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Original Article

Clinico-Haematological Profile and Risk Stratification in Patients with Essential Thrombocythemia in a Tertiary Care Center of North India

Dr. Renuka Verma[*1], Dr. Rachana Yadav[2], Dr. Rajnish Kalra[3], Dr. Veena Gupta[3], Dr. Sumiti Gupta[3], Dr. Jaideep Phougat[4], Dr. Sunita Singh[5]

[1] Associate Professor, Department of Pathology, PGIMS, Rohtak, Haryana
[2] Senior Resident, Department of Pathology, PGIMS, Rohtak, Haryana
[3] Professor, Department of Pathology, PGIMS, Rohtak, Haryana
[4] Senior Resident, Department of General Medicine, PGIMS,Rohtak, Haryana
[5] Senior Professor & Head, Department of Pathology, PGIMS, Rohtak, Haryana

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***Corresponding author:**
Dr. Renuka Verma
Associate Professor, Department of Pathology, PGIMS, Rohtak, Haryana

ABSTRACT

Background: Essential thrombocythemia is an uncommon clonal myeloproliferative neoplasm characterized by sustained thrombocytosis of more than $450x10^9$/L, marked megakaryocytic hyperplasia and high risk of vascular complications. Diagnosis is made by ruling out secondary causes of thrombocytosis, Polycythaemia Vera (PV), Primary Myelofibrosis (PMF) and Chronic Myeloid Leukaemia (CML) and by presence of somatic genetic mutations like Janus kinase-2 (JAK2)/ Calreticulin (CALR)/ Myeloproliferative leukaemia (MPL). **Aims and Objectives**: This study was designed to find out the prevalence of essential thrombocythemia among various patients of myeloproliferative neoplasms (MPNs), to analyse their clinical and haematological parameters, genetic mutations and to do risk stratification in patients with essential thrombocythemia. **Material and Methods:** This prospective observational study was conducted in the Department of Clinical Pathology, Pt. B. D. Sharma, PGIMS, Rohtak, Haryana. A total of 24 cases of essential thrombocythemia were enrolled from April 2020 to March 2023. Data analysis was done using Statistical Package for the Social Sciences Version 22.0. **Results:** Among 110 patients with myeloproliferative neoplasms, the prevalence of essential thrombocythemia was found to be 24 (21.81%). The mean age of patients was 52.20±12.46 years with age range of 32 to 78 years and male to female ratio of 1:1.4. In symptomatic patients, major complaints were generalised weakness in 16 cases (66.67%), headache in 11 cases (45.83%) and pain in abdomen in 8 cases (33.33%). The mean haemoglobin level was 11.10±2.70 g/dl, total leukocyte count was 13158.20±92185.40 cells/mm^3 and platelet count was 845000±372000 cells/mm^3. Risk stratification was done and majority of the patients, 13 cases (54.17%) were in intermediate risk group, followed by 8 cases (33.33%) in high-risk group and 3 cases (12.50%) in low-risk group. **Conclusion:** Essential thrombocythemia is uncommon in this region of North-India. Patients were usually symptomatic on presentation indicating delay in seeking medical attention. Platelet counts were high and bone marrow was hypercellular with megakaryocytic proliferation in cases with homozygous JAK2 mutation and these patients also presented with more severe complications. Majority of patients were in high and intermediate risk category indicating aggressive disease course

INTRODUCTION

Essential thrombocythemia (ET) is a myeloproliferative neoplasm (MPNs) with Philadelphia chromosome-negative. It characterized by sustained and isolated thrombocytosis along with megakaryocy-tic hyperplasia. It was first discovered in 1934 as a "Haemorrhagic thrombocythemia" by Epstein and Goedel.[1] It is a rare haematological malignancy with an incidence of 0.38-1.7 per 100,000 per year. Females have twice more prevalence than males.

The median age at diagnosis is 65 to 70 years; however, disease may occur at any age[2].

