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Abstract

Background: Acute myeloid leukemia (AML) is a malignant disease of the myeloid line that is caused by several chromosomal aberrations that include AML1–ETO and MLL–AF9. In the current study, the correlations of fusion gene quantitative RT–PCR and hematological parameters in patients with AML were examined to determine their prognostic value in clinical practice.

Methodology: This study was conducted at Alzaeim Alazhari University, Khartoum, Sudan. A total of 82 patients with AML (51 AML1–ETO and 31 MLL–AF9) were participated in the study. Quantitative RT–PCR was used to determine types of fusion genes

Results: The expression of MLL–AF9 was significantly higher than that for AML1–ETO (P < 0.01). In addition, with respect to FAB classification M2/M3 types were dominated in patients with AML1–ETO gene fusion, whereas M4/M5 types were dominated in MLL–AF9 subjects (P < 0.01). Finally, neither AML1–ETO nor MLL–AF9 quantitative RT–PCR gene expressions were correlated with the examined hematological parameters including: hemoglobin, total white blood count, platelets and blast cells (P > 0.05).

Conclusions: Significant variations in AML1–ETO and MLL–AF9 expression were observed in AML. No correlations between the expression of fusion genes and hematological parameters were detected.

Keywords: AML, RUNX1–RUNX1T1, MLL–MLLT3, RT–PCR, clinical parameters

Acute myeloid leukemia (AML) is a malignant disease of the myeloid line that starts in the bone marrow and then spreads into the blood. The lifetime risk of developing AML is about 0.5% 1. Several genetic defects have been implicated in the pathogenesis of AML that in most of the cases ends in the formation of a fusion oncogene as a result a chromosomal translocation 2 Among such genetic defects are t(8;21) RUNX1–RUNX1T1 and t(9;11) MLL–MLLT3 gene fusions. 3 The t(8;21) RUNX1–RUNX1T1 translocation is associated with high complete remission rate and a tendency of longer overall survival and progression free survival. 8–10

The t(8;21) RUNX1–RUNX1T1 translocation is very common in AML and caused by a translocation that involves chromosome 8 and chromosome 21 and the subsequent fusion of RUNX1 (AML1) gene with the RUNX1T1 (ETO) gene. 4 RUNX1 gene codes for runt–related transcription factor 1 that plays a role in the development of blood cells. 5 The RUNX1T1 gene on the other hand encodes the CBFA2T1 protein, a transcriptional corepressor that involved in the regulation of gene expression of several genes 6,7 The t(8;21) RUNX1–RUNX1T1 translocation is associated with high complete remission rate and a tendency of longer overall survival and progression free survival. 8–10

Original Article

Relationships between AML1–ETO and MLL–AF9 fusion gene expressions and hematological parameters in acute myeloid leukemia

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Quantitation of \textit{RUNX1–RUNX1T1} and \textit{MLL–MLLT3} fusion gene expression

Quantitation of \textit{RUNX1–RUNX1T1} and \textit{MLL–MLLT3} fusion gene expression was performed by real–time PCR using a Light cycler 480 Roche Diagnostics machine (Germany). The primers, probes and master mixes were obtained from Eurofins genomics (India). (Table 1).

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>F: GAAGTG AAGTCTCGAGTC&lt;br&gt;R: GAAGATG TGGATGGGATTTC&lt;br&gt;R probe: CGACTCTGCACCTCGAAG–TAMRA</td>
</tr>
<tr>
<td>AML1: ETO</td>
<td>F: AATCACAGTGGATGGGCCC&lt;br&gt;R: TGCGTCTTCACATCCACAGG&lt;br&gt;Probe: AMCTGAGAAGCACTCCACAATG CCAGACT–TAMRA</td>
</tr>
<tr>
<td>MLL–AF9</td>
<td>F: CCGCTCAAGCAGCCTAC&lt;br&gt;R: TGTC4TG6AGCTAGGTCG&lt;br&gt;Probe: AAGCCCGTCAGGAAAAG</td>
</tr>
</tbody>
</table>

Table 1: RT–PCR primers and probes

Amplification conditions were: 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 15 seconds at 95°C, 30 seconds at 65°C and 72°C for 15 seconds. GAPDH expression was an internal control. Expressions of \textit{AML1–ETO} and \textit{MLL–MLLT3} were normalized to that of GAPDH.

