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ORIGINAL PAPER

The detection of internal tandem duplication in the FMS-like tyrosine kinase 3 gene in a sample of Iraqi patients with acute myeloid leukemia

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ABSTRACT

Introduction and aim. Acute myeloid leukemia (AML) is a genetically heterogeneous malignancy significantly influenced by FMS-like tyrosine kinase 3 mutations. This study investigated the prevalence and clinical implications of internal tandem duplication (ITD) mutations in *FLT3* in patients with AML in Iraq.

Material and methods. The study involved blood samples collected from 50 newly diagnosed AML and 50 healthy subjects between April 2023 and January 2024 and used for conventional PCR. PCR products positive for the ITD mutation were subjected to fragment analysis using capillary electrophoresis (CF) to confirm ITD mutations.

Results. *FLT3-ITD* mutations were identified in 20% of patients with AML (10/50), with striking female predominance (90% of mutation-positive cases). Based on the FAB classification, M5 was the most prevalent FAB subtype among mutation carriers (40%), with no mutations detected in M7.

Conclusion. This study provides critical information on the prevalence and clinical implications of *FLT3-ITD* mutations in patients with AML in Iraqi, emphasizing the need for precise molecular diagnostic approaches. This finding revealed a distinct subset of patients with AML that harbor *FLT3-ITD* mutations, with a significant proportion exhibiting elevated allelic ratios, which reinforces their prognostic relevance.

Keywords. acute myeloid leukemia, capillary electrophoresis, FAB, *FLT3-ITD* mutation, sex disparity

Introduction

Acute myeloid leukemia (AML) is a hematologic tumor characterized by unrestricted proliferation of clonal myeloid progenitors in the bone marrow.[1,2] This arises from the accumulation of sequential mutations in hematopoietic stem cells, leading to impaired differentiation and excessive blast proliferation.[3] AML is the most common type of acute leukemia in adults and affects individuals of all ages.[4,5] In Iraq, leukemia is among the top ten malignancies in both sexes, accounting for 5.34% of all cancers (4.22 per 100,000 people) according to the Iraqi Cancer Registry.[6] The etiology of AML remains largely unknown, although various risk factors such as ionizing radiation, benzene exposure, smoking and prior cytotoxic chemotherapy have been implicated in its pathogenesis.[7] Fms-like tyrosine kinase 3 (*FLT3*) mutations are among the most frequent mutations, occurring in approximately 30% of cases. *FLT3* is a transmembrane receptor crucial for hematopoietic cell differentiation, survival, and proliferation through multiple signal transduction pathways.[8] The two primary *FLT3* mutations associated with AML are internal tandem dupli-

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cations (ITD) in the juxtamembrane domain (25%) and point mutations in the tyrosine kinase domain (TKD, 7–10%).[9,10] *FLT3-ITD* mutations lead to constitutive activation of downstream signaling pathways such as STAT5, RAS-MEK-MAPK, and PI3K-AKT, driving uncontrolled cell proliferation and inhibiting differentiation.[10] These mutations are associated with poor overall survival (OS) and high relapse rates following intensive therapy.[9]

Factors such as *ITD* length, allelic ratio, ITD site within *FLT3*, cytogenetic aberrations and genetic mutations, and overall patient variation in these areas suggest that *FLT3*-mutated AML is a highly ethnically heterogeneous disease with far-reaching prognostic implications.[11] Importantly, the allelic ratio of *FLT3-ITD* mutations has been identified as a critical prognostic factor influencing risk stratification and treatment decisions, particularly regarding the need for hematopoietic stem cell transplantation (HSCT).[12] Despite the well-established importance of *FLT3-ITD* mutations in the prognosis in Iraqi patients. Ethnic and genetic variabilities may influence the prevalence of mutation and its prognostic implications. Furthermore, standard diagnostic techniques such as gel electrophoresis may have limitations in detecting *FLT3-ITD* mutations and accurately determining the allelic ratio, which is essential for risk stratification. Fragment analysis offers greater sensitivity and accuracy in identifying *FLT3-ITD* mutations, allowing for a more precise assessment of their clinical impact.

