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Research Article

Impact of Cytogenetic and Molecular Genetics on the Outcome of Pediatric Acute Myeloid Leukemia: In KFSH-Dammam

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ABSTRACT

Objectives: The primary aim of this study is to study cytogenetic/molecular impact on the outcome of children diagnosed and treated for acute myeloid leukemia in KFSHD, from the year 2008-2018. Secondarily to review the clinical presentation of AML in our study population and evaluate the phenotype-genotype impact on the outcome. Assess the risk stratifications. The management and response to therapy were assessed, and the complications and relapse, and overall survival rates were identified.

Design: This is a retrospective cross-sectional study of all the pediatric patients below sixteen-year-old who diagnosed with acute myeloid leukemia and treated by chemotherapy alone or with allogeneic stem cell transplantation as well, in king Fahd specialist hospital in Dammam, pediatric haematology/oncology department in a decade between 1st January 2008 till end of December 2018, our study included 56 cases.

Setting: It is a single center study at King Fahad Specialist Hospital in Dammam which is 400 beds tertiary referral hospital with 27 beds pediatric oncology Ward, 4 beds bone marrow transplant and 18 bed pediatric oncology day care services.

Methods and Results: After obtaining the IRB approval, all data and information of patients were retrieved from patients' hard files and electronic medical records. Data analysis were done by using Statistical Package for the Social Sciences (SPSS) program version and stored in the Redcap system for confidentiality.

Results: Within our study period we diagnosed 56 cases that are diagnosed with acute myeloid leukemia and treated in king Fahad specialist hospital in Dammam. In this study the overall survival rate was around 75%, the Event free survival rate was 67% and the relapse rate was 32%.

Conclusion: Pediatric AML is a clinically and genetically heterogeneous disease with a low incidence, variable survival outcomes, and high frequency of relapse, treatment-related deaths, and long-term side effects. Despite all the improvement in the outcome of childhood acute myeloid leukemia during the last decades, yet the current survival of pediatric AML is around 70%, with a further intensification of chemotherapy might not be feasible and safe. Next-Generation Sequencing (NGS) seems promising in the diagnosis and monitoring of MRD of acute myeloid leukemia. It may extend the horizon toward discovering more underlying responsible genetic aberrations that could play a role in developing new immunotherapeutic approaches and target therapies in the future. However, extra attention to the standardization of the methods of testing and interpretation is crucial. Because of the disease's rarity, national and international collaboration is significantly essential to provide adequate numbers of patients for further local and global researches in the biological, genetics, and clinical aspects and for testing modern targeted therapies.

Keywords: Acute myeloid leukemia, Pediatric leukemia, Childhood leukemia, Cytogenetic and molecular impact on acute myeloid leukemia, Minimum residual disease

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Pediatric AML is a rare disease affecting children with a median age of 6 years, with an incidence of seven cases per million children younger than 15 years. Children have good outcomes compared to adults because of the occurrence of better genetics aberrations and less toxic effects of chemotherapy. Complete Remission (CR) is achieved in 90% of cases [1]. AML cells (blast cells) are malignant myeloid progenitor cells that fail to differentiate, increasing in the bone marrow and invading peripheral blood and other organs, such as the central nervous system. Clonal, acquired, somatic Cytogenetic Abnormalities (CAs) are detected in 75% to 80% of pediatric AML cases [1].

In recent years, analyses of molecular and cytogenetic aberrations have revealed the heterogeneity of pediatric Acute Myeloid Leukemia (AML), which is now partially, incorporated within the World Health Organization classification and current risk stratification systems. To date, most study groups have agreed on favourable prognostic factors such as inv (16) (p13.1q22)/CBFBMYH11, or t (16;16) (p13.1; q22), t (8;21)(q22;q22)/RUNX1-RUNX1T1, t (15;17)/PML-RARA, single NPM1 mutations or double mutated CEBPA [1]. In the last three decades, the outcome of childhood AML has significantly improved with Event-Free Survival (EFS) and Overall Survival (OS) rates now 60% and 70%, respectively, due to the high relapse rate. This success is related to the efforts of international collaborative groups that adapted a risk-based treatment approach in pediatric AML with the selective use of Hematopoietic Stem Cell Transplantation (HSCT). In addition, the advances in supportive care have further contributed to the improved outcome [2].

The outcome of pediatric AML is superior in developed countries due to ease in access to care, high level of socioeconomic status, supportive care measures, or inherent biological differences. This research evaluates the clinical presentation; risk Stratifying, treatment, treatment response, and impact of cytogenetic and molecular genetics on pediatric AML and compares the results with national and international studies and to recognize factors that need work on them [3].

