INTRODUCTION

Chronic myeloproliferative disorders (CMPDs) are clonal disorders of haemopoiesis that lead to an increase in the numbers of one or more mature blood cell progeny. CMPDs include polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF) and chronic myeloid leukemia (CML). These disorders share clinical, morphological and molecular features and can transform in their course to one another and into acute leukemia.1 CML results from an acquired genetic defect characterized by expansion of myeloid cell mass replacing normal haemopoiesis.1 CML is diagnosed on clinical and morphological features of blood and bone marrow, confirmed by presence of t(9; 22) “Philadelphia chromosome”. It results in juxtaposition of breakpoint cluster region (BCR), on long arm of chromosome 22, to the abelson (ABL) gene on the long arm of chromosome 9 (Bcr/ABL).1 There are only few case reports describing the presence of BCR/ABL gene rearrangement with JAK2V617F mutation in chronic myeloid leukemia.2-10 In contrast, JAK2V617F mutation is found in 95% cases of polycythemia rubra vera (PRV), 50% cases of ET and 40-50% cases of IMF, while occasional case reports are there for the other less common CMPDs like chronic eosinophilic leukemia, chronic neutrophilic leukemia, systemic mastocytosis, atypical CML, myelodysplastic syndrome/myeloproliferative disorders unclassifiable.11-15

ABSTRACT

Objective: To determine the association of JAK2V617F mutation along with BCR-ABL translocation or Philadelphia chromosome in chronic myeloid leukemia with early disease progression to advanced stages (accelerated phase or blast crisis) and poor outcome.

Study Design: Case series.

Place and Duration of Study: National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi, from February 2008 to August 2009.

Methodology: All the newly diagnosed cases of BCR-ABL or Philadelphia positive CML were tested for JAK2V617F mutation by Nested PCR. Demographic data, spleen size, hemoglobin levels, white blood cell and platelet counts were recorded. Independent sample t-test was used for age, haemoglobin level and spleen size. Fisher’s exact test was applied to compare disease progression in JAK2V617F mutation positive and negative cases.

Results: Out of 45 newly diagnosed cases of CML, 40 were in chronic phase, 01 in accelerated phase and 04 in blast crisis. JAK2V617F mutation was detected in 12 (26.7%) patients; 09 (22.5%) in chronic phase, none in accelerated phase and 03 (75%) in blast crisis. During a mean follow-up of 8 months, 03 patients in chronic phase transformed in blast crisis and 02 into accelerated phase. Overall 08 out of 11 (73%) JAK2V617F positive patients either had advanced disease or showed disease progression. Only 2 of 20 (10%) available patients, negative for the mutation, showed disease progression by transforming into blast crisis (p < 0.001). No statistically significant difference was seen in the age, spleen size, haemoglobin levels, white blood cells and platelets counts in JAK2V617F positive patients.

Conclusion: JAK2V617F mutation was detected in 26.7% cases of chronic myeloid leukemia. A significant proportion of them showed early disease progression.

Key words: Chronic myeloid leukemia. BCR-ABL translocation. JAK2V617F mutation. Blast crisis. Philadelphia chromosome.
JAK2V617F on exon 14 is the most common mutation in myeloproliferative disorders. JAK2 mutations on exon 12, mostly in-frame deletions and tandem point mutations, are less commonly reported. Both of these mutations result in the cytokine independent proliferation of the haemopoietic cells. JAK2V617F is a point mutation characterized replacement of the guanine by thymine at (1849 G→T) resulting in replacement of valine to phenylalanine at position 617 in JH2 domain of JAK2 protein. This leads to the loss of auto-inhibitory function of pseudokinase domain.

The role of JAK2V617F mutation is well established in myeloproliferative disorders other than CML. This study was planned to determine the frequency of JAK2V617F mutation in CML and whether it is associated with early progression of CML to advance stages.

