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Analyzing hematological indices and clinical associations of FLT3 mutation in acute leukemia

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***Abstract*---**Objective: The purpose of this research is to examine the relationship between FLT3 mutation and hematological indices and clinical correlates in patients with severe myelogenous leukemia &

acute lymphoblastic leukemia. Study Design: Descriptive Study. Place and Duration: Diagnostic and research Laboratory Liaquat university of Medical and health sciences jamshoro Hyderabad. January 2021 to December 2021. Methods: Total 79 cases of acute leukemia were included in this study. Patients provided written consent for detailed demographics and for self-structure questionnaire. All the data was obtained from laboratory records. The patients' clinico-hematologic and immunophenotypic characteristics were recorded, and the FLT3 mutation was analyzed molecularly. SPSS 24.0 was used to analyze all data. Results: Majority of the patients 44 (55.7%) were males and 35 (44.3%) cases were females. The included patients had mean age 37.21±14.59 years and had mean BMI 23.6±4.18 kg/m^2. We found that 48 (60.8%) cases had acute myeloid leukemia (AML) and 31 (39.2%) cases had acute lymphoblastic leukemia (ALL). Frequency of FLT 3 mutations in AML patients was 6 (12.5%) and in ALL patients was 1 (3.2%) after polymerase chain reaction. WBC was the most statistically significant hematological and clinical result connected with FLT3 mutation. Conclusion: The findings of this study led us to the conclusion that, aside from WBC, no other hematological correlates were found. Mutations in FLT3/ITD are highly prevalent among our adult AML patients of all FAB subtypes. To comprehend the pathophysiology of leukemias and their role as an invaluable prognostic marker in our patients, we need to conduct molecular mutation analysis in various cytogenetic groups with follow-up.

***Keywords*---**Hematological Indices, Clinical Correlates, Acute myeloid leukemia (AML), Acute lymphoblastic leukemia (ALL).

Introduction

The aberrant multiplication of clonal haemopoietic progenitor cells characterizes the spectrum of diseases known collectively as acute myeloid leukemia. Cytogenetic aberrations and molecular genetic abnormalities that play a role in leukemogenesis and illness progression have been identified as researchers gain a deeper grasp of the biology of disease. Some genetic anomalies are known to play a role in influencing prognosis, predicting response to treatment, and overall survival, whereas others characterize unique AML disease entities and are therefore necessary for diagnosis.[1] These genetic molecular indicators have the potential to serve as therapeutic targets and inform therapy choices. In addition, these can be employed in the evaluation of MRD.[2,3]

Nucleophosmin, or nucleolar phosphoprotein B23, shuttles nucleosomal proteins and core histones between the nucleus and the cytoplasm and has a role in regulating the ARF-p53 tumor suppressor pathway.[4] The nucleophosmin (NPM1) gene is particularly vulnerable to alterations in AML. Patients with these mutations have a better chance of responding to chemotherapy and living longer as a result.

Together with KIT, FMS, and PDGFR, FLT3 is classified as a type III receptor tyrosine kinase (RTK). The extracellular region of FLT3 is made up of five immunoglobulin-like domains, while the intracellular area is divided into a juxtamembrane (JM) domain, a tyrosine kinase (TK) domain, and a C-terminal (C-t) domain. Bone marrow stromal cells express the ligand (FL) for FLT3, and FLT3 is expressed on normal hematopoietic stem/progenitor cells. FL can be membrane-bound or soluble.[4,5] When FL binds to the extracellular domain of FLT3, the protein is dimerized and trans-phosphorylated at tyrosine residues in the activation-loop (A-loop). Multiple intracellular signaling pathways are triggered by activated FLT3, which ultimately results in the maintenance, proliferation, and differentiation of hematopoietic cells. Many acute leukemia cells also express FLT3, and when stimulated by FL, these cells become more proliferative and less likely to undergo apoptosis. In 1996, it was discovered that the FLT3 gene's JM domain coding region had an inner tandem duplication (FLT3-ITD) in cells with acute myeloid leukemia (AML). We then found a missense point mutation at the D835 position in the FLT3 TK domain (FLT3TKD), in addition to other points of mutation, deletions, and insertions in the surrounding codons.[4-6]