MATERIAL AND METHODS

The outcomes of 56 children with acute myeloid leukemia, who were treated with chemotherapy with or without stem cell transplantation in King Fahd specialist hospital in Dammam, pediatric haematology/oncology department, between 2008 and 2018 were retrospectively analysed. After obtaining the IRB approval (HAME 0318), required data extracted from patient's electronic medical records then computerized using Microsoft Excel sheet and revised instantaneously. Computerized data will be exported to SPSS (Statistical Package for Social Sciencesver.25). Frequency tables were drawn to explore the findings (frequencies, percentages, measures of central tendencies and dispersion and graphics). Cross-tabulation and ANOVA (Analysis of Variance) used to explore the magnitude of diagnostic interval and the most significant factors affecting it, and the percentages. Overall survival and event free survival were illustrated by the Kaplan Meier curves. Data stored in redcap system for confidentiality.

RESULTS

We studied the clinical characteristics of 56 patients who met the inclusion criteria as diagnosed with acute myeloid leukaemia involving the acute promyelocytic leukemia and secondary myeloid leukemia but excluding children with down syndrome who had acute myeloid leukemia from this study. We got 23 (41%) boys and 33 (58.9%) girls; most cases are below 5 years old (35.7%) with majority of patients at diagnosis were at young ages. Most of the patients (96.4%) had their AML as denovo, except for 2 cases (3.6%) were diagnosed as secondary myeloid leukaemia who are both cases of relapsed Wilms tumor and heavily treated with alkylating agents. In the front and second lines.

Regarding the AML predisposition syndromes, only 2 (3.6%) patients with one of the chromosomal instability syndromes: Fanconi anaemia was identified. Our cases had different complains at presentation with intermittent fever been noticed as the major presenting complain in 73%, followed with noticeable pallor in 62%, other symptoms occurred as shown in Table 1.

Table 1: Different symptoms at presentation.

Symptoms	Percentage (%)	Mean duration in days
Fever	73.2	15-21
Pallor	62.5	21-30
Fatigue	53.6	20-30
Mucosal bleeding	39.3	7-11
Nausea and vomiting	36	1-7
Bony aches	34	30-40
Weight loss	17.9	21-60
Headache	12	7-14
Skin nodules (Chloroma)	10.7	15-60
Seizure	1.8	1-3
Photophobia	1.8	1-3

The clinical signs were varied among the patients with hepatosplenomegaly been the most clinical finding in nearly more than half of them Table 2.

Table 2: Clinical signs at presentation.

Clinical sign	Percentage (%)
Hepatomegaly	53.6
Splenomegaly	42.9
Cervical lymphadenopathy	33.9
Inguinal lymphadenopathy	10.7
Axillary lymphadenopathy	8.9
Gum hypertrophy	12.5
Chloroma	10.7
Coagulopathy and DIC picture	7.1

The initial picture of complete blood counts for all the cases were shown on Table 3 with significant leucocytosis confirmed on 25% of the cases.

Table 3: Complete blood counts picture at presentation

Parameter	Value limit	Number of patients	Percentage (%)
WBC count (x109)	<50	34	60
	50-100	8	14
	>100	14	25
HB level (g/dl)	<10	45	80
	>10	11	20
Palettes count($X10^9$)	<50	37	66
	>50	19	33

Other lab parameters that done at initial workup setting showed 7.1% patients presented with DIC coagulopathy-like picture and none of them had frank picture of tumour lysis syndrome, probably due to effective preventive measures for candidate patients that were taken at diagnosis onset. Other abnormal laboratory findings were shown on Table 4.

Table 4: Other laboratory findings.

Abnormal laboratory test	Number of patients	Percentage (%)
Liver function	6	10.7
Elevated LDH	46	82
Elevated uric acid	2	3.6
Prolonged PT	1	1.8
Prolonged PTT	4	7.1
Elevated D-Dimer	25	44.6
Low fibrinogen	9	16.1
Deficient G6PD	1	1.8
Positive sickling test	3	5.4

With respect to the distribution for the patients as per the FAB system, we got 7 (12.5%) cases of M3 (Acute Promyelocytic Leukemia) and similar number of 8 cases under M5 (14.3%), with the majority of cases were unspecified and could not be allocated morphologically to certain French-American-British (FAB) classification System for childhood AML subgroup (26.8%) Table 5.

