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Detection of Janus Kinase2 Gene Mutation and some Hematological Parameters in Polycythemic Patients /Erbil Province

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By
Arkhawan Saifadin Aziz
BSc. in Biology 2015
Koya University

Supervised by: Assist Prof. Dr. Karim Jalal Karim
Dr. Hemn Rasul Salih

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بسم الله الرحمن الرحيم

(وَقُلْ رَبِّ زِدْنِي عِلْماً)

صدق الله العظيم

سورة طه آية [١١٤]

Dedication

I entirely dedicate this dissertation to my Father and merciful Mother, my dear Sisters and brothers. In addition, I want to thank my wonderful husband, Zryan, for being there for me every step of the journey and always encouraging me to do my best. To Baran and Tarza, my children, whose lives have been profoundly altered by this journey. I pray that God abundantly blesses every one of you.

Arkhawan

Supervisor's Approval

Hereby I'm, Karim Jalal Karim, state that this thesis as entitled (**Detection of Janus Kinase2 Gene Mutation and some Hematological Parameters in Polycythemic Patients /Erbil Province**) was prepared under my supervision at Biology Department / Koya University by (Arkhawan Saifadin Aziz) as a partial fulfillment for the degree of Master of Science (MSc.) in Biology.

I have read and reviewed this work and I confirm that it is an original work to the best of my knowledge.

Signature:

Name: Dr. Karim Jalal Karim

Academic title: Assistant Professor

Date: / / 2023

Co Supervisor's Approval

Hereby I'm, Hemn Rasul Salih , state that this thesis as entitled (**Detection of Janus Kinase2 Gene Mutation and some Hematological Parameters in Polycythemic Patients /Erbil Province**) was prepared under my supervision at Biology Department / Koya University by (Arkhawan Saifadin Aziz) as a partial fulfillment for the degree of Master of Science (MSc.) in Biology.

I have read and reviewed this work and I confirm that it is an original work to the best of my knowledge.

Signature:

Name: Dr. Hemn Rasul Salih

Academic title: Lecturer

Date: / / 2023

Head of Department Approval

Upon recommendation of the supervising faculty whose name and signature appear above, I have forwarded this thesis for review by the examining committee.

Signature:

Name: Dr. Hiwa Ramadhan Fatah

Academic title: Lecturer

Academic position: Head of Biology Department

Date: 11 / 10 /2023

Viva Examining Committee Approval

We, the viva examining committee confirm that we have read this thesis as entitled (**Detection of Janus Kinase2 Gene Mutation and some Hematological Parameters in Polycythemic Patients /Erbil Province).** We have examined the student **Arkhawan Saifadin Aziz** in relation to all aspects of this thesis. In our opinion, it meets the standards of a thesis for the degree of Master of Science (MSc.) in Biology.

Chairman

Signature:
Name: Dr. Sarmad Raheem Kareem
Academic title: Assistant Professor
Academic position: Faculty of Medicine-Koya University
Date: / /2023

Member

Signature:
Name: Dr. Kazhal Mohamad Sulaiman
Academic title: Assistant Professor
Academic position:Head of Biology Department Education Collage-Salahaddin University
Date: / /2023

Member

Signature:
Name: Dr. Shaymaa Hadi Ali
Academic title: Assistant Professor
Academic position: Collage of Science-Duhok University
Date: / /2023

Member and Supervisor

Signature:
Name: Dr. Karim Jalal Karim
Academic title: Assistant Professor
Academic position: Faculty of Science and Health-Koya University
Date: / /2023

Member, Co-Supervisor

Signature:
Name: Dr. Hemn Rasul Salih
Academic title: Lecturer
Academic position: Faculty of Medicine-Koya university
Date: / / 2023

Approved by the Council of the Faculty of Science and Health

Signature:
Name: Dr. Zrar Saleem Kareem
Academic title: Professor
Academic position: Dean of the Faculty of Science and Health
Date: / /2023

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Abstract

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.

The purpose of this study was to detect JAK2 mutations in patients with primary and secondary polycythemia. Additionally, erythropoietin hormone (EPO) levels were tested in polycythemic patients along with other hematological parameters were studies in 52 polycythemic patients (8 with PV and 44 blood donors). Samples were taken from patients attending Nanakaly Hospital, Directorate of Blood Bank in Erbil, and Shahid Dr. Khalid Hospital in Koya in Kurdistan region of Iraq.

Sanger sequencing of JAK2 exon 14 and polymerase chain reaction (PCR) results (500 bps) showed that 3 (5.8%) polycythemic patients had heterozygous Janus Kinase2 V617F mutations, while all other samples were negative. The average of age of the study participants was 38 years, and the male participants (94.2%) were higher than the females (5.8%). The percentage of patients who have abnormal EPO for secondary polycythemia group (75%) was higher than polycythemia group (15.4%). Abnormal level of EPO was observed in patients aged between 29 to 38 (30.8%). The average of each of the hemoglobin (HGB) and hematocrit (HCT) for negative JAK2V617F was higher than the positive JAK2V617F, while the average of each of the PLT and age for negative JAK2V617F were lower than the positive JAK2V617F. Furthermore, the negative nerghila, cigarette, and alcohol had higher rates of negative JAK2V617F than their positives.

Finally, most of the patients who had negative fatigue, prurtius, and headche higher rates of negative JAK2V617F than the positive JAK2V617F.

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List of Abbreviations

Symbols	Description
AML	Acute myeloid leukemia
Bp	base pairs
BM	Bone marrow
°C	Celsius degree
CKD	Chronic kidney disease
CFU-E	Colony-forming unit erythroid
CML	Chronic myeloid leukemia
CALR	Calreticulin
COPD	Chronic obstructive pulmonary disease
CFU-GEMM	Colony-forming-unit granulocyte, erythrocyte, macrophage, megakaryocyte
CBC	Complete blood count
DNA	Deoxyribonucliec acid
ddH2O	Double-distilled water
EDTA	Ethylenediaminetetraacetic acid
Epo-R	Erythropoitein receptor
ET	Essential thrombocythemia
ELISA	Enzyme-Linked immunosorbent Assay
EPO	Erythropoietin
G	Gram
GM-CSF	Granulocyte-macrophage colony-stimulating factor.
G-CSF	Granulocyte colonystimulating factor
HCT	Hematocrit
Hb	Hemoglobin
HSCs	Hematopoietic stem cells
HRP	Horseradish Peroxidase
IGF-1	Insulin- like growth factor
IL	Interleukin
JAK2	Janus kinase 2
JAK2V617F	Janus kinase 2 valine 617phenylalanine

JH	Janus homology
Kb	Kilobase
Ml	Milliliter
Min	Minute
MF	Myelofibrosis
MPL	Myeloproliferative leukemia protein
MPN	Myeloproliferative neoplasms
Ml	Microliter
Ng	Nanogram
NCBI	National Center for Biotechnology Information
OSA	Obstructive sleep apnea
OD	Optical density
Pg	Picogram
PMF	Primary myelofibrosis
PLT	Platelets
PCR	Polymerase chain reaction
PV	Polycythemia vera
RPM	Round per minute
RBCs	Red blood cells
SCF	Stem cell factor
STAT	Signal Transducer and Activator of Transcription
Sec	Second
TBE	Tris/Borate/EDTA
TPO	Thrombopoietin
TYK	Tyrosine kinases
TPOR	Thrombopoietin receptor
UV	Ultraviolet
WHO	World Health Organization
WBCs	White blood cells

Chapter One: Introduction

1. INTRODUCTION

Polycythemia or erythrocytosis is a disease marked by abnormal changes in the size and hemoglobin amounts of all RBCs in the blood. In secondary polycythemia, tissues don't get enough oxygen, so the body raises the level of erythropoietin hormone. This causes the body to make too many red blood cells (RBCs) and too much hemoglobin. In primary polycythemia, people have an inheritance or acquired mutation that causes the development of RBC progenitors (Kumar *et al.,* 2012). Those who have primary polycythemia typically have polycythemia vera (PV), an acquired myeloproliferative neoplasms (MPN) (Person and Messinezy , 1996; Huang *et al.,* 2010).

Erythropoietin (EPO), which acts on erythroid progenitors, is the main chemical that promotes erythropoiesis and is the cause of secondary polycythemia (Gordeuk *et al.,* 2005). A glycoprotein hormone called erythropoietin, also referred to as EPO, regulates erythropoiesis. It is a cytokine for bone marrow-based erythrocyte precursors. The molecular weight of human EPO is 30.4 kDa (Obeagu, 2015). It is produced by interstitial fibroblasts in the kidney in close cooperation with the peritubular capillary and tubular epithelial tubules and is also known as hematopoietin. Additionally, it is made in the liver's perisinusoidal cells (Siren *et al.,* 2001). Renal production predominates during adulthood, while liver production is prevalent during the fetal and perinatal period. Erythropoietin serves various biological purposes besides erythropoiesis. For instance, it is crucial for the brain's reaction to neuronal damage (Siren *et al.,* 2001). EPO contributes to the healing of wounds (Haron *et al.,* 2003).

A chronic myeloproliferative condition called polycythemia rubra vera (PV) causes an excess of red blood cells in the blood, bone marrow, liver, and spleen. A polycythemic phase is usually followed by a spent cytopenic phase, which includes extramedullary hematopoiesis, myelofibrosis, and hypersplenism (Cao *et al.,* 2006). Symptoms that may be present include pruritus after bathing, searing paresthesias in the distal extremities, gastrointestinal issues, or neurologic complaints like headache, weakness, or dizziness (Cao *et al.,* 2006).

