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Detection of Nucleophosmin (NPM-1) and FLT3-ITD mutations in 30 Iraqi pediatric acute myeloid leukemia patients

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Abstract

Background Mutation within the FLT 3 and NPM 1 genes ranked within the most frequent recurrent known genetic markers in acute myeloid leukemia (AML) and show apparently opposite prognostic significance.

Objective To detect the frequency of FLT3-ITD and NPM-1 mutations in Iraqi pediatric AML patients using conventional polymerized chain reaction (PCR), and to relate their prevalence with the clinical presentation and the response to induction therapy.

Methods A prospective study of 30 children presented with AML and 16 children who were age and gender matched served as negative control for the mutation. AML cases were classified according to FAB classification. WBC count, platelet count and hematocrit were measured at diagnosis and after 30 days. Molecular analysis was done on peripheral blood or bone marrow aspirate samples by conventional PCR technology.

Results FLT 3-ITD mutation was detected in 3/30 (10%) patients, whereas NPM1 mutation was detected in 4/30 (13.33%) patients. Both mutations were detected in older age patients and predominantly in male. No significant correlation between each mutation and various hematological parameters, however WBC count was significantly higher in FLT-ITD unmutated patients. FLT3-ITD mutation was detected in M3 and M3 variant whereas NPM-1 mutation was detected in M2 and M3v. The three patients having FLT-3-ITD mutation (100%) did not achieved complete hematological remission, whereas 3/24 (12.5%) patients without the mutation did not achieve remission. On the other hand 2 out of 4 (50%) patients with NPM-1 mutation had not achieved complete hematological remission and 4/22 (18.18%) patients without mutation did not achieve remission. Finally regarding the interrelation between the two mutations, the two children who had NPM1 mutation and no FLT3-ITD mutation had achieved complete remission on induction therapy whereas the three children who had FLT3-ITD mutation with or without NPM1 mutation did not achieved complete remission.

Conclusion Prevalence of FLT3-ITD and NPM-1 mutations in Iraqi pediatric AML patients is comparable to that recorded worldwide and both mutations were observed in older age children and mainly in male. FLT3-ITD mutation unlike NPM-1 mutation associate with poor response to induction therapy and the adverse effect of FLT3-ITD mutation overcome the favorable effect of NPM-1 mutation when they exist together.

Keywords Pediatric AML, Flt3-ITD mutation, NPM1 mutation, PCR

Introduction

Acute myeloid leukemia (AML) or acute non lymphocytic leukemia (ANLL) is group of marrow-based neoplasm that has clinical similarities but distinct morphologic, immunophenotypic and cytogenetic features, with wide spectrum of molecular genetic abnormalities [(1)]. In Iraq leukemia ranks the 1st cancer among the commonest ten childhood cancers according to Iraqi Cancer Registry 2008 with an incidence of 32.59%. Moreover, 85% of

Iraqi children with myeloid leukemia fall within the age group 5-14 years. AML constitutes 16.35% of all types of leukemia in all age groups [2]. Childhood leukemia rate have more than doubled over the last 15 years especially in the southern of Iraq and its rate are higher than that found in nearby countries, European Union and United States [3]. Generally AML account for about 15% of childhood leukemia and for approximately 80-90% of acute leukemia in adult [4].

In recent years several recurrent molecular markers were identified that allowed further sub classification and prognostic predictions in the vast majority of AML patients especially those with normal karyotype [5]. Of those markers, FLT3 and NPM1 aberrations were the most frequent genetic lesions and they show apparently opposite prognostic significance [6].

Fms-like tyrosine kinase (FLT3) is a cell surface tyrosine kinase [7] with important role in hematopoietic stem/progenitor cell survival and proliferation [8]. Length mutation/internal tandem duplications with the insertion of hundreds of nucleotides in the juxtamembrane domain-coding sequence of FLT3 gene (FLT3-ITM) has been found in approximately 20-25 % of adult [9] with lower prevalence in pediatric AML worldwide which may reach approximately 12% [10].

