FREQUENCY AND CLINICOMORPHOLOGICAL FEATURES OF JAK2V617F POSITIVE AND NEGATIVE POLYCYTHEMIA IN A TERTIARY CARE HOSPITAL OF PAKISTAN

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ABSTRACT

Background and Objectives: Polycythemia is a disorder characterized by raised hematocrit (HCT) level of ≥ 0.49 in males and ≥ 0.48 in females[1]. Although a hemoglobin (Hb) level of 16.5g/dl in males and 16g/dl in females is considered as an indication of absolute erythrocytosis, signs and symptoms related to polycythemia may be present at levels below that. WHO criteria for diagnosis of polycythaemia vera included a gain-of-function mutation JAK2V617F as the major diagnostic criteria[1].

Methods: A total of 40 patients of both primary and secondary polycythaemia were included in the study. Study is carried out over a period of 2 years i.e. from Jan 2014 – Jan 2016 at Allama Iqbal Medical College/Jinnah Hospital Lahore Pakistan. Clinicomorphological features and frequencies of different causes of polycythaemia were studied.

Results: In a total of 40 patients were diagnosed as primary polycythaemia or polycythaemia Vera (PRV). JAK2V617F mutation was not detected in 12 patients. Headache, erythromelalgia were the most common symptoms present. Patients were treated with phlebotomies and cytoreductive treatments in case of PRV and phlebotomies alone in secondary polycythaemia.

Conclusion: The most frequent symptoms in patients with polycythaemia were erythromelalgia, facial plethora and headaches. Patients with secondary polycythaemia should be investigated for the cause and treated accordingly.

Key Words: Polycythaemia, JAK2 mutation, erythropoietin.

INTRODUCTION

The polycythaemias are usually classified as relative and absolute.[1] Relative polycythaemia is a condition in which the patient characteristically has an elevation of the hematocrit level without an elevated red cell mass but it is due to contraction of the plasma volume, in contrast the absolute polycythaemias are accompanied by an actual increase in the red cell mass.[1] Polycythaemias can also be classified according to the response of their erythroid progenitor cells to growth factors like erythropoietin or the circulating levels of it.[1,2] Primary polycythaemias are characterized by increased sensitivity of the erythroid progenitors to regulatory growth factors, as a result of acquired somatic or inherited mutations expressed by progenitor cells. In contrast, secondary polycythemias are characterized by an increase in growth factors, primarily erythropoietin.[3] These conditions can usually be distinguished by *in vitro* assays of erythroid progenitor cells, quantitation of serum EPO levels, and detection of somatic JAK2V617F mutations.[1] Polycythemia is usually characterized by a HCT level of ≥ 0.49 in males and ≥ 0.48 in females. A hemoglobin level of 16.5g/dl in males and 16g/dl in females is considered as an indication of absolute erythrocytosis.[1] Early stages of polycythemia can be overlooked if this criteria for diagnosis of polycythemia is considered. Red cell mass is also included in recent criteria.[1] A gain–of-function mutation JAK2V617F is considered as major criterion for the diagnosis of polycythemia vera according to WHO[1,2]. Treatment modalities in both JAK2V617F positive and negative polycythemia are different.[2] The somatic V617F mutation in the Janus kinase (JAK2) gene which causes a valine to phenylalanine replacement at position 617 is found in 95% of cases of polycythemia Vera.[1,4]

In this work we have studied different aspects of clinicomorphological features and frequency of JAK2-V617F positive and negative polycythaemia so that morbidity and mortality resulting from this disorder can be prevented.

MATERIAL AND METHOD

This study is cross sectional and was carried out in Allama Iqbal medical college/Jinnah Hospital Lahore for a period of 2 years from Jan 2014 – Jan. 2016.

Inclusion criteria for this study were both genders

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with age above 18 years. It included cases with raised hemoglobin and hematocrit referred to Hematology department for evaluation or for bone marrow biopsy.

Cases of apparent polycythemia with history of dehydration and/or on diuretic therapy were excluded. These patients were tested subsequently after rehydrating them or after discontinuation of diuretic therapy. If Hb and HCT were still raised, those patients were also included.