This study aimed to evaluate the distribution of *FLT3-ITD* mutations in newly diagnosed Iraqi patients with AML and their association with sex and the French-American-British (FAB) subtype of AML. Furthermore, this study aimed to compare gel electrophoresis with fragment analysis to evaluate its effectiveness in *FLT3-ITD* detection and determine the allelic ratio.

Material and methods

The study included 50 patients (25 men and 25 women) with a new diagnosis of acute myeloid leukemia (AML) and 50 healthy controls matched for age and sex. Between April 2023 and January 2024, patients with AML were recruited from the Hematology Center at Medical City in Baghdad. All study participants provided their written informed consent and the study was approved by the institutional review board of the Hematology Center (approval number # 18982, 22 May 2023).

Control group subjects were randomly selected, ensuring gender distribution CLMSPAY distribution. The majority of controls were from the general population, and a small proportion were recruited from healthcare staff. Other exclusion criteria for AML patients were previous treatment (chemotherapy/radiotherapy) or a diagnosis of secondary AML. Controls were excluded if they had a history of blood disorders, malignancy, ongoing infections, use of immunosuppressive agents, autoimmune diseases or blood tests with abnormal results.

Blood samples (3 mL) from AML patients and controls were drawn into EDTA coated tubes and stored at -20°C for one week before processing. Genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. DNA quality and concentration were determined using NanoDrop spectrophometry. Flow cytometry data was used to classify subclasses according to French-American-British (FAB) criteria from a review of clinical records of all patients with AML diagnosed at UTMB until October 2023.

FLT3-ITD detection

Exons 14–15 of *FLT3* were amplified using conventional PCR. The following primer sequences were used.

- Forward primer: 5'-GCAATTTAGGTATGAAAGCCAGC-3'
- Reverse primer: 5'-CTTTCAGCATTTTGACGGCAACC-3'.[13]

The initial PCR was performed in a 25 µL volume consisting of 12 µL of green master mix (Promega, Madison, USA), 6 µL of nuclease-free water, 1 µL of the forward primer, 1 µL of the reverse primer, and 5 µL of genomic DNA (25 ng). The PCR cycling conditions were as follows: an initial denaturation step at 94 °C for 3 minutes; 35 cycles of 94 °C for 30 seconds (denaturation), 56 °C for 1 minute (annealing), and 72 °C for 2 minutes (extension); followed by a final extension at 72 °C for 7 minutes. The PCR products were resolved on 3% agarose gel. The wild-type *FLT3* allele produced a 329 bp fragment, whereas any fragment larger than 329 bp was classified as a mutant (*FLT3-ITD* positive). Fragment analysis was performed on *FLT3-ITD* positive samples using a SeqStudio Genetic Analyzer (Applied Biosystems, MA, USA) with the LeukoStrat® *FLT3* Mutation Assay 2.0 (Invivoscribe, San Diego, CA, USA), according to the manufacturer's instructions. Genomic DNA from *FLT3-ITD* positive cases (as determined by gel electrophoresis) was re-amplified in a 50 µL PCR reaction containing 45 µL of *FLT3 ITD* master mix, 0.25 µl of Taq DNA polymerase (Promega, Madison, USA), and 5 µL of gDNA (5 ng). The PCR cycling conditions for this step were: initial denaturation: 95°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min; and final extension: 72°C for 60 min. For the fragment analysis, PCR products were mixed in a 96-well PCR plate with 9 µL of Hi-Di formamide (Applied Biosystems, CA, USA), 1 µL of GeneScan™ 600 LIZ ™ Size Standard v2.0 (Thermo Fisher Scientific, MA, USA), a fluorescently labeled marker for accurate sizing, and 0.5 µL of each amplicon. The sam-

ples were denatured at 95°C for 3 minutes, then chilled on ice for 5 min. The plate was subsequently loaded into the SeqStudio Genetic Analyzer, and the run was initiated according to the manufacturer's instructions. In this assay, any electrophoretic peak equal to or greater than 330 bp was considered positive for the *FLT3-ITD* mutations. Gene Marker™ Software v 3.0.1 (SoftGenetics, PA, USA) was used to evaluate fragment profiles by quantifying the size, quantity, and area under the curve (AUC) of each peak. The allelic ratio (AR) was determined by dividing the AUC of the mutant allele by that of the wild-type allele. In cases where multiple mutant alleles were detected, their AUCs were summed. The difference in the total number of base pairs between the mutant and wild-type alleles was used to determine the mutation size.