Nucleophosmin (NPM) is a molecular chaperone that highly express in proliferating cells than quiescent ones and increase in response to mitogenic stimuli [11]. It has a proliferative and growth suppressive role by maintaining genomic stability, modulate the function of other tumor-suppressor transcription factors, regulate the function and stability of various nuclear proteins and promote the biogenesis of the ribosome [12]. NPM can function both as oncogenes and oncosuppressors.

NPM1 mutation result from insertion or combined insertion and deletion in one allele of NPM1. It is found in about 30 % of all adult AML [13] whereas it is much less common in childhood AML where in many study it occur in 8-12% [14,15]. There is a close association between these two markers, so that the incidence of FLT3-ITD mutation is increased in AML with NPM1 mutation, and they are seen twice as often in this group as compared with AML having wild type NPM1 [16].

Methods

This prospective study was conducted on 46 subjects including 30 children with AML and 16 children with benign reactive bone marrow aspirates which served as technical negative control for the mutation. Those patients were attending the Child Welfare Teaching Hospital from January 2011 to October 2011 and they were referred from different governorates of Iraq. This research was approved by the ethical committee at the College of Medicine, Al-Nahrain University Baghdad-Iraq, and informed consents, were obtained from all participants.

Patients were selected randomly in relation to age and sex. All patients were diagnosed as *de novo* AML and 26 out of 30 patients were newly diagnosed, whereas 4 patients were in relapse; all patients presented at diagnosis and were off treatment whether they were newly diagnosed or in relapse. From each patient and control subject peripheral blood sample and bone marrow (BM) aspirate were collected in 2 EDTA tubes. One ml of peripheral blood was used for analysis of hematological parameters by automated hematology analyzer (Sysmex KX-2N) in Al-Kadhimiya Teaching Hospital Laboratories. Of the 1 ml BM aspirate, 0.5 ml was kept in deep freeze (-70 ^{o}C) until the day of DNA analysis and the other 0.5 ml was equally divided into 2 eppendorff tubes, each contain 1 ml triozl reagent, mixed well and kept in deep freeze at -70 C until the day of RNA analysis. Peripheral blood and bone marrow aspirate smears were prepared and stained with leishman stain and special stains Sudan Black B and Periodic Acid Schiff using the standard procedures for staining [17] and were examined by two hematology consultants for diagnosis of AML and their sub-classification according to French-American-British (FAB) classification. Molecular analysis was performed in the Microbiology

Department/Al-Nahrain Medical College. The induction chemotherapy received by the patients consist of doxorubicine (Adriamycin) and cytosine arabinoside (Ara-C), while patients with M3 subtype received ATRA with daunorubicin and prednisolone. The initial response to induction chemotherapy was assessed in each patient after 30 days whether there is complete hematological remission (CR), treatment failure, or early death. Complete remission was defined as apparent recovery of hematopoiesis with < 5% blast cells on aspirate and near normal peripheral blood counts(hemoglobin > 10.0g/dl, neutrophil counts > 1.5 x10^9/l and platelet count > 100x10^9/l) [18].

For detection FT3-ITD Mutation**,** high molecular weight DNA was extracted according to the kit protocol (Promega) following the instruction manual [19]. All samples were analyzed for FLT3 mutation on chromosome 13, exon 11 using conventional PCR. The use of exon 11 specific primers allowed covering the whole juxtamembrane and the first part of tyrosine kinase-1 domain where most of the reported mutations are located [20].

Fifty to 100 ng of DNA(5 µl)was amplified in a 50 µl reaction mixture containing 1.5 mM MgCl, 50 mM KCl, 200 µM for each deoxyribonucleotide triphosphate (dNTP), 2.5 units Taq polymerase, 40 picomol of each primer which have the following sequences (Forward Primer **11F: 5'-CAATTTAGGTATGAAAGCC-3',** Reverse Primer **12 R: 5'-CAAACTCTAAATTTTCTCT-3'**). A positive reaction was assessed in duplicate and a negative control was included in each reaction. PCR amplification was performed using PCR Thermal cycler (Eppendorf Master cycler, France).Amplification process consisted of 40 cycles of 30 sec at 94 ^oC for denaturation. 45 sec. at 50 ^oC for annealing, 1 minute at 72 ^oC for extension and 1 cycle of 7 minutes at 72 ^oC for the final extension. (20) Twenty µl of the PCR product was electrophoresed on 2.5% agarose gel (Promega), using 100 bp DNA ladder (Promega) as molecular weight marker and was stained with ethedium bromide (Promega).