Table 5: FAB classification subgroups.

FAB category	Frequency (f)	Percentage (%)
M0	4	7.1
M1	5	8.9
M2	9	16.1
M3	7	12.5
M4	6	10.7
M5	8	14.3
M6	1	1.8
M7	1	1.8
Unspecified	15	26.8
Total	**56**	**100.0**

The immunophenotyping tests for most of the patients showed classical myeloid markers with apparent markers been noticed in 20 cases (35.7%) as shown in Table 6, we found that 2 cases showed apparent lymphoid markers of CD 19 and both cases has t(8,21) in their Fish studies. A further common apparent marker was the presence of CD 7 in 14 patients (25%) ,50% (7 patients) of the cases who express CD7 had leucocytosis at their initial presentation with WBC more than 100,000 and the majority of risk group is intermediate risk to high risk, and only 2 cases out of them were classified as low risk group. In addition to that about 57% of this group of patients showed disease refractory status or relapse during their course of therapy.

Table 6: Initial apparent immunophenotyping markers for the patients

Immunophenotyping marker	Number of patients	Percentage (%)
CD19	2	3
CD2	4	7
CD4	4	7
CD7	14	25

The classical Auer rods were manifested in 9 cases (16%) among them is the seven cases of APL patients. CSF examinations confirmed CNS positive cases in 5 patients (8%), with spinal chloroma detected in 1 (1.8%) case and 2 (3.6%) other cases had orbital involvement Basic imaging's done for all the cases that involved US abdomen which confirmed the organomegaly in form of hepatosplenomegaly in around 54% of the patients and initial echocardiogram were normal in the majority of the cases but only 2 (3.6%) patients showed declining of their cardiac functions ,and both of them presented with picture of severe overwhelming sepsis at presentation. The data of cytogenetic testing for our study population showed that, the most common cytogenetic abnormality was t (8:21) in 11 cases (19%), followed in second place by 14.2% which about of 8 cases with FLT 3 high ITD , Third frequent abnormality noticed was t (15;17) in 7 cases (12.5%) , INV 16 that was noticed in 4 cases (7%) ,and the rest of cytogenetic abnormality were at less frequencies as shown on Table 7.

Table 7: The cytogenetic/molecular profile of the patients

FAB category	Frequency (f)	Percentage (%)
t (8;21)	11	19
Inv. 16	4	7.1
t (15;17)	7	12.5
t (9;11)	2	3.6
t (11 q 23) MLL	2	3.6
Monosomy 7	3	5.4
Trisomy 8	1	1.8
Complex	2	3.6
NPM1	2	3.6
FLT3	8	14.2
t (7:12)	1	1.8
NORMAL	13	23.2
Total	**56**	**100**

The majority of our sample patients were stratified as low risk patients based on their cytogenetic test around 42.9%, followed by high risk patients which involved about 32.1% of the cases and lastly the intermediate risk patients which diagnosed in 25%,

since our data was started from 2008 where the criteria of risk stratification of the acute myeloid leukemia was based on the FAB system initially and the response to therapy, it was revaluated again at data analysis time as per the new system for risk stratification 2016 World Health Organization (WHO) Classification of Myeloid Neoplasms and Acute Leukemia.

Table 8: The risk stratification for the patients.

Risk	Number (N) of patients	Cytogenetic data	Total patients number (%)
Low risk	11	t (8;21)	24 (42.9%)
	4	Inv 16	
	7	t (15;17)	
	2	NPM 1	
Intermediate risk	2	t (9;11)	14 (25%)
	11	Normal cytogenic data	
	1	Trisomy 8	
High risk	2	MLL	18 (32.1%)
	3	Monosomy 7	
	2	Complex cytogenetic	
	8	FLT3/ITD	
	1	t (7;12)	
	2	Therapy-related AML	
Total	56		56 (100%)

All the patients were assigned to chemotherapy protocol as per risk category with most of the cases 49 patients (87.5%) received MRC 12/15 protocols, and APL directed therapy protocols in 7 cases (12.5%). Among the patients of the low risk group around 50% of them were given total of 4 cycles while the rest were given total of 5 cycles based on their responses and the treating consultant's decisions. The remission status were evaluated either by morphology in earlier years of the study or by Multi-color Flow Cytometry (MFC) in the latest years of the study, it was assessed in two points post cycle 1 and cycle 2 induction, and the remission status for all the cases were shown as per the risk group at the 2 evaluation points as in Table 9 and as expected we found that more than 80% of the low risk group achieved complete remission post first cycle of induction compared to less than 40% from the high risk group.

