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RESEARCH PAPER

Molecular Identification of Janase kinase 2- V617F gene mutation (exon14) in Polycythemia patients in Erbil Governorate

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A B S T R A C T:

Polycythemia vera (PV) is a clonal chronic myeloproliferative disease that causes cytokine-independent proliferation of myeloid precursors and primarily impacts the elderly. This primarily affects the erythroid lineage and causes an abnormally high number of circulating erythrocytes. Increased numbers of circulating granulocytes and platelets were also seen in many cases. This investigation aimed to identify JAK2 mutations in individuals with primary and secondary polycythemia. Furthermore, investigating some hematological parameters among polycythemic patients. 52 polycythemic patients, 8 with PV and 44 with secondary—donated blood. Samples were taken from patients at two hospitals and a blood bank center in the Kurdistan Region of Iraq. Sanger sequencing of JAK2 exon 14 and PCR results (500 bps) show that 3 (5.8%) polycythemic patients had heterozygous Janus Kinase 2 V617F mutations, while all other samples were negative. The average age is 38 years, and male participants (94.2%) out number females (5.8%). The average of each of the HGB and HCT for negative JAK2V617F was higher than the positive one, while the average of each of the PLT and age for negative JAK2V617F were lower than the positive JAK2V617F. Next, the negative nerghila, cigarette, and alcohol had higher rates of negative JAK2V617F than their positives.

KEY WORDS: Polycythemia vera, JAK2V617F, Exon14, HGB, HCT, PLT
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1.INTRODUCTION:

Polycythemia, also known as erythrocytosis, is characterized by an abnormal rise in the volume and hemoglobin amounts of all circulating red blood cells (RBCs). Secondary polycythemia, in which patients have decreased oxygen supply to tissues, causing the body to compensate by raising the level of erythropoietin hormone, which causes excessive RBC synthesis and increased hemoglobin levels, and Primary polycythemia, in which affected individuals have an inherited or acquired mutation that results in an abnormal proliferation of RBC progenitors (Kumar *et al*., 2012).

Those who have primary polycythemia typically have Polycythemia vera (PV), an inherited Myeloproliferative neoplasms (MPN) (Person and Messinezy, 1996; Huang *et al*., 2010).

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Secondary polycythemia results from improper control of substances that promote erythropoiesis, primarily erythropoietin (EPO), Which acts on erythroid precursors (Gordeuk *et al*.,2005). A chronic myeloproliferative disease called polycythemia rubra vera (PV) causes an excess of red blood cells in the blood, bone marrow, spleen, and livers. An exhausted cytopenic period typically follows a polycythemic phase. which includes extramedullary hematopoiesis, myelofibrosis, and hypersplenism. Symptoms that may be present include pruritus after bathing, burning paresthesias in the distal extremities, gastrointestinal issues, or neurologic complaints like headache, weakness, or vertigo. After unintentional results of erythrocytosis on a complete blood count, patients are given another diagnosis (Cao *et al*., 2006).

The most frequently documented genetic abnormality in PV patients is the gain of function

mutation in the Janus kinase 2 (JAK2) gene. About 95% of PV cases have the JAK2 V617F exon 14 mutation (Xia & Hasserjian, 2016). JAK2 exon 12 variants may be present in PV patients who are JAK2 V617F negative (Shi *et al*., 2016).

The JAK2 gene is found in human DNA chromosome 9p24.1 ~~on the short arm of chromosome 9 at the p24.1 locus~~. It is a 25-exon gene that encodes a 130.7 kDa protein with 1132 amino acids. (Yamaoka *et al*., 2004). The Janus kinase (JAK) is a family of non-receptor tyrosine kinases, which has four members, includes (JAK1, JAK2, JAK3, and TYK2) (Purandare, 2010). Seven different JAK homology (JH1–JH7) domains are shared by the Janus kinases (Wilks, 2008). When Janus kinase 2 (JAK2) is activated, tyrosine residues are carefully phosphorylated at Janus homology1 (JH1) and Janus homology 2 (JH2), the kinase and negative regulatory JAK homology regions, respectively. JAK2 V617F, which is the product of a point mutation (1849GT) in exon 14, changes the codon in the JH2 domain 617 from phenylalanine to valine. This change in a single amino acid results in the uncontrollable activation of the JAK2 enzyme (Purandare, 2010). The four JAK2 mutations in exon 12 are simultaneous point mutations or in-frame deletions. Both JAK2 V617F and the codon 12 increase erythropoietin receptor-expressing cell lines' proliferative potential and make those cells more sensitive to cytokines (Tefera, 2007).