White blood cell (WBC) count, patient age, and the percentage of blasts are all factors in the prognosis and stratification of AL, as are immunophenotyping, cytogenetics, and molecular genetic characteristics, according to the current WHO diagnostic guidelines for AL (Swerdlow et al., 2008). Patients are categorized as having a low, intermediate, and high risk of getting cancer based on cytogenetic risks and molecular genetic variations such FLT3, KIT, NPM1, and CEPBPA. Notable mutations in the gene that codes for the FMSlike tyrosine kinase 3 (FLT3) receptor are found in about 30% of individuals with recurrent myeloblastic leukaemia (AML) (Koh et al., 2009), and are linked with a poor prognosis.[7,8]

Recently, it has been shown that over 20% of adult AML patients carry FLT3 gene internal tandem duplication (ITD) mutations.It has been observed that the prevalence of leukemia in children is between 5 and 16.5 percent [9].[10] The juxtamembrane domain of the FLT3 protein is encoded by exons 11 and 12 of the human FLT3gene on chromosome 13q12. Typically, mutations affect the part of the FLT3 protein between AA 575 and AA 613, resulting in an in-frame insertion sequence.[11] When transfected into 32D or BA/F3 cells, this modification triggers constitutive activation of the protein, which in turn activates downstream signal molecules like STAT5, Ras, and MAP kinase.[12] FLT3-ITD mutations were discovered to be common in AML patients without any other cytogenetic abnormalities and were found to be related with elevated leukocyte counts. It suggests that FLT3 abnormalities are linked to a poor clinical outcome.Assessment of the possible prognostic significance of this mutation is complicated by the small sample sizes and inconsistent treatment protocols of the previous research [13]. About 7% of patients with AML have been found to have point mutations in FLT3's codon 835.These mutations [14] in the activation loop of FLT3's second tyrosine kinase domain (TKD) cause the protein to be permanently activated.[15] The significance of this change in terms of prognosis is not yet known.

Materials and Methods

This Descriptive study was conducted at Diagnostic and research Laboratory Liaquat university of Medical and health sciences jamshoro Hyderabad and comprised of 79 patients. Haematology collected blood samples from 79 adults with various French-American-British (FAB) classifications of AML/ALL. AML/ALL was confirmed through morphological analysis and FAB classification. Insufficient funding prevents the collection of cytogenetic and immunophenotypic data on these people at the present time. Patients gave their consent to commence therapy after receiving all relevant information. Proteinase K and Phenol were used to extract DNA from patient blood samples. The patient's DNA was extracted and frozen at -20 degrees Celsius for further analysis. Primers for amplifying exons 14 and 15 of FLT3/ITDs were previously reported.

In the case of EcoRV, the amino acids D835 and I836 are encoded by the GATATC recognition sequence. The PCR product was digested at 37 degrees Celsius for three hours in a reaction volume of 15 ml using 5U of EcoRV (New England BioLabs). Mutants were found in digestion products separated on a 3.5% agarose gel by looking for the absence of the GATATC site. Chi2 and Fisher's exact tests in SPSS 24.0 were used to examine differences in variable distributions between patient groupings.

Results

Majority of the patients 44 (55.7%) were males and 35 (44.3%) cases were females. The included patients had mean age 37.21±14.59 years and had mean BMI 23.6±4.18 kg/m^2. There were 62 (78.5%) patients had hemoglobin ≤10 mg/dl. WBC Count x 109/L >50 was found in 42 cases and Platelet count x109/L ≤50 was found in 58 cases.(table 1)

Table-1: Demographics of the enrolled cases

Variables	Frequency	Percentage
Gender		
male	44	55.7
Female	35	44.3
Mean age (years)	37.21±14.59	
Mean BMI (kg/m^2)	23.6±4.18	
Hemoglobin		
>10 mg/dl	17	21.5
≤10 mg/dl	62	78.5
WBC Count x 109/L		
>50	42	53.2
>10-50	25	31.6
≤10	11	13.9
Platelet count x109/L		
>50	21	26.6
≤50	58	73.4

