Dissecting Primary Erythrocytosis Among Polycythemia Patients Referred to an Indian Armed Forces Hospital

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Abstract Referrals for evaluation of polycythemia cases have increased since the hemoglobin (Hb) thresholds for diagnosis of Polycythemia Vera (PV) have been lowered by WHO. The current study enrolled patients of age > 18 years from the Indian Armed Forces or their family members with polycythemia from November 2016 to October 2018. After exclusion of secondary causes, 49 patients were diagnosed as Primary Erythrocytosis (PE). The patients were classified into two groups: PV and Idiopathic Erythrocytosis (IE) and a systematic comparison of clinical and laboratory features of the two groups was done. The prevalence of PV in PE was 20.4% (10 of 49) while the rest 39 (79.6%) had IE. Seven PV patients had *JAK2*^{V617F} mutation, one had *JAK2*^{Exon12} mutation, and two were *JAK2* negative PV. Nine of 10 (90%) PV patients had Hb > 18.5 g/dl, while only 21 of 39 (53.8%) IE patients had Hb > 18.5 g/dl ($p = 0.06$). None of the *JAK2* mutated patients had Hb < 18.5 g/dl. We conclude that PV is more prevalent in patients of PE with Hb > 18.5 g/dl. Most patients with Hb between 16.5–18.5 g/dl would still be classified as IE. We advocate the need for further studies evaluating the utility of investigating all patients of PE with the revised WHO Hb threshold as well as studies on genetic profile of IE patients from India.

Keywords Polycythemia · Erythrocytosis · Erythropoietin · *JAK2* mutation

Introduction

‘Polycythemia’ or ‘Erythrocytosis’ refers to disorders characterized by elevated hemoglobin (Hb). Erythrocytosis is classified as ‘Primary Erythrocytosis’ (PE) when there is an intrinsic defect in erythropoietic lineage with inappropriately low serum erythropoietin (EPO) levels, or ‘Secondary Erythrocytosis’ (SE), when the increased red cell production is driven through EPO with normal or increased EPO levels [1, 2]. Among PE patients, those with evidence of clonal myeloproliferation are referred as ‘Polycythemia Vera’ (PV) [1]. The etiology of SE is usually established through history, clinical examination and routine investigations. Despite exclusion of PV and SE, no cause is identified in a group of patients who are labelled as ‘Idiopathic Erythrocytosis’ (IE) [3]. Increasing number of genetic defects in the oxygen sensing, erythropoiesis and oxygen transport pathways are being described in patients with IE [3]. The prevalence of *JAK2* mutated PV in patients of PE has been variably reported from 1.4% to 40% and continues to change with the changing WHO definitions of PV [4, 5]. There is scarce data available regarding the prevalence of PV and IE in patients of PE from India.

We performed a prospective single centre cross sectional study to determine the prevalence of PV and IE among PE patients and compared their characteristics.

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Patients and Methods

All patients of age > 18 years referred to our armed forces hospital, who fulfilled WHO 2016 definition of polycythemia i.e. Hb > 16.5 g/dl in men or > 16.0 g/dl in women; Hct > 49% in men or > 48% in women confirmed on two occasions one week apart, were enrolled in the study after informed consent [6]. All identifiable causes of SE due to hypoxia, sleep apnoea, excessive smoking, alcohol abuse, high altitude, renal disease, paraneoplastic causes and use of androgens were ruled out based on history, clinical findings and relevant investigations. Family history was obtained to rule out inherited causes. Majority of them had previously documented normal Hb at the time of joining the military and mandatory annual medical examinations carried out thereafter.

Serum EPO levels were estimated by chemiluminiscent immunometric assay by using immulyte analyser (Siemens-1000), with normal range being 5.4 and 31 mIU/ml as per the reference kit. All patients with PE were tested for *JAK2*^(V617F/Exon12) mutations using recommended real time polymerase chain reaction technique [7]. Bone marrow (BM) examination was done for all patients (46 of 49), except three who already fulfilled the WHO 2016 criteria for PV based on Hb, *JAK2* mutation positivity and sub-normal EPO.

Patients with low/normal serum EPO levels and no other identifiable secondary or primary cause were classified as IE and were compared with patients of PV [6].

Descriptive statistics were presented as median with inter quartile range (IQR), and comparison of variables among the PV and IE groups was done by calculating percentages.

Results

A total of 87 patients presenting with polycythemia over a period of 24 months were enrolled. Thirty five patients (40.2%) who were classified as SE were excluded from the analysis, 3 patients (3.5%) refused to participate in the study, and the remaining 49 patients (56.3%) were included for analysis. The consort diagram for distribution of all polycythemia patients enrolled in the current study is as given in Fig. 1. All patients except one were males as the study was conducted in an armed forces hospital.

The overall prevalence of PV was 20.4% (10 patients), while 79.6% (39 patients) belonged to IE group. *JAK2*^(V617F/Exon12) mutation was detected in 80% (8 out of 10) of PV patients. Seven of these patients had *JAK2*^{V617F} mutation, while one patient had *JAK2*^{Exon12} mutation. Two

patients were diagnosed as *JAK2* negative PV based on BM panmyelosis with low EPO levels.

Thirty of 49 PE patients (61.2%) had Hb > 18.5 g/dl and 19 patients (38.8%) had Hb 16.5 to 18.5 g/dl. Ninety percent (9/10) of PV patients had Hb > 18.5 g/dl, while only 21 of 39 (53.8%) IE patients had Hb > 18.5 g/dl ($p = 0.06$). None of the *JAK2* mutated patients had Hb < 18.5 g/dl.