This study was aimed to identifying the JAK2 V617F exon (14) mutation in both primary and secondary polycythemic patients. Furthermore, investigating some hematological parameters among polycythemic patients**.**

2. MATERIALS AND METHODS

2.1 Patient specimens

The prospective case-control research was conducted in December 2021 and completed on September 2022. It enrolled 44 blood donors who attended the Directorate of Blood Bank in Erbil city and Shahed Dr. Khalid Hospital in Koya, in addition, a control group of 8 PV patients was included in this research, Collected from Nanakaly Hospital, Kurdistan region, Iraq. The blood samples were divided in to two aliquots. An aliquot was used to evaluated some hematological parameters, and the other one to detection JAK2 gene (exon 14). Furthermore, we detect the JAK2 V617F gene (exon 14) for all samples, Furthermore, a questionnaire was used to collect demographic characteristics for patients including age, gender, smoking cigarette, narghile smoking and using alcohol consumption.

2.2 Blood Sampling.

Polycythemia vera (PV) patients and blood donors provided three ml of peripheral blood, which was drawn into a fresh, sterile EDTA-containing tube as an anticoagulant and stored at 4°C for DNA extraction for the detection of the JAK2 V617F (exon 14) mutation and complete blood count (CBC).

2.3 DNA extraction.

The AddPrep genomic DNA extraction kit was used to extract total genomic deoxyribonucleic acid (DNA) from peripheral blood samples in accordance with the manufacturer's directions. The DNA samples' quality was then evaluated using a spectrophotometer (Nano Drop) [NanoVue Plus, UK] before being stored at -20 °C for later downstream applications.

Exon 14 of the JAK 2 V617F gene was checked for mutations in all cases. JAK 2 V617F exon 14 forward primer is 5'-GCTACATCCATCTACCTCAG-3' and JAK 2 reverse is 5'-CTGACACCTAGCTGTGATCC-3'. Polymerase Chain Reaction (PCR) amplification (500 bps) of the JAK2 transcript was performed using 200 ng/µl of patient DNA. By adding the Taq Master Kit (ADDBIO INC, Korea) according to the manufacturer's protocol, in a PCR tube, 20 µl of the Master Mix, 1µl of each primer, 8µl of nuclease-free water, and 10µl of template DNA were mixed. The JAK2 genes were then amplified under the following PCR conditions.

The samples were amplified using the thermal cycler, starting with an initial denaturing phase for 5 minutes at 94°C, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 5 minutes afterward. The PCR product was stored at -20 °C until it had been loaded, and 1% agarose gel electrophoresis was used to isolate the PCR amplification products for 60 minutes at 80 volts. In the Immunogene Center in Erbil, Iraq, on the 3130 Genetic Analyzer, the PCR products were purified and read using the Sanger method. (Applied Biosystems, Hitachi High-Technologies, Tokyo, Japan). Software such as Finch TV, BioEdit, and Molecular Evolutionary Genetics Analysis were used to analyze the sequence data in order to find known and novel mutations.

2.4 Statistical analysis:

IBM SPSS Statistics version 25 was used for the statistical analysis.

2.4.1 Normality Test

Before running an independent sample t-test, the assumptions of normality and homogeneity of variances of the variables have to be tested. A normality test is used to determine whether a sample of data was drawn from a population with a normally dispersed distribution. (Aroian *et al.*, 2017). Kolmogorov-Smirnov (K-S) normality test examines if variables are normally distributed. K-S test is used if the sample is 50 or more but Shapiro-Wilk is used if sample size less and equal to 50.

