Distribution of fusion transcripts and its clinical impact in patients with acute myeloid leukemia in Sudan

**Abdel Rahim Mahmoud Muddathir[1,2],
Tarig A. M. Hamid[3],
Elwaleed M. Elamin[4],
Omar F. Khabour[5]**

[1]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Taibah University, Medina, Kingdom of Saudi Arabia, [2]Department of Hematology and Blood Transfusion, Faculty of Medical Laboratory Science, Alzaeim Alazhari University, Khartoum, Sudan, [3]Department of Hematology and Immunohematology, Sharq Elnile College, Khartoum North, Sudan, [4]Department of Histopathology and Molecular Biology, Alzaeim Alazhari University, Khartoum, Sudan, [5]Department of Medical Laboratory Sciences, Jordan University of Science and Technology, Irbid, Jordan

Address for correspondence:
Abdel Rahim Mahmoud Muddathir, Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Taibah University, Medina, KSA.
E-mail: abdelrahimm@gmail.com

ABSTRACT

Objective: Acute myeloid leukemia (AML) is a common malignant disorder of hematopoietic progenitor cells that caused by chromosomal translocation and the formation of fusion oncogenes. This study determined the frequencies of fusion genes in Sudanese patients with AML and their clinical impacts.

Methods: This study was conducted at Alzaeim Alazhari University, Khartoum, Sudan. A total of 97 patients with AML were recruited in the study from different clinics in Khartoum state. Quantitative real-time polymerase chain reaction was used to determine types of fusion genes.

Results: The highest frequency of genetic defects was observed for *AML1-ETO* fusion gene (57.6%) followed by *MLL-AF9* (35.1%) and *FUS-ERG* (7.2%). No significant differences in blast cells, hemoglobin, total white blood cells, and platelets were found between different gene fusion groups ($P > 0.05$). In addition, no differences in the frequency of splenomegaly, hepatomegaly and lymphadenopathy were observed between different gene fusion groups ($P > 0.05$). With respect to French-American-British (FAB) classification, the M2 and M3 were significantly higher in patients with *AML1-ETO* fusion (86%, $P < 0.01$) whereas M4 and M5 were higher in patients with *MLL-AF9* fusion (76.5%, $P < 0.01$).

Conclusions: The study concluded that *AML1-ETO* and *MLL-AF9* fusion genes were predominant in AML Sudanese patients. None of the examined clinical parameters were different between different fusion genes except for FAB stages.

Keywords: Acute myeloid leukemia, *AML1-ETO*, *MLL-AF9*, real-time polymerase chain reaction, Sudan

Introduction

Acute myeloid leukemia (AML) is a common malignant disorder of hematopoietic progenitor cells.[1,2] AML is characterized by the accumulation of abnormal blood cells in the bone marrow and blood stream that interfere with normal blood functions.[3] The etiology of AML is heterogeneous and includes several genetic defects such as *AML1-ETO*, *MLL-AF9*, and *FUS-ERG*.[4,5] The *AML1-ETO*, also known as t(8;21), was historically the first chromosomal defect observed in AML.[6,7] The t(8;21) fuses the chromosome 21 AML1(*RUNX1*) gene with the *ETO* gene that encodes the CBFA2T1 protein on chromosome 8.[8] The *MLL-AF9* gene defect is formed as a result of a t(9; 11) reciprocal translocation.[9] The expression of the *MLL-AF9* fusion gene is associated with extramedullary tumor infiltration, high relapse frequency, and low survival rates.[10-13] The *FUS–ERG* fusion gene is caused by t(16;21) (p11;q22) chromosomal aberration. The fusion gene codes for a hybrid protein that contains the N-terminal domain of FUS the C-terminal domain of ERG.[14,15]

The types of genetic defects are among the most important prognostic factors and are used in the diagnosis and treatment of AML as each type is associated with specific clinical, morphological, and immunological disease characteristics.[16,17] In this study, the distribution of genetic defects that are associated with AML was investigated among Sudanese patients. In addition, the impact of genetic defects on hematologic parameters and clinical remarks of AML was also examined. The study findings are expected to improve the management and prognosis of AML in Sudan.