Measurements of Hematological parameters

Hematological parameters (platelets counts, white blood cells count and hemoglobin) were analyzed using a KX-21N automated hematology analyzer (Sysmex Corporation, Japan).

FAB classification

Bone marrow samples were obtained from patients using disposable lumbar puncture needle size 16 for adults and size 18 for kids. Staining procedure of samples for FAB classification was as previously described.\textsuperscript{17} Based on recommended criteria,\textsuperscript{18} cells were classified according to the FAB into eight AML subtypes as follows: M0, M1, M2, M3, M4, M5, M6, and M7.

Statistical analysis

The GraphPad statistical analysis software was used for statistical analysis. Chi square was used to compare frequency data, whereas linear regression was used to examine correlations between level of fusion genes expression and hematological parameters. A \( P \) less than \( .05 \) was used to indicate significant differences.
Results

In the current study, 82 patients with AML (51 t (8;21) \textit{RUNX1–RUNX1T1} and 31 with t(9;11) \textit{MLL–MLLT3}) were included in the study. The mean age of the participants was 40.06 ± 2.58 for \textit{RUNX1–RUNX1T1} patients and 32.10 ± 3.50 for \textit{MLL–MLLT3} patients (\(P > .05\), Table 2). No significant differences were found between the two groups in terms of age categories (\(P = .1\)) and gender distribution (\(P = .69\)).

The expression of fusion genes in blood samples obtained from patients was examined using real time PCR techniques (Figure 1). The expression of \textit{MLL–MLLT3} was significantly higher than that for \textit{RUNX1–RUNX1T1} (\(P < .01\)).

![A) MLL1-ETO Expression](image1.png)

![B) MLL-AF9 Expression](image2.png)

Table 2. Demographics of the study population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AML1–ETO (n=51)</th>
<th>MLL–AF9 (n=31)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (± SEM)</td>
<td>40.06 ± 2.58</td>
<td>32.10 ± 3.50</td>
<td>0.063</td>
</tr>
<tr>
<td>Age groups: n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>11 (21.6)</td>
<td>12 (38.7)</td>
<td></td>
</tr>
<tr>
<td>21–40</td>
<td>10 (19.6)</td>
<td>8 (25.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;40</td>
<td>30 (58.8)</td>
<td>11 (35.5)</td>
<td></td>
</tr>
<tr>
<td>Males/females ratio</td>
<td>1.13: 1</td>
<td>0.94: 1</td>
<td>0.690</td>
</tr>
<tr>
<td>Place of Residency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Capital</td>
<td>33 (64.7%)</td>
<td>10 (32.3)</td>
<td>0.016</td>
</tr>
<tr>
<td>– North–East</td>
<td>15 (29.4%)</td>
<td>18 (58.0)</td>
<td></td>
</tr>
<tr>
<td>– South–West</td>
<td>3 (5.9%)</td>
<td>3 (9.7)</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 1. Expression of fusion genes in AML.](image3.png)

![Figure 2. Expression of fusion genes according to gender.](image4.png)

![Figure 3. FAB classifications among fusion genes.](image5.png)

Fusion genes were quantified using real time–PCR techniques. No significant differences were observed in the expression of \textit{AML1–ETO} (A) and \textit{MLL–AF9} (B) among male and female subjects (\(P > 0.05\)).

Figure 3 shows FAB classifications of AML and their distribution according to the type of gene fusions. The results showed that M2/M3 types were dominated in patients with \textit{RUNX1–RUNX1T1} gene fusion, whereas M4/M5 types were dominated in patients with \textit{MLL–MLLT3} gene fusion (\(P < .01\)).

![Figure 3. FAB classifications among fusion genes.](image6.png)
Fusion genes and hematological parameters in AML, Abdel Rahim Mahmoud Muddathir, et. al.

The M2/M3 types were dominated in patients with AML1–ETO gene fusion whereas M4/M5 types were dominated in MLL–AF9 subjects. * indicates significant difference P < 0.01.