Serum EPO levels were normal in 26 of 49 (53.1%) and low in 23 (46.9%) PE patients. Seventy percent of PV patients (7/10) and 41% (16/39) of IE patients had low EPO.

Two of 10 (20%) PV patients [1 myocardial infarction (MI), and 1 deep vein thrombosis (DVT)], while 6 of 39 (15.4%) PE patients (1 thrombotic stroke, 1 MI, and 4 DVT) had evidence of thrombosis.

Table 1 summarises the clinical and laboratory findings of PV and IE patients.

Discussion

There is scarce data on the epidemiology and clinical profile of polycythemia from our country, particularly using the revised WHO 2016 Hb threshold for polycythemia [8]. The prevalence of *JAK2* mutation in general population is 0.2% and increases to 0.8% in patients who meet WHO 2016 criteria for polycythemia [9].

Using Polycythemia Vera Study Group Criteria, Usman et al. [10], reported 43.3% prevalence of PV from Pakistan in patients of PE, while Chacko et al. [11], reported 40.2% prevalence of PV using WHO 2008 criteria among PE patients. The lower prevalence of PV (20.4%) in the current study is likely because of the redefined WHO 2016 Hb threshold used to define polycythemia.

The PV group had higher prevalence of splenomegaly, fatigue; higher Hb, Hct, and leucocyte count similar to other studies [12–14]. In the PV group, 70% patients had low EPO, comparable with other studies [15, 16]. On the contrary in the IE group, 41% patients had low EPO levels but none of them had any family history to support an inherited cause of PE. Notably, 90% of the PV patients had a Hb > 18.5 g/dl. None of the patients with Hb < 16.5–18.5 g/dl had *JAK2* positivity. We therefore conclude that *JAK2* mutated PV is more prevalent in patients of PE with Hb > 18.5 g/dl, while most patients of PE with Hb ranging from 16.5 to 18.5 g/dl would continue to be classified as IE based on routine investigations.

Referrals for evaluation of polycythemia have increased ever since the WHO lowered the Hb threshold for diagnostic criteria of PV. Using the current WHO 2016 criteria, 4.1% of the unselected males and 0.35% of the females from Canada; and 5.6% males and 0.22% females in Brazil

Fig. 1 Distribution of polycythemia patients in the current study **Table 1** Comparison of clinical and laboratory characteristics of Polycythemia Vera (PV) and Idiopathic Erythrocytosis (IE) patients

Parameters	Polycythemia Vera (PV) (n = 10, 20.4%)	Idiopathic Erythrocytosis (IE) (n = 39, 79.6%)
Total patients of Primary Erythrocytosis (PE) (n = 49)		
Age (years), median (IQR ^a)	58 (36–73)	39 (32–50)
Splenomegaly (n, %)	2 (20%)	None
Headache (n, %)	2 (20%)	4 (10.3%)
Fatigue (n, %)	4 (40%)	1 (2.6%)
Hypertension (n, %)	5 (50%)	7 (17.9%)
Thrombotic complications (n, %)	2 (20%)	6 (15.4%)
a. Venous	1	4
b. Arterial	1	2
Hemoglobin (g/dl), Median (IQR ^a)	19 (18.6–20.8)	18.6 (18–19.9)
Hematocrit (%), Median (IQR ^a)	57.8 (54.5–58.7)	52.7 (51.2–56.0)
TLC (cumm), Median (IQR ^a)	11,700 (7800–15,775)	7800 (6200–8650)
Platelets (cumm), Median (IQR ^a)	263,000 (210,750–375,750)	243,000 (197,000–287,500)
EPO level (mIU/ml), Median (IQR ^a)	4.5 (3.2–10.8)	7.1 (4.53–9.7)

^aIQR inter quartile range

were classified as suspected PV [17]. However similar data were lacking from India. The benefits of diagnosing masked PV cases who have increased risk of thrombosis as advocated by revised WHO guidelines [6] need to be weighed against the economic burden of unnecessary testing of patients with borderline high Hb in the Indian context with resource constraints.

There is considerable heterogeneity in the management of erythrocytosis among the clinicians across the world [18]. With better understanding of physiologic pathways and molecular mutations that lead to erythrocytosis, IE is a shrinking entity in today's world. Sensitive molecular techniques like targeted next generation sequencing may detect novel or variant mutations in *JAK2* genes in apparent *JAK2*^(V617F/Exon12) negative PV [19, 20]. Genetic mutations in the erythropoiesis and hypoxia sensing pathways have been detected in upto 20% patients of IE [21]. Studies, including one from India suggest that patients with EPO level below normal may be screened for mutations involving the EPO signalling pathway (EPOR exon 7–8) while those with an inappropriately normal EPO level with respect to their Hb may be screened for mutations in the oxygen sensing pathway (*VHL*, *PHD2*, *HIF2 α* , β globin and α globin gene) [22, 23].

The cost effectiveness and clinical utility of using EPO directed algorithms to dissect PE remains unknown and needs validation in future multicentric studies with adequate representation from both sexes and general population.

Conclusion

The prevalence of PV has changed with the revised WHO 2016 criteria. In this prospective study, 20.4% of PE patients were classified as PV. The prevalence of PV was higher in PE patients with Hb > 18.5 g/dl, while majority of patients with Hb between 16.5 and 18.5 g/dl were classified as IE. Further studies are required to assess the utility of investigating all PE patients with the revised WHO Hb threshold as well as to determine the genetic profile of IE patients in India.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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