Table 9: The remission status post induction 1, 2 cycles.

Risk Group	RM_1 Number of Patients	RM_2 Number of Patients	NR Number of Patients
LR=24	20 (83.3%)	3 (12.5%)	1 (4.1%)
IR=14	11 (78.5%)	2 (15.3%)	1 (7.6%)
HR=18	7 (38.8%)	2 (10.5%)	9 (47.3%)

RM_1: Remission status post cycle 1 induction, RM_2: Remission status post cycle 2 induction, NR: Not in Remission after cycle 2, LR: Low Risk, IR: Intermediate Risk, HR: High Risk

Considering the Stem cell transplantation, it was done in total of 25 cases (44.6%) which mainly as allogeneic in 22 (88%) cases, and autologous in 3 cases (12%) where all done in CR2 and involved within the low risk group, the indication for stem cell transplantation as per risk stratification is illustrated in Table 10. One case had double BMT due to second relapse and offered the second BMT in CR2.

Table 10: The BMT data

Risk	Total patients N (%)	BMT done at CR 1	BMT done at CR 2
High Risk	13 (52%)	11(84.6%)	2(15%)
Intermediate Risk	8 (32%)	5(62.5%)	3(42.8%)
Low Risk	4 (16%)	1(25%)	3(75%)

However, the radiation therapy is not a well-established modality of therapy in treatment protocols for acute myeloid leukemia but still if can be offered for serious chloroma with pressure symptoms as it was been given for one of our patients who presented with spinal chloroma and paraplegia and also offered for the two cases of the Fanconi syndromes as part of their conditioning therapy preparation for the BMT. Evaluating the complications during therapy courses, we had around 43 cases had documented complications ranging between moderate to severe and the most frequent adverse events happened is explained in Table 11, 15 patients (26.7%) developed fungal infection of lungs or deep organs, another 5 (8.9%) cases had their blood cultures showed streptococci bacteremia and CD toxins were isolated from stool specimen for 9(16%) patients.

Table 11: The complications during therapy.

Complication	Number of patients	Percentage (%)
Septic Shock	22	39.2
Infection	43	76.7
Major Bleeds	10	17.8
Typhlitis	9	16.1
Cardiac Dysfunction	9	16.1
Pseudotumor-Cerebri	1	1.8

In our centre we adopted the policy of placing most of the acute myeloid leukaemia patients on prophylactic antimicrobial agents to minimize the risk of infections during their prolonged neutropenia, in this study we found that almost 100% of our cases were given proper PCP -prophylaxis and antifungal coverage during neutropenia, while 92.9% were given antibacterial agents during their neutropenic nadir, with no certain policy for prophylactic antiviral coverage but provided only for therapeutic reasons or as part of BMT course. Unfortunately 18 (32%) patients in our study experienced disease recurrence, with almost all of them had it as medullary relapse of the disease and the majority of them belongs to the high risk group as 7 cases out of the 18 patients (38.8%) belongs to this group experienced disease recurrence, surprisingly we got 6 patients out of the 24 cases (25%) in the low risk group also had had disease recurrence ,probably overestimated because the acute promyelocytic leukaemia was initially involved in this group in this study, again we noticed 5 case out of the 14 patients (35.7%) in the intermediate risk group had disease relapse the details of the cryptogenic features for all the relapse cases with relation to their risk stratification is illustrated in Table 12.

Table 12: The relapse data in relation to the risk and cytogenetic.

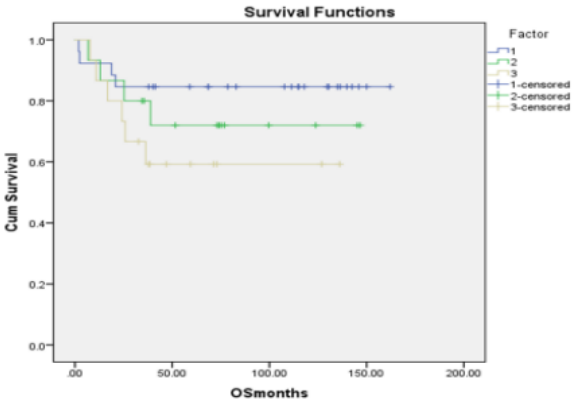
Risk	Number of Patients	Cytogenetic Features
Low Risk (6/24) 25%	1	INV 16
	2	T (8;21)
	3	T (15:17)
Intermediate Risk (5/14) 35.7%	4	Normal cytogenetic
	1	Trisomy 8
High Risk (7/18) 38.8%	3	FLT3/ITD
	2	Monosomy 7
	1	Complex cytogenetic
	1	T(AML)

We had 14 (25%) out of total cases from our study population passed away, the causes of death were identified in the mortality cases and documented as shown in Table 13 with around 60% died with disease progression from the low-risk group 2 cases (8.3%) both with t (8,21) died one with relapse and the second with bowel ischemia. Data of death from intermediate group showed that 4 cases (28.5%) passed away 2 out of them had disease relapse, nearly 8 cases (44.4%) from the high risk group died 5 out of them had disease recurrence.

