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Clinico-Hematological Patterns of Patients Presenting With Erythrocytosis

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ABSTRACT

Objective: To assess the frequency of erythrocytosis and compare the clinical features and haematological parameters in patients presenting with primary and secondary erythrocytosis in the Pakistani population.

Study Design: Cross-sectional study.

Place and Duration of Study: Armed Forces Institute of Pathology, Rawalpindi Pakistan, from May 2020 to May 2021.

Methodology: Eighty-three subjects presenting with marked erythrocytosis (Hb-17 g/dl or more, HCT – 50% or more) were included in the study, while those admitted with conditions leading to spurious polycythaemia or patients on diuretics were excluded. Polycythaemia vera was diagnosed as per revised WHO criteria 2016.

Results: Only 23 subjects (27.71%) fulfilled the diagnostic criteria for Polycythaemia vera, while 60 subjects (72.29%) had secondary erythrocytosis. Significant differences were observed in haematological patterns between Polycythaemia vera and Secondary erythrocytosis patients. Of the 23 Polycythaemia vera subjects, 20(86.9%) were males and only 3(13.1%) were females, whereas 35(58.3%) out of 60 subjects with secondary erythrocytosis were males and 25(41.6%) were females (p=0.01). Males showed a higher frequency of Polycythaemia vera and, hence, of Janus Kinase2 positivity than females (Odds ratio=0.23, p=0.02).

Conclusion: Polycythemia vera is less common than secondary erythrocytosis in the local population. Males showed a higher frequency of Polycythemia vera and, hence, of Janus Kinase2 positivity than females.

Keywords: Bone marrow, Erythrocytosis, JAK2 Exon 12 mutations, Polycythaemia vera. Polycythemia, Janus kinase 2, Erythropoietin.

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INTRODUCTION

Erythrocytosis is characterized by an expansion in red cell mass to more than 125% of the specified value for a particular age and gender.[1] It usually manifests as a persistent increase in haemoglobin or venous haematocrit for more than two months. Literature reports a prevalence of 44-57 per 100,000 cases for primary erythrocytosis, while the prevalence of secondary erythrocytosis, although relatively higher, is difficult to quantify due to a lack of credible data.[2,3]

Primary and secondary erythrocytosis can be further divided into acquired or congenital types. Primary acquired erythrocytosis mainly involves classic Polycythaemia vera (PV). It is caused by the expression of JAK2V617F or JAK2 exon 12 mutations resulting in erythroid hyperplasia with hypercellular bone marrow.[4] Although in 1% of PV patients these markers cannot be detected, Secondary acquired erythrocytosis occurs due to risk factors outside of bone marrow like increased erythropoietin (EPO) production that can be due to EPO producing tumours or due to exogenous sources.[5] It may also occur as a physiological response to hypoxia–due to high altitude, smoking, respiratory, cardiac or renal disorders or due to sleep apnea.[6] Primary congenital erythrocytosis results from EPOR gene mutation, while secondary congenital erythrocytosis is seen in disorders characterized by high-affinity hemoglobin variants, diseases with 2,3-BPG deficiency or defects in oxygen-sensing pathways.[7] Another type is the idiopathic erythrocytosis which is even more rare and lacks a definite etiology.[8]

Erythrocytosis is frequently encountered in clinical settings. A recent study reported the JAK2 exon 12 mutations in 79.2% of the Pakistani population suffering from PV.[9] This study aimed to assess the frequency of erythrocytosis and compare the clinical features and haematological parameters in patients presenting with primary and secondary erythrocytosis in the Pakistani population. Study results will help gain insight into the disease presentation of these relatively common haematological disorders in the

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local population and add to the rather scarce data bank.

METHODOLOGY

The cross-sectional study was conducted at the Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan, from May 2020 to May 2021 after approval from IRB (Letter no.FC-HEM18-9/READ-IRB/20/357). The sample size was calculated using the WHO sample size calculator, keeping anticipated population proportion (P) of PV at 0.057.10 Non-probability consecutive sampling was used.

Inclusion Criteria: Patients presenting with marked erythrocytosis (Hb- 17 g/dl or more. HCT–50% or more) were included.

Exclusion Criteria: Patients admitted with acute dehydration, sepsis, shock and other conditions leading to spurious Polycythaemia or patients on diuretics were excluded.