For detection of NPM1 mutation, total RNA was extracted from bone marrow cells using bioZOLTM-G RNA Isolation Kit (bioWORLD-US) following the instruction manual of the kit [21]. To express different types of NPM1 mutations on chromosom 5 exon 12, Single Strand Confirmatory Polymorphisim-Reverse transcriptase-Polymerase Chain Reaction (SSCP-RT- PCR) was used. Approximately 1 µg of RNA and 5 picomoles of each primer **"NPM-F, 5_-ATCATCAACACCAAGATCA and NPM-R, 5_-CATGTCTGACCACCGCTACT 3_** were added to 0.2 ml lyophilized reaction tube (Single step *Accu power*® RocketScript RT/PCR Premix Kit (BiONEER-Korea) and the volume was completed to 50 µl using nuclease free water. For the negative control tube, nuclease free water was added instead of the template RNA, whereas for the positive control tube 1 µg of RNA extracted from OCI-AML3 cell line was added. The PCR reaction conditions adopted by Brown et al study [12] was applied (table 1) and PCR product was visualized by electrophoresis on 3% agarose gel (Promega, US).

Table 1. PCR reaction for NPM1 mutation analysis

Step	Temperature	Time	Cycles
c DNA synthesis	42 ^oC	60 min	1
Inactivation	95 ^oC	5 min	1
Pre-denaturation	94 ^oC	3 min	1
Denaturation	94 ^oC	45 sec	35
Annealing	57 ^oC	1 min	
Extension	72 ^oC	1 min	
Final extension	72 ^oC	10 min	1

The data were analyzed using SPSS program (Statistical Package for Social Sciences) version 16 and Microsoft Office Excel 2007. Numeric data were expressed as mean±SE or SD, frequency was used to express discrete data. Student T-test was used to analyze numeric data while Chi-square was used to analyze discrete data, and Fischer Exact test was used when Chi square test was not valid. P-value was considered significant when it was less than < 0.05.

Results

In this prospective study both patients and control group were age matched ($P > 0.05$). The age of AML patients ranged between 1.5-12 years, with mean of 6.82±3.78 (Mean ± SD) and male to female ratio was 1:1. Whereas the age range of control children was 1-12 years and the mean age was 6.48±4.64 with male to female ratio of 2.2:1 ($P > 0.05$) .The most common FAB subtype in pediatric AML patients included in this study, was M3 (M3 and M3v) which constitute 43.33% of the cases followed by M2 which constitute 36.67% of the cases, lastly M1 and M5 each was detected in 10% of cases.

FLT3-ITD mutation was detected in 3 out 30 pediatric AML patients (10%), by applying conventional PCR on patients and control cases. The amplified DNA product of the wild type (i.e.) unmutated, was approximately 133 bp band whereas the mutated type showed additional band > 133bp (approximately 180 bp) as shown in figure 1. All the control children enrolled in this study showed wild type FLT3 gene and they served as negative control for the mutation.

NPM1 mutation was found in 4/30 cases (13.33%) by applying Single Strand Confirmatory Polymorphisim-Reverse transcriptase-Polymerase Chain Reaction (SSCP-RT- PCR) on extracted RNA. The mutated cases showed hetero duplex formed from mutant allele and wild type allele, both presented as 2 bands, the first band was approximately 550 bp, whereas the second band was approximately 320 bp. Patients negative for NPM1 mutation and control children did not showed hetero duplex formation (i.e.) they showed wild type; The OCI/AML3 cell line which was used as positive control had showed hetero duplex formation on agarose gel electrophoresis (Figure 2).