Table 13: The causes of death.

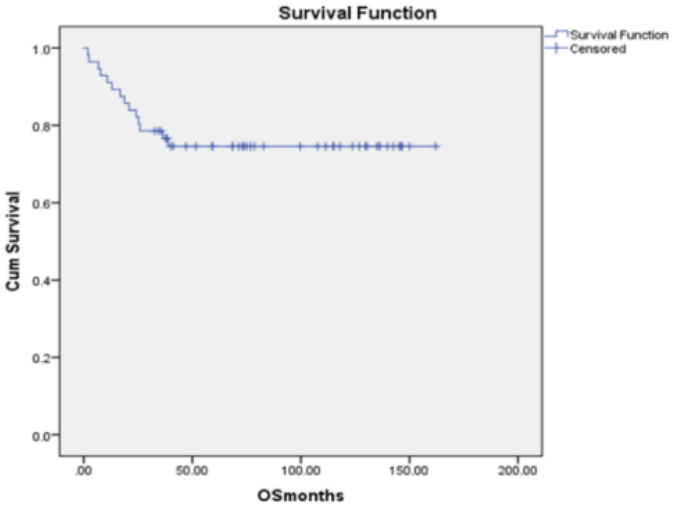
Cause of Death	Number of Patients	Percentage (%)
Sepsis While in remission	2	14.2
Disease Progression with Multi-Organ Failure ± sepsis	8	57.1
Typhlitis with Bowel Ischemia	1	7
BMT related complications	3	21.4

The overall survival varies among different risk groups with best survival as expected was on the low-risk group that was nearly above 85%, and around 70% to the intermediate risk, with the worse outcome in the high-risk group which was about 60% Kaplan Meier Curve 1.

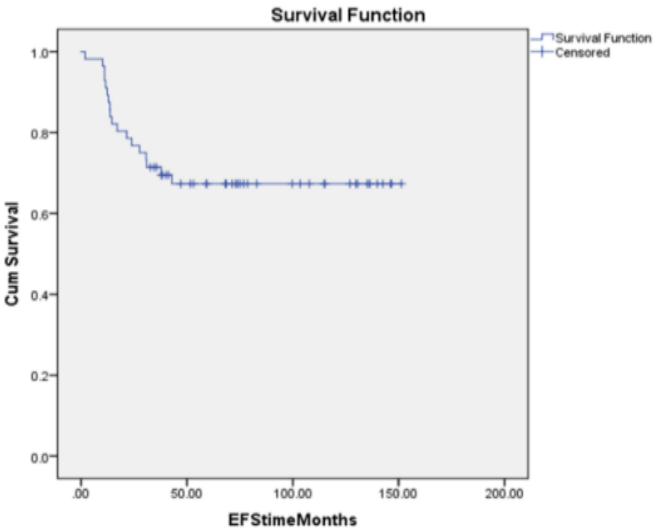


Kaplan Meier Curve 1: Overall survival as per risk groups; 1 High Risk Group; 2 Intermediate Risk Group; 3 High Risk Group.

Our Overall survival=74.6% at 162 months Kaplan Meier Curve 2, the EFS is 67.3% at 151 months Kaplan Meier Curve 3, the relapse rate as mentioned earlier as 32%.



Kaplan Meier Curve 2: Overall survival 74.6%



Kaplan Meier Curve 3: Event free survival 67.3.