We found that 48 (60.8%) cases had acute myeloid leukemia (AML) and 31 (39.2%) cases had acute lymphoblastic leukemia (ALL).(figure 1)

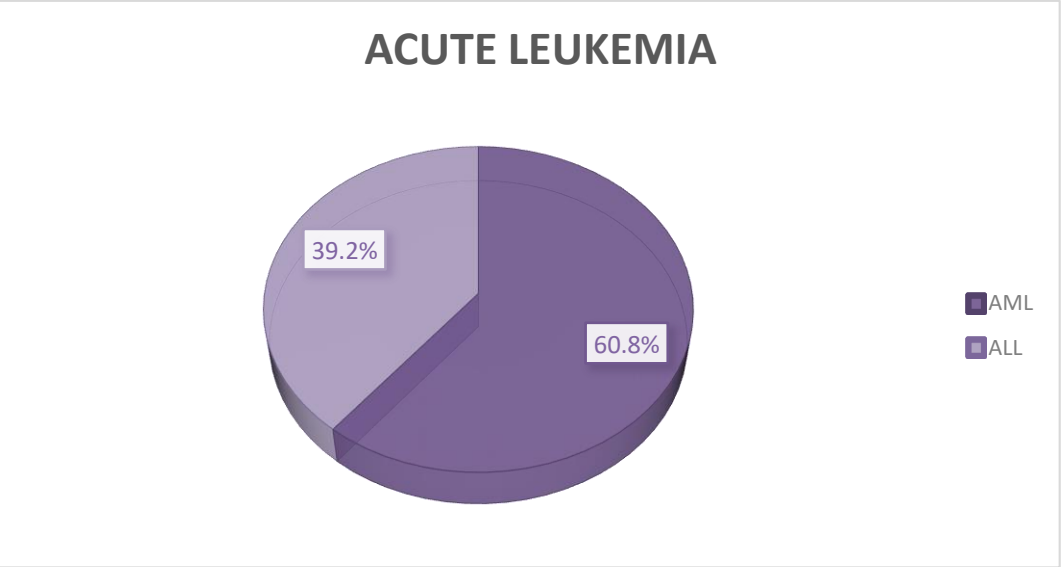

Figure-1: Association Of Acute Leukemia

Frequency of FLT 3 mutations in AML patients was 6 (12.5%) and in ALL patients was 1 (3.2%) after polymerase chain reaction.(table 2)

Table-2: FLT3 mutations among all cases

Variables	AML (48)	ALL (31)
FLT3 Mutation		
Yes	6 (12.5%)	1 (3.2%)
No	42 (87.5%)	30 (96.8%)

There was a significant correlation between white blood cell count and FLT3-ITD in patients with AML (p=0.006) and ALL (p=0.004). The correlation between FLT3 gene changes and six-month survival was also evaluated. Patients with adult acute myeloid leukemia (AML) who expressed CD34 and had FLT3-ITD had a trend toward slower progression (p=0.057).

Table-3 FLT3-ITD mutation status in individuals with acute leukemia: correlation with predictive variables

Variables	AML FLT3-ITD (P values)	ALL FLT3-ITD (P values)
LDH	0.106	0.490
CD34 Expression	0.065	0.132
WBC count at diagnosis[a]	0.006*	0.004*
% Blasts	0.086	0.785

In the presence of FLT3/ITD, the difference between the white blood cell (WBC) counts of ITD+ patients and FLT3/WT patients (mean 150x109/L) was not statistically significant. Hyperleukocytosis was clearly linked to FLT3/ITD. Although white blood cell counts were higher (mean 34.8x109/L) than in individuals with wild-type blood, the link between clinical variables and D835 mutations could not be investigated because of the limited number of samples.(table 4)

Table-4: Incidence of FLT3/ITD Mutation in Patients with French-American-British (FAB)-Classified Acute Myeloid Leukemia and Acute Lymphoblastic Leukemia (n= 79)