The Janus Kinase 2 (JAK2) gene gain of function mutation is the most often documented genetic abnormality in PV patients. About 95% of PV patients have the JAK2 V617F exon 14 mutation (Xia and Hasserjian, 2016). Patients with PV who are JAK2 V617F negative can have mutations in the JAK2 exon 12 area (Shi *et al.,* 2016).

The Janus kinase (JAK) family of non-receptor tyrosine kinases, which consists of 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 in the kinase and negative regulatory JAK homology domains JH1 and JH2, respectively are phosphorylated in a tightly controlled manner. JAK2 V617F, which results from a point mutation (1849GT) in exon 14, causes the substitution of phenylalanine for valine at codon 617 in the JH2 domain. The JAK2 enzyme becomes constitutively active as a result of this alteration to a single amino acid (Purandare, 2010). The four JAK2 mutations in exon 12 are tandem point mutations or in-frame deletions. Exon 12 of JAK2 V617F and the proliferation of erythropoietin receptor-expressing cell lines result in cytokine hypersensitivity in these cells (Tefferi, 2007).

We hypothesized that patient with Polycythemia Vera and blood donors who has secondary polycythemia have mutation in exon 14. Furthermore, cigarette smoking, narghile and alcohol consumption are major risk factors for mutation in exon 14.

This study aimed to identify JAK2 mutation in both primary and secondary Polycythemic patients. Another aim was investigating some hematological parameters among polycythemic patients, including change in erythropoietin hormone level, and also study of some factors.

Chapter Two: Literature Review

2. LITERATURE REVIEW

2.1 Hematopoiesis

All of the blood's cellular components are produced as a result of the highly organized process known as hematopoiesis, which also produces clotting factors and immunological host defenses as shown in figure 2-1 (Ghiaur and Jones, 2019).

There are over ten distinct lineages types of blood cells, each of which performs a unique job in the body. In both innate and acquired immunity, leukocytes play a crucial role. Platelets are produced by megakaryocytes to aid in blood clotting and wound healing, while oxygen and carbon dioxide are carried by erythrocytes. All of the many kinds of blood cells originate from hematopoietic stem cells (HSCs), which are mostly located in the bone marrow (BM), the major location of adult hematopoiesis. Millions of "old" blood cells are replaced by new ones every second, making the blood one of the most adaptable and regenerative tissues (Ghiaur and Jones, 2019).

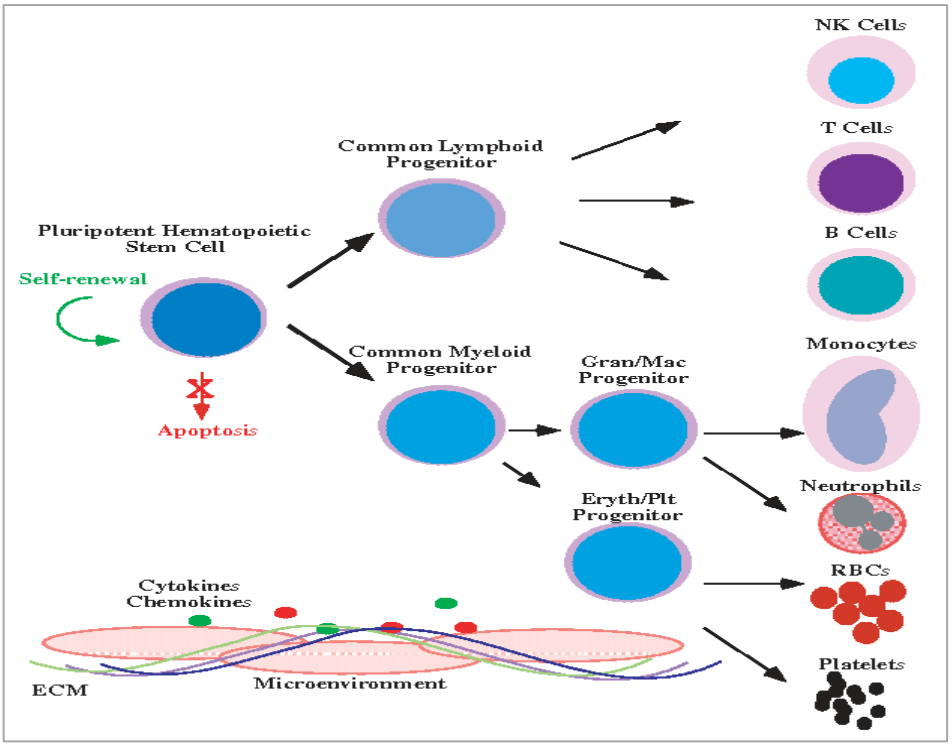


Figure 2-1: Hematopoiesis's main differentiation pathways. ECM = extracellular matrix (Smith, 2003).

In extreme circumstances, such as anaemia or infections, the number of blood cells increases rapidly. When the body is recovered, the number of cells gets back to normal level. The average lifetime of an adult blood cell ranges from hours to years, depending on the kind of cell (Rieger and Schroeder, 2012).

Pluripotent hematopoietic stem cells differentiate into mature blood cells. As a continuous process, the differentiation of mature blood cells from hematopoietic stem cells entails discrete modifications brought on by the local microenvironment as well as cumulative signals from soluble glycoprotein factors. To produce the number of blood cells required for life, signals that inhibit blood cell formation from being excessive and signals that drive the production of mature blood cells must be properly regulated. Not all regulatory procedures are completely understood (Kawahara and Shiozawa, 2015). When transplanted into an irradiated host, early progenitor cells such as the colony-forming-unit granulocyte, erythrocyte, macrophage, and megakaryocyte (CFU-GEMM) can develop into a variety of lineages but cannot fully restore the hematopoietic system (Kawahara and Shiozawa, 2015).

2.2. Polycythemia

An increase in hemoglobin levels above normal is referred to as polycythemia. This rise might actually exist or might only be visible due to spurious or relative polycythemia, which causes a reduction in plasma volume (Jameson *et al.*,2022). Erythrocytosis and polycythemia are sometimes used interchangeably, but some people distinguish between the two. Polycythemia refers to any increase in red blood cells, but erythrocytosis necessitates evidence of increased red cell mass. Patients with polycythemia are frequently discovered accidentally when their hemoglobin or hematocrit levels are found to be elevated. Typically, men's Hb levels are considered excessively high at 17 g/dL (170 g/L), and women's levels are considered lower which is at 15 g/dL (150 g/L). Atypical hemoglobin levels may be less than 50% in men or 45% in women (Jameson *et al.*,2022).

2.2.1 Classification of Polycythemia

2.2.1.1 Relative Polycythemia (Gaisbock Syndrome, Spurious, or Stress Erythrocytosis)

Stress erythrocytosis, also known as relative polycythemia, is rather frequent. Patients are frequently hard-working middle-aged executives who have a propensity for hypertension,

coronary artery disease, and early stroke. Their red cell count is often less than 6 million/mm3, although their hemoglobin and hematocrit levels are elevated. Despite their plasma volume contracting, their red blood cell mass is normal. Although the cause of the plasma volume shrinkage in these patients is unknown, abnormalities in catecholamine metabolism may be a contributing factor. Phlebotomy should never be used while treating stress erythrocytosis. Management aims to reduce vascular problems and hypertension (Silverstein, 2016).

Relative polycythemia is caused primarily by two factors. 1- Plasma volume can be depleted by dehydration (such as from vomiting, diarrhea, heavy perspiration, or diuretics). 2- Stress erythrocytosis (Gaisböck's polycythemia) is a misnomer because it is caused by the contraction of plasma volume. Most hypertensive and obese men have this benign condition (Saint and Chopra, 2018).

2.2.1.2 Absolute Polycythaemia

Haemoglobin, haematocrit, or the number of red blood cells are all higher in absolute polycythaemia, which is a real increase in red cell mass. It can be developed as a main or secondary phenomenon, and it is clinically significant (Butler and Maxwell, 2018).

2.2.1.2.1 Primary Polycythaemia

When aberrant erythropoiesis results from a congenital or acquired disease, primary polycythemia develops. The most frequent ailment is polycythaemia vera (PV), a clonal Myeloproliferative neoplasm (MPN) typically associated with low serum (EPO) levels and virtually always accompanied by the JAK2 V617F mutation (Butler and Maxwell, 2018). Intrinsic factors in the generation of red blood cells result in an increase in the red cell count in primary polycythemia (polycythemia vera) (Tefferi, 2003).

2.2.1.2.2 Secondary Polycythemia

The most frequent cause of secondary polycythemia is an erythropoietin-driven increase in hematocrit in response to persistent hypoxemia. The most frequent causes of hypoxemia, which results in secondary polycythemia, include chronic obstructive pulmonary disease (COPD), obese hypoventilation syndrome, and obstructive sleep apnea (OSA) (Nadeem *et al.,* 2012). No strong clinical evidence exists to support secondary polycythemia's association with an elevated risk of thrombosis and test indicators of coagulation activation are significantly less enhanced in secondary polycythemia than in PV (Nadeem *et al.,* 2012).

Secondary polycythemia is caused by external factors like smoking, hypoxia from chronic lung illness, and other frequent causes of polycythemia. Smoking can therefore be a serious risk factor for polycythemia (Potula and Hu, 1996; Iso *et al.*, 2005).

2.3 Myeloproliferative Neoplasms (MPN)

Classic (MPN) that don't have the Philadelphia chromosome (Ph-) are essential thrombocythemia, polycythemia vera, and primary myelofibrosis. (Kralovics *et al*., 2005; Pikman *et al*., 2006; Klampfl *et al*., 2013) It is being demonstrated that almost ninety percent of MPN individuals have a driving mutation in the genes that code for (JAK2), calreticulin (CALR), or the thrombopoietin receptor (TPOR, MPL).

