JAK2 Mutations in Chronic Myeloproliferative Neoplasm; Towards the Application of Personalized Treatments for Saudi Patients

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ABSTRACT

The chronic myeloproliferative neoplasms (CMPN) are a group of clonal hematopoietic stem cell disorders in which large numbers of red blood cells, white blood cells, or platelets grow and spread excess in the bone marrow and the peripheral blood. Cytogenetic analysis of the t(9;22) and molecular detection of BCR/ABL is the main diagnostic criteria in Philadelphia positive CMPN (CML). The identification of non-receptor tyrosine kinase JAK2 mutations (exon 14 JAK2 V617F and exon 12) have significantly contributed to our understanding of the molecular mechanisms in the pathogenesis of Philadelphia negative CMPN such as polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) patients. According to the revised WHO classification, JAK2 mutation is considered as a major diagnostic and clonal marker in Philadelphia negative CMPN which will play a major role in designing personalized treatments for the disease. JAK2 V617F mutation frequency is unknown in Saudi Arabia. Therefore, investigation of the JAK2 V617F mutation was carried out in DNA samples from 78 peripheral blood specimens corresponding to patients with polycythemia vera (PV) (n = 11), Chronic myeloid leukemia (CML) (n = 45), essential thrombocythemia (ET) (n = 10), idiopathic myelofibrosis (MF) (n = 12). We used polymerase chain reaction and direct DNA sequencing to detect the JAK2 mutation. Overall, the incidence of the JAK2 V617F mutation was 91% in PV, 40% in ET, and 25% in MF. This approach proved to be reliable and more sensitive in detecting the mutation. Two essential findings arose from our study. First, this technique could be carried out with DNA samples, even partially degraded, from routinely processed BM or peripheral blood specimens. Second, after correlation with morphological features, it turned out that the characteristics of the megakaryocytes were more specific than the mutational status of JAK2 in characterizing ET and PMF. Concerning PV, as expected, the incidence of the JAK2 mutation was higher, but the morphological criteria were misleading in some cases, strongly suggesting that the combination of both morphology and molecular data would enable the characterization of virtually all cases. JAK2 V617F mutation frequency along with accurate morphological characterization is very reliable tool in diagnosing and classifying CMPN in Saudi patients.

Keywords: Leukemia; CMPN; PV; ET; PMF; JAK2 Mutation

1. Introduction

Chronic myeloproliferative neoplasms (CMPN) including chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are a phenotypically diverse category of malignancies that are thought to derive from stem cells in the myeloid lineage [1]. CMPN is characterized by an overproduction of mature myeloid cells, bone marrow hypercellularity, extramedullary hematopoiesis, a tendency for thrombosis and or hemorrhage, and may sometimes progress to a leukemic transformation. Characterization of the molecular lesions underlying CMPN has broadened our understanding of the disease mechanism and has advanced diagnostic, prognostic, and therapeutic applications in this disease class. The first genetic alteration identified in CMPN was the translocation (9;22) that creates the BCR-ABL fusion gene resulting in a constitutively activated tyrosine kinase that is responsible...
for the activation of signal transduction pathways leading
to CML [2,3]. The identification and characterization of the
BCR-ABL chimeric protein led to the development of small molecules that target the tyrosine kinase activity of the chimeric protein. The genetic basis of Philadelphia negative CMPN was not clear until the year 2005 when it was first demonstrated that an acquired somatic mutation in the Janus kinase 2 gene (JAK2), causes a valine to phenylalanine substitution at position 617 (JAK2 V617F) resulting in a constitutively activated tyrosine kinase in most cases of CMPN [4-6]. In addition, the JAK2 gene is located on the short (p) arm of chromosome 9 at position 24. [7] More precisely, the JAK2 gene is located from base pair 4,985,244 to base pair 5,128,182 on chromosome 9 [7]. However, JAK2-STAT signaling pathway is essential for mediating cytokine dependent proliferation, survival and apoptosis, a disruption in this pathway in hematopoietic stem cells lead to myeloproliferative dis-eases. Activating mutations in JAK2 have been found in the majority of BCR-ABL-negative myeloproliferative neoplasms. The JAK2 (V617F) mutation has been previously reported in approximately 95% of patients with PV, 23% - 57% in ET and in 35% - 50% in PMF [8-10]. The identification of activating JAK2 mutations has raised considerable interest in the development of JAK2 inhibitors for the treatment of Philadelphia negative CMPN [11]. The aim of the current study was to determine the frequency of JAK2 mutations in the CMPN patients from Saudi Arabia.

2. Materials and Methods
2.1. Patients and Samples
The current study includes 78 patients diagnosed with CMPN collected at King Abdul Aziz University Hospital and King Abdulaziz Hospital and Oncology Center in the Western Region of Saudi Arabia between 2001 and 2010. The study population included 45 CML cases (57%), 10 ET cases (13%), 12 PMF cases (16%) and 11 PV cases (14%) (Figure 1). Patient characteristics are shown in Table 1. The current study included 78 CMPN patients which comprises of 45 CML cases (57%), 10 ET cases (13%), 12 PMF cases (16%) and 11 PV cases (14%) (Figure 1). Median age at diagnosis was 60 years. Patients were diagnosed and categorized using WHO 2001 criteria [15]. The basic investigations included complete blood count, arterial
Table 1. Clinical characteristics of Ph-negative CMPN patients and their JAK2 status.