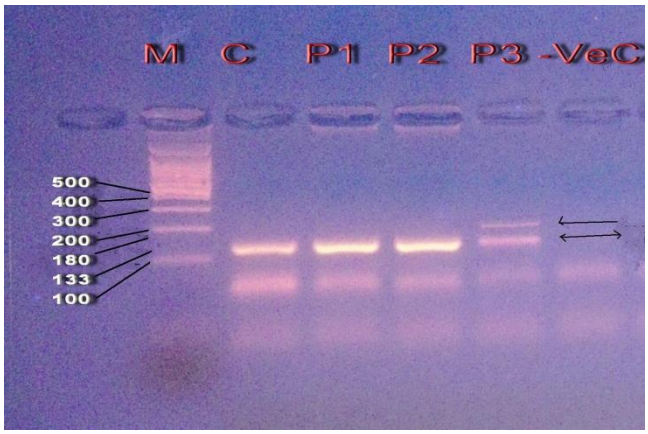

Fig. 1. PCR detected FLT3-ITD mutation. Lane C: Amplified product from healthy control. Lanes P1, P2: amplified product from patients' wild type (app.133 bp, double head arrow). Lane P3: amplified products from patients show extra mutated band (app180 bp arrow) of FLT3-ITD. Lane –VeC: negative control (no template). M: Molecular weight marker (DNA ladder). Electrophoresis was carried in 2.5% agarose gel at (4V/cm) for 60 min.

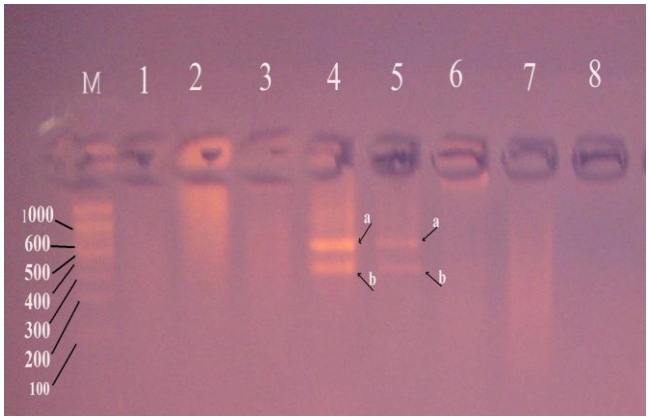

Fig. 2. Detection of NPM1 mutations using Single Strand Confirmatory Polymorphism- RT-PCR in pediatric AML patients. Lanes 1 and 2: control children show absence of hetero duplex formation. Lanes 3, 6, and 7: amplified products from AML children showed absence of hetero duplex formation. Lane 4: positive control OCI/AML3 cell line show hetero duplex formed from mutant and wild type alleles appear as 2 bands (band a app.550 and band b app. 320 bp). Lane 5: mutated AML patients show hetero duplex formation from mutant and wild-type allele of NPM1 gene, arrows a and b. Lanes 8: negative control (no template). M: Molecular weight marker (DNA ladder). Electrophoresis was carried in 3% agarose gel at (4V/cm) for 120 min.

Although equal numbers of male and female were included in the study, the FLT3-ITD mutation was found only in male and NPM1 mutation was predominantly in male M/F ratio 3/1. The mean age of patients was not significantly higher in mutated than non-mutated patients. Regarding FAB classification, the three FLT3-ITD mutated cases were detected only in M3 and M3v, whereas one case carrying NPM1 mutation was detected in M2 and the other three cases were detected in M3 and M3v subtype (Table 2 and 3).

The current study showed that there was no significant correlation between the presence or absence of these two mutations in relation to hematological parameters as shown in table 2 and 3. Although mean peripheral blood and bone marrow malignant cells percent were higher in FLT3 mutated compared to non-mutated patients but both correlations did not reach the level of significance. Also mean peripheral blood and bone marrow malignant cells percent in NPM mutated patients were higher compared to that of non-mutated and this relation was significant in the former but not in the latter. The three FLT3 mutated cases were newly diagnosed and *de novo*-AML cases. To assess the response to induction therapy regardless of the regimen used , the three children with FLT3-ITD mutation (100%) did not achieved complete hematological remission on induction therapy, whereas 4/23 (12.5%) patients without the mutation did not achieved complete hematological remission (Table 2).