Myelofibrosis is a serious illness that can be found either prior to or following essential thrombocythemia or (PV) is diagnosed. Both critical thrombocythemia and PV are chronic-phase myeloproliferative neoplasms. MPN have been broken up into groups based on clinical and lab features (Arber *et al.,* 2016; Harrison *et al.*,2014; McMullin *et al.*,2007) However, it is unclear where and how to draw the boundaries of separation between them (Wilkins *et al.,* 2008; Barbui *et al.,* 2014).

The protein (JAK2), a nonreceptor tyrosine kinase, is the target of the most frequent mutation in these situations. JAK2 aids in the mediated transmission of signals that promote cell growth and differentiation. Erythropoietin [Epo], thrombopoietin, or interleukins are examples of ligands that cytokine receptors bind to. This binding results in the phosphorylation of JAK2, which subsequently activates downstream signaling cascades to drive the transcription of new genes (Anderson and McMullin, 2015).

The most prevalent mutation, JAK2V617F (in exon 14), renders the protein's inhibitory domain inactive and causes cytokine-independent proliferation. Nearly all PV cases, as well as more than half of all ET and MF cases, have this mutation. Alternative JAK2 exon 12 mutations often exist in the 4% of PV cases that lack this common mutation (Azzato, 2015).

The prevalence of JAK2 mutations in ET and primary myelofibrosis is believed to be between 50% and 60%, which is lower than the prevalence of mutations in PV. An additional 25% of mutations are attributed to calreticulin (CALR) mutations and 5% to mutations in the myeloproliferative leukemia protein (MPL). Triple negative refers to the remaining 15% of cases, and these three mutations are mutually exclusive (Aruch, and Mascarenhas, 2016; Tefferi and Barbui, 2015; Tefferi and Pardanani, 2015).

2.3.1. Clasification of Myeloproliferative Neoplasms

2.3.1.1 Essential Thrombocythemia(ET)

A Myeloproliferative Neoplasms called is characterized by excessive platelet production in the bone marrow. Small fragments called platelets (PLT), also known as (Thrombocyte) join together to create blood clots that delay or stop bleeding and aid in wound healing. If there are too many platelets, they could be grouped together and obstruct blood flow (DeGennaro, 2017). A thrombus, or blood clot that forms in a blood vessel, can result from an abundance of platelets. Serious health issues, including a stroke, heart attack, or pulmonary embolism, may result from this primary thrombocythemia, idiopathic thrombocythemia, and primary thrombocytosis are among the additional names for ET (DeGennaro, 2017).

Essential Thrombocythemia epidemiology is 1 to 50 per 100,000 individuals. The prevalence of women. 60s as the median age at diagnosis. A familial pattern only occasionally occurs (Aruch and Mascarenhas, 2016; Tefferi and Barbui 2015).

The cause of ET is not fully understood. The majority of instances of ET are linked to one or more acquired genetic abnormalities in hematopoietic stem cells that cause an excess of megakaryocytes, the bone marrow's progenitor cells for platelets. These mutations take place during a person's lifespan and are not inherited. ET is less frequently inherited (DeGennaro, 2017).

The JAK2, MPL, or CALR genes are mutated in the great majority of ET patients.These mutations have the following approximate frequencies: 60% JAK2 mutation ,CALR mutation (20–35%) , MPL mutation: 1-4% JAK2, MPL, or CALR gene mutations are not present in 10% of ET patients (DeGennaro ,2017).

2.3.1.2Myelofibrosis (MF)

Chronic MF is a clonal hematopoietic stem cell disorder characterized by marrow fibrosis, extramedullary hematopoiesis, and splenomegaly. It is also known as idiopathic myelofibrosis, agnogenic myeloid metaplasia, or myelofibrosis with myeloid metaplasia. Since myelofibrosis and splenomegaly are also characteristics of both PV and chronic myeloid leukemia (CML), it can be challenging to diagnose PMF without a particular clonal marker (Jameson *et al.*, 2022).

The overproduction of "megakaryocytes," the word for the enormous bone marrow cells that fragment and create hundreds to thousands of platelets, is a crucial continuous component of MF. Platelets are one-tenth the size of red blood cells in terms of volume. In the body, used

platelets are often replaced by fresh ones (Hobbs and Rampal, 2015). In MF, additional megakaryocytes are produced, resulting in an excess of platelets being discharged into the blood and "cytokines" being released into the bone marrow. In the marrow, the cytokines promote the growth of fibrous tissue. Ironically, some patients' platelet production can decrease when the number of megakaryocytes is too high (The Leukemia and Lymphoma Society, 2012).

The prevalence of myelofibrosis, which affects about 1 in 100,000 people worldwide, is equivalent to that of other MPNs. Genetic investigations revealed JAK2 (65%), CALR (25%), and MPL (7%) mutations, with the remaining mutations being classified as triple negative. This mutational spectrum is mostly similar to that of ET (Tefferi and Pardanani, 2015; Hobbs and Rampal, 2015).

Despite being documented in people of various ages, myelofibrosis is most frequently detected in middle-aged and elderly patients, with the majority of patients being older than 50 at the time of diagnosis (Lui and Hao, 2018; Takenaka *et al.,* 2018). While some research has shown that men have symptoms more frequently than women, other studies have shown that both sexes experience symptoms almost equally (Lui and Hao, 2018). The median overall survival for PMF is typically 5-7 years after diagnosis, with the most common reasons for mortality being infections, vascular events, and leukemia transformation (Lui and Hao, 2018;Mascarenhas *et al.,* 2018).

2.3.1.3 Polycythemia Vera (PV)

French physician Louis Henri Vaquez (1860-1936) originated the name "maladie de Vaquez," or (PV), in 1892 (Tefferi *et al*., 2021). The Polycythemia Vera Study Group (PVSG) reclassified and refined the definition of PV for 65 patients in 1967 (Regimbeau *et al*., 2022). The World Health Organisation reclassified and refined the definition of PV three times in rapid succession, in 2001, 2008, and 2016. The hallmark of PV, like all MPNs, is unchecked cell proliferation. Erythroid hyperplasia, myeloid leukocytosis, thrombocytosis, and splenomegaly are all features of PV, a myeloproliferative illness (Tefferi and Barbui, 2020).

Clonal proliferation of an abnormal hematopoietic stem or progenitor cell characterises the so-called "classic" BCR-XY group of diseases, which includes ET, MF, and PV. The estimated annual incidence is 2.3-2.8 per 100,000 persons (Ruggeri *et al*., 2003), with a median age at diagnosis of about 60 years and a male-to-female ratio of 1.2:1. In particular, elevated red blood cell counts, thrombotic and hemorrhagic tendencies, a variety of symptoms, and a

cumulative risk of developing MF and/or progressing over time into acute myeloid leukaemia are the hallmarks of this condition (Iurlo *et al.,* 2020).

Polycythemia Vera is considered a clonal disease since the first genetic mutation that initiates disease development takes place in a single bone marrow cell. However, the underlying cause of this malignant change remains unknown. It has been shown that a driver mutation in the JAK2 gene is present in 98% of PV patients (Tefferi *et al*., 2016). In the remaining individuals, the JAK2 gene encoding the pseudokinase domain is largely altered in exons 12–15 (Lee *et al.,* 2009; Sofi *et al*.,2022).

These shared phenotypes are the consequence of constitutive activation of JAK2, the receptor tyrosine kinase for EPO, TPO, and granulocyte colony-stimulating factor (GCSF). Activation of JAK2 occurs directly when a point mutation (V617F in JAK2 exon 14) or, less often, insertions or deletions in JAK2 exon 12 occur (James *et al*., 2005; Scott *et al*., 2007). Insertions and deletions in the ER chaperone calreticulin (CALR) and point mutations in the thrombopoietin receptor MPL are two mechanisms that indirectly activate JAK2. As a result, it may bind MPL and accidentally activate JAK2 (Nangalia *et al.,* 2013; Klampfl *et al.,* 2013).

Studies have seen an unexpected rise in the molecular complexity of MPN due to the coexistence of several additional mutations that can exist with or without JAK2V617F or MPL mutations. The most commonly affected genes are those involved in RNA splicing (SRSF2) or epigenetic gene regulation (TET2, DNMT3A, EZH2, ASXL1, IDH1, and IDH2). Proteins (TP53, NRAS, IKZF) are favored during the leukemic transformation stage (Vannucchi and Guglielmelli, 2013).

2.4. Factor affecting Polycythemia

2.4.1Physiologic Factor

2.4.1.1Age and Sex Distribution

JAK2V61F expression is age-independent; therefore, PV can happen at any age (Xie *et al.,* 2014), but it happens earlier in women and becomes more common after age 60 (McNally *et al.,*1997) with male predominance (Steensma *et al.,* 2015).

The majority of PV patients do not have a family history of the condition. Though the illness sometimes affects more than one family member. Compared to other Europeans or Asians, Jews of Eastern European heritage are more likely to have PV. New cases of PV occur

at a rate of around 2.8 per 100,000 males and 1.3 per 100,000 females of all racial and ethnic backgrounds. Prevalence (the proportion of a population with a disease diagnosis) for PV is around 22 cases per 100,000, (The Leukemia and Lymphoma Society , 2015).

