Coexistence of Ten-Eleven Translocation 2 and Calreticulin Mutations in Myeloproliferative Neoplasms: Possible Prognostic Value

Abstract

Background: Mutations in the genes regulating epigenetic factors such as Ten-Eleven Translocation 2 (TET2) along with other mutations are highly effective in the patient’s prognosis. The aim of this study was to evaluate the prevalence of TET2 and calreticulin (CALR) mutations among myeloproliferative neoplasms (MPNs) patients to determine if there is a prognostic value for these mutations coexistence. Materials and Methods: Blood sample was collected from patients. For the evaluation of mutations, polymerase chain reaction (PCR) was performed on the patient’s DNA and PCR products were subsequently sequenced. Results: No significant correlation between coexistence of TET2 and CALR mutations with complete blood count parameters in patients was seen. However, platelet count was lower in patients with TET2 and CALR mutations compared with patients with CALR mutations only. Conclusion: The coexistence of CALR and TET2 mutations in MPN patients can be used as a prognostic factor.

Keywords: Calreticulin, Janus kinase 2, mutation, myeloproliferative neoplasms, Ten-Eleven Translocation 2

Introduction

Although recent results have indicated that mutation in genes involved in signaling pathways such as Janus kinase 2 (JAK2), calreticulin (CALR), and myeloproliferative leukemia virus (MPL) is a major reason for the incidence of myeloproliferative neoplasms (MPNs), the latest findings have shown that the mutations in genes involved in epigenetic regulation and the disruption of epigenetic processes and DNA methylation play an essential role in the occurrence of abnormalities in replication, differentiation, and expression of genes in MPNs, which can be considered as a key factor in the onset of disease. Ten-Eleven Translocation 2 (TET2) is a member of TET family of proteins whose encoding gene has 13 exons and lies in 4q24 locus. The expression of TET2 is different in various cells, but it seems to be highly expressed in hematopoietic cells. TET2 has catalytic activity and plays a crucial role in the process of 5-methylcytosine conversion to 5-hydroxymethylcytosine (5-hmC), controlling the expression of genes through epigenetic processes. Mutation in TET2 decreases 5-hmC production and subsequently increases DNA methylation. Various mutations such as missense, nonsense, and frameshift occur in TET2, which are randomly distributed across all TET2 exons; nevertheless, a majority of mutations occurs in exons 4 and 12. These mutations have been identified in several hematological malignancies, including MPNs, chronic myelomonocytic leukemia, and acute myeloid leukemia (AML). Some recent studies have shown that mutations in TET2 by itself cannot be considered as a factor of malignancy but have been detected in many hematologic malignancies along with mutations in other genes such as JAK2, MPL, and FLT3. As well as somatic mutations in exon 9 of CALR gene were identified in a majority of patients with ET and primary myelofibrosis (PMF) who were negative for JAK2 and MPL mutations that are known as driver mutations for MPN. It has also been noted that MPN patients with mutations in CALR have better clinical outcomes and survival than those with mutations in JAK2 or MPL. It was found that initially CALR mutations occur in about 70% of MPN patients who do not have mutations in JAK2 and MPL. [13]
However, co-occurrence of both the mutations has been reported in few MPN patients. Nonetheless, precise studies have not been conducted to detect mutations in both TET2 and CALR genes in MPNs. Hence, mutations in TET2 and CALR genes appear to cause changes in blood cell count and response to treatment in patients. Therefore, for the first time, we examined TET2 and CALR mutations in MPN patients and their association with hematological parameters of patients in this study.

Materials and Methods

Patients

In a year period (since 2017 August until 2018 September), all MPNs patients who referred to Shafa Hospital of Ahvaz, Southwest of Iran (including 260 persons), were evaluated for JAK2 and CALR mutations. After collection and analysis of 5 ml peripheral blood from each patient that was collected in ethylenediaminetetraacetic acid, 212 patients who had JAK2 mutations were excluded from the study. Since our studies inclusion criteria were the presence of CALR mutation and no previous history of treatment, patients lacking CALR mutation were excluded. Finally, 19 patients who had CALR mutation were recruited for our study, including 14 patients with essential thrombocythemia (ET) and 5 PMF patients [Figure 1]. Diagnosis of patients was based on the World Health Organization criteria.[14]

We obtained ethical approval for the study from the local ethics committee of Ahvaz Jundishapur University of Medical Science (IBR Number: IR. AJUMS. REC.1396.733).

Polymerase chain reaction and sequencing

DNA extraction from samples was performed using salting out method,[15] and polymerase chain reaction (PCR) was performed using specific primers [Table 1] to amplify exon 4 and 12 mutations. The PCR reaction was carried out in a total volume of 25 µl containing 1× reaction buffer, dNTP (0.25 mM), primer (2 pmol/l), genomic DNA templates (0.4 µg), and Taq DNA polymerase (1.5 U). Thermocycler was programmed as first denaturation for 3 min at 95°C followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 4 min. Negative control was considered in each PCR reaction which defined as a sample without a DNA template. All the PCR products were checked on 1.5% agarose gel electrophoresis staining with safe stain, and product bands were visualized under ultraviolet light. Subsequently, PCR products were sequenced by the Sanger method.