ET is a clonal stem cell disorder. Approximately 90% of cases have a somatically acquired genetic mutations such as JAK2, CALR, or MPL that results in the upregulation of JAK-STAT pathway. Among different genetic mutations JAK2V617 is the most frequent driver mutation occurring in approx. 50-65% of cases followed by CALR mutation in 15-30% of cases and MPL mutation in 4-8% of cases while 10-20% of the cases lack all three mutations and are known as triple negative[3].

Patients of ET usually present with sustained and progressive thrombocytosis which can be found incidentally associated with no symptoms. A variable proportion of patients have mild splenomegaly and leukocytosis[4]. Up to 40% of patients are symptomatic with fatigue, early satiety, inactivity, concentration issues, and abdominal discomfort, with a consequent decrease in quality of life. In addition, patients, may experience arterial and/or venous thrombotic complications and bleeding risk with potential of transformation to myelofibrosis or alternative aggressive myeloid neoplasm[5].

The therapeutic goal for ET patients is to avoid the occurrence of major vascular events while minimizing the side effects induced by medication. Consensus regarding the management of adult ET follows a risk-adapted strategy. Low risk patients are usually managed with low-dose aspirin, whereas treatment of high-risk ET is based on the use of cytoreductive therapy[6].

The aim of this study was to find out the prevalence of essential thrombocythemia among patients with myeloproliferative neoplasms, to analyse various clinico-haematological parameters and to do risk stratification in patients with essential thrombocythemia.

MATERIAL AND METHODS

This was a prospective observational study conducted in the Department of Clinical Pathology, Pt. B. D. Sharma, PGIMS, Rohtak, Haryana over the period of three years from April 2020 to March 2023.

Case Selection: A total of 110 patients with myeloproliferative neoplasms were screened out of which 24 cases had essential thrombocythemia and were analysed. Informed consent was obtained from all the study participants.

Clinical analysis: Detailed clinical history, physical examination findings and results of previous investigations were obtained from all the cases. Patients who did not have sufficient information including reports of a mutation test were excluded from the study.

Laboratory analysis: For complete hemogram 2ml of EDTA blood sample was collected and analysed on BC-6800 MINDRAY. Peripheral blood film examination was also done. Reports of kidney and liver function tests were analysed. Bone marrow aspiration and/or trephine biopsy were performed to look morphology of megakaryocytes. Reports of mutation analysis were also tabulated.

The diagnosis of essential thrombocythemia was made according to 2016 revised WHO diagnostic criteria requiring all four major criteria or the first three major and the minor criteria:[7]

Table 1: WHO Essential Thrombocythemia Criteria

HO ET criteria
• **Major criteria**
1. Platelet count ≥450 × 109/L
2. BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyper lobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibres
3. Not meeting WHO criteria for BCR-ABL1+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of JAK2, CALR, or MPL mutation
• **Minor criteria**
1. Presence of a clonal marker or absence of evidence for reactive thrombocytosis
2. Diagnosis of ET requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion

Risk Stratification: Patients were classified into high risk, intermediate risk and low risk category according to Myeloproliferative neoplasm working group guidelines. Patients were categorised as high risk if they were older than 60 years or they had a previous history of thrombosis or if platelet counts were $\geq 1500x10^9/L$. Patients with low-risk and intermediate risk ET were <40 years and 40-60 years respectively, with absence of prior thrombotic manifestations and they had platelets counts $<1500x10^9/L$.[8]

Statistical analysis: The data were entered in Microsoft Excel 2010. With the help of Statistical Package for the Social Sciences (SPSS) version 22 for windows the results were statistically analysed. Data were expressed as mean ± standard deviation. For quantitative and qualitative variables, range was presented as frequency & percentage.

RESULTS

Among 110 cases of myeloproliferative neoplasm screened, 24 cases were of essential thrombocythemia during the period of three years with a prevalence of 21.81%.