Thrombosis, which can be arterial or venous and can occur in unusual places like the splanchnic bed, is the main cause of morbidity and mortality in individuals with PV (Sekhar *et al.*, 2013). Additionally, there is a tendency for the condition to advance to post-PV myelofibrosis (PPV-MF), which happens in 4.9–6% of cases at 10 years. Additionally, at 10 years, 2.3–14.4% of patients develop acute myeloid leukemia (AML) (Cerquozzi and Tefferi, 2015) When compared to age- and sex-matched populations, life expectancy in patients with PV is lower because of these two significant problems (Passamonti *et al.*, 2004).

2.4.1.2Hormonal Factors

Several cytokines control the process of erythropoiesis. Granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-6, stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF), insulin-like growth factor (IGF-1), and, of course, EPO are among the growth factors involved (Fisher, 2003). EPO functions in erythroid progenitor cells that are further along in their development. The colony-forming unit erythroid (CFU-E) cells in the bone marrow are its main target cells. These cells are stimulated by EPO to multiply and develop from normoblasts into reticulocytes and then into mature erythrocytes (Figure 2-2) (Lombardero *et al.*, 2010) . Actually, CFU-E cells have the highest density of EPO-R on their surfaces, making them the most EPO-sensitive cells (Jelkmann, 2004).

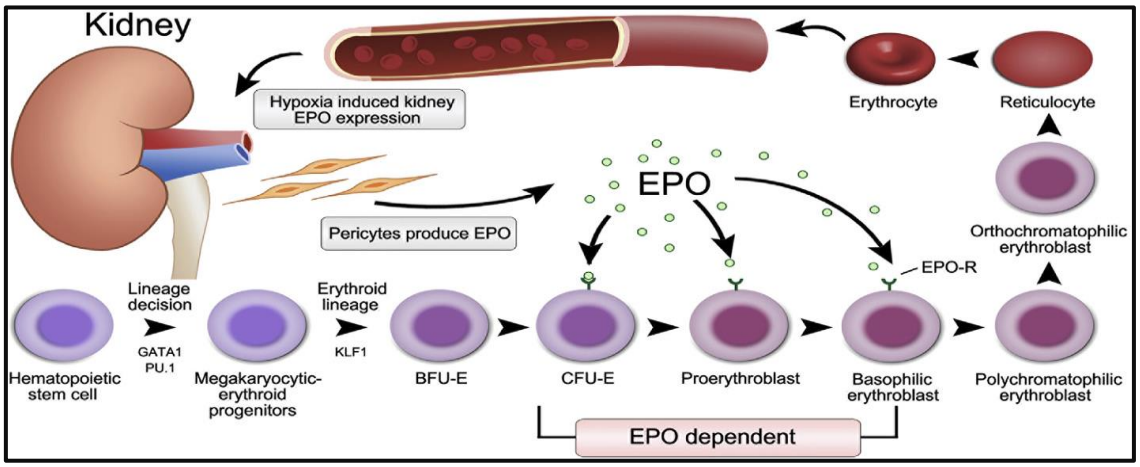


Figure 2-2 Erythroid development stages and the kidney's EPO-controlled oxygen-dependent feedback loop (Shih *et al.*, 2018).

2.4.1.2.1Erythropoietin Hormone

The primary factor controlling RBC production is (EPO). EPO is linked to Epo-R on red cell progenitors and produced by the kidney in response to hypoxia, where it stimulates differentiation and Hb production (Ma *et al*., 2004).

A glycoprotein hormone with a molecular weight of 30.4 kDa, erythropoietin has 165 amino acids, three N-linked carbohydrate chains, one O-linked chain, and two disulfide linkages. The main regulator of erythropoiesis is erythropoietin, whose levels are negatively correlated with oxygen availability (Fisher, 2003). Less than 10% of given erythropoietin is eliminated in the urine; metabolism is assumed to take place in the kidney, liver, and bone marrow. Plasma half-lives vary between 2 and 13 hours. Serum concentrations vary between 6 and 32 U/l (Foley, 2008).

A particular trans-membrane dimeric receptor that has been identified in both erythroid and non-erythroid cell types is how erythropoietin functions. When EPO interacts with its receptor, the receptor's shape changes, it is phosphorylated and the genes that code for anti-apoptotic proteins are expressed (Foley, 2008), Thus, the main function of EPO is to prolong the survival of erythroid cells by protecting them from programmed cell death (apoptosis). When combined with SCF, GM-CSF, 1L-3, and IGF-1, EPO promotes the maturation and proliferation of erythroid progenitor cells (Fisher, 2003).

Peritubular fibroblasts in the adult renal cortex and hepatocytes during the fetal state of the human produce EPO. In adult humans, the liver produces a small quantity of extra-renal EPO. Although EPO mRNA can also be found in the brain, liver, spleen, lung, and testes, chronic kidney disease (CKD) prevents these organs from replacing renal EPO. EPO generated from the brain has local neuroprotective effects (Jelkmann, 2011).

2.4.1.2.1.1Mechanism of Erythropoietin Action

EPO's primary purpose is to stop apoptosis in erythroblasts and CFU-E cells that are dependent on EPO but have not yet begun to synthesize hemoglobin. Epo-R, a hypoxia-induced receptor for erythropoietin, (Haase, 2010), is produced as a protein with a kDa of between 66 and 78. The dimerization of two EPO receptors by one molecule has served as evidence that is activated. Studies have revealed that neither the reticulocyte nor the mature erythrocyte possess EPO receptors, and that the number of EPO receptors per cell gradually declines during erythroid cell differentiation (Fisher, 2003).

The activation of Janus kinase 2 (JAK2) by self-dimerization requires a conformational change in the EPO receptor triggered by erythropoietin binding to the receptor. Despite the

absence of endogenous tyrosine kinase activity in the EPO receptor, EPO nonetheless triggers intracellular signalling through the rapid tyrosine phosphorylation of numerous proteins (Lacombe and Mayeux, 1999). When EPO binds to an Epo-R, it triggers the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and JAK-signal transducers and activators of transcription (STAT) pathways (Elliott *et al.*, 2008).

It is well known that JAK kinases like to bind to the protein known as Signal transducer and activators of transcription (Stathatos). When tyrosin is phosphorylated, STATs go to the nucleus and start the transcription of particular cytokine target genes (Ammarguellat *et al.*, 2001), where it up-regulates expression of the antiapoptotic gene. Thus, activation of the STAT5 signaling pathways is essential for the antiapoptotic activity of EPO (Um and Lodish, 2006).

2.4.1.2.1.2 Erythropoietin in Polycythemia

Hematopoietic growth factor, or EPO, is necessary for the efficient development and differentiation of erythroid progenitors. When there is hypoxia or anemia, it produces more of it. Estimating serum EPO levels may be able to help identify the various kinds of polycythemia (Cario, 2005).

If the kidneys are not significantly affected, EPO levels typically vary inversely with hematocrit. EPO is released in response to hypoxia, which in turn increases erythrocyte synthesis in the bone marrow. The release of EPO is inhibited by high blood levels of RBC, hemoglobin, hematocrit, or oxygen (Obeagu, 2015). A neoplastic (clonal) blood condition known as primary polycythemia (polycythemia vera) is characterized by the independent generation of hematopoietic cells. Erythrocyte production is increased, while EPO levels are suppressed as a result. When hemoglobin levels above 18.5 g/dL, chronic leukocytosis, persistent thrombocytosis, atypical thrombosis, splenomegaly, and erythromelalgia (dysesthesia and erythema involving the distal extremities) are all signs of PV (Obeagu, 2015).

Secondary polycythemias may result from a healthy or unhealthy increase in red cell mass. Hypoxia and a corresponding rise in red cell mass are characteristics of appropriate secondary polycythemias, such as high-altitude life and pulmonary illness. In an effort to enhance the amount of oxygen delivered by increasing the number of oxygen-carrying RBCs, EPO synthesis is increased. Some cancers, including those of the kidney, liver, lung, and brain, generate EPO or proteins that resemble it. These increases cause inappropriate secondary polycythemias (Obeagu, 2015).

The majority of secondary polycythemias are acquired, frequently as a result of an Epo response to hypoxia that is oxygen-sensitive; they can also be brought on by a tumor that

secretes Epo. Erythropoietin receptor mutations, for example, or mutations affecting the von Hippel-Lindau tumor suppressor or hypoxia-inducible factor, which regulate intracellular oxygen sensing, are associated with polycythemias. Congenital secondary polycythemia is an uncommon disorder caused by mutations in the HIF-1a prolyl hydroxylase gene, congenital methemoglobinemia, and 2,3-bisphosphoglycerate insufficiency (Prchal, 2003).

Since EPO controls erythrocyte production, checking the blood EPO level may tell you whether the erythrocytosis is hormone-mediated or self-regulatory. When individuals have erythrocytosis due to hypoxia, their serum EPO levels tend to rise. However, EPO levels are often lower in patients with PV and remain low in the majority of cases even after adequate venesection (Messinezy *et al*., 2002). EPO values below the standard range may be seen in idiopathic erythrocytosis, lowering the specificity of low EPO levels for PV. Even if the serum EPO level is normal, the erythrocytosis might still be due to PV or hypoxia. However, precise, sensitive, and repeatable EPO tests have made the use of low blood EPO levels a secondary criterion in the diagnosis of PV (Messinezy *et al.,* 2002).