Regarding NPM1 mutation, the four mutated patients were newly diagnosed and *de novo*- AML cases. Regardless to the regimen used, 2/ 4 (50%) patients having NPM1 mutation did not achieve complete remission and 4/22 (18.2%) without the mutation did not achieved complete remission (Table 3). Table 4 showed the correlations between the two mutations, it revealed that two patients had both mutation (group 1), two patients had only NPM1 (group 2), one patient had only FLT3-ITD (group 3) and 25 patients did not have any mutations (group 4). Most of these mutations were detected in male (4:1). Moreover 4/5 (80%) of those mutated cases were of M3 subtype. All other hematological parameters were reduced particularly in group 3 whereas peripheral blood and bone marrow malignant cells percent were increased in all AML cases particularly in group 1. The current study revealed that patients harboring FLT3-ITD mutation with or without NPM1mutation, in group 1 and 3 did not achieved response to induction therapy whereas the two patients harboring only NPM1 mutation in group 2 had achieved complete response to induction therapy.

Table 2. Demographic characters and laboratory features of mutated and non-mutated FLT3-ITD pediatric AML patients

Clinical Presentation		FLT3-ITD - ve N = 27	FLT3-ITD +ve N = 3	N	%	*P*-value
Gender	**Male**	12	3	15	50	0.224
	Female	15	0	15	50	
Age (years)		6.72 ± 2.84	9.25 ± 2.07	30	100	0.408
FAB subtype	**M1**	3	0	3	10	
	M2	11	0	11	36.67	
	M3	7	2	9	30	
	M3v	3	1	4	13.33	
	M5	3	0	3	10	
WBC count $X10^9$/L		42.32 ± 10.35	11.55 ± 4.02	30	100	0.039*
Platelet count $X10^9$/L		25.67 ± 5.27	19.00 ± 6.94	30	100	0. 743
Hematocrit %		21.73 ± 1.37	18.00 ± 2.44	30	100	0. 486
Peripheral blood blast %		35.78 ± 5.36	50.50 ± 15.38	30	100	0.491
Bone marrow blast %		56.94 ± 3.80	64 ± 11.42	30	100	0.638
Response*	**Remission**	24	0	24	76.67	0.009*
	Failure	3	3	6	23.33	

* Response to induction therapy

Table 3. Demographic characters and laboratory features of mutated and non-mutated NPM1 mutation pediatric AML

Clinical Presentation		NPM - ve N = 26	NPM +ve N = 4	N	%	P-value
Gender	**Male**	12	3	15	50	0.602
	Female	14	1	15	50	
Age (years)		6.79 ± 0.75	7.00 ± 2	30	100	0.753
FAB subtype	**M1**	3	0	3	10	
	M2	10	1	11	36.67	
	M3	8	1	9	30	
	M3v	2	2	4	13.33	
	M5	3	0	3	10	
WBC count $X10^9$/L		41.35 ± 2.63	27.26 ± 7.79	30	100	0.354
Platelet count $X10^9$/L		24.11 ± 1.14	30.00 ± 12.52	30	100	0. 563
Hematocrit %		21.85 ± 1.80	18.56 ± 1.23	30	100	0. 362
Periphral blood blast %		32.00 ± 1.26	67.00 ± 3.78	30	100	0.037
Bone marrow blast %		56.00 ± 1.96	67.00 ± 3.32	30	100	0.453
Response*	**Remission**	22	2	24	80	0.169
	Failure	4	2	6	20	

* Response to induction the

Table 4: The interrelation between FLT3-ITD and NPM1 mutations in pediatric AML patients

Feature		G1 (NPM+ & FLT3+)	G2 (NPM+ & FLT3-)	G3 (NPM- & FLT3+)	G4 (NPM- & FLT3-)
Number		2	2	1	25
Gender	**Female**	0	1	0	14
	Male	2	1	1	11
Age		11.00±3.00	5.00±2.00	7.50	6.93±0.98
FAB subtype		M3	M2, M3	M3	M1(3), M2(10), M3(9), M5(3)
WBCX10^9/L		16.40±2.50	32.70±2.30	6.70	43.52±4.10
Platelet $X10^9$/L		8.00±3.20	41.00±3.00	30.00	23.75±6.19
Hematocrit (L/L)		21.00±0.22	17.35±0.35	15.00	22.28±1.86
Peripheral blood malignant cells %		73.00±3.00	64.00±4.00	28.00	32.25±6.89
BM malignant cells (%)		78.00±5.00	61.50±6.50	50.00	56.37±5.20
Response*	**Failure**	2	0	1	4