Methods

Subjects

The study design is cross-sectional and descriptive in nature that was conducted between January 2013 and January 2017 in Alzaeim Alazhari University, Khartoum, Sudan to investigate the frequency of common fusion genes in AML and their impact on hematological parameters. The study included 97

AML patients (52 male and 45 female) from different clinics in Khartoum state. The age range of patients was between 15 and 75 years old. The study was approved by the Institutional Review Boards of Alzaeim Alazhari University, Khartoum, Sudan. Informed consent was obtained from participants after full explanation of study aims and procedures. A total of 5 mL of venous blood was collected in ethylenediaminetetraacetic acid (EDTA) evacuated tubes and used for hematological and molecular assays as described below.

RNA extraction

Total RNA was extracted from peripheral blood collected in EDTA using R & A blue kit (Intron Biotechnology, Korea). The mRNA was converted to cDNA using Maxime Reverse Transcriptase PreMIx kit (Intron Biotechnology, Korea) according to the manufacturer's protocol. In summary, 1 µg of isolated RNA was used in the reaction (12.5 µl total volume) that contains 0.5 µg of oligo (dT18) primers and 1 µl of 10 mM dNTPs. The reaction was incubated at 65°C for 5 min and then immediately cooled down on ice. Then, the reverse transcriptase mixture (4 µl of 10XRT buffer and 2 µl of 0.1 mol/L DTT and 20U of RNase Block) was added. The mixtures were incubated at 37°C for 2 min. Then, 50 U of RT enzyme was added and the mixture was incubated at 42°C for 50 min. The mixture then incubated at 75°C for 15 min to terminate the reaction. The cDNA quantity/quality was assessed using ND-100 spectrophotometer (Nano Drop Technologies, Wilmington, USA). Samples were stored at −80°C until used.

Molecular detection of fusion genes

Molecular detection of fusion gene expressions was performed by real-time polymerase chain reaction (RT-PCR) using a thermos-cycler machine from Roche Diagnostics machine (LightCycler 480, Germany). Commercial probes and primers for the detection of fusion genes were from Eurofins genomics (Ebensburg, Germany). The used primers for AML1-ETO translocations were AML1-A: CTACCGCAGCCATGAAGAACC, ETO-B: AGA GGA AGG CCC ATT GCT GAA, AML1-C: ATG ACC TCA GGT TTG TCG GTC G, ETO-D: TGA ACT GGT TCT TGG AGC TCC T, and AML1-E: TGG CTG GCA ATG ATG AAA ACT ACT as previously described.[18] With respect to *MLL-AF9*, the used primers were MLL6S: GCA AAC AGA AAA AAG TGG CTC CCC G and AF9AS3: TCA CGA TCT GCT GCA GAA TGT GTC T as previously described.[19] Finally, the used primers for *FUS-ERG* were FUS: CAG CGG TGG CTA TGG ACA G and ERG: GGT GCC TTC CCA GGT GAT G.[19] The RT-PCR was performed as the followings: 50°C for 120 s and 95°C for 5 min followed by 40 cycles of 15 s at 95°C, 30 s at 65°C and 15 s at 72°C. *GAPDH* was used as a control for gene expression.

Laboratory parameters

Measurements of platelets, white blood cells, and Hb level were performed using a KX-21N automated hematology analyzer (Sysmex Corporation, Japan). With respect to French-American-British (FAB) classification, bone marrow samples were stained as previously described.[20] Samples were grouped according to the morphological appearance and types and number of the blast cells into the following categories: M0, M1, M2, M3, M4, M5, M6, and M7.[21]

Data processing

All data were entered and analyzed using statistical analysis software SPSS (Statistical Package for the Social Sciences) version 21. Chi-square was used to associate fusion genes with the demographics of participants. ANOVA test was used to associate the fusion genes with hematological and clinical parameters. A $P < 0.05$ was considered as statistically significant.

Results

Table 1 shows characteristics of participants. The mean age was 36 years (range: 2–84). Male to female ratio was 1.15:1. Most of the patients were from Middle and West part of Sudan. Clinical remarks of AML patients include splenomegaly (18.6%), hepatomegaly (19.6%), and lymphadenopathy (5.2%). About half of the patients were receiving combinations of anti-AML therapeutic drugs, whereas 41% were taking dexamethasone.