2.4.2 Independent Sample T Test

Age, WBC, RBC, HGB, HCT, and PLT were used as independent variables, and JAK2V617F was used as an independent variable to examine the association between the two. (positive and negative).

3. Ethical consideration:

The Faculty of Science and Health's professional study committee gave ethics approval (25, December 2021). With their consent, the patients' venous blood was used for our research prior to the collection of samples.

4. RESULT:

4.1 Sociodemographic characteristic of the study population

In the present study, the majority of the patients had secondary polycythemia (82.7%) compared to those who have polycythemia vera (17.3%). Most of the participants were aged between 29 and 38 years (32.7%), followed by those aged 19–28 (23.1%), 39–48 (23.1%), 49–58 (11.5%), and more than 58 years (9.6%), since the average of their ages was 38 years.

4.2 Blood samples

Data analysis showed a significant difference in mean value of HGB, HCT, PLT and age between the two study groups (p=0.05). Furthermore, the mean values of HGB and HCT in the negative JAK2V617F group were higher (17.812, and 52.164) than the positive JAK2V617F group (14.718 and 39.450) respectively. On the other hand, the average of the PLT and age for the negative JAK2V617F group (228.830, and 37.041) were lower than the positive JAK2V617F group (332.533 and 54.333) respectively.

Additionally, in regard to individuals' WBCs and RBCs , no significant differences were observed between the positive and negative JAK2V617F (Table 1).

Table 1 Comparison positive and negative of the JAK2V617F with each of the (Age, WBC, RBC, HGB, HCT, and PLT)

JAK2V617F		N	Mean	Std. Deviation	T	p-value
WBC	Positive	3	6.721	3.474	1.233	0.224
	Negative	47	8.694	2.648		
RBC	Positive	2	6.528	0.598	1.242	0.22
	Negative	48	6.035	0.548		
HGB	Positive	3	14.718	4.306	2.878	**0.006**
	Negative	49	**17.812**	1.624		
HCT	Positive	2	39.450	11.332	4.151	**0.000**
	Negative	47	**52.164**	3.949		
PLT	Positive	2	**332.533**	82.777	2.518	**0.015**
	Negative	47	228.830	56.343		
Age	Positive	3	**54.333**	9.074	2.41	**0.020**
	Negative	49	37.041	12.171		

4.3 Comparison of polycythemia patients with Cigarette,Nergilla and Alcohol factors.

The percentages of Secondary Polycythemia patiens with smoking nergilla and cigarette were (21.2% and 51.9%) respectively compared to Polycythemia Vera (3.8% and 3.8%) respectively as well as the percentage of patient with Secondary Polycythemia was detected to be higher among the alcohol consummers (23.1%) compared to Polycythemia Vera (3.8%) as shown in (Fgure3).

4.4 JAK2 V617F

In the present study, exon 14 of the JAK2 gene and the PCR product were sequenced using the Sanger method (500 bps). Three (5.8%) of the overall cases of polycythemic patients had the Janus Kinase2 V617F mutation, while other samples were negative for the Janus Kinase2 V617F mutation. A 500 bp fragment from exon 14 of the Janus kinase 2 gene among polycythemia patients was successfully amplified with the precise primers as shown in (Figure 1).

Chromatograms of DNA sequences in three samples showed as heterozygous mutant but in all the other samples were in the form of wild-types as shown in (Figure 2).