METHODOLOGY

This study was conducted from February 2008 to August 2009 at National Institute of Blood Diseases and Bone Marrow Transplantation (NIBD & BMT). This was a prospective observational study of all newly diagnosed CML patients who presented during above mentioned period. The study was approved from the institutional ethics committee. Informed consents were obtained from patients. Data was recorded on case report forms. Age, gender, symptoms, signs, bone marrow biopsy reports, phase of the disease, BCR-ABL gene rearrangement or Philadelphia chromosome (for the confirmation of diagnosis), Hb levels, white blood cell counts, platelet counts and spleen size were recorded. Patients were classified into chronic phase, accelerated phase or blast crisis as per WHO criteria. Peripheral blood samples were collected and Nested PCR method was applied to detect JAK2V617F mutation (Figure 1).

For Nested PCR the RNA for the analysis was obtained from the clotted blood sample. The RNA was extracted by using Blood RNA Mini kit from Qiagen, Germany. The cDNA were amplified by using forward primer of set one (JAK2F) by RT-PCR enzyme MMLV (nitrogen, USA). The cDNA target template was first amplified by first set of primers of Nested PCR. In first PCR run JAK2F and JAK2R primers were used. Then PCR products from the first PCR reaction were subjected to a second PCR run, however, with a second new set of primers which included JAK2F2 and JAK2R2. As these primers were nested within the first PCR product, it was made very unlikely that non-specifically amplified PCR product would contain binding sites for both sets of primers. This Nested PCR amplification ensured that the PCR product from the second PCR reaction and no contamination from non-specifically amplified PCR products from alternative primer target sequences. Following sequences of primers were used for detection in our laboratory from Integrated DNA technologies (IDT California, USA).

Sequence JAK2F (5'-GAT GAG CAA GCT TTC TCA CAA GC-3')
Sequence JAK2R (5'-GCA TGG CCC ATG CCA ACT GTT T-3')
Sequence JAK2F2 (5'-ACG GTC AAC TGC ATG AAA GTT T-3')
Sequence JAK2R2 (5'-CCA TGC CAA CTG TTT AGC CA-3')
Sequence Jak2R (5'-GCA TGG CCC ATG CCA ACT GTT T-3')
Sequence Jak2F (5'-GAT GAG CAA GCT TTC TCA CAA GC-3')
Sequence JAK2F2 (5'-ACG GTC AAC TGC ATG AAA GTT T-3')
Sequence JAK2R (5'-GCA TGG CCC ATG CCA ACT GTT T-3')
Sequence JAK2R2 (5'-CCA TGC CAA CTG TTT AGC CA-3')

Each sample was analyzed for the mutation using appropriate negative and positive controls. The quality of the results was also assured by physical isolation of PCR reagents and products for nucleic acids, autoclaving solutions, avoiding splashes, use of separate pipettes for pre- and post-PCR steps and aliquot reagents.

Mean age, Hb levels, WBC counts, platelet counts and spleens size were measured in all the samples. Mann-Whitney U-test was used for platelet and WBC counts. Independent sample t-test was used to compare disease progression in JAK2V617F mutation positive and negative cases. The p-value < 0.05 was considered significant. SPSS version 17 was used to analyze the data.

RESULTS

Of the 45 patients studied, 22 were males and remaining females. Mean age of patients was 38.8 years (range from 10 to 71 years). Fifteen patients showed t(9;22) while 30 patients had BCR-ABL gene rearrangement. Forty patients were in chronic phase, one in accelerated phase and four in blast crisis. JAK2V617F was detected in 12 (26.7%) patients; 09 (22.5%) in chronic phase, none in accelerated phase and 03 (75%) in blast crisis. Table I shows the mean haemoglobin of all the subjects which was 9.14 gm/dl. The mean platelet count was 494.3x 10^9/L. Total leucocyte count 250.9x10^9/L. Mean spleen size was 7.4 centimeter below the costal margin. Table II shows the differences in the haematological parameters and spleen size in the patients with JAK2V617F mutation from those who were negative for the mutation. The independent sample t-test was applied for the Hb level, age and spleen size, while Mann Whitney U-test was applied for the WBC and platelet counts due to skewed data. Statistically, no significant difference was seen in the age (p=0.743), haemoglobin (p=0.083), WBC count (p=0.55), platelet count (p=0.09) and spleen size (p=0.233) between the patients with JAK2V617F positive and negative at the time of diagnosis.