Age and sex distribution: The mean age of patients was 52.20±12.46, with age range of 32 to 78 years. Majority of the patients were in age range of 41-50 years (11 cases; 45.83%), followed by 4 cases each (16.67%) in age group of 51-60 years and 61-70 years. Three patients (12.5%) were < 40 years of age and 2 patients (8.33%) were > 70 years of age. Out of 24 patients, 10 were male (41.67 %) and 14 were females (58.33%) with male to female ratio of 1:1.4.[Table 2]

Table 2: Age & Sex Distribution (n= 24)

Age Group	Male (%)	Female (%)	Total no. of cases (%)
31-40	02 (66.67)	01 (33.33)	03 (12.50)
41-50	04 (36.36)	07 (63.64)	11 (45.83)
51-60	02 (50.00)	02 (50.00)	04 (16.67)
61-70	01 (25.00)	03 (75.00)	04 (16.67)
71-80	01 (50.00)	01 (50.00)	02 (8.33)
Total	**10 (41.67%)**	**14 (58.33%)**	**24 (100%)**

Clinical features and physical examination findings: Most common symptom at the time of presentation was generalised weakness or fatigue in 16 cases (66.67%) followed by headache in 11 cases (45.83%), pain in abdomen in 8 cases (33.33%), weight loss in 7 cases (29.17%), visual disturbance in 5 cases (20.83%), fever in 4 cases (16.67%), joint pain in 2 cases (8.33%) and 1 case (4.17%) each of shortness of breath and excess menstrual bleeding.

The arterial and venous thrombosis was seen in 6 cases (25.00%) and 4 cases (16.67%) respectively. Among comorbidities, 9 cases (37.50%) had hypertension, 4 cases (16.67%) had diabetes mellitus and 1 case (4.17%) of each had tuberculosis and HIV in the past. Out of 24 patients, 13 cases (54.17%) smoked, 9 cases (37.50%) consumed alcohol, and 8 cases (33.33%) consumed non-vegetarian diet, while none of the patient had history of ET in family.

On physical examination, 10 cases (41.67%) had pallor, 3 cases (12.50%) had edema, 2 cases (8.33%) had icterus and 1 case (4.17%) each had cyanosis and clubbing. Ten cases (41.67%) had splenomegaly, 06 cases (25.00%) had hepatomegaly and lymphadenopathy was seen in 2 cases (8.33%). [Table 3]

Table 3: Clinical Features and Physical Examination Findings (n= 24)

SYMPTOMS AT PRESENTATION	TOTAL NUMBER OF CASES (n)	PERCENTAGE %
GENERALIZED WEAKNESS/ FATIGUE	16	66.67
HEADACHE	11	45.83
PAIN IN ABDOMEN	08	33.33
WEIGHT LOSS	07	29.17
VISUAL DISTURBANCE	05	20.83
FEVER	04	16.67
JOINT PAIN	02	8.33
SHORTNESS OF BREATH	01	4.17
EXCESS MENSTRUAL BLEEDING	01	4.17

PRIOR THROMBOSIS	CASES NUMBER	CASES NUMBER
ARTERIAL THROMBOSIS	06	25.00
VENOUS THROMBOSIS	04	16.67
COMORBIDITIES	**CASES NUMBER**	**PERCENTAGE %**
HYPERTENSION	09	37.50
DIABETES MELLITUS	04	16.67
TUBERCULOSIS	01	4.17
HIV	01	4.17
PERSONNAL HISTORY	**CASES NUMBER**	**PERCENTAGE %**
SMOKING	13	54.17
ALCOHOL	09	37.50
NON-VEGETARIAN	08	33.33
FAMILY HISTORY OF ET	00	00
EXAMINATION	**CASES NUMBER**	**PERCENTAGE %**
PALLOR	10	41.67
ICTERUS	02	8.33
CYANOSIS	01	4.17
CLUBBING	01	4.17
EDEMA	03	12.50
ORGANOMEGALY	**CASES NUMBER**	**PERCENTAGE %**
SPLEENOMEGALY	10	41.67
HEPATOMEGALY	06	25.00
LYMPHADENOPATHY	02	8.33