All means were express as mean ± SD

* Response to induction therapy

Discussion

In this study FLT3-ITD mutation was detected in 3 out 30 pediatric AML patients (10%), this result was in agreement with many studies [8,10,22,23], and was in line with Zaker *et al* study from Iran who had reported that the frequency of this mutation in Iranian pediatric AML patients was 7.7% [24]. This study showed that the three patients with FLT3-ITD mutation were male in spite the male:female ratio of the patients included in the study was 1:1, this male predominance was in agreement with many studies and may add an adverse prognostic effect to FLT3-ITD mutation [10,22-25]. Furthermore those patients were older than patients without mutation (p>0.408). Meshinchi *et al* [22] had showed that there was a stepwise increase in the prevalence of FLT3/ITD by age as the prevalence of FLT3/ITD increased from 1.5% in infant AML to 7% for patients aged 1 to 5 years

to nearly 17% in patients aged 10 to 20 years. Such an age-associated increase in prevalence may offer clues to the pathology of FLT3/ITD in AML. FLT3/ITD is considered a cooperating event in the evolution of AML, so that an early molecular event (ex, translocation) may occur in a minor clone leading to maturation arrest. This subpopulation may remain quiescent until such a time when FLT3/ITD is acquired. Such a time-dependent process provides a proliferative advantage and subsequent evolution of AML in the preleukemic clone [22].

FLT3-ITD mutations was detected in M3(M3&M3v) subtype patients, this was in agreement to other studies [10,23-26], however Meshinchi et al [22] and Chang et al [10] had reported that there was no predominance of a particular FAB class in Flt3-ITD + cases. The mean WBC count at the time of diagnosis of those patients with FLT3-ITD mutation was significantly lower than that in patients without mutation, which is expected since FLT3-ITD mutation was detected in M3 and M3v and M3 subtype usually present with lower WBC count, added to this the small sample size [27]. This result differs from that of Meshinchi et al who reported that WBC count at diagnosis was higher in mutated than unmutated cases [22]. Furthermore, the mean malignant cells percent in peripheral blood and bone marrow in patients with FLT3-ITD mutation was non-significantly higher than in patients without this mutation which was in agreement to many studies [22,25]. This was explain by Piacibello *et al* who had propose that FLT3 expression may play a role in the survival or proliferation of leukemic blasts, and that FL (FLT3 Ligand) may induce dose-dependent proliferation of leukemic blasts [28].

Those patients with FLT3-ITD did not achieve remission on induction (0%) as compared to non-mutated cases where 24/30 (80%) had achieved remission, this result was confirmed by other studies [22,23,25] who had stated that the presence of the FLT3/ITD was a significant prognostic factor for induction failure or relapse.

In the current study, NPM1 mutations were found in 4/30 (13.33%) of cases, which was comparable with other studies in which the incidence of NPM1 mutations ranges between 8-12% [12,29-31]. Furthermore the mean age of children harboring NPM1 mutations in this study was higher than those without the mutation (P = 0.753) [12,23], Brown et al had stated that the mutation appears not to occur in children younger than 3 years. On the other hand the incidence of the mutations is high in adult where It ranges between 25.4-41 % as stated by many studies [15,23,31,32]. These epidemiologic data suggest that the risk of acquiring a mutation such as NPMc+ or FLT3/ITD in a myeloid stem/progenitor cell is cumulative and the latency between the acquisition of NPMc or FLT3/ITD and the acquisition of the cooperating mutation(s) for development of AML may be on the order of years. An alternative explanation is that the myeloid stem/progenitor cells in young children are relatively resistant to the acquisition of NPMc+ or FLT3/ITD mutations [12].

In the current study NPM1 mutations were detected mainly in male where male/female ratio was 3/1. Other studies found that there was no significant difference in the prevalence of NPM1 mutations among sexes [29,33]. Whereas, other studies reported that there was a significant presence of NPM1 mutation in female (12, 14).This difference may be due to smaller sample size.