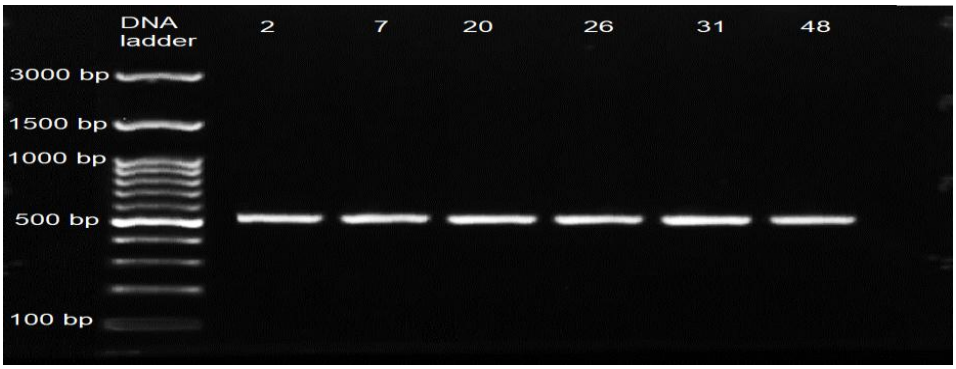
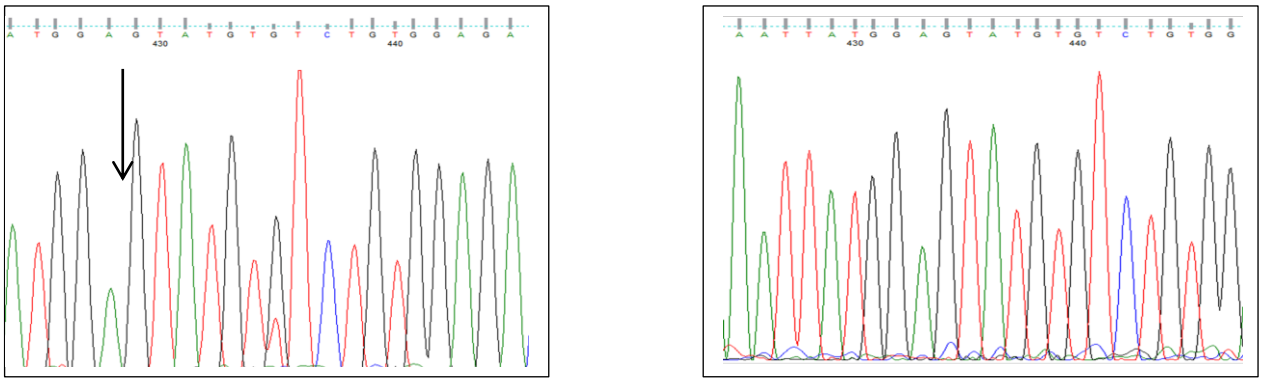


Figure 1: PCR products of the Janus kinase 2 gene. Lane 1 contains DNA ladder (100bp). Lane 2 through 48 contain the PCR products of the amplified DNA from six samples of patients with polycythemia(500bp). The DNA samples were run in 1.5% agarose at 80V for 1hour.



(A) (B)

Figure 2: Chromatograms of the DNA sequences of the c.1849GT (JAK2V617F) mutation in exon 14 of the JAK2 gene. (A) c.1849G→T heterozygous mutant; (B) wild-type. The arrow indicate mutation locations.

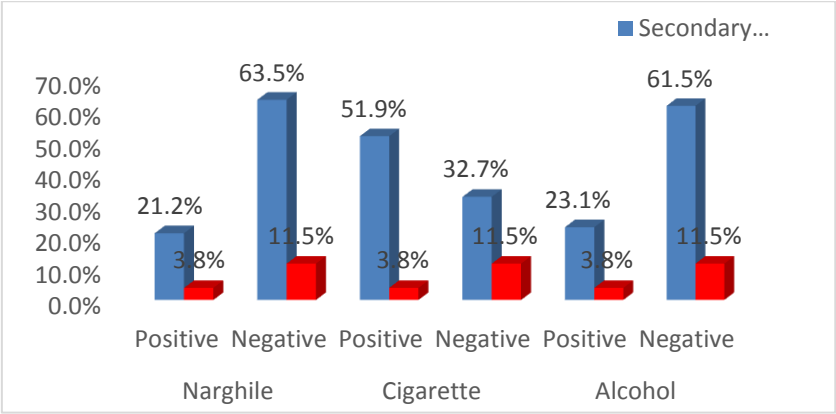


Figure3 Comparison between Secondary Polycythemia and Polycythemia Vera with each of Narghile, Cigarette, and Alcohol.