Laboratory investigations: The mean haemoglobin level was 11.10±2.70 g/dL. The mean TLC was 13158.20±92185.40 cells/mm^3 and mean platelet count was 845000±372000 cells/mm^3. The mean blood urea, serum creatinine and serum uric acid were 46.45±8.20 mg/dL, 1.8±0.9 mg/dL and 5.2±2.6 mg/dL respectively. The mean total bilirubin and albumin levels were 2.1±1.6 mg/dL and 3.3±2.2 g/dL respectively. [Table 4]

Table 4: Laboratory Investigations (n= 24)

LABORATORY PARAMETERS	MEAN ± SD
HEMATOLOGICAL PARAMETERS	
HAEMOGLOBIN (g/dL)	11.10 ± 2.70
TOTAL LEUCOCYTE COUNT (cells/mm ^{3})	13158.20 ± 92185.40
NEUTROPHILS (%)	65.8 ± 4.4
LYMPHOCYTES (%)	26.5 ± 6.9
MONOCYTES (%)	4.2 ± 1.9
EOSINOPHILS (%)	2.5 ± 1.4
PLATELET COUNT (cells/mm ^{3})	845000 ± 372000
RETICULOCYTE (%)	2.6 ± 1.3
KIDNEY FUNCTION TEST	
BLOOD UREA (mg/dL)	46.45 ± 8.20
SERUM CREATININE (mg/dL)	1.8 ± 0.9
SERUM URIC ACID (mg/dL)	5.2 ± 2.6
LIVER FUNCTION TEST	
SERUM Total bilirubin (mg/d L)	2.1 ± 1.6
SERUM ASPARTATE AMINOTRANSFERASE (AST) (U/L)	35.2 ± 6.3
SERUM ALANINE TRANSFERASE (ALT) (U/L)	32.9 ± 4.4
SERUM ALKALINE PHOSPHATASE (ALP) (U/L)	115.2 ± 82.6
SERUM PROTEIN (g/dL)	6.3 ± 3.7
Albumin (g/ dL)	3.3 ± 2.2

Peripheral blood film examination revealed thrombocytosis in all the cases (100%) [Figure 1]. Bone marrow examination revealed hypercellular marrow with megakaryocyte proliferation in 22 cases (91.67%) with presence of large megakaryocytes in clusters and hyper lobated nuclei, [Figure 2, 3] while 2 cases (8.33%) had normal cellularity. On immunohistochemistry these megakaryocytes were CD 41 positive. [Figure 4]