Forty patients were included in the study. They were diagnosed as having polycythaemia on the basis of history, physical examination findings and with HCT ≥ 0.49% in males or ≥ 0.48% in females. A few patients with Hb within normal range were also included if other parameters indicating polycythemia were present such as raised HCT. Polycythemia vera is diagnosed according to WHO criteria including JAK2V617F mutation, serum erythropoietin levels and bone marrow biopsy. JAK2V617F mutation was detected by using allele specific PCR done on DNA from granulcytes in peripheral blood. Bone marrow biopsy was done in patients with high suspicion of clonal polycythemia.

Cases of secondary erythrocytosis who were negative for JAK2V617F and had raised serum erythropoietin levels were tested through different tests for determination of etiology. They were asked about some important points in the history which included H/O visit to hilly area, H/O smoking, signs of dehydration. Family history of erythrocytosis etc. was also inquired. Patients were examined for important signs like erythromelalgia, facial plethora, numbness and splenomegaly. Patients were examined for signs of arterial or venous thrombosis. Investigations were done for secondary causes which included chest X-RAY, CT scan Chest, pulse oximetry for oxygen saturation, echocardiography, detection of high affinity hemoglobins with Hb-electrophoresis, ultrasonography abdomen, CT scan abdomen. CT scan brain was done for suspected meningioma. Data was analysed with SPSS version 21 and results are expressed as mean, standard deviation and frequency/ percentages.

RESULTS

A total of 40 patients were included in the study. Among them 30 were males and 10 were females (figure 1). Mean age of the patients was 58 ± 22 years. Mean Hb was 19 ± 3 and 20 ± 3 while mean HCT was 55 ± 3 and 56 ± 3 in JAK2 positive and negative polycythaemia cases respectively (Table 2).

JAK2V617F mutation was done in all patients included in the study. It was positive in 28 patients. It was done with RT-PCR. Mean erythropoietin levels in cases of polycythaemia vera were 1.5IU/L while mean erythropoietin levels in secondary erythrocytosis cases was 14IU/L (normal value 2.4-5.2IU/L). Bone marrow biopsy was done in all such patients who were suspected to have polycythaemia Vera which were 30 in number. Panmyelosis was present in all of the biopsies and characteristic megakaryocytic pleomorphism was appreciated.