5. DISCUSSION:

The primary purpose of the current study was to identify the JAK2 V617F exon (14) mutation in both primary and secondary polycythemia patients. In the present study, 3/52 (5.8%) of the study participants had polycythemia vera (PV) with an average age of 32.7, our result agree with those of Srour *et al*., (2016), out of 44 cases of PV patients (13.82%) were positive whom aged between 61-70 years (Srour *et al*., 2016). According to previous studies, the chances of getting PV increases with age (Xie *et al*., 2014; Ruggeri *et al*., 2003).

In regards to age, the majority of the study participants were male, and the PV was more frequent in males than females. Our results are in agreement with the study conducted by Yadav *et al*., (2018), they showed hat out of 142 cases of polycythemic patients, the majority were male (68.18%) while females (31.82) (Yadav *et al*., 2018). We hypothesize that, this could be due to the fact that the gene that is responsible for PV is more frequent in males than females (Steensma *et al*., 2015).

In PV patients, a rise in red blood cell production led to elevation in hematocrit and hemoglobin levels (Bellucci & Michiels, 2006). There was a substantial age difference between JAK2 V716F mutant PV and ET harboring CALR variants, as well as differences in hemoglobin, WBC, and PLT counts (Rumi *et al*., 2014).

Our results show that the average of each of the HGB and HCT for negative JAK2V617F (17.812, and 52.164) were higher than the average of positive JAK2V617F (14.718 and 39.450) respectively but the average of each of the PLT and age for negative JAK2V617F (228.830, and 37.041) were lower than the average of positive JAK2V617F (332.533 and 54.333) respectively. Compared to an earlier study conducted on the same subject found that 1.7% of potential blood donors were rejected to donate blood because of their high Hb levels. According to a study conducted in Turkey, 2.8% of potential blood donors were turned away because of the elevated hemoglobin levels (Arslan, 2007 ; Rabeya *et al*., 2008). Similarly, according to an iranian report, 2.4% of blood donors were turned away due to the high levels in Hb (Kamaruzzaman *et al*., 2018).

Patients with chronic Philadelphia-negative classical MPNs, who typically have increased Hct and/or WBC, frequently carry the JAK2 mutation JAK2V617F. Those previously thought to be healthy but later proven to have no hematologic disorders carry the gene as well (Xu *et al*., 2007).

The results of smoking cigarettes for the most of the patients were positive (55.8%), but most of them were negative for smoking hookah (75%), and drinking alcohol (73.1%). On the contrary, a study conducted by All-Rubaie *et al*., (2014) showed that 79 out of 94 blood donors smoked cigarettes, hookah, or both, and that one of the causes of their elevated normal HCT was their blood donation. 90% of the 20 donors found the JAK2V617F mutation were smokers, and 22.8% (18/79) of all smokers tested positive for the mutation, compared to 13.3% (2/15) of non-smokers.

Smokers have been shown in multiple studies to have a greater prevalence and frequency of the JAK2 V617F mutation than nonsmokers, which could be due to the accelerated erythropoiesis that makes hemopoietic cells susceptible to this mutation (All-Rubaie *et al*., 2014).

There is no evidence to suggest that the presence of the JAK2 V617F mutation is linked to a lower RBC, HCT, or WBC level. In smokers, the JAK/STAT signaling system is constantly activated, which contributes to accelerated erythropoiesis, myelopoiesis, and thrombopoiesis (Scott, 2011).

Out of the 227 participants, 121 (53%) had polycythemic hemoglobin (>172 g/L), and 85 (70%) of them were smokers. Of these, 14.8% (18 out of 121) smoked cigarettes, 12% (15 out of 121) smoked shisha, and 2% (3 out of 121) smoked both cigarettes and shisha. Additionally, among non-smokers, 2.5% and 3.5%, respectively, noted daily exposure to cigarettes and shisha, respectively (AlQahtany *et al*., 2020).

In the current work, we sequenced the JAK2 exon 14 gene and PCR product using the Sanger method. (500 bps). The Janus Kinase2 V617F mutation was detected as heterozygous mutant forms were found in 3 (5.8%) of the total cases in polycythemia patients, and all other samples were negative for Janus Kinase2 V617F. Similarly research that looked at PV patients and blood donors revealed that 20/94 blood donors had the JAK2V617F mutation, or 21.3% of them (AL-Rubaie *et al*., 2014).