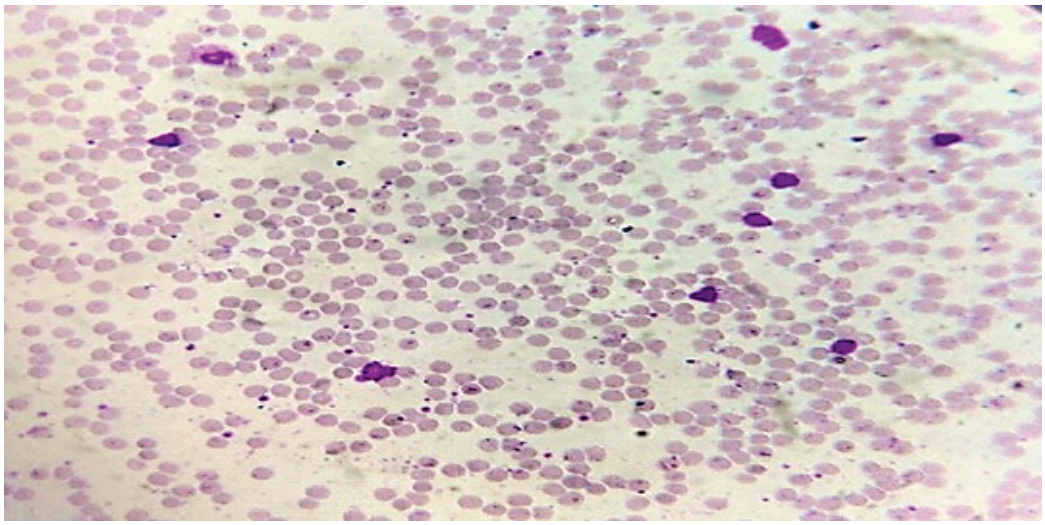

Figure 1: Peripheral blood film with thrombocytosis (Leishman stain, x200)

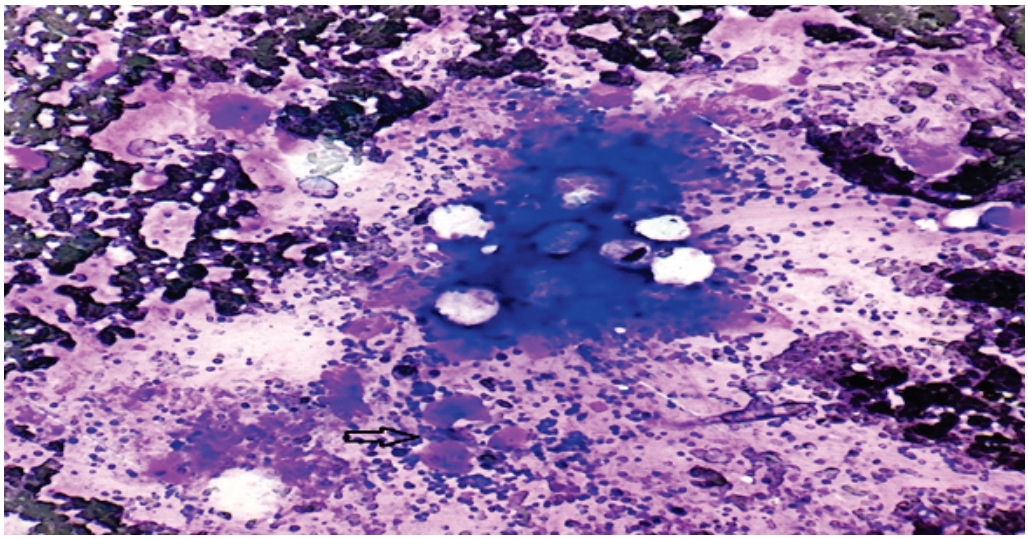

Figure 2: Hypercellular bone marrow aspiration smear with clusters of large megakaryocytes (Giemsa stain, x100)

