CORRELATION BETWEEN JANUS KINASE 2 (JAK 2-V617F) POSITIVITY, LAP SCORE AND BONE MARROW FIBROSIS IN CASES OF THROMBOCYTOSIS

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ABSTRACT

Objective: Raised platelets count may result from essential thrombocythemia (ET) and also seen in cases of secondary thrombocytosis. It is essential to confirm the diagnosis of ET and differentiate it from secondary causes of raised platelet count. Molecular testing has become part of protocols in the diagnosis of myeloproliferative disorder including cases of thrombocytosis to confirm the diagnosis of ET. JAK2 V617F mutation is seen with a frequency of 55% in cases of ET and in many cases this mutation is negative. Molecular studies are very expensive and beyond the reach of majority of patients in developing countries. The aim of present study is to establish correlation between JAK-2 positivity, Leucocyte Alkaline Phosphate (LAP score) and bone marrow fibrosis in the cases of thrombocytosis to confirm the diagnosis of Essential Thrombocythemia.

Material and Methods: Between Jan 2019 to August 2019, 27 patients with platelet counts more than 600x109/l were enrolled in the study. Median age was 56 (39-75) years, Male to Female ratio 3:1. Investigations carried out were blood complete picture, LAP score, bone marrow aspiration and trephine biopsy stained with both H&E and reticulin stain. PCR for JAK2 V6187F was performed on real time PCR.

Results: LAP score was raised (>150) in 22 cases, bone marrow fibrosis more than grade II was present in 20 cases. JAK2 V617F was positive in 20 cases. In this study cases with raised LAP score and bone marrow fibrosis more than grade II JAK2 positivity was seen in 74% cases in contrast to 55% JAK2 V617F positivity seen in cases of thrombocytosis.

Conclusion: LAP score and bone marrow examination are cost effective and widely available procedures at a District Head Quarter Hospitals are sensitive indicator of JAKV617F positivity, the facility for molecular studies are available in a few specialized centers in the country and these studies are very expensive as well. These simple tests should be performed before proceedings to molecular studies.

Key Words: ET, JAK2V617F, LAP score, Bone marrow fibrosis.

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INTRODUCTION

Myeloproliferative disorders (MPD) are group of colonels and haematological disorders characterized by aberrant haemopoietic proliferation and increased tendency towards leukaemic transformation. The World Health Organization (WHO classified MPD into two general categories. Chronic myeloproliferative diseases and myelodysplastic/ myeloproliferative diseases [1].

The four major classic MPD are BCR-ABL positive (Chronic Myeloid Leukaemia (CML) Polycythemia Rubra Vera (PV), ET and Myelofibrosis (MF) [3]. These disorders are characterized by specific clinicopathologic criteria. PV, ET and MF share several clinical and laboratory features including increased number of one or more circulating mature cell types, hepatosplenomegaly, colonel marrow hyperplasia without extensive dysplastic changes [2] ET is characterized by increased production of megakaryocytes in bone marrow resulting in increased platelet count in peripheral blood. The platelet count is more than 600 X10 9 /L. There is no identifiable cause of secondary thrombocytosis and bone marrow examination show increased megakaryocytes.

Janus Kinase -2(JAK-2) mutation is a major criterion for the diagnosis of ET [4,8]. JAK-2 belongs to family of non-receptor tyrosine kinases and plays a role in transducing signals via JAK signal transducer and activator of transcription pathway (JAK-STAT) JAK 2 is essential in signaling pathways for hormone like cytokines such as growth hormone, erythropoietin (EPO) thrombopoietin (TPO), granulo

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macrophage colony stimulating factor (GMCSF) [5]. JAK2 V617F mutation is seen with a frequency of 55% in cases of ET and in many cases this mutation is negative. Molecular studies are very expensive and beyond the reach of majority of patients in our country. The aim of present study is to establish correlation between JAK-2 positivity, Leucocyte Alkaline Phosphate (LAP score) and bone marrow fibrosis in the cases of thrombocytosis to confirm the diagnosis of ET.