Out of 45 patients, follow-up data of 14 patients was not available. The characteristics of the remaining patients of both the groups were compared which are depicted in Table III showing the response to the treatment either
with Imatinib mesylate / nilotinib or hydroxyurea during the mean follow-up of 8 months. This table also shows statistically significant reduction in the mean platelet count, WBC count and in the spleen size in JAK2V617F mutation negative patients when compared to results at the time of diagnosis. However, in patients who were positive for the JAK2V617F mutation except for the reduction in the WBCs count, which was statistically significant but clinically insignificant, all other parameters remained statistically insignificant. During a mean follow-up of 8 months (range 3-19 months), out 12 JAK2V617F positive patients, one was lost to follow-up, 03 patients died due to transformation into blast crisis and 5 of the remaining 8 (62.5%) alive patients in chronic phase showed disease progression; 02 into accelerated phase, and 03 into blast crisis. Advanced disease and progression was noted in 73% (08 out of 11) patients of JAK2V617F positive CML cases, whereas only 10% (02 of 20 available) JAK2V617F negative patients showed disease progression with both being transformed into blast crisis (p < 0.001).

### DISCUSSION

Chronic myeloproliferative disorders have long been considered as orphan diseases except for chronic myeloid leukemia. Since 2005, JAK2V617F mutation has been reported in different CMPDs. There are scattered case reports about the presence of this mutation in chronic myeloid leukemia.\(^2\)\(^-\)\(^10\) In this study, 26.7% had shown the presence of JAK2V617F mutation. Seventy five percent (3 out of 4) of patients who presented in blast crisis and 22.5% (9 out of 40) patients in chronic phase had this mutation. Another interesting observation was early progression of disease to accelerated phase and blast crisis in 62.5% (5 out of 8) JAK2V617F positive patients during the follow-up, who initially presented in chronic phase. This study was planned with the assumption that this mutation might be detected in those CML patients who had high platelet counts, raised Hb levels and massive splenomegaly at the outset.\(^20\)\(^21\) These results showed a lower mean Hb level and platelet count, while a higher white blood cell count and spleen size in JAK2V617F positive cases when compared with cases negative for the mutation. But the difference in all parameters was statistically insignificant (Table II).

All the patients were newly diagnosed cases of CML who had never received Imatinib mesylate or any other treatment like hydroxyurea and interferon before the analysis of JAK2V617F mutation. Its presence in 3 out of 4 cases in blast crisis and early disease transformation in 5 of the 8 cases, has suggested that it might have a role in disease progression. At the moment, it cannot be ascertained whether JAK2V617F mutation in 5 of the 8 cases, occurred in BCR-ABL clone or whether it was a separate clone. How do these two interact or operate together is not known at present. In other published case studies, with co-existing BCR-ABL positive and the JAK2V617F, it has been suggested that BCR-ABL clone remained the dominant clone, when suppressed by Imatinib mesylate therapy, competing clone manifested clinically (JAK2V617F mutation).\(^6\)\(^8\) Campiotti and his colleagues have reported the complete absence of mutation when they repeated the mutation status after 6 months of

### Table I: Characteristics of all the patients at the time of diagnosis (n=45).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>95%CI</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.8 ± 13.8</td>
<td>34.7 to 42.9</td>
<td>71</td>
<td>10</td>
</tr>
<tr>
<td>HB (gm/dl)</td>
<td>9.14 ± 2.12</td>
<td>8.50 to 9.8</td>
<td>15.5</td>
<td>5.5</td>
</tr>
<tr>
<td>WBC (x10⁹/L)</td>
<td>250.9 ± 160.1</td>
<td>202.9 to 299.1</td>
<td>744.0</td>
<td>17.3</td>
</tr>
<tr>
<td>Platelet (x10⁹/L)</td>
<td>494.3 ± 319.3</td>
<td>398.4 to 590.2</td>
<td>1600</td>
<td>18</td>
</tr>
<tr>
<td>Spleen size (cm/bcm)</td>
<td>7.4 ± 3.7</td>
<td>6.3 to 8.51</td>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>

Hb: Haemoglobin; WBC: White Blood Cell Count; bcm: below costal margin.