2.4.2Genetic Factors

2.4.2.1 Janus Kinase (JAK2)

The activating mutation V617F in the JAK2 gene, which codes for tyrosine kinase, was discovered in 2005 (Stein *et al*., 2015), and since then, the genetic basis of PV has been better understood. Increased red cell mass due to a JAK2 mutation is diagnostic of PV. Multiple investigations have shown that between 95% and 97% of PV patients have the V617F mutation in exon 14 of the JAK2 gene, which is absent in both normal individuals and those with secondary polycythemia. Thus, this mutation allows doctors to distinguish individuals with PV apart from those with secondary polycythemia (Landolfi *et al*., 2010). However, the JAK2 V617F mutation is seen in 50% of those with ET and primary myelofibrosis, therefore it is not exclusive to PV. Three percent of individuals with PV do not have the typical JAK2 exon 14 mutations, but do have a mutation in exon 12, suggesting that all PV patients have a mutation in either exon 14 or exon 12 (Landolfi *et al.,* 2010).

The cytokine receptors interleukin (IL)-3, IL-5, and IL-6, as well as the EPO, TPO, GM-CSF, and granulocyte colony-stimulating factor receptors of hematopoietic cells, rely on JAK2, a member of the Janus tyrosine kinase family (Verma *et al.,* 2003).

Humans have the JAK2 gene on chromosome 9p24.1. The protein it encodes is 130.7 kDa and contains 1132 amino acids (Yamaoka *et al*., 2004). The gene has 25 exons. Janus

kinases (JAKs) are a family of non-receptor tyrosine kinases (TYK) that play crucial roles in erythroid and megakaryocytic lineage development, proliferation, and apoptosis. EPO, TPO, and GM-CSF residues are all cytokines that signal via the JAK/STAT pathway (dos Santos *et al.,* 2011).

JAK homology (JH) domains 1 through 7 are the 7 distinct regions of preserved homology that define the structure of JAK2. JH2, a pseudokinase that lacks kinase activity, inhibits the JAK2 kinase domain (Figure 2-3). (Michiels, 2013) JAK2V617F is located in the JH2 domain(Baker *et al.,* 2007). JH2 controls the catalytic activity of JH1 (Bandaranayake *et al.,* 2012). JAK2V617F functions as an oncogenic factor in mutant forms, promoting myeloproliferative neoplasms (MPNs) such as primary MF, ET, and PV. (Patel *et al.,* 2019).

Exons 14 and 12 of the JAK2 gene, which code for particular peptides, can become mutated, leading to mistakes in protein translation and the insertion of erroneous amino acid sequences into the JAK2 protein kinase. The single DNA point mutation G1849T of exon 14 from the transversion of guanine to thymine is related to the first protein mutation JAK2 kinase V617F. When measured in granulocyte assays, this results in the amino acid valine being replaced by the amino acid phenylalanine at position 617 in protein JAK2 kinase. However, only 27% of pediatric patients with sporadic PV experience this (Putter and Seghatchian, 2021) . In addition, up to 12% of adult individuals with JAK2 V617F exon 14 negative mutations had multiple JAK2 exon 12 mutations. Exon 12 mutations are more likely to result in solitary erythrocytosis than exon 14 V617F-associated mutations, which have a documented propensity for erythrocyte, platelet, and granulocyte cell excess (Putter and Seghatchian, 2021).

V617F mutation present either in heterozygous or homozygous status(Vannucchi *et al.,* 2006). This particular amino acid mutation results in constitutive phosphorylation activity on the 14th exon, which supports cytokine hypersensitivity and growth factor independence while giving the mutant clones an edge in terms of proliferating. Later, it was proposed that a certain clinical phenotype and result are connected to the homozygous or heterozygous V617F mutation theory (Regimbeau *et al.,* 2022). Patients with JAK2 V617F homozygosity had significantly higher haemoglobin levels, increased pruritus incidence, stimulated erythropoiesis and myelopoiesis, a higher prevalence of splenomegaly, and increased progenitor cells in peripheral blood associated with a higher risk of fibrotic transformation than heterozygote PV patients (about 30% depending on the cohorts (Regimbeau *et al*., 2022). Abnormal activation of the JAK-STAT signalling system is the result of mutations in the JAK2 gene, which may cause a wide range of symptoms (Maddali *et al.,* 2020).

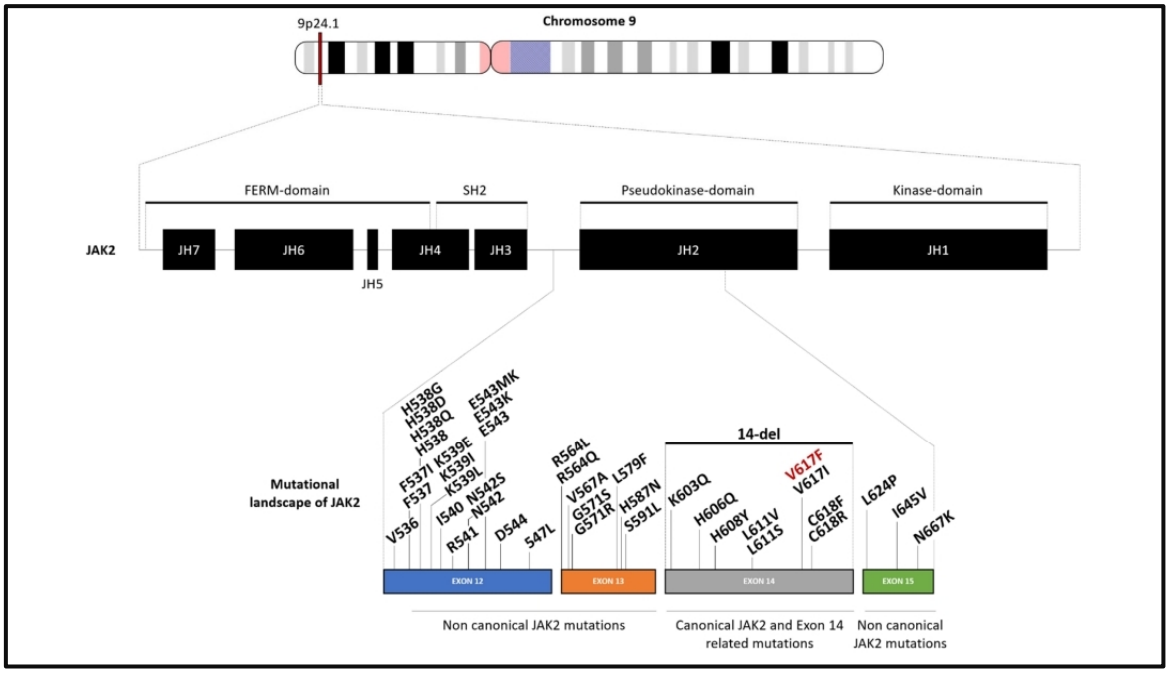


Figure 2-3 JAK2 gene architecture and PV mutation landscape: Schematic (Regimbeau *et al.*, 2022).

2.4.2.2 Calreticulin Gene (CALR)

The CALR gene has 9 exons that span 4.2 kb and found on chromosome 19q13.2. CALR mutations may influence the phenotype of MPN through JAK-STAT (janus kinase-signal transducer and activator of transcription) dependent or independent processes (Tefferi *et al.*, 2014). Exon 9 frameshift mutations caused by somatic insertions, deletions, and complex insertions/deletions make up the majority of CALR mutations (Klampfl *et al.*,2013; Sofi and Hidaya, 2022).

Calreticulin, a 46-kDa chaperone protein found in the endoplasmic reticulum (ER) lumen, is the protein that CALR encodes. Protein folding and calcium homeostasis are both crucially regulated by CALR (Regimbeau *et al.*, 2022). Calreticulin is also found in other cellular structures where it controls a wide range of biological processes, such as apoptosis and proliferation (Rosso *et al.*, 2017).

The calreticulin protein's typical physiological role includes chaperoning the appropriate folding of proteins and glycoproteins and maintaining homeostatic levels of cytosolic and ER calcium molecules, which are crucial for processes like adhesion, migration, phagocytosis, and immunoregulatory functions (Gold *et al.*, 2010). All of the CARL variants

discovered to yet have been identified in exon 9, with the exception of a few uncommon point mutations (Wu *et al.*,2014).

The two most prevalent CALR variants are Type I mutations, which involve a 52 base pair deletion in exon 9, and Type II mutations, which involve an insertion of 5 bp TTGTC (Tefferi *et al.*, 2014). At the time of diagnosis, peripheral granulocytes and BFU-E included the majority of type-1 CALR mutations (Broséus *et al.*, 2014). It is important to note that early in the disease, CALR changes were more frequent (Ortmann *et al.*, 2015). In individuals with JAK2/MPL-negative disease, CALR mutations have been shown to be mutually exclusive with JAK2 and MPL mutations and to be present in 56 to 88% of cases (Rosso *et al.*, 2017).

2.4.2.3 Myeloproliferative Leukemia Protein (MPL)

Megakaryocyte and platelet formation, as well as the self-renewal of hematopoietic stem cells, are all influenced by the human c-mpl gene (MPL). Haematopoietic disorders, however, have been linked to a large number of MPL mutations. The typical regulatory systems are altered by these mutations, which results in autonomous activation or signaling deficits (He *et al.*, 2013).

The TPO receptor is known to be encoded by the MPL oncogene of the myeloproliferative leukemia virus. Tryptophan-to-leucine and tryptophan-to-lysine substitutions, which result in constitutive, cytokine-independent activation of the JAK-STAT pathway, are the two most often found MPL acquired mutations (Pardanani *et al.*, 2006).

Although JAK2V617F is the most frequent mutation in MPNs that are BCR-ABL-negative, over 50% of ET and PMF patients do not have this mutation (He *et al.*, 2013). In 2006, MPL-W515L was identified by (Pikman *et al.*, 2006) and MPLW515K was discovered several months later (Pardanani *et al.*, 2006). MPL-W515L/K occurs often in ET and PMF patients with frequencies of about 1% and 5%, respectively, although it is uncommon in PV patients (He *et al.*, 2013).