All positive samples were analyzed in duplicate to confirm the results, and negative control was included in each run to ensure the validity of the assay.

Statistical analysis

The Statistical Analysis System software (SAS Institute, Cary, NC, USA), version 9.4 was used for data analyses. For continuous variables, comparisons were performed by using Student t-test or the least significance difference (LSD) method. For categorical variables, the chi-square test was used. Pearson correlation coefficients were computed to assess relationships between variables. Statistical significance was determined by a probability value (p) less than 0.05.

Results

This study included 50 newly diagnosed AML patients and 50 healthy subjects. The AML patients had a median age of 43.5 years (range: 15–80 years), with a mean age of 43.06 years. In the control group, age ranged from 16 to 67 years, with a median age of 44 years and a mean age of 42.02 years. There were no significant differences in age between groups. *FLT3-ITD* mutations were detected in 10 of 50 AML patients (20%), while none of the healthy controls exhibited these mutations. In *FLT3-ITD*–positive patients, mutant clones were identified by the presence of one or two additional fragments, along with the wild-type fragment. The duplication region varied in size from 33 to 73 bp, as determined by agarose gel electrophoresis. Among the 10 patients with the ITD mutation, 9 (90%) had a single ITD (one mutated clone), and 1 (10%) had double ITDs (two mutated clones) and lane 4 (~380 bp and ~400 bp). None of the patients had more than two ITD mutations. In one patient (lane 9), there was very weak or absent amplification of the wild-type *FLT3* allele on agarose gel, with only a single band (~400 bp) corresponding to the mutant allele, as illustrated in Figure 1.

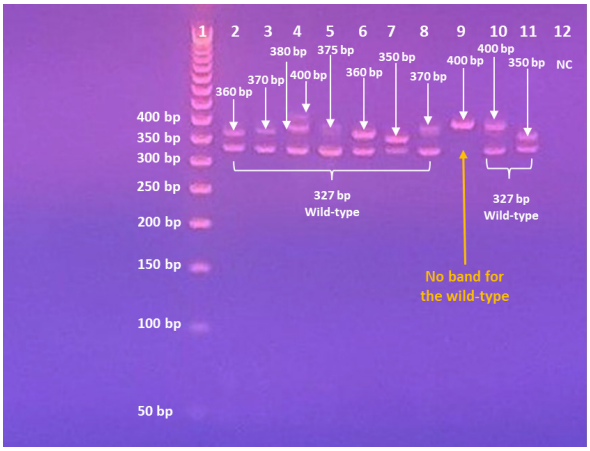

Fig. 1. Image of a 3% agarose gel stained with ethidium bromide displaying mutations in *FLT3-ITD*. Lane 12 served as a negative control. Lane 1 is a 50 bp DNA ladder; lanes 2–11 show patient samples that were positive for the *FLT3*-ITD mutation

Table 1. The nature of ITD in the *FLT 3* gene in Individuals with AML according to CE

	Size (bp)		
	Wild-type	Mutant -type	Inserted nucleotides
1	327	363	36
2	327	387	60
3	327	381	54
		408	81
4	327	390	63
5	327	363	36
6	327	353	26
7	327	378	51
8	327	391	64
9	327	390	63
10	327	347	20

Table 2. Comparison of the ITDs sizes in both agarose gel electrophoresis and CE methods

	ITD size via capillary electrophoresis (bp)	ITD size via agarose gel electrophoresis (bp)
1	363	~ 360
2	387	~ 370
3	381	~ 380
	408	~ 400
4	390	~ 375
5	363	~ 360
6	353	~ 350
7	378	~ 370
8	391	~ 400
9	390	~ 400
10	347	~ 350

Fragment analysis of *FLT3-ITD* revealed 11 distinct types, with one patient exhibiting double ITDs. The median ITD size was 50.3 bp, with sizes ranging from 21 bp to 81 bp, as determined by capillary electrophoresis (CE) (Table 1).

