually a group of heterogeneous morphological subtypes. Each subtype has its own morphological, cytogenetic and phenotypic variation. Regarding categorizing of AML patients, the latest WHO classification is mostly used all over the world. In present study, AML with maturation was the most important observed morphological subtype. This finding was consistent with the previous study conducted in Karachi Pakistan.[15]

The survival statistics of AML patients in current study was very bad as 100% included patients were died because of disease. No patient could survive for more than 10 months. However, in United States, the 5 years' survival statistics of AML patients was 27.4% and its percentage was high among young age group. The main reason behind this poorer prognosis in studied AML patients was the late diagnosis and incomplete treatment and old age had worst prognosis, as old patients respond poorly to therapy and mostly die during the induction therapy. Similar finding was also observed in another study conducted in Bosnia .[16]

In present study, both peripheral blood samples as well as bone marrow aspirate slides were incorporated for the detection of gene mutations at both exons 11 and 17 of c-kit gene. But incidentally, none of the collected samples showed any type of mutation in these exons. Our findings were remarkably similar to a Turkish study, in which they also did not find c-kit gene mutations in any of their AML case .[17] Our findings were also consistent with those of a Brazilian study published in 2012 on the detection of mutations in acute myeloid leukemias. They discovered mutations in exon 8 of the c-kit gene, but none in exon 17. This could be owing to the fact that these populations have a low prevalence of C-Kit gene mutations .[18]

Our findings, on the other hand, were in contrast with the majority of previously published studies. Exon 17 of the c-kit gene was shown to be mutated in 36.5% of AML patients in a Chinese study published in 2014 and likewise, a Korean study revealed 31% of mutations in exon 17 of the c-kit gene. [19]Another German study discovered 7% of mutations in exon 11 of the c-kit gene.[20] Exons 11 and 17 mutations were found in 46% and 38% of the Indian population, respectively. We began working on AML patients to explore for mutations in these exons, keeping in mind that India and Pakistan are neighboring countries and have same indigenous populations. However, no mutations were obtained in these two exons. Therefore, more prospective study should be undertaken in future to reduce such bias and gain more information in this subject.

Conclusion

In current study two patients revealed polymorphisms: an intronic mutation upstream of the coding sequence and a base pair deletion in the coding region of exon 11. On exon 17, no SNPs had been observed. Similar findings were reported in a research on minor insertions and deletions in the human genome conducted in the United States of America, where only intronic SNPs of exon 11 were reported. [21]

Limitations of the Study: Ideally, the whole gene should be screened to know the actual prevalence of mutations in any gene. Due to budget constraints only two exons were selected based upon the results of previous studies that reported majority of mutations in exon 11 and 17.

Future Perspective: For future, more comparison studies with bigger sample size should be done on whole c-kit gene comprising of 21 exons, to know the actual prevalence of mutations of c-kit gene in AML patients.

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