Variables	AML (48)	ALL (31)
Mo	1	0
M1	2	1
M2	3	1
M3	2	1
M4	1	2
M5	1	1
M6	0	1

Discussion

Cytogenetic karyotyping and molecular analysis are two forms of genetic testing that play an important role in the classification and diagnosis of patients with AML. Differentiating cytogenetic and molecular aspects of various disease entities allows for the classification of AML patients. Patients with acute myeloid leukemia can benefit from the use of these genetic molecular markers because they show how likely they are to go into complete remission and remain disease-free for an extended period of time. AML treatment regimens should take into account the prognostic importance of the disease's genetic landscape.[16,17]

Multiple studies [18,19] have found that FLT3/ITD is associated with a poorer prognosis in AML patients. However, in Pakistan, no research has been conducted on the prevalence and prognostic significance of the D835 and FLT3 mutations in AML patients with different FAB subtypes. Similar to a previous Pakistani study (32.26 percent), this one also indicated that FAB-M2 was the most common type (37.50 percent), followed by M1 (22.58 percent) and M4 (22.58 percent) [19]. However, AML-M4 was found to be the most prevalent FAB subtype in a second investigation of 116 patients [18]. The frequency of FLT3/ITD mutations was highest in FAB-M4 patients, and the frequency of D835 mutations was the same in FAB-M1, M2, and FAB-M4 patients. Our cohort of AML patients had mutation rates in the ITD that are comparable to those identified in previous studies conducted around the world [17]. Most often, FLT3 mutations have been linked to the M2, M3 subtype of FAB [20]. All other FAB subtypes seem to share a similar frequency of FLT3/ITD mutations [21,22]. Our study is the first attempt to measure the frequency with which these mutations occur in our patients without using cytogenetic data. Cytogenetic disorders are associated with a much increased mutation rate in AML subtypes. The large variation in reported

frequency of ITD mutations (13%-27%) may be due to differences in study sample sizes.

A poor prognosis awaits patients with AL who also have increased LDH activity. Researchers found that patients with AL who had FLT3 gene mutations also had elevated LDH activity. However, neither this inquiry nor a prior one turned up any evidence of a similar phenomenon. Patients with FLT3 gene mutations had LDH activity that was higher than the 200.0 U/L cutoff in both the adult and juvenile populations.[23,24]

Although Liu et al. (2007) could not find a statistically significant association between the existence of FLT3 mutations in genes and blast cell percentages, patients with FLT3 gene mutations were more likely to have high blast cell percentages across both categories.[25]

Another crucial prognostic factor is whether or not blast cells express CD34. Regardless of FLT3-ITD status, Zhu et al. (2013) demonstrated that CD34 expression is a poor prognostic predictor. However, multiple studies (Barragán et al., 2011; Zhu et al., 2013) have found a correlation between the FLT3-ITD mutation and the CD34 marker. The hypothesis that FLT3 gene changes connect with CD34 expression was not supported by the data presented here. Five people who tested negative for FLT3-D835 also expressed high levels of CD34.[26,27]

AML/ALL patients with FLT3-ITD mutations have elevated leukocyte counts. Consistent with previous findings [28], we observed this to be the case when contrasting individuals with FLT3/ITD mutation to those having wild-type FLT3. According to the results of this investigation, the prevalence of mutations is strongly influenced by the age of patients. No association between patient age and FLT3 mutation status has been found, in contrast to the findings of earlier investigations [28].

This study found no association between the FLT3-ITD mutation and a poor outcome (death or relapse) amongst adult patients. FLT3-ITD was associated with an elevated mortality risk, indicating a poor prognosis, among the subset of children and adolescents who were diagnosed with AML. Complete remission, disease-free survival, or overall survival are not linked to the FLT3-D835 mutation, according to the available data. These hypothesis are supported by the data from this inquiry.[29,30]

Conclusion

The findings of this study led us to the conclusion that, aside from WBC, no other hematological correlates were found. Mutations in FLT3/ITD are highly prevalent among our adult AML patients of all FAB subtypes. To comprehend the pathophysiology of leukemias and their role as an invaluable prognostic marker in our patients, we need to conduct molecular mutation analysis in various cytogenetic groups with follow-up.

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