Finally, we examined the relationship between the magnitude of fusion gene expression as determined by real–time PCR and hematological parameters. The results (Table 3) showed that neither RUNX1–RUNX1T1 nor MLL–MLLT3 gene expressions were correlated with the examined hematological parameters including: hemoglobin, total white blood count, platelets and blast cells (P > .05).

Table 3: correlation between expression of the fusion gene and hematological parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AML1ETO</th>
<th>MLL–AF9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>$r^2 = 0.007$, $P = 0.54$</td>
<td>$r^2 = 0.018$, $P = 0.47$</td>
</tr>
<tr>
<td>Platelets</td>
<td>$r^2 = 0.006$, $P = 0.57$</td>
<td>$r^2 = 0.000$, $P = 0.98$</td>
</tr>
<tr>
<td>Total white blood cells</td>
<td>$r^2 = 0.011$, $P = 0.46$</td>
<td>$r^2 = 0.005$, $P = 0.70$</td>
</tr>
<tr>
<td>Blast cells</td>
<td>$r^2 = 0.001$, $P = 0.90$</td>
<td>$r^2 = 0.000$, $P = 0.92$</td>
</tr>
</tbody>
</table>

Discussion

In the current study, the relationship between expression of RUNX1–RUNX1T1 and MLL–MLLT3 fusion genes in AML and clinical parameters were examined. The level of MLL–MLLT3 expression was significantly higher than that of RUNX1–RUNX1T1. However, neither RUNX1–RUNX1T1 nor MLL–MLLT3 expression was correlated with hematological parameters in AML patients.

The t(8;21) RUNX1–RUNX1T1 translocation is a major cause of AML worldwide. This fusion creates a hybrid oncogene of RUNX1 gene that codes for runt–related transcription factor 1 and RUNX1T1 gene that codes a transcriptional corepressor. The fusion protein initiates leukemogenesis and the subsequent impairment of the development of blood cells.16,20 The t(9;11) MLL–MLLT3 translocation is another common cause of AML. The fusion gene codes for a hybrid protein of myeloid/lymphoid leukemia protein and human homolog of Drosophila melanogaster trithorax. The resulting fusion protein miss–regulate several developmental and cell cycle genes, including HOX genes.21 In the current study, the expression level of the MLL–MLLT3 fusion gene was higher that detected for RUNX1–RUNX1T1 gene. The observed differences in the expression of fusion genes could contribute to the clinical outcomes of AML. In agreement with this, previous studies have shown that the t(8;21) RUNX1–RUNX1T1 translocation is associated with high complete remission rate and a tendency of longer overall survival and progression free survival, whereas t(9; 11) MLL–MLLT3 translocation is associated with AML infiltration, high relapse frequency, and low survival rates.13,15,26

The results showed a lack of associations between examined fusion gene expression and hematological parameters in AML patients that include hemoglobin, platelets, white blood cell counts and blast cells. Since the current study is among the few studies that examined correlations between real–time PCR results and hematological parameters in AML, the present findings are difficult to compare with previous literature. A previous study from India on 16 patients with RUNX1–RUNX1T1 fusion has reported no correlation between fusion gene expression with hematologic parameters.22 On the other hand, monitoring expression of fusion genes in AML has been shown to be useful for the prediction of outcomes of AML. For example, in a study that include 70 AML patients with RUNX1–RUNX1T1 fusion gene, RT–PCR expression results were correlated with a probability of relapse.23 Similarly, RUNX1–RUNX1T1 transcripts on real–time quantitative polymerase chain reaction has been shown to be a good marker of relapse in pediatric AML.24 More studies are thus required to confirm the present findings.

Conclusion

In conclusion, differences in the magnitude of RUNX1–RUNX1T1 and MLL–MLLT3 fusion gene expressions were detected in patients with AML. However, no correlations between real–time quantitative polymerase chain reaction expression data of fusion genes and hematological parameters in AML were found. More studies are required to confirm the present findings.

Acknowledgments

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Conflict of interest: Authors have nothing to declare.

References