Table 2 shows frequency of different types of gene fusion observed in AML patients. The highest frequency was observed

Table 1: Characteristics of the study participants

Parameter	Value
Age	
Mean±SEM	36.2±2.9
Range (years)	15–75
Gender: *n* (%)	
Male	52 (53.6)
Female	45 (46.4)
Region: *n*(%)	
Middle	35 (36.1)
North	14 (14.4)
South	3 (3.1)
East	8 (8.2)
West	37 (38.2)
Complications: *n* (%)	
Splenomegaly	18 (18.6)
Hepatomegaly	19 (19.6)
Lymphadenopathy	5 (5.2)
Treatment: *n* (%)	
Combinations	47 (48.5)
Dexamethasone	40 (41.2)
Atra	6 (6.2)
Others	4 (4.1)

for *AML1-ETO* gene fusion (57.6%) followed by *MLL-AF9* (35.1%). However, *FUS-ERG* fusion gene accounted for 7.2%. The normalized expression of different fusion genes ranged between 0.23 for *AML1-ETO* to 0.36 for *FUS-ERG*.

The effects of different gene fusions on hematological parameters are shown in Table 3. According to ANOVA, no differences in blast cells ($P = 0.93$), hemoglobin ($P = 0.52$), total white blood cells ($P = 0.43$), and platelets count ($P = 0.33$) were found between different fusion genes. In addition, no differences in the frequency of splenomegaly, hepatomegaly, and lymphadenopathy ($P > 0.05$) were observed between different gene fusion groups.

We also examined the distribution of different gene fusions according to geographical areas, gender, and age [Table 4]. The results showed similar distribution of different gene fusions in AML patients in the different part of Sudan ($P = 0.589$), age groups ($P = 0.155$), and according to gender ($P = 0.239$).

Finally, the distribution of AML patients according to gene fusion type and FAB classification is shown in Table 5. The M2 and M3 FAB stages were significantly higher ($P < 0.01$) in patients with *AML1-ETO* fusion (86%) compared to patients with *MLL-AF9* fusion (23.5%). On the other hand, M4 FAB stage was higher ($P < 0.05$) in patients with *MLL-AF9* fusion (47.1%) compared to patients with *AML1-ETO* fusion (8.9%).

Discussion

AML is a mixed group of clonal hematopoietic stem cell disorders. The progress of AML is associated with accumulation of acquired genetic alterations in hematopoietic progenitor cells. In the current study, we examined the frequency of different fusion transcripts in patients with AML from Sudan. The results showed that *AML1-ETO* gene fusion is the most common type (57.6%) followed by *MLL-AF9* (35.1%) and FUS-ERG (7.2%). In a previous study from Sudan, the *AML1: ETO* gene mutation was also found to be the most common one.[22] A similar distribution was also observed in studies from Indian,[23] China,[24] Egypt,[25] and Japan.[11]

In the present study, significant differences in the distribution of AML patients according to FAB classification and type of gene fusion were observed. The M2 and M3 FAB stages were significantly higher in patients with *AML1-ETO* fusion

Table 2: Distribution of chromosomal aberrations seen in AML patients

Type of chromosomal aberration	Frequency	Expression (Δ Cq)
AML1-ETO	57.6	0.23±0.09
MLL-AF9	35.1	0.31±0.05
FUS-ERG	7.2	0.36±0.13

AML: Acute myeloid leukemia

Table 3: Hematological parameters of AML patients according to type of chromosomal aberration

Parameter	*AML1-ETO* (*n*=56)	*MLL-AF9* (*n*=34)	*FUS-ERG* (*n*=7)	*P*-value
Blast cells (mean%±SD)	25.5±3.1	23.4±4.9	24.2±9.9	0.934
TWBCs (x10³/µL)	23.1±5.9	15.52±4.9	11.93±4.5	0.520
Hb (g/dL)	8.90±0.28	9.31±0.4	9.77±0.63	0.427
Platelets (×10³/µL)	54.23±7.2	72.68±11.7	54.90±13.7	0.337
Splenomegaly (%)	10 (17.9)	6 (17.6)	2 (20)	0.741
Hepatomegaly (%)	11 (19.6)	6 (17.6)	3 (30)	0.122
Lymphadenopathy (%)	2 (3.5)	2 (5.9)	1 (10)	0.324