When comparing the allele sizes obtained via agarose and CE, the ITD sizes were nearly identical (Table 2).

Regarding the patient in lane 9 (Figure 1), no wild-type allele band was detected by gel electrophoresis in lane 9 (Figure 1); however, CE revealed the presence of both wild-type and mutant alleles (Figure 2). For *FLT3-ITD* positive patients, the mean age was 44.7 years, with ages ranging from 23 to 74 years and a median age of 44 years. There were no significant differences in age distribution among patients with these mutations.

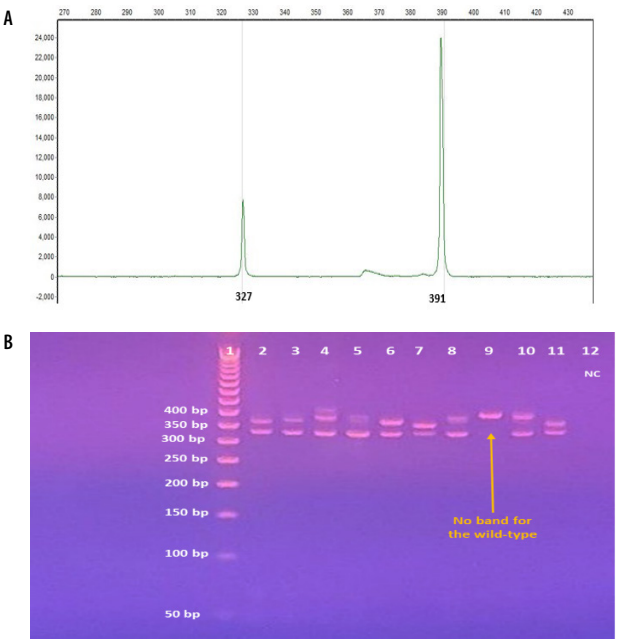

Fig. 2. A: Capillary electrophoresis for case number 8, B: Gel electrophoresis for case number 8 (lane 9)

AML patients without *FLT3-ITD* mutations were predominantly male. Conversely, in the group with *FLT3-ITD* mutations, females were predominant, with mutations detected in nine females (90%) and one male (10%), as shown in Table 3. The odds ratio (OR) was calculated to assess the association between sex and *FLT3* gene mutation status in AML patients. An OR greater than 1 indicates higher odds of the event (mutation) occurring in females compared to males, while an OR less than 1 suggests lower odds. In this study, the OR for the mutant *FLT3* type was 1.372, indicating that females had higher odds of carrying the FLT3 mutation compared to males. The associated p-value (p=0.01) suggests that this difference is statistically significant. Conversely, the OR for the wild-type *FLT3* gene was 0.317, indicating lower odds of females having the wild-type allele compared to males, although this was not statistically significant (p=0.206). These findings suggest a potential sex-related difference in the distribution of *FLT3* mutations among AML patients.

In the distribution of AML patients with and without the *FLT3-ITD* mutation across FAB subtypes, neither group included individuals with the M1 subtype and no *FLT3-ITD* mutations were observed in the M7 subtype. Among patients with wild-type *FLT3-ITD* (n=40), the most prevalent subtype was M5, accounting for 17 cases (42.5%), followed by M4 in 9 patients (22.5%). In contrast, in the *FLT3-ITD*–positive cohort (n=10), M5 remained the most common subtype (four patients, 40%), followed by M0 (two patients, 20%) (Table 4). The OR was calculated to assess the association between FLT3 mutation status and the different FAB subtypes of AML. The OR analysis revealed a significant association between *FLT3* mutations and FAB subtypes M5 and M4, indicating higher mutation frequency in these groups. In contrast, subtypes M2 and M3 showed lower, non-significant odds. OR values could not be determined for some subtypes (M0, M1, M6) due to limited sample size.