Polycythaemia vera was diagnosed per revised WHO criteria 2016, including "major criteria – i) Hb > 16.5 gm/dl or HCT >49% for males and Hb >16 gm/dl or HCT >48% for females; ii) bone marrow biopsy showing hypercellularity for age with trilineage growth including permanent erythroid, granulocytic and megakaryocytic proliferation with pleomorphic mature megakaryocytes; iii) presence of JAK2V617f or JAK 2 exon 12 mutation and minor criteria - subnormal serum Erythropoietin levels.[10] Diagnosis required meeting all three major criteria or the first two major and minor criteria. In addition to demographic data and clinical findings, laboratory features, including Hb, PCV, total WBC count, RBC and platelet count, ESR, EPO levels and JAK2 V617F mutation analysis (done by real-time PCR technique), were documented.

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 27.0. Quantitative variables were expressed as Mean±standard deviation (SD) and were compared using the independent sample t-test. Categorical variables were expressed as frequency and percentages and were compared using Fisher's exact test. The *p*-value of ≤0.05 was taken as significant. Logistic regression (odds ratio) was used to determine the likelihood of having a positive JAK2 mutation based on gender.

RESULTS

The study cohort comprised of 83 subjects. Of these, 28(33.73%) were females and 55(66.27%) were males. The mean age of the study sample was 53.93±4.8 years. Only 23 subjects (27.71%) fulfilled the diagnostic criteria for Polycythaemia vera, while 60 subjects (72.29%) had secondary erythrocytosis. A statistically significant difference was observed between PV and SE patients regarding clinical presentation with symptoms (p<0.001). Thirty-nine (65%) of the subjects with SE were asymptomatic, while 19(82.6%) of PV patients presented with symptoms. Around 22(95%) of PV patients presented with plethora, 17(73.9%) with splenomegaly, and 14(61%) had hypertension. In contrast, none of the SE patients had splenomegaly (p=0.001), but 32(53.3%) had a plethora. The most common complication observed in both PV and SE patients was ischemic heart disease. Clinical characteristics of the patients at

Table-I: Clinical Characteristics of Study Subjects at the time of Presentation (n=83)

Characteristics	Polycythaemia vera (n=23) n(%)	Secondary Erythrocytosis (n=60) n(%)	*p*-value
Symptoms			
Asymptomatic	4(17.39)	39(65)	<0.001
Headache	7(30.43)	16(26.67)	0.78
Dizziness	8(34.78)	10(16.67)	0.08
Generalized weakness	13(56.52)	14(23.33)	0.008
Chest Pain	5(21.74)	0 (0)	0.001
Claudication in limbs	3(13.04)	0(0)	0.01
Signs			
Plethora	22 (95.65)	32(53.33)	<0.001
Splenomegaly	17 (73.91)	0 (0)	<0.001
Hypertension	14 (60.86)	28(46.67)	0.33
Complications			
Nil	11(45.16)	52(86.67)	<0.001
Ischemic Heart Disease	5(21.74)	7(11.67)	0.29
Cerebrovascular Accident	2(8.69)	0(0)	0.07
Peripheral Arterial Thrombosis	3(13.04)	0(0)	0.01
Deep Vein Thrombosis	2(8.69)	1(1.66)	0.18

Table-II: Comparison of Haematological Patterns of Polycythaemia Vera and Secondary Erythrocytosis Study Subjects (n=83)

Haematological Parameters (Mean±SD)	Polycythaemia vera	Secondary Erythrocytosis	*p*-value
Hb (g/dl)	18.3±0.97	17.2±0.87	<0.001
HCT (%)	58.2±1.79	52.3±2.53	<0.001
Total WBC Count (x109/L)	13.3±4.53	6.4±1.24	<0.001
ESR (mm/hr)	3.45±0.6	11.15±2.56	<0.001
RBC Count (x109/L)	9.3±1.3	8.3±0.74	0.003
Platelet Count (x109/L)	528.35±133.64	232.55±66.95	<0.001
Serum Erythropoietin Levels n(%)			
Normal	6(26.09)	29(48.33)	<0.001
Low	17(73.91)	0(0)	
High	0(0)	31(51.67)	
JAK2 V617F mutation	22(95.65)	0(0)	<0.001
EXON 12	1(4.35)	0(0)	0.27
Bone Marrow Aspirate n (%)			
Normal	03 (13.04)	N/A	-
Trilineage Hypercellularity	20 (86.96)	N/A	-

Table-III: Gender-wise Mutation Analysis of Polycythaemia vera Study Subjects (n=83)

Mutation	Male	Female	Odds Ratio	*p*-value
JAK 2 Positive	19(82.61)	03(13.04)	0.23	0.028
JAK 2 Negative	1(4.35)	-		
EXON 12 Positive	1(4.35)	-	-	0.998
EXON 12 Negative	-	-		

the time of presentation are highlighted in Table-I.