2.4.3Environmental Factors

In the case of PV or primary polycythemia, no definitive causative environmental factors have been identified. However, increased risk has been noted in embalmers, refinery

workers, and from exposure to either benzene or occupational exposure to low-dose radiation (Tefferi, 2003). A viral etiology has also been proposed (Sassaki *et al.,* 2000).

Secondary polycythemia has a wide range of causes, including paraneoplastic syndromes, chronic pulmonary and cardiac conditions, circulatory shunts, smoking, high altitude exposure, and exogenous erythropoietin use (Marvi and Lew, 2011). Such factors ultimately result in elevated serum EPO levels and reactive erythrocytosis. Abnormalities in erythropoietin receptor sensitivity, such as Chuvash polycythemia, or in the hemoglobin molecule, may also result in increased red blood cell production (Marvi and Lew, 2011).

2.4.3.1Cigarate and Narghile Smoking

Smoking tobacco is thought to be a major cause of death throughout the world. Smoking exhibited detrimental impacts on hematological limitations that were both severe and long-lasting. According to the figures from the World Health Organization, approximately 5 million people worldwide pass away each year from diseases brought on by smoking. Smoking cigarettes is associated with a higher risk of cardiovascular illnesses (AlQahtany *et al.*, 2020).

Smoking tobacco in cigarettes is a highly effective way to deliver the addictive chemical nicotine. It creates an aerosol of extremely fine particles that transports nicotine to the lungs, where it is swiftly absorbed, travels to the left heart, and eventually reaches the brain in a matter of seconds (Glantz and Bareham, 2018). Breathing in the poisonous smoke produced by tobacco products, particularly cigarettes, is the tragic cause of tobacco-associated disease and death worldwide (Abrams *et al.,* 2018).

Narghile smoking, a kind of tobacco use that originated in the Middle East and is also known as narghile, argileh, hookah, hubble-bubble, and goza, has become more and more commonplace throughout the world (Aslam *et al.,* 2014). Smoking cigarettes and cigarette can cause high levels of carboxy hemoglobin, which can cause hypoxemia and eventually lead to polycythemia (AlQahtany *et al.,* 2020).

According to studies, hookah smokers may inhale more smoke in a single session than cigarette smokers (Akl *et al.,* 2010). The hookah pipe, which is placed in the mouth and includes more than 30 recognized substances, including 3-bromooctane, benzaldehyde, zinc, cadmium, and 1-methylcycloheptene, according to experts, is where the hookah risk is concentrated.

These substances induce numerous genetic alterations and cancer because they interact with DNA (Sadiq *et al.*,2019).

The main risk factor for hypoxia brought on by secondary polycythemia is cigarette smoking. More than 7000 substances, particularly tar, nicotine, and carbon monoxide, are thought to be responsible for a smoker's increased risk of developing a number of ailments. Because of the effects of nicotine's peripheral vasoconstriction and carbon monoxide intake, hypoxia is associated with poor gas exchange and decreased oxygen supply (Sung *et al.*, 2022). Smoking depletes the bone marrow's oxygen supply, which inhibits the generation of red blood cells (RBCs), which are the oxygen transporters. The consequences of raised hematocrit (HCT) due to excessive erythrocytosis include increased RBC mass, increased blood viscosity, slower blood return via the veins (slow blood flow), and increased platelet adhesion. Clot development occurred due to an increase in blood viscosity and the activation of platelets at the vessel wall (Shawky, 2020). Additionally, it may result in an increase in white blood cells, particularly when immune responses to vascular injury and inflammatory alveolar tissue occur in succession (Malenica *et al*., 2017).

Compared with PV, wherein the increased incidence of thromboembolic events has been well established, the association between thromboembolic events and secondary polycythemia is relatively uncertain (Bhatt, 2014). In a case-control study of polycythemia vera and polycythemia caused by smoking, 60% of patients with polycythemia vera and 41% of patients with smoking-related polycythemia had at least one thromboembolic problem. Additionally, that study demonstrated that PV was associated with a lower risk of thromboembolic events than smoking-related polycythemia (Sung *et al.*, 2022).

2.4.3.2Alcoholism

Alcohol has several deleterious effects on the different kinds of blood cells and the methods in which they operate. Overconsumption of alcohol, for instance, has been linked to a decline in overall blood cell production and the production of blood cell precursors with structural faults that prevent them from developing into functional cells. In alcoholics, abnormal red blood cells are common and are often destroyed at an earlier age, which may lead to anaemia (Ballard, 1997). White blood cells, which help prevent bacterial infections, are severely affected by alcohol's effects on their formation and function. Therefore, alcoholics are very susceptible to bacterial infections. Finally, alcohol use has a deleterious effect on platelets and other parts of the blood clotting system. A higher incidence of stroke has been linked to heavy alcohol use (Ballard, 1997).

2.5. Some Hematological Parameter Changes in Polycythemia

2.5.1Red Blood Cell Count

Red blood cells are one of the most abundant three types of blood cell, its anucleated biconcave disk shaped the production of RBCs in the blood controlled by erythropoietin. This is also known as haematopoietin which is a kidney secreted glycol protein cytokine in response to hypoxia its stimulates bone marrow erythropoiesis (Ifeanyi and Getrude Uzoma, 2018).

Polycythemia is characterised by RBC count or haemoglobin (Hb) level that is higher than the reference range after adjusting for age, sex, and altitude (Jalowiec *et al*., 2022). The plasma volume decreases in erythrocytosis due to hypoxia or inadequate EPO production because the body is trying to keep the total blood volume the same while the red cell mass increases. It is not possible to differentiate between absolute erythrocytosis and plasma contraction using hematocrit values alone (Spivak, 2018).

The converse occurs in PV, when neither tissue hypoxia nor excessive erythropoietin synthesis are present. As the red cell mass increases on its own, the plasma volume increases or remains constant, disguising the rise in red cell numbers as measured by the hematocrit (Spivak, 2018).

2.5.2 Hemoglobin& Hematocrit

Heterotetrameric hemoglobin is made up of globin subunits that resemble a-like and b-like and are each attached to a heme prosthetic group. Hb primarily transports carbon dioxide (CO2) from tissues to the lungs and oxygen (O2) from the lungs to peripheral tissues (Thom *et al.*, 2013).

According to the most recent classification by World Health Organisation (WHO) for myeloid neoplasms, certain blood values are needed to diagnose PV in (2016). These include a haemoglobin level of 16.5 g/dL in men and 16 g/dL in women; a hemocrit level of 49% in men and 48% in women; or RBC mass level of 25% above the mean normal predicted value (Jalowiec *et al.,* 2022)**.**

2.5.3 White Blood Cells(WBCs)

White blood cells (WBCs), also called leukocytes, are a component of the immune system that may be further subdivided into granulocytes and agranulocytes based on whether or not their cells contain granules. All three of these cell types are granulocytes (Fathima and Khanum, 2017). Agranulocytes may also be subdivided into lymphocytes and monocytes. T

lymphocytes and B lymphocytes are two types of lymphocytes, both of which perform an essential role in the body. T lymphocytes (cells reliant on the Thymus) directly attack many different types of infected cells and cancers via cell-mediated immunity. B lymphocytes (Bursa dependent cells) are in charge of humoral immunity when they make antibodies to fight against bacteria, viruses, and other pathogens (Fathima and Khanum, 2017).

Red, white, and platelet cells build up as a result of a multipotent haematopoietic progenitor cell's proliferation in PV (Di Nisio *et al.*,2007). Patients with PV frequently experience the serious complication of thrombosis, which has an effect on clinical outcomes and reflects the severity of the disease. The elevated mortality rate among patients with PV is caused by fatal cardiovascular events, which affect about one-fifth of patients with PV and are diagnosed with an arterial or venous thrombotic event as a presenting characteristic (Kroll *et al.*, 2015). Patients with PV are classified into groups with low, intermediate, or high thrombosis risk based on age, thrombosis history, and cardiovascular risk factors. Additionally, patients may have elevated WBCs and platelet counts, which will raise their risk of thrombosis (Keohane *et al.*, 2013).

2.5.4 Platelets Count(PLT)

The main physiological regulator of platelet synthesis is TPO, which is synthesized by the bone marrow megakaryocyte in a controlled manner before entering the bloodstream. By interacting with the TPO receptor and activating the Janus kinase (JAK) and (STAT) pathways, TPO promotes platelet formation and megakaryocyte proliferation. The liver produces TPO at a steady pace, and the rate of PLT production, which is primarily responsible for blood clotting, is inversely related to the rate of TPO synthesis (Kuter, 2013).

2.6 DNA sequencing

DNA sequencing is the process of determining the nucleic acid sequence – the order of nucleotides in DNA. It includes any method or technology that is used to determine the order of the four bases: adenine, guanine, cytosine, and thymine (Heather and Chain, 2016). The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. Establishing the sequence of DNA is key to understanding the function of genes and other parts of the genome (Shendure *et al*., 2017).

Several methods have been developed since the 1970s. The chemical method was developed by Alan Maxam and Walter Gilbert and the dideoxy method by Fred Sanger (McGinn and Gut, 2013) .The Sanger method was the most commonly used method in molecular biology until the advent of mass-sequencing technologies, also called next-generation DNA sequencing methods. The latter include pyrosequencing, Illumina, and SOLiD platforms. These technologies allowed generating millions of reads of 50–1000 nucleotides long in a single run, which are assembled in large DNA molecule thanks to powerful bioinformatic programs (Slatko *et al*., 2018).