Table 3. Distribution of sample study according to ITD mutation in the *FLT 3* gene and sex

FLT 3 gene	Individuals with AML				
	Male	Female	χ2	Odds ratio (95% CI)	p
Wild Type	24 (96%)	16 (64%)	1.60	0.317 (0.22–0.78)	0.206
Mutant Type	1 (4%)	9 (36%)	6.40	1.372 (0.84–1.75)	0.01
Total	25 (100%)	25 (100%)	–	–	–

Table 4. Relationship between ITD mutation in the *FLT 3* gene and FAB classification

FAB n (100%)	Wild type	Mutant type	χ2	Odds ratio (95% CI)	p
M0	2 (5%)	2 (20 %)	0.00	–	>0.999
M1	0 (0%)	0 (0%)	0.00	–	<0.001
M2	3 (7.5%)	1 (10%)	1.00	0.294 (0.17–0.63)	0.317
M3	4 (10 (%)	1 (10%)	1.80	0.387 (0.22–0.93)	0.179
M4	9 (22.5%)	1 (10%)	6.40	1.372 (0.84–1.75)	0.01
M5	17 (42.5%)	4 (40%)	8.047	1.502 (0.92–2.26)	0.0046
M6	1 (2.5%)	1 (10%)	0.00	–	>0.999
M7	4 (10%)	0 (0%)	3.72	0.86 (0.47–1.59)	0.0419
Total	40 (100%)	10 (100%)	–	–	–

Table 5. Allelic ratio (AR) for the *FLT 3* – ITD mutations

FAB		Capillary band area		AR (%)
		Wild-type	Mutant-type	
1	M5	232121	108325	0.47
2	M2	82303	21559	0.26
3	M4	265237	100480	0.58
			54190	
4	M3	67911	3750	0.05
5	M5	171245	1128722	0.75
6	M5	190602	181357	0.95
7	M6	37533	12512	0.33
8	M0	52943	222877	4.2
9	M0	248057	162024	0.65
10	M5	11599	4123	0.36

Table 5 presents the allelic ratio (AR) for each patient with ITD mutation. The distribution was balanced, with 50% of the patients exhibiting a high AR and 50%

exhibiting a low AR. For patients with two mutant alleles, the band areas for each allele were summed.

Regarding the correlation of the hematological parameters between the patients harboring *FLT3*-ITD and other individuals with AML without mutation (wild-type) who were enrolled in this study, the mean counts of white blood cells and peripheral blood blast cell percentages in patients harboring the mutations of *FLT3*-ITD were insignificant compared to other patients without mutations (Table 6).

Table 6. Relationship between the *FLT 3* gene ITD mutation and hematological parameters

FLT 3 gene ITD mutation	Mean±SE	
	WBC count $\times 10^9$/L	Peripheral blood blast cell
Wild type	29.09±2.05	49.72±4.19
Mutant type	30.78±2.81	49.37±3.62
p	0.873	0.902

Discussion

FLT3 is a proto-oncogene that plays a critical role in hematopoietic cell survival, differentiation, and proliferation.[14] ITD mutations in *FLT3* occur in approximately one-third of patients with AML and are frequently associated with poor prognosis and diminished responses to chemotherapy.[15] In this study, the investigated the prevalence and characteristics of *FLT3-ITD* mutations in a cohort of 50 patients with AML in Iraq, using both gel electrophoresis and CE for mutation detection. Despite the clinical significance of these mutations, research on *FLT3* in Iraq is limited. no prior regional study has employed highly sensitive fragment analysis to detect *FLT3-ITD* mutations.