Statistically significant differences were also observed in haematological patterns between PV and SE patients. Mean Hb levels observed for PV patients were 18.3±0.97 g/dL, and for SE patients were 17.2±0.87 g/dL (p=<0.001). The mean HCT in PV subjects was 58.2±1.79%, while for SE subjects, the mean HCT was 52.3±2.53% (p=<0.001). Seventeen (73.9%) of the PV patients had low serum EPO levels, whereas 31(51.67%) of SE subjects showed high serum EPO levels. In the present study cohort, real-time PCR revealed that 22(95.65%) of PV subjects had positive JAK2 V617F mutations while only 1(4.35%) had EXON12 mutations. Table-II depicts the haematological patterns of the study subjects along with *p*-values suggesting statistically significant differences between the 02 groups. Table-III shows gender-wise mutation analysis for PV patients. The statistically significant difference was observed between males and females for JAK 2 mutation, where males were 0.227 times more likely to exhibit JAK2 positive mutation than females.

DISCUSSION

In the present study, secondary erythrocytosis was more frequently encountered than polycythaemia vera (PV). PV was, however, more common in males (87%) than in females (13%), suggesting a gender preference. Comparable results have been reported in the Indian population, where majority of the subjects presenting with PV were males.[4,11-13] Owing to the redefined WHO criteria for diagnosing PV, the prevalence of PV encountered in this study is lower than that reported in Pakistani population in 2004 by Usman *et al*.[14] prior to the revised diagnostic criteria.

The majority (83%) of the patients in the PV group mainly presented with symptoms, while those with secondary erythrocytosis (SE) were largely asymptomatic (p=0.01). This can be justified by the fact that PV patients have hyperviscous blood owing to an increase in mean Hb, Pack Cell volume and a higher number of blood cells reflecting the "trilineage marrow hypercellularity". As a result, these patients are more prone to developing symptoms associated with hyper-viscosity, such as ischaemic heart disease, cerebrovascular accidents, etc. Other studies have also implicated the role of leukocytosis in thrombotic potential in PV.[15,16] Leukocytosis and thrombocytosis warrant the use of cytoreductive therapy in vera in addition to periodic phlebotomies to maintain Hb <16 gm/dl to reduce the risk of complications.

Low serum EPO levels were found in 73.9% of PV patients and none among SE patients (p=0.012). High serum EPO levels were observed in 51.67% of SE patients but not in PV patients (p=0.01). These findings

highlight a statistically significant difference between the 02 groups, suggesting that low EPO levels favour PV significantly. These results of the present study are endorsed by Nevrekar *et al*.[4] who arrived at the same conclusion of low EPO levels favoring PV. However, the literature suggests that EPO levels are a minor diagnostic criterion for PV and are not reliable as a stand-alone diagnostic marker due to physiological variations associated with obesity and smoking.[17]

In the present study, Jak 2V617F positivity was seen in 95.65% of patients of Polycythaemia vera (p=.005), while Exon 12 positivity was seen in only one patient. Males showed a higher frequency of PV and, hence, of JAK 2 positivity as compared to females (OR=0.23). The frequency of JAK2 positivity as found in the present study was comparable with the reported in literature.[18-20] Nevrekar *et al*. documented a JAK2 positivity prevalence of 47% in PV patients.[4] In a study by Sazawal *et al*. JAK2 was found positive in 82% patients with PV in Indian patients.[12] Moreover, positive JAK2 mutation has been reported to demonstrate a strong predictive value for PV both alone and in combination with other variables.[17]

The present study aimed to assess the frequency and clinical features of patients presenting with primary and secondary erythrocytosis. The strength of the study is that it objectively assessed the patients presenting with erythrocytosis and did not rely on subjective findings.

LIMITATION OF STUDY

Although the present study had a decent sample size, more accurate results may be achieved by sampling from diverse healthcare centres with an even larger sample size across the country. Samples may be sought from different racial and ethnic groups to cater these effect modifiers specifically. Since the study only focused on disease presentation, the need, type and frequency of treatment required were needed to be evaluated.

CONCLUSION

Based on the findings of this study, it can be concluded that Polycythaemia vera is less frequently encountered than secondary erythrocytosis in the local population. The majority of patients with Polycythaemia vera mainly presented with symptoms, while those with secondary erythrocytosis were largely asymptomatic. JAK2 positivity was seen in 95.65% of patients with Polycythaemia vera, while Exon 12 positivity was seen in only one patient. Males showed a higher prevalence of PV and, hence, of JAK 2 positivity as compared to females.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SA & AMA: Conception, study design, drafting the manuscript, approval of the final version to be published.

JZ & HMR: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

AK & SA: Data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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