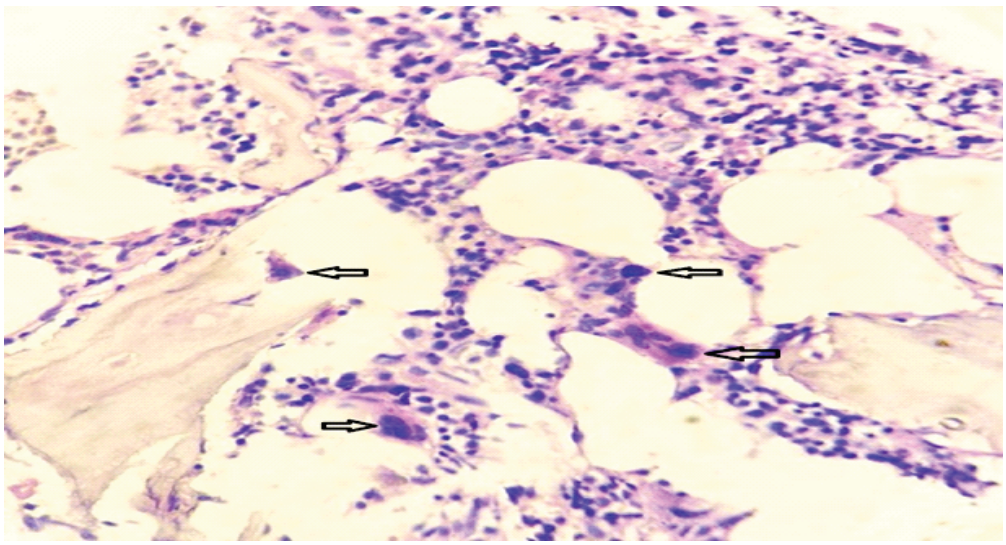

Figure 4: Megakaryocytes were positive for CD 41 on bone marrow biopsy section (IHC, x200)

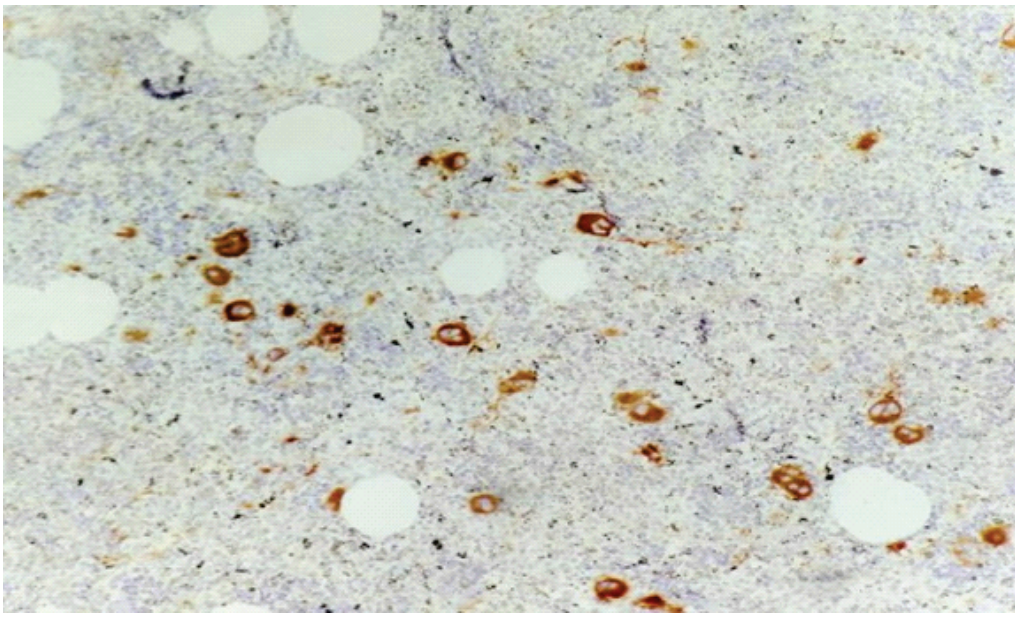

Figure 4: Megakaryocytes were positive for CD 41 on bone marrow biopsy section (IHC, x200)

Mutation analysis: Out of 24 cases, 17 cases (70.83%) had JAK2V617 mutation, followed by CALR exon 9 mutation in 5 cases (20.83%) and MPL exon 10 mutation in 2 cases (8.34%). [Table 5]

Table 5: Mutation analysis (n= 24)

TYPE OF MUTATION	NUMBER OF POSITIVE CASES (%)
JAK2V617	17 (70.83)
CALR exon 9	05 (20.83)
MPL exon 10	02 (8.34)

Risk stratification: According to Myeloproliferative neoplasm working group guidelines, 8 cases (33.33%) were in high-risk category, 13 cases (54.17%) were in intermediate risk while 3 cases (12.50%) were in low-risk category. [Table 6]

Table 6: Risk stratification (n= 24)

RISK STRATIFICATION	NUMBER OF CASES (%)
High risk	08 (33.33%)
Intermediate risk	13 (54.17%)
Low risk	03 (12.50 %)

DISCUSSION

Essential thrombocythemia is breakpoint cluster region protein/Abelson murine leukaemia viral oncogene homolog 1 (BCR/ABL1) -negative myeloproliferative neoplasms. It is a clonal hematopoietic stem cell disorder that is characterized by isolated thrombocytosis. Complications of ET includes thrombosis, haemorrhage, and progression to myelofibrosis or acute myeloid leukemia[9] Clinico-Haematological profile, metabolic results, mutation analysis and risk stratification of ET patients were examined in our study. To the best of our knowledge, this is the first kind of study from this region of country.