Regarding the frequency of NPM1 mutation within the FAB classification, one of the four mutated children was of M2, two of M3V and one of M3 subtype. Brown et al [12] had stated that NPM1 mutations have been found in all FAB morphologic subtypes of AML but to less extend in M3 and rare in M5. Thus we may propose that the detection of the mutation in M3 and M3v reflect the high no. of M3 and M3v patients (43.33%) included in this study and the small sample size. Regarding the relation of NPM1 mutations to the hematological parameters, children with NPM1 mutations had lower WBC count at diagnosis than that in patients without mutations, (P = 0.354) which was in line with Brown et al (12)who stated that the median white blood cell count at diagnosis did not

significantly differ between NPMc+ and NPMc- cases. The mean platelet count was higher in mutated patients (p = 0.563) which was in line with Rau and Brown et al [34] and Cazzaniga *et al* [29]. Chou *et al* [35], found non-significant difference in the platelet count between mutated and non-mutated patients. Hematocrit % was lower in mutated patients as compared to that in patients without mutation, (P = 0.362) which was expected since three of the mutated cases were of M3 and M3v subtype. This conflicted correlations between mutated and non-mutated patients due to that the exact role of NPMc+ in leukemic transformation is still unknown and more research is needed to clarify how mutated NPM1 promotes leukemogenesis and how it interacts with other mutations, and why it confers a more favorable prognosis [34]. The mean peripheral malignant cell percent was significantly higher in children with mutations than in children with wild type NPM1 (P = 0.037) and the mean BMA malignant cell percent in mutated cases was higher than non-mutated cases (P = 0.453), this result was in agreement with previous studies [12,29,33,34]. The 4 NPM1 mutated pediatric patients were newly diagnosed and *de novo* cases .This result was in agreement with other studies [12,34].

The interrelationship between those two mutations was shown in table 4, Flt3-ITD mutation was detected in 2 out of 4 cases harboring NPM1 mutation , this was similarly observed in many studies [12,31,33,34] which stated that *NPM1* mutations and FLT3 ITD mutations frequently occur together, therefore one might speculate that the two cooperate to cause leukemic transformation. However, a mechanistic link has yet to be established [34,35]. Furthermore both those patients did not achieve early remission on induction therapy which clarifies the adverse effect of FLT-ITD which overcomes the favorable effect of NPM1 mutation [34]. In the current study the two children having NPM1 mutations and no FLT3-ITD mutations had achieved complete remission on induction, this result is supported by Brown *et al* (12) study who had found that within the FLT3/ITD- subgroup, the presence of NPMc+ is associated with a near doubling of 5-year EFS (event-free survival) (from 35-69%), and a greater than 50% increase in5-year OS (overall survival) (from 51-77%) and they had suggested that the NPMc+, FLT3/ITD-negative subset of childhood AML may be prospectively identifiable as a favorable risk group, similar to patients with favorable risk cytogenetics (t(8;21) and inv [16]. The same results were observed by Falini *et al* [31]. Further in the present study , patients who had FLT3-ITD mutation whether they had NPM1 mutation or not , did not achieved remission on induction therapy which clarify the negative prognostic effect of Flt3-ITD mutation within NPM1 + and NPM1- subgroups. This result was supported by study of Rau and Brown (34) Boonthimat *et al* [36] and Brown *et al* [12] who had suggested that there was a possible pathogenic link between these two gene mutations in a way that the FLT3-ITD adverse effect will overcome the favorable effect of NPM1 mutations. Finally within the FLT3/ITD-negative subgroup there was a trend toward improved EFS and 5 years OS, for NPMc positive patients compared with the NPMc negative patients i.e., patients having NPM1c +, had an advantage over those having FLT3-ITD+ or lacking NPM1 [12].

We may conclude that in this pioneer study, the incidence of FLT3-ITD mutations and NPM1 mutations in pediatric AML was 10% and 13.33% respectively in a sample of Iraq patients and both mutations tend to appear in older age group (>3 years). Patients having NPM1 mutation had better response to induction therapy over those harboring FLT3-ITD mutations whether they were NPM1c+ or NPM1 c-.

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