The median age of AML patients in this study was 43.5 years (range: 15–80 years), which aligns with the results of previous Iraqi studies. For example, Latif et al. reported a median age of 47 years (range: 14–86 years)[16], whereas Al-Shammari et al. found a median age of 46 years (range: 15–85 years).[17]

FLT3-ITD mutations were detected in 10 of 50 patients (20%), a prevalence consistent with regional reports (21.88% in one study, 20.15% in a Saudi Arabian study, and 20.4% in another).[18,19] However, lower frequencies have been reported, such as 16%, 17.4%, and 14.54%.[20–22] These discrepancies may be attributed to differences in the sample source (peripheral blood vs. bone marrow), nucleic acid type (DNA vs. RNA), and the assay used for amplification and detection.[13]

Since the detection of the *FLT3*-ITD mutation can facilitate the diagnosis, prognosis, and treatment of patients with AML, a pivotal component of current research is the comparative analysis of *FLT3-ITD* mutation detection using traditional gel electrophoresis versus CE.[23] While gel electrophoresis is widely utilized owing to its accessibility and cost-effectiveness, it relies on the visual interpretation of bands and is prone to errors, particularly when bands are faint or when the tumor cell content in a sample is low. Unlike traditional agarose gel electrophoresis, CE provides precise measurement of amplified DNA fragment sizes while eliminating distortions like band shifting and inconsistencies between different gels. Additionally, CE superior resolution enables distinguishing PCR products differing by as little as a single base pair. These advancements enhance analytical accuracy, proving particularly valuable for detecting tandemly duplicated regions, which may be nearly identical in length to non-duplicated (wild-type) alleles.[24]

The findings highlight these limitations. One patient was initially classified as homozygous (*FLT3-ITD/ITD*) by gel electrophoresis because of the apparent absence of a wild-type band, which was later correctly identified by CE as heterozygous (*FLT3-ITD*/WT), displaying two distinct peaks, one for the mutant allele and another for the wild-type allele.

It is crucial to differentiate between homozygous and heterozygous *FLT3-ITD* mutations because homozygosity is linked to a more aggressive progression of the disease, higher relapse rates, and worse prognosis than heterozygous or wild-type cases.[25] The *FLT3-ITD* allelic burden plays a pivotal role in disease outcomes, with patients lacking or having low levels of the wild-type allele exhibiting particularly poor survival rates.[26]

AML treatment achieves complete remission in 60–80% of patients with induction therapy, Over recent decades, advancements in targeted therapies and supportive care have significantly improved the 5-year survival rate for de novo AML, increasing from 13% to 55%.[27,28] Therefore, a highly accurate *FLT3-ITD* detection method is required for optimal risk stratification and treatment planning of AML.

When conducting gel electrophoresis, careful analysis of FLT3-ITD testing outcomes is crucial. This technique requires visual examination of band patterns to draw conclusions, and since human judgment is involved, there remains an inherent risk of inaccuracies in result interpretation. In present study, the wild-type band was not observed on gel electrophoresis but was clearly detected using CE. When the gel electrophoresis band is very faint, it is difficult to determine by visual inspection whether it represents a typical band for *FLT3*-ITD. CE provides higher resolution and consistent measurement of amplicon sizes, enabling the detection of subtle differences between mutant and wild-type alleles.[29] Therefore, it is essential to conduct fragment analysis during screening for *FLT3-ITD* to ensure accurate mutation detection and minimize misclassification. This issue is particularly pertinent in the Iraqi clinical context, where many laboratories continue to rely on PCR followed by gel electrophoresis for *FLT3-ITD* detection, primarily because of financial and logistical constraints. A study involving

25 Iraqi patients with acute lymphoblastic leukemia used conventional PCR techniques for *FLT3-ITD* detection, identifying mutations in 8% of cases. The study recommended the incorporation of advanced and potentially more sensitive methods, such as CE, to improve diagnostic accuracy.[30] Additionally, a comparative study found that CE detected *FLT3-ITD* mutations at lower allele ratios, reducing false negatives.[24] Given these advantages, integrating fragment analysis into routine AML diagnosis in Iraq is crucial for more precise risk stratification and treatment decisions.