Another study found that PV patients had significantly higher counted mutant ratio of JAK2V617F than did blood donors with positive

JAK2V617F mutations, with mean values of 58±26.9 (range >1.3 - 100) and 11.10±12.6 (range >1.3 - 42.6), respectively (All-Rubaie *et al*., 2014).

Mutations in JAK2's exons 12–15 are particularly common in MPN cases. Nevertheless, approximately 95% of PV cases and 50-60% of ET or PMF cases have the JAK2 V617F variant in exon 14 (Chauffaille, 2010). A 2015 review study showed that the rate of MPN diagnoses had changed since 2005, when screening for the JAK2 mutation started (Deadmond & Smith-Gagen, 2015). There has been a shift in the proportion of individuals correctly diagnosing themselves with PV (21% less) and ET (31% more) (Mohammed & Zrari, 2020).

6.CONCLUSIONS

In conclusion, our findings in this study emphasized that most of our patients had secondary polycythemia, a JAK2V617F mutation in exon 14 for PV patients proved by molecular methods. Our society showed a higher risk for males compared to female individuals. Finally, other variations require additional research. Future research can take non-JAK2 V617F patients with PV who may have mutations in the JAK2 exon 12 region, as well as family history and environmental factors, into account.

REFERENCES

AlQahtany, F.S., Algahtani, F.H., Alshebly, M.M., Madkhaly, F.M., Ghandour, M.K., Almalki, J.H., AlOtaibi, W.S., Batarfi, A.S. & Mendoza, F.C., 2020. Association between cigarette & shisha smoking and the severity of polycythemia: A cross sectional study. *Saudi Journal of Biological Sciences*, 27:460-464.

Arslan, Ö., 2007.Whole blood donor deferral rate and characteristics of the Turkish population. *Transfus Med*. 17(5):pp. 379-83.

AL-Rubaie, H.A., Khudeir, M.A. & Al-Bayaa, I.M., 2014. Detection of JAK2V617F tyrosine kinase mutation and estimation of serum erythropoietin in blood donors who have high hematocrit. *Journal of the Faculty of Medicine Baghdad*, 56(4), pp.395-400.

Aroian, K., Uddin, N. & Blbas, H., 2017. Longitudinal study of stress, social support, and depression in married Arab immigrant women. *Health care for women international*, 38(2), pp.100-117.

Bellucci, S. & Michiels, J.J., 2006. The role of JAK2 V617F mutation, spontaneous erythropoiesis and megakaryocytopoiesis, hypersensitive platelets, activated leukocytes, and endothelial cells in the etiology of thrombotic manifestations in polycythemia vera and essential thrombocythemia. In Seminars in thrombosis and hemostasis, 32:381-398. Copyright© 2006 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA

Cao, M., Olsen, R.J. & Zu ,Y., 2006. Polycythemia vera. New clinicopathologic perspectives. *Arch Pathol Lab Med*, 130. pp.1126–1132.

Chauffaille, M.D.L.L., 2010. Myeloproliferative neoplasms: a review of diagnostic criteria and clinical aspects. *Revista Brasileira de Hematologia e Hemoterapia*, 32:308-316.

Deadmond, M.A. & Smith-Gagen, J.A., 2015. Changing incidence of myeloproliferative neoplasms: trends and subgroup risk profiles in the USA, 1973–2011. *Journal of cancer research and clinical oncology*, 141:2131-2138.

Gordeuk, V.R., Stockton, D.W., & Prchal, J.T., 2005.Congenital polycythemias/ erythrocytosis. *Haematologica*, 90(1),pp.109-11.

Huang, L.J., Shen, Y.M. & Bulut, G.B., 2010. Advances in understanding the pathogenesis of primary familial and congenital polycythemia. *British Journal of Haematology*, 148.pp.844–52.

Kumar, V., Abbas, A. & Aster, J. 2012. Robbins Basic Pathology, ninth ed.