DISCUSSION

Acute leukemia is the most common childhood malignancy, with Acute Myeloid Leukemia (AML) comprising 15% to 20% of it and is therefore relatively uncommon compared to Acute Lymphoblastic Leukemia (ALL), which represents approximately 80% [4]. In this study, we evaluated; the outcome of 56 pediatric patients with acute myeloid leukemia diagnosed over a decade in our center. About 96% of the cases were *de novo*. In comparison, we had only less than 4% were secondary leukemia, which is precisely like some comparative data of biologic properties and genetic abnormalities in pediatric (children and adolescents <18 years of age) and adult (age<60 years) AML by Ursula Creutzig, et al. [5] who reported that the incidence of de novo AML in the pediatric population is more than 95% compared to only 83% in adults. The incidence of secondary AML in children is 1% compared to 17% in adults. The mean age at presentation for our cases was 7.9 years however, the effect of age on prognosis in childhood acute myeloid leukemia is less clear than its effect on acute lymphoid leukemia with some studies reported that younger children were more likely to have an intermediate cytogenetic risk and but less favourable cytogenetic features, and they tend to have a high incidence of translocations involving chromosome 11 with breakpoints at 11q23 [6]. We

diagnosed around 8% of the patients to have CNS positive disease, which is around the same range that reported in the literature by Johnston DL, et al. in A Children's Oncology Group Study that incidence of CNS disease at diagnosis in pediatric acute myeloid leukemia (AML) is between 6%-29%.

On the other hand, we had around 10.7% of our cases presented with cutaneous chloroma, which is near to what was reported in another study by Kobayashi R, et al. [7], where they found that the extra medullary infiltration isolated as skin involvement in 13 (23%) of their cases, however, they stated that this extra medullary infiltration tends to be associated with higher white cell count, a higher proportion of M4/M5, and a higher incidence of inv16 and 11q23 abnormalities, but despite that the overall survival did not differ between patients with or without extra medullary infiltration [7]. In a study by Hanslik G et al., they suggested that over 30% of the pediatric patients with different types of malignancies showed abnormalities in at least one clotting parameter, with the highest risk to develop a coagulopathy which is about 77.2% [8] occur in leukemia, Our data showed that 7% (4 patients) of our acute myeloid leukemia presented with coagulopathy and DIC picture with all of them belong to the group of the acute promyelocytic leukemia which known with its propensity to cause Disseminated Intravascular Coagulopathy (DIC) [9]. As it is well known that the Final diagnosis of acute myeloid leukemia is made by confirmation of at least 20% malignant myeloid blasts in the peripheral blood and/or bone marrow and/or the presence of classic AML chromosomal abnormalities even when malignant blasts are below 20% [10]. In our study all the patients were diagnosed with blasts more than 20% infiltrating the bone marrow.

Nowadays, Multiparameter Flow Cytometry (MFC) is widely used to assess the Leukemia-Associated Immunophenotype (LAIP), to assess the cell differentiation and maturation, and confirm the diagnosis of ALL or AML based on lineage-specific markers. It can also be used to evaluate the response measurements of Minimal Residual Disease (MRD) after the therapy or for detection of an emerging disease recurrence [11]. Beside the classical AML cell markers as the MPO positivity or the monocytic differentiation some of our patients had apparent markers that known in the literature to be associate with AML, 2 of our cases with t (8,21) found to express the B-lymphoid marker CD 19, and as in a study done by K Walter, et al., they stated that the Coexpression of lymphoid and myeloid molecules is a well-known feature of Acute Myeloblastic Leukemia (AML) with t (8,21) and added that there is a positive correlation between PAX5 and CD19 expression in t (8,21)- positive AML cells and demonstrate that PAX_5 binds to the promoter and enhancer of CD19 gene and remodels chromatin structure at the promoter [12].

Around 25% of our patients demonstrated the presence of A pan T-cell antigen CD 7, which thought to be arising from primitive stem cells with multilineage potential [9], half of our cases of positive CD 7 had significant leucocytosis at their presentation and more than half of them showed disease refractory or relapse course during their therapy. In the current study, we found that the presence of apparent marker CD 7 in pediatric patients with acute myeloid leukemia might be associated with an unfavourable prognosis; reviewing the literature, we found that in one study by Giovanni Del Poeta, et al. [13], they had a nearly similar incidence of about 26% of their AML patients was positive for the CD7 antigen. They found that this group of patients tended to have a white blood cell count (WBC) greater than 100x IO'VL and showed poor results when treated with the conventional therapeutic regimens. Risk-group stratification followed by risk-based treatment carried out by most international pediatric AML groups and is now based on cytogenetic and molecular disease characteristics, together with the response to induction therapy detected by MRD [14], the molecular landscape for pediatric AML is significantly different from the adult population characterized by more frequent incidence of core-binding factor, nucleophosmin 1, and CEBPA gene mutations that comprise approximately one-third of pediatric AML, a quite higher than seen in adults [14,15]. On the other hand, the cryptic translocations are significant contributors to childhood and declines in adulthood [14,15]. Currently, the WHO considers the Cytogenetic characterization as an independent prognostic factor in both adults and children with AML. And reclassify the disease based on them into good, intermediate, and unfavourable groups. Favourable AML cytogenetic include the core-binding factor (CBF) leukaemia's t (8,21) (RUNX1-ETO) and inv (16) (MYH11-CBFB), as well as t (15;17) (PML-RARA). On a molecular level, AML with mutations in the nucleophosmin 1 gene (NPM1) or CEBP gene were more recently acknowledged as favourable aberrations, while for example, the Unfavourable cytogenetic include, for example, the complex cytogenetic (three or more distinct cytogenetic abnormalities in a leukemic clone), monosomy 7, monosomy 5, del (5q) and abnormal chromosome 3 all the remaining chromosomal considered within the intermediate-risk cytogenetic [16].