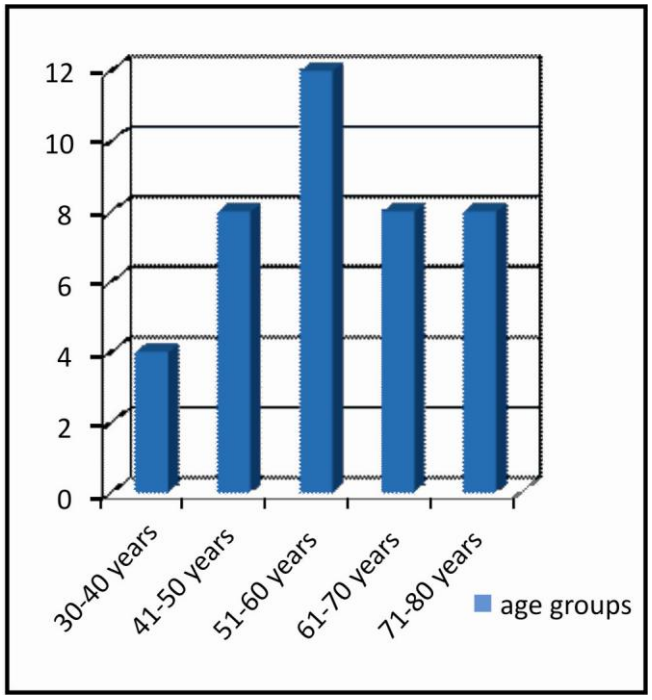

Fig. 1: *Age Groups in Study.*

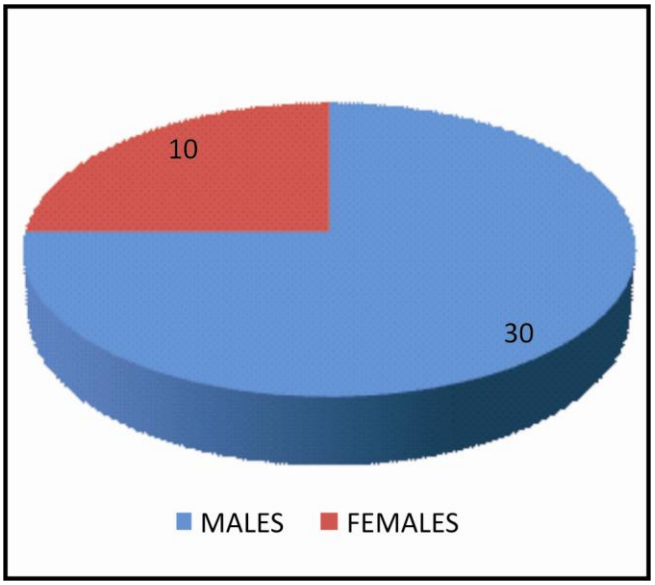

Fig. 2: *Gender Distribution.*

Frequency of different signs and symptoms are analysed in these patients. Headache, erythromelalgia and facial plethora is present in 100% of the patients (Table 1). Patients with JAK2 negative polycythaemia were tested for the cause of polycythemia. There were total 12 patients who were negative for JAK2V617F.

One of JAK2V617F negative male patient who presented in surgical department for operating inguinal hernia, incidentally diagnosed as having polycythemia and had low serum erythropoietin levels. His Hb was 20g/dl at presentation with HCT 60%. JAK2V617F mutation was negative in that patient. A female patient who was JAK2V617F mutation negative developed DVT. Bone marrow biopsy was done in these cases which was highly suggestive of polycythaemia Vera. Therefore these patients were also included in polycythaemia Vera.

Among cases of secondary polycythemia there were 4 cases of renal cysts, 3 case of congenital heart disease, 2 of adrenal tumour and 1 case of renal cell carcinoma. In all these cases of secondary erythrocytosis EPO levels were raised (Table 3).

Haematological parameters of polycythemia vera and secondary erythrocytosis are compared. There is statistical significant difference between primary and secondary polycythaemia in TLC, platelets, MCV, MCH and basophil count.

Patients with secondary erythrocytosis were treated for underlying cause and phlebotomies while those with polycythaemia Vera were treated with hydroxyurea and phlebotomies.

Table 1*: Signs and Symptoms in 40 Patients with Polycythemia.*

Sr. No.	*Signs and Symptoms*	*No. of Patients/ Frequency*
1.	H/O Visit to Hilly Area	0%
2.	H/O Smoking	33% (13)
3.	Dehydration	0%
4.	Family H/O Polycythemia	23% (9)
5.	H/O Congenital Heart Disease	8% (3)
6.	Intake of Hematinics	11% (4)
7.	Renal or Pulmonary Disease	13% (5)
8.	H/O Renal Transplantation	0%
9.	Headaches	100% (40)
10.	H/O Erythromelalgia	100% (40)
11.	Aquagenic Pruritis	43% (17)
12.	Numbness	33% (13)
13.	Dyspnea	23% (9)
14.	Oliguria	11% (4)
15.	Flacial Plethora	100% (40)
16.	Hypertension	33% (13)
17.	Splenomegaly	43% (17)
18.	Signs of Arterial or Venous Thrombosis	11% (4)
19.	Signs and Symptoms Related to Some Malignancy	8% (3)

DISCUSSION

Polycythaemia can be primary, secondary or idiopathic. Diagnosis of polycythaemia is revolutionized as a gain-of-function mutation JAK2V617F was found in more than 90% of the patients with polycythemia vera.[2] Differentiation between primary and secondary polycythaemia can be done more precisely. But there are still cases in which no cause for polycythemia can be demonstrated.[3,4] In this study we analysed clinicomorphological features in different patients of polycythemia.