2.6.1 Sanger sequencing

Sanger sequencing was the first method of DNA sequencing, developed by Fred Sanger in 1975. It was the method used for the ground-breaking Human Genome Project, completed in 2003 (Kumar *et al*., 2019).

In the sanger sequencing method, the Patient DNA is used as a template in a polymerase chain reaction (PCR). A mix of normal bases (dNTPs) and chain terminating bases (ddNTPs) is used in the PCR reaction. When a chain terminating base is randomly incorporated into a growing DNA chain, it cannot grow any further. This means that DNA fragments of different lengths are generated. Each fragment ends in a chain-terminating base (Le Gallo *et al*., 2017). The DNA fragments are then separated by size using capillary electrophoresis. Each of the four chain terminating bases (A/T/C/G) has a different fluorescent label. A laser is used to excite these fluorescently labelled bases at the end of each fragment. Shorter fragments come first in the sequence followed by increasingly longer fragments. The fluorescence of the base that terminated each length of fragment is recorded, and a chromatograph is generated showing which base is present at which position along the DNA fragment. The chromatogram is compared to a reference file to identify any variants (Slatko *et al*., 2018).

Sanger sequencing remains the most accurate form of DNA sequencing. It is still widely used in clinical laboratories for diagnostic sequencing of a single gene, testing for a specific familial sequence variant, prenatal testing for known familial variants, to confirm variants that are identified by next-generation sequencing (NGS), to fill gaps in NGS data(Le Gallo *et al*., 2017).

Chapter Three: Materials and Methods

3-MATERIALS AND METHODS

3.1 Materials:

3.1.1 Tools

Table 3-1: List of the tools used in the current study.

No.	Instruments	The Company	Manufacture
1	Micro plate reader EXL 800	BioTek	USA
2	Micro plate washer ELX50	BioTek	USA
3	Incubator + shaker	Heidolph	Germany
4	Incubator 37°C	Memmert	Germany
5	Spectrophotometer (Nano drop)	NanoVue plus	UK
6	Vortex	ScientificIndustries	USA
7	Autoclave	HICLAVE	Japan
8	UV – Transilluminator	Syngene	UK
9	Thermal Cycler	Biorad	USA
10	Electrical balance	AND	Japan
11	Micro centrifuge I	Thermo scientific	Germany
12	Micro centrifuge II	LabTech	Korea
13	General purpose centrifuge	Hettich	Germany
14	Deep freezer (-80°C)	GFL	Germany
15	Electrophoresis	Biorad	USA
16	Microwave	Striling	China
17	Thermo shaker	Grant Bio	UK
18	Mythic 22 Coulter Counter	Orphee Medical	Switzerland
19	Freezer	LG	USA

3.1.2 Items

Table 3-2: List of items used in the current study.

No.	Item	Company	Manufacture
1	Plastic disposable syringe, 5ml	Changzhou	China
2	Tourniquet	GXin	China
3	EDTA blood collection tube, 2 ml	MGROUP	India
4	Gel separator tube, 5 ml	BD Vacutainer	UK
5	Gloves	ForPro	China
6	Micro pipette, 10, 100, 100 µl + Tips	Eppendorf	Germany
7	Eppendorf tubes, 1.5 ml	IndiaMart	India
8	Plastic zip bags	VINAYAKMART	USA
9	Permanent marker	GXin	China

3.1.3 Chemicals, Reagents and Kits

Table 3-3: Chemicals, kits, and reagents utilized in the current study are listed below.

No.	Chemicals	Company	Manufacture
1	Human EPO(Erythropoietin) ELISA Kit	Elabscience	USA
2	ABO kit	TULIP DIAGNOSTICS (P) LTD	India
3	AddPrep Genomic DNA Extraction	Addbio	South Korea
4	Agarose Powder	KBC	Spain
5	DNA Ladder	SMOBIO	Taiwan
6	DNA Loading buffer	Genetbio	South Korea
7	Add Taq Master	ADDBIO INC.	Korea
8	Nuclease free Water	Inno-train diagnostik	Germany
9	10X TBE buffer	Sinacolon	Iran
10	Ethidium Bromide	Inno-train diagnostik	Germany
11	Primer	Macrogen	South Korea
12	Absolute Ethanol(100%)	Scharlau	Spain

3.2 Sample collection

Blood samples were collected from 52 polycythemic patients in which 8 of them have PV and 44 of them have secondary polycythemia were blood donors from December 2021 to September 2022. Samples were collected from patients admitted to Nanakaly Hospital, Directorate of Blood Bank in Erbil city and Shahid Dr. Khalid Hospital in Koya , Kurdistan Region, Iraq. The blood samples were divided into two groups. Hematological parameters, Erythropoietin hormone, and detection were evaluated for mutation in JAK2 gene (exon 14). Furthermore, a questionnaire was used to collect demographic characteristics of each patients including age, sex, smoking cigarette , narghile smoking, alcohol consumption, Fattigue, pruritis, and headache. The questionnaire data was typically gathered by filling out the direct questionnaire form for the current investigation. The exclusion criteria for the study participants were patients with hemoglobin levels lower than (16 g/dl), red blood cells (5 million/mm3), and hematocrit (46%).

3.2.1 Ethical approval

The sample collection of the current study was approved by Ethical Committee of Koya University, Erbil General Directorate of Health and Koya General Directorate of Health.

3.3 Methods

3.3.1Blood Sampling

Five milliliters of peripheral blood were collected from PV patients and blood donors, using disposable syringe and transferred into new sterile ethylenediaminetetraacetic acid (EDTA) and gel separator tubes. Three ml of blood was dispensed in EDTA containing tube as anticoagulant for Complete Blood Count (CBC) detection and then kept immediately at 4°C for DNA extraction for JAK2 V617F(exon 14) mutation detection and Complete Blood Count. While 2 ml was dispensed in gel separator tube, and serum specimens were separated and then stored in deep freezer at −80°C until using in the determination of Erythropoietin hormone.

3.3.2Study design

In general, the study's design may be summed up as seen in figure (3.1).

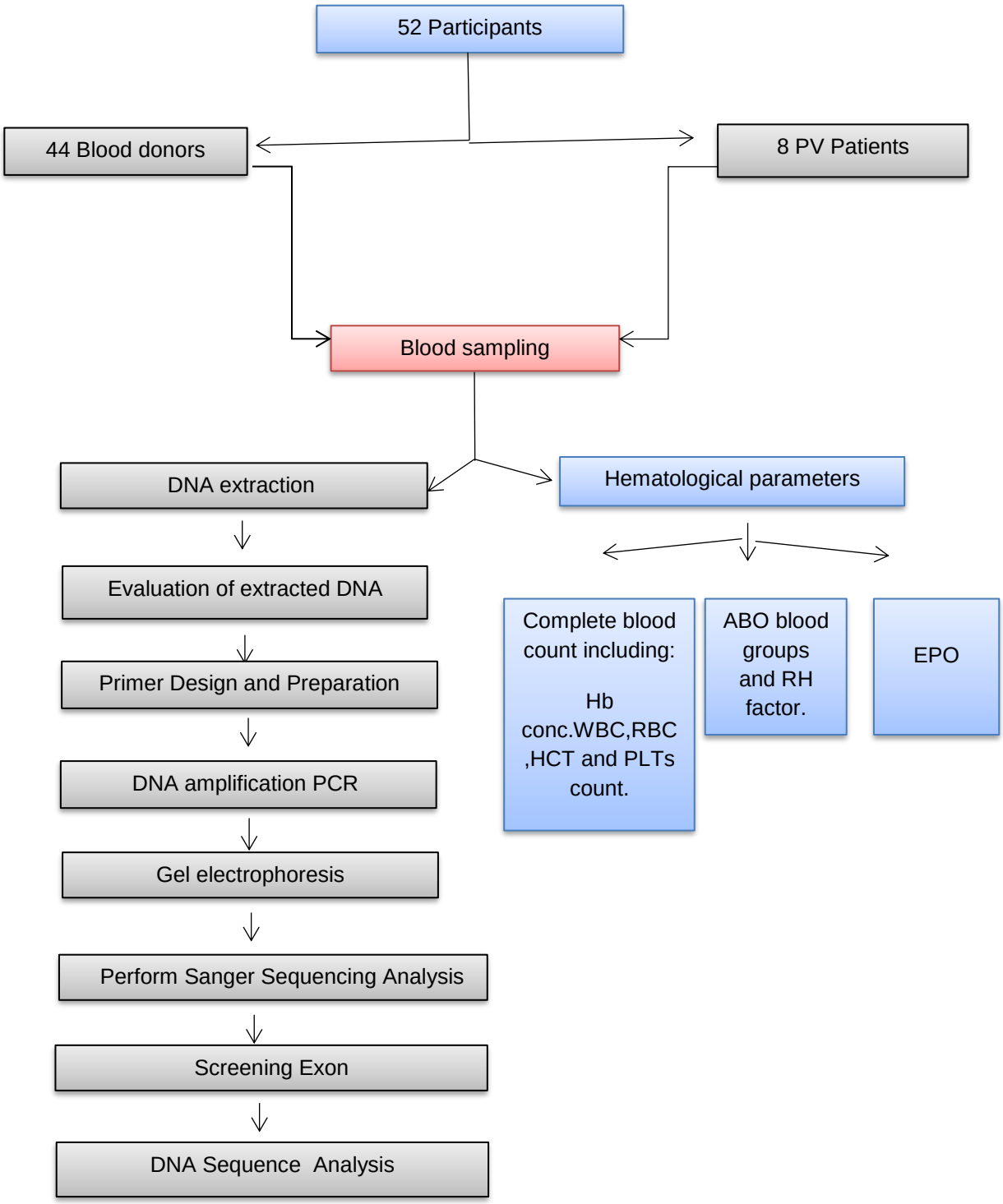


Figure 3-1: Design of the current study were all included. Sample collection, blood samples, DNA analysis, and hematological tests .