### Table II: Comparison of characteristics between JAK2V617F positive and JAK2V617F negative patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Positive (n=12)</th>
<th>Negative (n=33)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.7 ± 15.08</td>
<td>39.21 ± 13.49</td>
<td>0.743</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>8.23 ± 1.36</td>
<td>9.47 ± 2.26</td>
<td>0.083</td>
</tr>
<tr>
<td>WBC (x10⁹/L)</td>
<td>272.75 ± 173.04</td>
<td>243.07 ± 157.13</td>
<td>0.055</td>
</tr>
<tr>
<td>Platelet (x10⁹/L)</td>
<td>423.58 ± 415.22</td>
<td>298.4 ± 157.13</td>
<td>0.09</td>
</tr>
<tr>
<td>Spleen size (cm/bcm)</td>
<td>8.50 ± 3.53</td>
<td>7.00 ± 3.7</td>
<td>0.233</td>
</tr>
</tbody>
</table>

Hb: Haemoglobin; WBC: White Blood Cell Count; bcm: below costal margin.

### Table III: Comparative analysis of the patients with JAK2V617F mutation positive and negative patients after the median follow-up of 8 months (paired sample t-test).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre Post (8th months)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n=20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>9.43 ± 2.42</td>
<td>10.37 ± 2.16</td>
</tr>
<tr>
<td>Plt</td>
<td>496.55 ± 318.38</td>
<td>298.4 ± 157.13</td>
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<tr>
<td>WBC</td>
<td>256.21 ± 185.37</td>
<td>298.4 ± 157.13</td>
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<tr>
<td>Spleen size</td>
<td>6.80 ± 3.75</td>
<td>7.00 ± 3.7</td>
</tr>
<tr>
<td>Positive (n=11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>8.12 ± 1.37</td>
<td>8.55 ± 2.36</td>
</tr>
<tr>
<td>Plt</td>
<td>435.64 ± 433.28</td>
<td>298.4 ± 157.13</td>
</tr>
<tr>
<td>WBC</td>
<td>282.11 ± 178.27</td>
<td>298.4 ± 157.13</td>
</tr>
<tr>
<td>Spleen size</td>
<td>8.36 ± 3.67</td>
<td>8.64 ± 7.12</td>
</tr>
</tbody>
</table>

Hb: Haemoglobin; WBC: White Blood Cell Count; bcm: below costal margin.

Figure 1: Showing the 2% gel electrophoresis on 3 samples, ladder of 100 bp, negative control and a positive control.
treatment with Imatinib mesylate.7 The JAK2V617F positive blast crisis patients in the present study, did respond to Imatinib mesylate. Patients who progressed to blast crisis only showed haematological response initially, but transformed within few months. Two JAK2V617F positive chronic phase patients transformed despite Imatinib therapy, while remaining 3 did poorly on hydroxyurea.

These findings need confirmation in a larger cohort. If established, they have a major implication in CML management; JAK2V617F positive CML at diagnosis may be considered for upfront haematopoietic stem cell transplantation. It remains to be seen whether this mutation reflects two diseases in one patient or simply genetic aberration in a single disease. Follow-up of JAK2V617F mutation analysis in JAK2V617F positive patients is required to observe the behaviour of the disease for early disease progression and response to therapy.

CONCLUSION

JAK2V617F mutation was detected in 26.7% cases of chronic myeloid leukemia. A significant proportion of them showed early disease progression.

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REFERENCES