<table>
<thead>
<tr>
<th></th>
<th>ET JAK2 V617F</th>
<th>ET JAK2 WT</th>
<th>MF JAK2 V617F</th>
<th>MF JAK2 WT</th>
<th>PV JAK2 V617F</th>
<th>PV JAK2 WT</th>
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<td>Number of Patients</td>
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<td>6</td>
<td>3</td>
<td>9</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>WBC ($\times 10^9/l$)</td>
<td>10.07</td>
<td>6.05</td>
<td>14.67</td>
<td>5.92</td>
<td>9.24</td>
<td>7.8</td>
</tr>
<tr>
<td>Platelet ($\times 10^9/l$)</td>
<td>873.75</td>
<td>884.67</td>
<td>336</td>
<td>89.12</td>
<td>559.00</td>
<td>251</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.77</td>
<td>12.47</td>
<td>8.9</td>
<td>11.39</td>
<td>19.76</td>
<td>17.4</td>
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<td>3</td>
<td>2</td>
<td>8</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Thrombosis</td>
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<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Haemorrhage</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Homozygous V617F cases</td>
<td>0</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

Essential Thrombocythaemia (ET); Myelofibrosis (MF); Polycythaemia vera (PV); Wild type (WT).

Figure 1. Percentage of patients with JAK2 V617F in each subtype of CMPN; Chronic myelogenous leukemia (CML), Essential Thrombocythaemia (ET), Myelofibrosis (MF), Polycythaemia vera (PV).

Sequence analysis revealed 5 cases (29%) with homozygous JAK2 V617F mutation among all cases V617F positive cases. Figure 2 shows sequences of CMPN patients with wild type JAK2, heterozygous and homozygous V617F mutations.

4. Discussion and Conclusion

The association of JAK2 V617F (exon 14 mutation) and CMPN such as PV, ET and PMF was first reported in 2005 [4,16]. This discovery was soon followed by a number of studies reporting the incidence of JAK2 V617F mutation in CMPN [5-6,8]. The identification this activating mutation generated considerable interest in the search for JAK2 inhibitors for the treatment of Philadelphia negative CMPN [11]. The current study was aimed at evaluating the status of JAK2 mutations in CMPN cases from the Western region of Saudi Arabia. Our results indicate that the percentage of JAK2 V617F exon 14 mutations in different categories of Philadelphia negative CMPN patients is similar to the previously reported frequencies elsewhere in the world [6,17]. Acquired mutations targeting exon 12 of JAK2 are reported in most JAK2 V617F-patients [18,19]. In our study, the analysis of exon 12 sequences revealed no mutations, which could be explained by the low number of patients studied. Another aspect that our study highlights is the homozygosity observed in 29% of cases with JAK2 V617F mutations. Kralovics et al, reported a significant association between homozygosity of the mutant allele is a time-dependent clonal evolution [6]. In addition, the presence of homozygous JAK2 V617F is associated with a two-fold increase risk of thrombosis in CMPN [10]. It has been reported that the presence of homozygous
Table 2. Clinical characteristics of Ph-negative CMPN patients and their JAK2 status.

<table>
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<tr>
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<th>ET JAK2 WT</th>
<th>MF JAK2 V617F</th>
<th>MF JAK2 WT</th>
<th>PV JAK2 V617F</th>
<th>PV JAK2 WT</th>
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</thead>
<tbody>
<tr>
<td>Number of Patients</td>
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<td>6</td>
<td>3</td>
<td>9</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>WBC (×10^9/l)</td>
<td>10.07</td>
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<td>Platelet (×10^9/l)</td>
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<td>89.12</td>
<td>559.00</td>
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<tr>
<td>Hb (g/dl)</td>
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<td>19.76</td>
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<td>2</td>
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<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Thrombosis</td>
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<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Haemorrhage</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Homozygous V617F cases</td>
<td>0</td>
<td>-</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Essential Thrombocythaemia (ET); Myelofibrosis (MF); Polycythaemia vera (PV); Wild type (WT).

mutation in PV and ET patients reflect a higher leukocyte count and hemoglobin value at diagnosis, and these patients have a larger spleen volume and are also older in age. Moreover, they develop myelofibrotic transformation more than the others [20].

In conclusion, our findings strongly support the view that peripheral blood mutation screening for JAK2 V617F be introduced into the initial evaluation of patients with suspected CMPN in Saudi Arabia. An early identification of the mutation status helps in excluding a large number of secondary causes. However, since the mutation may be absent in a few cases of PV, ET and IMF, it cannot be used as a single test for making the diagnosis. Additionally, we recommend carrying this study in the whole Kingdom to include a much larger population.

5. Acknowledgements

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REFERENCES


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