In present study the prevalence of ET was 21.81% among various myeloproliferative neoplasms. The results were similar to study done by Sah et al with a prevalence of 23.61%[10]. The mean age of patient was 52 years, indicating that ET is a disease of advancing age, findings were similar to study done by Sazawal et al with the mean age of 49 years.[11] However, there was higher average age in western countries (65-70 years), reason being different racial groups and genetic makeup[12]. The incidence of ET was higher in females similar to study done by Sah et al and Fabris et al[10, 13].

In our study, majority of patients (66.67%) were symptomatic and presented with generalized weakness or fatigue, indicating that patients present to OPD only when they acquire symptoms, findings were similar to study of Sah et al, in which 82.4% presented with the symptoms of fatigue[10]. However, results were different from study of Sultan et al, in which 61.9% patients were asymptomatic and were diagnosed inadvertently[14].

In our study splenomegaly and hepatomegaly was present in 41.67% and 25% cases respectively indicating that patients here neglect the disease in its earlier phase.[15]

A large series from Thailand found splenomegaly and hepatomegaly in 10.8% and 8.4% of the population, respectively[16]. In our study, the mean platelet count was 845000 ± 372000 cells/mm^3, results from other regional studies of Nepal (677000±262067.70 cells/ mm^3), Thailand ($1191x10^9$ /l) and China ($1000x10^9$ /l) were similar to our study[10, 16, 17].

Majority of the patients in our study had JAK2V617 mutation (70.83%), followed by CALR exon 9 mutation (20.83%) and MPL exon 10 mutation (8.34%). Platelet counts were high and bone marrow was hypercellular with megakaryocyte proliferation in cases with homozygous JAK2 mutation and these patients also presented with more severe complications. Similar findings were noted by Sah et al with 88.23% having JAK2V617 mutation, 11.76% having CALR exon 9 mutation while none of the patients were tested positive for MPL exon 10. The lack of an adequate number of samples in the study population may be the reason[10].

Risk stratification was done in our study, with 33.33% cases in high risk, 54.17 % cases in intermediate risk cases and 12.50% cases in low-risk category, indicating aggressive disease course along with concomitant factors of delay in seeking medical attention. In contrast to us, in a previous study from Nepal and United States, only 17.64% cases and 17% case were found in high-risk category[10, 18].

STRENGTHS AND LIMITATIONS

This is the first kind of study from this region of North-India in patients with essential thrombocythemia. Detailed clinic-haematological parameters were discussed. Mutation analysis was discussed and risk stratification was also done. However, sample size was small and follow up data for clinical outcome was not available as during Covid-19 pandemic, patients were not able to visit for regular follow-up. Also, this study was done at a single centre which might have led to selective bias. Hence, studies with larger sample size, long-term follow-up assessing the clinical outcome and inclusion of novel prognostic molecular tests would be better in near future.

CONCLUSION

Essential thrombocythemia is uncommon in this region of North-India. Patients were usually symptomatic on presentation indicating delay in seeking medical attention. Platelet counts were high and bone marrow was hypercellular with megakaryocytic proliferation in cases with homozygous JAK2 mutation and these patients also presented with more severe complications. Majority of patients were in high and intermediate risk category indicating aggressive disease course.

Contribution Details:

All the authors have contributed to concept, literature search, data acquisition, data analysis, manuscript editing and review.

NO CONFLICT OF INTEREST EXISTS; NO FINANCIAL DISCLOSURE.

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