The majority of our patients in this study, about 23% have normal karyotyping and no identifiable cytogenetic aberration, which goes along with similar data by Mohamed Radhi et al. [16] that cytogenetic abnormalities are not detected in about 20% of children with AML. The most frequent detected cytogenetic in our patients was t (8,21) in about 19% of the cases, which is similar to a national study from Saudi Arabia where they found the incidence of t (8,21) is around 18.9% [17] but higher to what reported in international studies which are about 7%-16% [18]. Despite this translocation is associated with a good prognosis, yet we had around two patients out of 11(18%) belong to this group had disease relapse with one death (10%) with the disease. Interestingly in a national multicenter retrospective study from Saudi Arabia by Jastaniah et al. studying the prognosis of t (8,21) over Saudi children with AML, they concluded that this translocation carries inferior survival and resistance to salvage therapy in the local population compared to other reports from international groups [17]. Other favourable cytogenetic and molecular aberrations occur to a lesser frequency in our study population with INV 16 in (7%) which is within a range of 3%-8% that reported in a similar study by Kalliopi, N. Manola, who also mentioned this incidence is noticeably less than Chinese pediatric patients (11.6%) [18]. Unfortunately, 25% (one out of 4) of our cases with INV16 experienced disease relapse, but no death among this group. On the other hand, the nucleophosmin (NPM) gene NPM 1 mutation was found in around 3.6% of our patients. This group had excellent remission and survival in our study.

Generally, Acute Promyelocytic Leukemia (APL) represents -6% to 11% of children with AML [19]; in this study, the incidence was a little higher 12.5%, all our cases treated with ATRA based chemotherapy protocols, but later about 3 out of 7 patients (42.8%) showed disease recurrence significantly higher than what reported

in a study by Oussama Abla, et al. that the Relapse occurs in 17%-27% of children with APL [20]. All our relapsed acute promyelocytic leukemia underwent autologous BMT in CR 2 with 100% overall survival. The prognostic impact of FLT3-ITD is influenced by the allelic ratio, with higher ratios associated with poorer outcomes. Around 14% of our study population were diagnosed to have FLT 3-ITD, this incidence is near to data reported by Rhadi, et al. [16] when he mentioned that the FLT3/ITD comprising about 12% and FLT3/ALM, about 8% with the overall prevalence of FLT3 mutations was about 20%, and other similar results by Patrick Brown et al., who reported its incidence of 10%-17% in pediatric AML cases [21]. However, all of them were treated with Sorafenib, which is a first-generation pan-kinase inhibitor with activity against FLT3 in addition to their front line chemotherapy, yet 3 out of 8 (37.5%) of our patients of this group of the FLT3/ITD had disease relapse with one death among them, Despite none of our cases received Gemtuzumab Ozogamicin (GO) still the percentage of relapse among our FLT 3/ITD patients is nearer to the group reported with less Relapse Rate (RR) after initial CR which was 37% for GO recipients *vs.* 59% for NoGO recipients as per a report from the Children's Oncology Group [22]. The worse group of cytogenetics identified in this study was monosomy 7, which occurs in about 5% (3 patients), going along with data reported by Kalliopi N. Manola(21), which stated it occurs in 2% to 7% in pediatric AML. We got zero survival for the patients of this group despite allogeneic stem cell transplantation. This is far below what was mentioned in the previous study that the 5-yr survival rate of children with monosomy 7 was found to be 39%. 2 out of 3 of our patients of this group had disease relapse after BMT and died with disease progression, and one had BMT-related mortality. Treatment-related AML (t-AML) t-AML can be a result of earlier treatment for leukemia or solid tumor and is known to have associated poor outcomes. The 2 cases of t-AML in this study were primary cases of Wilms tumor, treated with alkylating agents, and both died.