MATERIAL AND METHODS

Currently, patients with ET are classified on the basis of clinical and laboratory criteria. The biological factors underlying these distinctive MPD are poorly understood. Between Jan 2019 to August 2019, 27 patients with platelet count more than 600 X10/L were enrolled in the study. Median age was 56 (39-75) years, male to female ratio 3:1. Investigations carried out were blood complete picture, LAP score, bone marrow aspiration and trephine biopsy stained with both H&E and reticulin stain. PCR for JAK2 V617F was performed on real time PCR. Determination of LAP score was based on enzyme reaction with leukocyte alkaline phosphatase liberating naphtol or a substituted naphthol compound which then couples with fast blue RR or other chromogen to form an insoluble precipitate. Colour of the precipitate relates to the type of substituted naphthol substrate and diazonium dye used (colour is reagent dependent). Cells are scored as to the degree of phosphates activity present 0 to 4+. One hundred cells are counted and the score totaled. Selection criteria for patients was sustained platelet count >600,000 U/L, absence of conditions associated with secondary thrombocytosis, normal stainable iron on marrow biopsy, normal hematocrit and MCV, cytogenetic without (9;22) and no evidence of BCR-ABL gene rearrangement. No morphologic or evidence of myelodysplastic syndrome and leukoerythroblastic changes on peripheral blood and bone marrow examination.

RESULTS

LAP score was raised (>150) in 22 cases, bone marrow fibrosis more than grade II was present in 20 cases. JAK2 V617F was positive in 20 cases. In this study cases with raised LAP score and bone marrow fibrosis more than grade II JAK2 positivity was seen in 74% cases in contrast to 55% JAK2 V617F positivity seen in cases of ET.

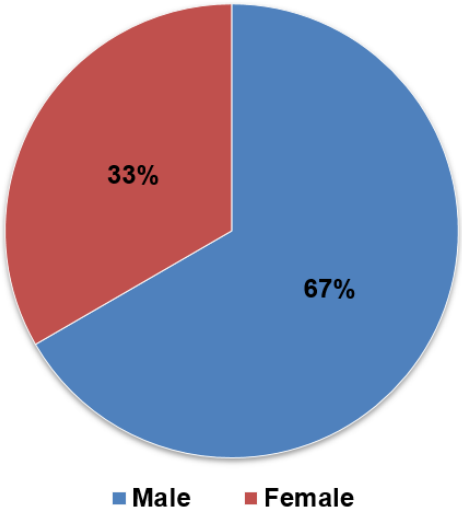

Figure-1: No of patients according to gender.

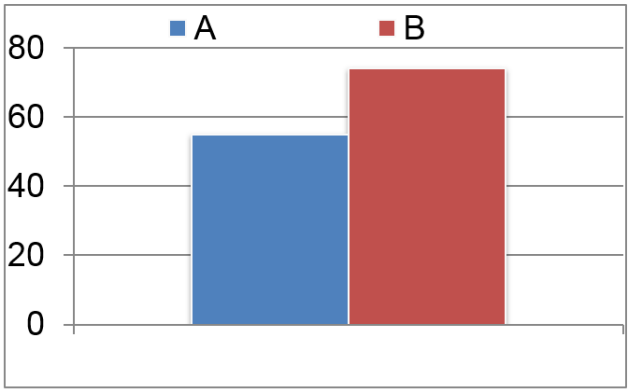

Figure-2: A: JAK2 V617F positivity in cases of thrombocytosis. B: JAK2 V617F positivity in cases with raised LAP score and bone marrow fibrosis more than grade II.

DISCUSSION

JAK2 was first described in 2005 and now molecular testing has become part of protocols in the diagnosis of ET [11]. Frequency of JAK-2positivity in case of ET is 55% and, in many cases, JAK-2 is negative. JAK-2 mutation is the major criteria for the diagnosis of MPD [7]. The discovery that JAK 2 mutation is detected in ET and other MPD has proved turning point in understanding pathophysiology of these disorders.