Statistical analysis

The results were fed into SPSS version 20 software (IBM, Armonk, NY, USA), and statistical analysis was conducted using t-test, Chi-square, and Kolmogorov–Smirnov tests. Statistical significance was indicated as P ≤ 0.05.

Results

Patient information

From 19 patients with CALR mutation, 14 patients were afflicted with ET and had an average age of 52.71 (range: 32–76) and the remaining five patients had PMF with an average age of 51.20 (range: 43–58 years). Moreover, 12 patients were male and 7 were female. Data on laboratory and hematological parameters of these patients that were recorded at the time of diagnosis are presented in Table 2, including hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), and platelet [Table 2].

Prevalence of Ten-Eleven Translocation 2 gene mutations in calreticulin-positive patients

After analysis of samples, it was found that 35.7% of the ET patients had mutation in TET2 gene, 7.1% of whom showed mutations in exon 4, 21.4% in exon 12, and 7.1% in both exons 4 and 12. On the other hand, 80% of PMF patients had mutations in TET2 gene, among whom 20% showed mutation in exon 4, 40% in exon 12, and 20% in both exons 4 and 12 [Table 3].

Correlation between Ten-Eleven Translocation 2 mutations with hematological parameters

After investigation, no significant correlation was found between Hb (P = 0.347), RBC (P = 0.117), and WBC (P = 0.329) with mutations occurring in TET2 gene. However, platelet count was lower in patients with a mutation in TET2 gene compared to those without mutation in it, which was statistically significant (P = 0.03) [Tables 4 and 5].

Discussion

MPNs are disorders characterized by imbalance in the production of erythroid series, which leads to monoclonal
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MPNs are divided into three groups: polycythemia vera, essential thrombocythaemia (ET), and PMF. MPNs are different in terms of clinical symptoms so that some patients have no complications and others develop advanced disease causing secondary AML. Furthermore, 75% of the MPN patients have \( JAK2^{V617F} \) mutation and most of the time show similar molecular manifestations. Nonetheless, a diagnostic challenge is created when patients lack the \( JAK2^{V617F} \) mutation. Hence, recent studies have shown that identification of new mutations can be effective in differentiating between MPNs as well as determining the prognosis based on mutations for treatment of disease.

TET2 is a factor controlling the expression of many genes by regulating epigenetic processes. Investigations have shown that mutation in TET2 gene alone cannot lead to malignancy. Therefore, the coexistence of TET2 gene mutation along with mutation in other genes has been shown in many malignancies.

<table>
<thead>
<tr>
<th>Primer pairs</th>
<th>Primer</th>
<th>Primer sequence (5’-3’ )</th>
<th>Product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Exon 4 F</td>
<td>TTTTTGTTCATGCTTTTAGAA</td>
<td>740</td>
</tr>
<tr>
<td></td>
<td>Exon 4 R</td>
<td>TTCACCATGTTGTGGTCCA</td>
<td>665</td>
</tr>
<tr>
<td>2</td>
<td>Exon 4 F</td>
<td>GTCTATAGGTCAATATGCTGCT</td>
<td>880</td>
</tr>
<tr>
<td>3</td>
<td>Exon 4 R</td>
<td>AGGAAGCTGAGGAACCTGTG</td>
<td>849</td>
</tr>
<tr>
<td>4</td>
<td>Exon 4 F</td>
<td>TTCTGCCCACACAACACCA</td>
<td>884</td>
</tr>
<tr>
<td>5</td>
<td>Exon 4 R</td>
<td>ACCTGTGGTGGAGGTGGTTTG</td>
<td>912</td>
</tr>
<tr>
<td>6</td>
<td>Exon 12 F</td>
<td>CAAGTCACAAATGTACCAAGTTA</td>
<td>744</td>
</tr>
<tr>
<td>7</td>
<td>Exon 12 R</td>
<td>TGATATTGTAGAAGGTTGA</td>
<td>725</td>
</tr>
</tbody>
</table>

Table 1: Primers used for analysis Ten-Eleven Translocation 2 mutations

F: Forward, R: Reverse

Table 2: Disease type, gender, age, and cell count information of 19 recruited patients