Although fragment analysis has not yet been widely adopted in Iraq, it provides critical quantitative data on the mutant-to-wild-type AR, which has significant prognostic implications. High AR is associated with aggressive disease and poor outcomes. The European Leukemia Net (ELN 2017) set an AR cutoff of 0.5, but the revised ELN guidelines now classify all *FLT3-ITD* mutations as intermediate-risk, leaving the clinical significance of AR under debate.[31]

The current study found that 50% of *FLT3-ITD*-positive patients had high AR, consistent with Zhou et al., who reported 51.1% high AR cases, while Kivioja et al. found a high AR in only 36% of patients.[32,33]

Regarding AML subtypes (FAB classification), we found no *FLT3-ITD* mutations in M7 cases, whereas M5 exhibited the highest mutation frequency (40%). This aligns with Fahed et al. and regional studies by Hamed et al. and Sarojam et al., who reported *FLT3-ITD* prevalence rates of 50% and 25.4% in M5 cases, respectively.[20,21,34] The M2 subtype had the second highest mutation rate (20% and 23.9% in different studies), and these variations were potentially influenced by sample size and ethnic diversity.

The findings revealed a significantly higher frequency of *FLT3-ITD* mutations in females (90% of *FLT3-ITD* positive cases) than in males. This sex-related difference in incidence, molecular presentation, and outcomes is being increasingly recognized. Studies have shown that *FLT3-ITD* mutations are more prevalent in females with co-occurring mutations in NPM1 and DNMT3A, in contrast, males are more predisposed to possess supplementary mutations in splicing of RNA and methylation modifier genes. These molecular differences may contribute to variations in disease progression and treatment response.[35] The exact mechanisms underlying these sex-based disparities remain poorly understood, but may involve hormonal influences, environmental stressors, or socioeconomic factors. Further research is needed to elucidate the impact of sex on *FLT3-ITD* driven AML and develop sex-specific therapeutic strategies.

Study limitations

The research has several constraints, such as a comparatively limited participant pool and a single-site methodology, which could reduce the broader applicability of the outcomes. Although fragment analysis and allelic ratio assessment were performed for *FLT3-ITD* detection, sequencing of *FLT3-ITD* mutations was not conducted. Future studies should incorporate sequencing techniques to characterize ITD length, insertion sites, and sequence variations, which may have prognostic significance. Furthermore, collaborative research across multiple institutions involving expanded patient groups, along with combined genetic and RNA expression profiling, may enhance the understanding of both molecular mechanisms and clinical implications of FLT3-ITD mutations in Iraqi AML patients.

Conclusion

This study provides critical insights into the prevalence and clinical implications of *FLT3-ITD* mutations in AML patients in Iraqi, emphasizing the need for precise molecular diagnostic approaches. This finding revealed a distinct subset of AML patients harboring *FLT3-ITD* mutations, with a significant proportion exhibiting elevated allelic ratios, reinforcing their prognostic relevance. A striking sex-based disparity was observed as these mutations were predominantly detected in female patients, suggesting potential sex-specific molecular pathways in AML pathogenesis that merit further investigation. CE has been found to provide better resolution than conventional gel electrophoresis, especially in ambiguous cases, and to distinguish between heterozygous and homozygous mutations. This methodological advancement is critical for improving risk stratification and therapeutic considerations, including targeted therapies and transplantation eligibility. These findings were in line with mutation frequencies reported elsewhere, but highlight the need for standardized, high-sensitivity diagnostic protocols in clinical practice, which should help to reduce uncertainties in molecular diagnostics.

Declarations

Funding

This study received no funding.

Author contributions

Conceptualization, S.A.L. and I.A.A.; Methodology, S.A.L.; Software, S.A.L.; Validation, S.A.L. and I.A.A.; Formal Analysis, S.A.L. and I.A.A..; Investigation, S.A.L. and I.A.A.. Resources, S.A.L.; Data Curation, S.A.L. and I.A.A.; Writing – Original Draft Preparation, S.A.L.; Writing – Review & Editing, S.A.L. and I.A.A.; Visualization, S.A.L.; Supervision, I.A.A.; Project Administration, I.A.A.; Funding Acquisition, S.A.L.

Conflicts of interest

The author declare that they have no competing interests.

Data availability

Relevant datasets from this investigation are available from the corresponding author upon substantiated request.

Ethics approval

The study protocol, along with the participant information sheet and consent form, was reviewed and approved by the Hematology Center of Medical City, Baghdad, Iran. Ethical approval was granted (document number: 18982) on May 22, 2023.

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