The Janus Kinase (JAK) family is one of the ten recognized families of non-receptor tyrosine Kinases. JAK family is intracellular that plays a role in transducing signals via the JAK Signal transducer and Activator of Transcription Pathway (JAK-STAT) [12]. The JAK-STAT system consists of three components. Receptor penetrates the cell membrane, JAK is located intracellularly and is bound to the receptor. STAT carries the signal into nucleus and DNA. Transgenic mice that do not

express JAK1 have defective response to some cytokines such as interferon-gamma [6].

JAKs have seven regions of homology called Janus homology domains 1 to 7 (JH 1-7). JH 1 is a kinase domain. JH 1 contains typical features of tyrosine kinase and is important for enzymic activity necessary for the activation of JAK-2, JAK-3 and Ty K2. JH2 is a pseudokinase domain and lacks enzymatic activity. The JH 3 JH 4 domains of JAK share homology with sre-homology 2 (SH2) domains. The amino terminal and JH 4-JH7 of JAKS are called FERM domain (band 4.1 Ezrin, Radixin and Moesin). This domain in association with other JAKs and serve as cytokine receptor.

JAK-2 is essential for hormone like cytokines such as growth hormone erythropoietin (EPO) thrombopoietins (TPO), GM-CSF and IL2. Binding of EPO to its cell membrane receptor of developing normoblasts activates the JAK 2 which in turn stimulate signal transduction pathways to increase proliferation [9].

ET is strongly associated with acquired mutations in an auto inhibitory domain of the JAK 2 tyrosine Kinase. The specific JAK 2 mutation closely associated with MPD V617F causes constitutive activation of JAK 2 Kinase domain which results in thrombopoiesis losing its dependency on growth factors signaling and becoming virtually autonomous [10].

Normal to elevated levels of TPO are observed in most patients with ET. JAK2V617F is relevant in ET as MPL signals through JAK. A large number of cytokines are dependent on JAK intracellular signaling.

The discovery that JAK 2 mutation is detected in patients with ET is a great breakthrough in understanding the pathophysiology of these disorders [13] Ruxolitinib is a JAK 2 inhibitor which has been approved by FDA for the treatment of ET. Ruxolitinib effectively control systemic symptoms, reduces, splenomegaly and also prolongs overall survival in ET patients [14]. Molecular studies are very expensive, require expertise and available only in few centres. In this study we have established correlation between LAP score and bone marrow fibrosis which are less expensive investigation and are easily available at District Head Quarter Hospital. JAK2 V617F mutation is seen with frequency of 55% in cases of ET and remaining cases this mutation is negative. In this study out of 27 cases with raised platelet count LAP score was high in 22 cases and bone marrow fibrosis more than grade II was present in 20 cases. JAK2V617F positivity in cases with raised LAP Score and bone marrow fibrosis was 74%. We recommend that easily and cost-effective investigations should be completed first before embarking on expensive investigation as most of our patients are non-affording.

CONCLUSION

Molecular techniques like Real time PCR for JAK2v617F mutation detection is an expensive modality for screening of suspected cases of thrombocytosis and facility for these studies are available only in specialized centers. LAP score and bone marrow examination are cost effective and widely available procedures at a District Headquarter Hospital are sensitive indicator of JAKV617F Positivity. These simple tests should be performed before proceedings to molecular studies. This study has shown that LAP score. Bone marrow aspiration and trephine biopsy is sensitive surrogate marker for screening of patients with raised platelet count.

AUTHORS CONTRIBUTION

Muhammad Adil Ayub: Investigator and writing original draft preparation
Muhammad Usman: Supervisor
Fahim Akhtar: Literature review
Muhammad Khan: Data curator
Maria Mumtaz: Proof reading
Iram Nazir: Statistical analysis and supervision
Maeesa Wadood: Methodology and supervision

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