Many factors play an important role in improving the survival rates for childhood acute myeloid leukemia significantly during the last decades, strict risk stratification, intensified chemotherapy, allogeneic hematopoietic stem cell transplantation, improved supportive care, and the development of new target therapies. Currently, the two most essential drugs included in most of the protocols for acute myeloid leukemia are Cytarabine and anthracycline, with the MRD level at the end of induction being the most significant predictor of relapse [23]. Aggressive supportive care is mandatory in managing patients with acute myeloid leukemia due to the serious complication of the disease and the toxicities from the medications; therefore, many centers adopted strict policies regarding the need for blood products transfusion and antimicrobial coverage during the neutropenia period to minimize the risk of infection which is considered a major cause of morbidity and mortality in AML [24]. Fortunately, none of our cases showed a frank classical picture of life-threatening Tumour Lysis Syndrome (TLS) due to the full supportive care preventive measures that we started before the onset of chemotherapy. Specifically, hyper hydration, uric acid lowering agents, and the close monitoring of urine output, renal function, and biochemistry. The value of hematopoietic stem cell transplantation (SCT) in pediatric AML as a front line is still under discussion, and the procedure-related deaths need to be balanced carefully against reducing relapse risk. SCT in first CR is therefore currently only recommended for a selected subset of high-risk cases in most European and North-American treatment protocols [25]. Apart from cytogenetic and molecular aberrations, Response to induction therapy is intuitively a significant prognostic factor in AML. Traditionally the morphological Response after one or two courses of chemotherapy, i.e., achieving complete remission (CR), is still considered the most crucial outcome predictor. CR is defined as less than 5% malignant blast in the bone marrow, combined with haematological remission and the independence of blood products transfusion need. More recently, the LAIP defined by multiparameter flow cytometry added to the response criteria in many protocols aiming for strict adjustment and tailoring of the provided therapy. Our study showed that the rate of complete remission achieved after induction chemotherapy is quite different in patients with high risk versus low; it almost doubled from about 40% in the high-risk group to more than 80 % in the low-risk group. Despite intensified treatment still, 25%-35% of pediatric patients with AML relapse [26]. Similarly, this study showed a relapse rate of 32%, with around 40% out of them belongs to the high-risk group.

According to risk group stratification, we found that the survival rates varied significantly among our different risk groups for low, intermediate, and high-risk groups were 85%, 70% and 60%. In comparison to what reported in the United Kingdom Medical Research Council's 12nd AML trial (MRC AML12) as 84%, 76%, and 47% for low, intermediate, and high-risk groups. The overall survival=74.6% at 162 months, and the EFS is 67.3% at 151 months. In a study by Hyery Kim [19], they found that the 5-year survival rate ranging from 65% to 75%, and Event-Free Survival (EFS) rates from the time of diagnosis ranged from 50% to 65%. Interestingly, when comparing the results of this study to previous similar old study that was done on the same institute over the same patient population between May 2008 and Sept 2012, the current study data showed better EFS, OS, and Relapsed Free Survival (RFS) from 54%,59.9%,59.1% to 67.3%,74.6%,73.1% respectively. This improvement might probably be due to applying more precise, refined risk stratification parameters, improving supportive care, and starting to perform the HSCT procedures locally in our centre instead of referring the eligible cases to other national institutes [27,28].

CONCLUSION

Pediatric AML is a clinically and genetically heterogeneous disease with a low incidence, variable survival outcomes, and high frequency of relapse, treatment-related deaths, and long-term side effects. Despite all the improvement in the outcome of childhood acute myeloid leukemia during the last decades, yet the current survival of pediatric AML is around 70%, with a further intensification of chemotherapy might not be feasible and safe. Next-Generation Sequencing (NGS) seems promising in the diagnosis and monitoring of MRD of acute myeloid leukemia. It may extend the horizon toward discovering more underlying responsible genetic aberrations that could play a role in developing new immunotherapeutic approaches and target therapies in the future. However, extra attention to the standardization of the methods of testing and interpretation is crucial. Data from our institute showed noticeably improved survival results over the last few years, reflecting the more efficient implementation of supportive care and therapy strategies. Because of the disease's

rarity, national and international collaboration is significantly essential to provide adequate numbers of patients for further local and global research in the biological, genetics, and clinical aspects and for testing modern targeted therapies.

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