<table>
<thead>
<tr>
<th>MPNs subgroup</th>
<th>( n )</th>
<th>Gender</th>
<th>Mean age</th>
<th>Hb (g/dL)</th>
<th>RBC (x10^6 mm^3)</th>
<th>WBC (x10^3 mm^3)</th>
<th>Platelet (x10^3 mm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET</td>
<td>14</td>
<td>Male</td>
<td>52.71</td>
<td>2.23±11.67</td>
<td>1.01±4.427</td>
<td>17.71±16.075</td>
<td>381.19±670.271</td>
</tr>
<tr>
<td>PMF</td>
<td>5</td>
<td>Male</td>
<td>51.20</td>
<td>2.75±9.82</td>
<td>0.92±3.56</td>
<td>22.08±16.184</td>
<td>53.15±168.6</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>Male</td>
<td>52</td>
<td>2.44±11.184</td>
<td>1.04±4.199</td>
<td>18.53±16.541</td>
<td>355.73±606.842</td>
</tr>
</tbody>
</table>

MPNs: Myeloproliferative neoplasms, Hb: Hemoglobin, WBC: White blood cell, RBC: Red blood cell, ET: Essential thrombocytopenia, PMF: Primary myelofibrosis, SD: Standard deviation

Table 3: Frequency of Ten-Eleven Translocation 2 mutations in patients

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>Patients with TET2 mutation, ( n ) (%)</th>
<th>Only exon 4 mutated, ( n ) (%)</th>
<th>Only exon 12 mutated ( n ) (%)</th>
<th>Exon 4 and exon 12 mutated, ( n ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET</td>
<td>5 (35.7)</td>
<td>1 (7.14)</td>
<td>3 (21.42)</td>
<td>1 (7.14)</td>
</tr>
<tr>
<td>PMF</td>
<td>4 (80)</td>
<td>1 (20)</td>
<td>2 (40)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (47.36)</td>
<td>2 (10.52)</td>
<td>5 (26.31)</td>
<td>2 (10.52)</td>
</tr>
</tbody>
</table>

Table 4: Association between Ten-Eleven Translocation 2 mutation and hematological parameters in calreticulin mutated patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>TET2 mutation (mean±SD)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>10.6±2.62</td>
<td>0.347*</td>
</tr>
<tr>
<td>RBC</td>
<td>3.801±1.17</td>
<td>0.117*</td>
</tr>
<tr>
<td>WBC</td>
<td>25.468±24.321</td>
<td>0.329**</td>
</tr>
<tr>
<td>Platelet</td>
<td>432.88±361.73</td>
<td>0.039*</td>
</tr>
</tbody>
</table>

*\( P \) value calculated by \( t \)-test, **\( P \) value calculated by Mann-Whitney test.

Table 5: Association between Ten-Eleven Translocation 2 mutation and platelet count in studied patients

<table>
<thead>
<tr>
<th>Patients with TET2 mutation</th>
<th>Patients without TET2 mutation</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>432.88±361.73</td>
<td>0.039</td>
</tr>
<tr>
<td>736.4±281.58</td>
<td>TET2: Ten-Eleven Translocation 2</td>
<td></td>
</tr>
</tbody>
</table>
in the genes encoding epigenetic process regulators such as TET2, IDH1 and 2, and DNMT31 was higher in PMF patients than in ET patients. Furthermore, in a study by Ivanova et al. on 16 MPN patients, seven patients had mutations in ASXL1, CALR, RUNX1, SRSF2, TET2, and U2AF1 genes. In the current study, however, only 47.4% of the PMF patients had mutations in both CALR and TET2 genes, and the frequency of mutation in exons 4 and 12 of TET2 gene in PMF patients was higher than ET patients. In addition, the prevalence of the mutation in exon 12 compared to exon 4 was higher in both groups of PMF and ET patients [Table 3]. Studies have shown that the coexistence of mutations in MPN patients can affect the progression of disease, as well as prognosis and response to treatment. For this purpose, in the study of Limsuwanachot et al., it was found that platelet count was significantly higher in ET patients lacking CALR mutation relative to those carrying CALR mutation, which was statistically significant (P = 0.04). In addition to platelets, Hb and WBC were higher in nonmutated patients compared to those with CALR mutation, which was statistically significant (P = 0.01 for Hb and P = 0.04 for WBC). Moreover, in the study of Ha et al., it was found that platelet count was higher in PMF and ET patients with TET2 mutation than those without TET2 mutation, but no significant relationship was found between them (P = 0.2 for ET and P = 0.05 for PMF). In PMF patients, WBC count was higher in those with mutation in TET2 compared to those lacking it, which was statistically significant (P = 0.03). On the other hand, among ET patients, it was also found that those with TET2 mutation had higher WBC count than nonmutated ones, which was not statistically significant (P = 0.4). In our study, platelet count was found to be lower in patients with mutation in TET2 than those lacking TET2 mutation, which was statistically significant (P = 0.039). In contrast, there was no significant difference between the levels of RBC, Hb, and WBC with TET2 mutation among patients [Table 4].

**Conclusion**

According to the present study, given the decrease in platelet count in patients having TET2 and CALR mutation, it can be argued that the coexistence of CALR and TET2 mutations can be considered as a prognostic factor in MPNs patients.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

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