AML: Acute myeloid leukemia

Table 4: Distribution of chromosomal aberrations seen in AML patients according to geographical area, gender, and age

Type of chromosomal aberration	*AML1-ETO n* (%)	*MLL-AF9 n* (%)	*FUS-ERG n* (%)	*P*-value
Geographical area				0.589
Middle	23 (41.1)	10 (29.4)	2 (28.6)	
West	18 (32.1)	15 (41.7)	4 (57.1)	
Other areas	15 (26.8)	9 (25)	1 (14.3)	
Gender				0.239
Male	29 (51.7)	18 (52.9)	3 (42.9)	
Female	27 (48.3)	16 (47.1)	4 (57.1)	
Age (Mean: 36)				0.155
Above mean	33 (58.9)	13 (38.2)	4 (57.1)	
Below mean	23 (41.1)	21 (61.8)	3 3 (42.9)	

AML: Acute myeloid leukemia

Table 5: Distribution of AML patients according to FAB classification and type of gene fusion

Type of chromosomal aberration	M1	M2	M3	M4	M5 and above	*P*-value
AML1-ETO	1.8%	55.4%	30.4%	8.9%	1.8%	<0.01
MLL-AF9	0%	5.9%	17.6%	47.1%	29.4%	
FUS-ERG	28.6%	28.6%	14.2%	28.6%	0%	

AML: Acute myeloid leukemia, FAB: French American British

(86%), whereas the M4 FAB stage was higher in patients with *MLL-AF9* fusion. These findings are in agreement with what previous reports from Sudan[22] that the majority of AML patients with *AML1-ETO* fusion was in M2/M3 FAB stages. In India study done by Sazawal *et al.*,[23] the majority of the patients with *AML1-ETO* had the M2 subtype. A similar observation was reported in studies that were conducted in Iranian[26] and Korea.[27] The data of *MLL-AF9* were also in agreement with previous studies that were conducted in Egypt,[25] China,[28] and Netherlands.[29]

In the current study, the result showed that the number of male patients with AML was found to be relatively higher than females. This trend was previously observed in previous studies from Sudan,[22] Pakistan,[30] and Korea.[27] In the present study, the observed clinical remarks (splenomegaly, hepatomegaly, and lymphadenopathy) were found in 19% of participants. This is also similar to that observed in previous studies that were conducted in Pakistan,[30] Palestine,[31] and Pakistan.[32] However, higher percentages of such complications were reported in an Indian study.[33]

With respect to the impact of different gene fusions on hematological parameters, no differences in blast cells, hemoglobin, total white blood cells, and platelets count were found between different fusion genes.

In addition, no differences in the frequency of splenomegaly, hepatomegaly, and lymphadenopathy ($P > 0.05$) were observed between different gene fusion groups. These findings are in agreement with a study that was conducted in Iran.[34] More studies are required to confirm the present findings.

The present findings provide insight regarding the frequency and distribution of translocations that underline the etiology of AML in Sudan. In addition, the implementation of molecular diagnosis in AML can predict FAB stages and some characteristics of the neoplastic cells, and the subsequent overall prognosis of the disease.

Conclusions

AML1-ETO and *MLL-AF9* fusion genes were predominant in AML Sudanese patients. None of the examined clinical parameters were different between different fusion genes except for FAB stages.

Authors' Declaration Statements

Ethics approval and consent to participate

Availability of data and material

The data used in this study are available and will be provided by the corresponding author on a reasonable request.

Competing interests

The authors have no competing interests.

Funding statement

Authors' Contributions

All authors contributed to study design, laboratory work, data analysis, report writing. Tarig A. recruited subjects and conducted the laboratory work. Abdel Rahim M and Omar F participated in writing and critically revised the manuscript with important intellectual content, and Elwaleed M approved the final version of the manuscript.

Acknowledgments

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