In all patients headache was the most common symptom present. In a Chinese study this symptom was present in 17% of the patients.[6] Facial plethora was present in 100% of our patients while in another study it was present in 54% of the patients[5].Among different signs, erythromelalgia was most common. Splenomegaly is appreciated in 43% of our patients. In studies performed in other centers splenomegaly either radiological or clinically palpable is present in 68%.[6] Aquagenic pruritis is present in 50% of polycythemia patients in another study[6] and 43% of all patients in our study while another study done in France revealed its presence in 67% of PRV patients.[7]

JAK2V617F was detected in 28 patients and EPO levels were low in them. However in 2 other patients who were negative for JAK2V617F but clinically and morphologically resembles PRV should be tested for JAK2Exon12 mutation as pointed out in a study done on patients of polycythemia with unmutated JAK2 V617F. In that study JAK2Exon12 mutation was detected in 3.7% of the patients.[4] AK2EXON12 mutation is not done as it is available in only a few centers worldwide. One patient with JAK2V617F negative polycythemia in our study was isolated erythrocytosis but bone marrow biopsy was suggestive of polycythaemia vera. It is in accordance with other studies also showing isolated raised Hb and haematocrit with normal WBC and platelet count.[8,9]

Evidence of thrombosis was present in 4 of the patients who was found to be JAK2V617F mutation positive and also in one patient who was JAK2V617F mutation negative. That patient developed myocardial infarction and repeated episodes of digital ischemia of

feet. In another study thrombosis was reported in 7.6% of the patients.[10] The use of cytoreduction significantly reduced thrombotic risk in these cases.[10] The patients in our study did well after initiation of cytoreduction and no evidence of cardiac or digital ischemia was reported afterwards.

In all these cases of secondary erythrocytosis EPO levels were raised. However in some studies it was normal in a few cases with secondary polycythemia as well.[11]

It is ***concluded*** that the most frequent symptoms in patients with polycythemia are erythromelalgia, facial plethora and headaches. Although polycythaemia vera diagnosis requires JAK2V617F positivity and low serum erythropoietin levels, there are some patients with polycythemia who did not fulfill this criteria but bone marrow morphology strongly favored the diagnosis of polycythemia vera. However cases with clinicomorphological and laboratory features favoring polycythemia vera should be tested for other mutations. Patients with secondary erythrocytosis should be investigated for the cause and treated accordingly.

Table 2: *Hematological parameters in 40 patients of polycythemia.*

Sr. No.	*Hematological Parameters*	*Mean (JAK2V617F positive)*	*Mean (JAK2V617F Negative)*	*P value (t test)*
1.	Hb g/dl	19 ± 3	20±3	0.1
2.	TLC x10^9/l	16 ± 4	08±2	0.02*
3.	Plts x10^9/l	500 ± 100	200±50	0.01*
4.	MCV fl	75 ± 6	90±3	0.05*
5.	MCH pg	20 ± 3	28±2	0.04*
6.	MCHC g/dl	22 ± 3	30±4	0.6
7.	HCT %	55 ± 3	56±3	0.7
8.	Neutrophils x10^9/l	7 ± 2	5±4	0.6
9.	Lymphocytes x10^9/l	2 ± 1	2±2	0.9
10.	Monocytes x10^9/l	0.8 ±0.2	0.7±0.2	0.8
11.	Eosinophils x10^9/l	0.3 ± 0.1	0.2±0.1	0.5
12.	Basophils x10^9/l	0.4 ± 0.2	0.04±0.01	0.04*

*= Statistically significant p value

Table3: *Frequency of different disorders in patients with secondary polycythemia.*

Sr. No.	*Cause of Secondary Polycythemia*	*Number of Patients*
1.	Renal Cyst	4
2.	Renal cell carcinoma	1
3.	Adrenal tumor	2
4.	Congenital cardiac disease	3

Conflict of Interest

There is no conflict of interest among authors.

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Authors' Contributions

Conceived and designed the experiments: Azmi R. Analysed the data: Azmi R, Natiq M. Wrote the first draft of the manuscript: Azmi R, Khalid A. Contributed to the writing of the manuscript: Azmi R. Jointly developed the structure and arguments for the paper: Azmi R, Khalid A. Made critical revisions and approved final version: Natiq M. All authors reviewed and approved of the final manuscript.

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