Kamaruzzaman, S.F.M., Noor, N.H.M., Hassan, M.N., Yusoff, S.M.& Abdullah, W.Z., 2018. Detection of JAK2 V617F mutation among donors with erythrocytosis. *Journal of Clinical and Health Sciences*, 3(2), pp.19-25.

Mohammed, S.K. & Zrari, S.A., 2020. A single developing country's Hematology-Oncology Centre retrospective analysis of the Janus Kinase 2 V617F mutation in Philadelphia negative Myeloproliferative neoplasms. *KUWAIT MEDICAL JOURNAL*, 52:412-416.

Person, T.C. & Messinezy, M., 1996. Investigation of patients with polycythemia, 72: pp.519-24.

Purandare, A. V., Lorenzi, M. V. & Lombardo, L. J. 2010. Chapter 13: Janus Kinase (JAK2) Inhibitors for the Treatment of Myeloproliferative Neoplasm (MPN). Annual Reports in *Medicinal Chemistry*. v. 45:pp.210-227.

Ruggeri, M., Tosetto, A., Frezzato, M., & Rodeghiero, F. 2003. The rate of progression to polycythemia vera or essential thrombocythemia in patients with erythrocytosis or thrombocytosis. *Ann. Intern. Med.* 139, 470–475.

Rabeya, Y., Rapiaah, M., Rosline, H., Ahmed, S.A., Zaidah, W.A. & Roshan, T.M., 2008. Blood pre-donation deferrals-a teaching hospital experience. *Southeast Asian journal of tropical medicine and public health*, 39:571.

Rumi, E., Pietra, D., Ferretti, V., Klampfl, T., Harutyunyan, A.S., Milosevic, J.D., Them, N.C., Berg, T., Elena, C., Casetti, I.C. & Milanesi, C., 2014. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood, *The Journal of the American Society of Hematology*, 123:1544-1551.

Steensma, D.,P., Bejar, R., Jaiswal ,S,. *et al*., 2015. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* .126:9-16.

Srour, S.A., Devesa, S.S., Morton, L.M., Check, D.P., Curtis, R.E., Linet, M.S. & Dores, G.M., 2016. Incidence and patient survival of myeloproliferative neoplasms and myelodysplastic/myeloproliferative neoplasms in the United States, 2001–12. *British journal of haematology*, 174:382-396.

Scott, L.M., 2011. The JAK2 exon 12 mutations: a comprehensive review. *American journal of hematology*, 86:668-676.

Shi, J., Yuan, B. Hu, W. & Lodish, H. 2016. JAK2 V617F stimulates proliferation of erythropoietin-dependent erythroid progenitors and delays their differentiation by activating Stat1 and other nonerythroid signaling pathways, *Exp Hematol* ,44 (11) :pp.1044–1058.e5.

Tefferi**,** A**.** 2007. JAK2 Mutations in Polycythemia Molecular Mechanisms and Clinical Applications. The New England *Journal of Medicine*,365(5).pp.444-445.

Wilks, AF.,2008. The JAK kinases: not just another kinase drug discovery target. *Semin Cell Dev Biol* ,19,pp.319–28.

Xie, M., Lu, C., Wang, J., *et al*.,(2014). Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat.Med. 20:1472-1478.

Xia, D. & Hasserjian, R.P. 2016. Molecular testing for JAK2, MPL, and CALR in myeloproliferative neoplasms. *Am J Hematol* .91 (12) :pp.1277–1280.

Xu, X., Zhang, Q., Luo, J., Xing, S., Li, Q., Krantz, S.B., Fu, X. & Zhao, Z.J., 2007. JAK2V617F: prevalence in a large Chinese hospital population. *Blood*, 109:339-342

Yadav, S., Chaudhary, J., Kumar, N., Kannauje, P.K., Kumar, K., Bhattnagar, R. & Tiwari, R., 2018. Distribution of ABO and rh blood group in myeloproliferative diseases. *Acta Medica International*, 5:39.

Yamaoka, K., Saharinen, P., Pesu, M., Holt, V., E., Silvennoinen, O., & O'Shea, j. j., 2004. The janus kinases (jaks). *Genome biology*. 5(12): pp.1-6.