3.4Laboratory testes

3.4.1Complete Blood Count

The complete blood count (CBC) is a clinical assay that enumerates five major leukocyte classes in blood based on cell shape and size (automatic cell counters rely on cell size and isoelectric focusing). The Coulter method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator.

Hematological parameters including (Hb level, red blood cell (RBC), HCT, white blood cell (WBC) count, platelet count) were measured for all blood samples by coulter counter (Mythic22), Orphee Medical, Switzerland.

ABO blood groups and Rh type were tested according to manufacturer's instructions (TULIP DIAGNOSTICS (P) LTD/ INDIA).

3.4.2Erythropoietin Hormonal Assay

3.4.2.1Principles

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human EPO. Samples (or Standards) were addeded to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human EPO and Avidin-Horseradish Peroxidase (HRP) conjugate were addeded successively to each micro plate well and incubated. Free components were washed away. The substrate solution was addeded to each well. Only those wells that contain Human EPO, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of Human EPO.

3.4.2.2 Assay procedure

1. Standard, blank, and sample wells were identified for diluting. All samples and standards should be analysed in triplicate, thus 100 µl of each dilution was put to the appropriate wells. The sealant that included with the package was used to cover the plate. 90 minutes at 37 degrees celsius should be enough time for incubation. Care was taken to prevent getting solution on the inner wall of the micro ELISA plate well or it may cause foaming.

2. The liquid was decanted. The Biotinylated Detection Ab working solution was added to each well at once. The sealant was reapplied to the plate's surface and incubated at 37°C for one hour.

3. The solution was decanted from each well and replaced with 350 µl of wash buffer. After soaking for 1 minute, the solution was removed by aspirating or decanting and wipe each well dry with absorbent paper. This wash procedure was done three times.

4. Each well was then filled with 100 µl of HRP conjugate working solution. Reapply the sealant to the plate's surface. The recommended incubation time is 30 minutes at 37°C.

5. The wash solution was removed from each well and step 3 is repeated five more times.

6. Each well was supplemented with 90µl of the Substrate Reagent. The sealant was reapplied to the plate's surface. 15 minutes of incubation at 37°C. The plate was shelled from the sun. One thing to keep in mind is that the 30 minute maximum response time is flexible depending on how long the actual colour shift takes. The Micro plate was preheated for 15 minutes before taking an OD reading.

7. Each well was supplemented with 90 µl of the substrate reagent..

8. A micro-plate reader was used with the wavelength set to 450 nm, calculate the OD value of each well simultaneously.

3.4.2.3Calculation of Results

The results of the ELISA assay was calculated using MyAssay software version 2.8. The concentration of each sample were calculated by four parameter logistic (4PL). The standard curve for the ELISA test was created using the same software.

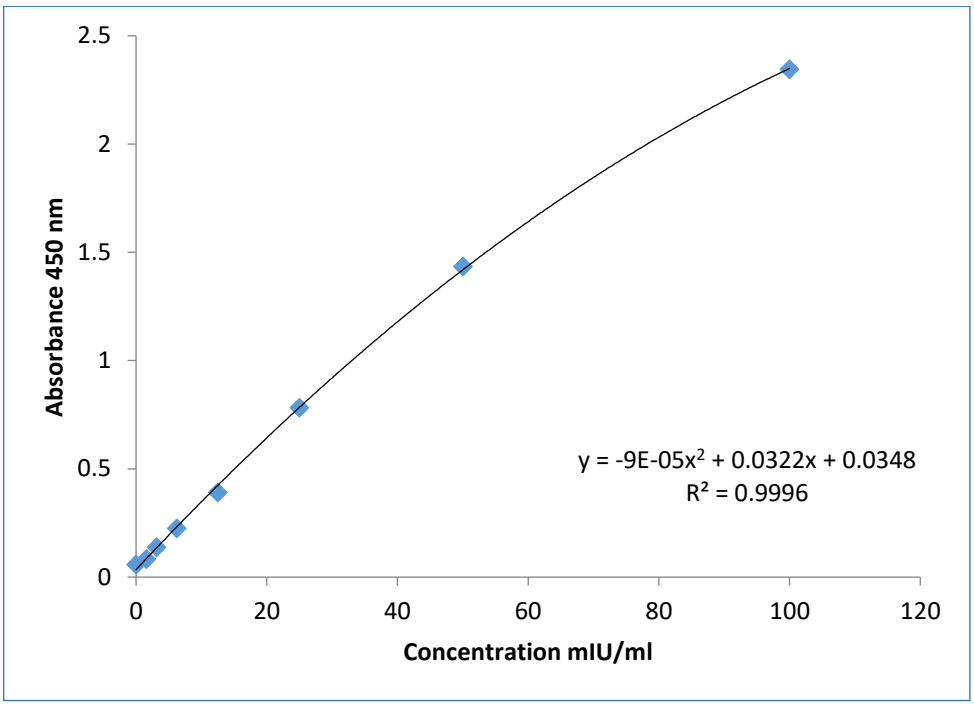


Figure 3-2: EPO concentration standard curve in mlU/ml.

3.4.3Extraction of Genomic DNA

According to the manufacturer's procedures and company instructions, genomic DNA was extracted from whole blood samples preserved in EDTA anticoagulant tubes for molecular investigations using the AddPrep Genomic DNA Extraction Kit (Addbio, South Korea). To easily, quickly, and affordably extract genomic DNA from blood, AddPrep Genomic DNA Extraction Kit was used. It allows for the quick isolation of genomic DNA from blood samples as little as 200µl and is compatible with whole blood that has been treated with citrate or EDTA. High-quality PCR and agarose gel analysis may be performed using the extracted genomic DNA (Miller *et al*., 1988).

Whole blood DNA extracted with the use of the AddPrep Genomic DNA Extraction Kit, which has the following components: Proteinase K (20 mg/ml) 1.2 ml X 2 tubes, Spin column 100 ea, Binding solution 25 ml, Washing 1(30 ml), Washing 2(12 ml), and Elution (25 ml). To prepare the kit for use, 22.5 ml and 48 ml of absolute ethanol were added to wash buffer 1 and buffer 2, respectively.

3.4.3.1Extraction Protocol for Blood

1. A 20 µl solution of Proteinase K (20 mg/ml) was poured into a 1.5 ml micro-centrifuge tube.
2. 200 ul of the whole blood and 1 ml of Proteinase K solution was added to a micro - centrifuge tube.
3. 200 µl of Binding Solution was added to the sample tube and pulse-vortexed for 15 seconds, to ensure proper mixing
4. The mixture was incubated for 10 minutes at 56 °C.
5. Then 200 µl of absolute ethanol (100 percent) were added and pulse-vortexed for 15 seconds.
6. Carefully transferred the lysate into the top reservoir of the spin column with the 2.0 ml without wetting the rim..
7. After spinning for one minute at 13,000 rpm, the flow-through was removed and replaced it with the 2.0 ml collecting tube.
8. After filling the spin column and collection tube with 500 µl of Washing 1 Solution and centrifuged at 13,000 rpm for 1 minute, the flow through was drained out and reinstalled the spin column and 2.0 ml collection tube.
9. After assembling the spin column with the 2.0 ml collecting tube, 500 µl of Washing 2 Solution was introduced and centrifuged at 13,000 rpm for 1 minute. The flowthrough was poured off.
10. The column was dried by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in spin column.
11. The spin column was transferred to a new 1.5 ml micro-centrifuge tube .
12. After waiting at least 1 minute, 100–200 µl of Elution Solution was injected to the spin column using the micro-centrifuge tube.
13. Centrifuged at 13,000 rpm for 1 minute to separate genomic DNA.
14. The extracted genomic DNA was frozen at -20 °C (Rohland *et al*., 2018).

3.4.3.2 Evaluation of Purity and Concentration of DNA

To detect quantitative and qualitative analysis of the extracted DNA after extraction, concentration and purity of the extracted DNA were checked by Spectrophotometer (Nano drop)[NanoVue plus, UK] at Science and Health Research Center of Koya University. This instrument estimates the light absorbance at specific frequencies (The concentration of DNA was calculated by ng/µl and the purity of DNA was estimated depending on the ratio of OD260/OD280). The device was calibrated to zero using 1µl Elution solution was placed on

the sensor of the instrument, then measured by Nanodrop software program. An absorbance quotient value of 1.8 and a ratio (R) 2.0 considered to be good, purified DNA. A ratio of less than 1.7 shows protein contamination (Ghatak *et al*., 2013; Gupta, 2019).

3.5 Primer Preparation

3.5.1 Design of the Primer

Primers for polymerase chain reaction (PCR) are short pieces of single-stranded DNA (usually between 18 and 25 nucleotides in length) that are complementary to sequences found at the 3'-ends or inside the target DNA (Erlich,1989). Primers shown in table (3.4) were developed by hand in this study using primer-design software and the genomic sequence of the Janus kinase 2 (JAK2) gene obtained from the Primer-BLAST database at the National Center for Biotechnology Information (NCBI).

Table 3-4: Primer sequence for PCR amplification used in present study.

Exon	Primer name	Primer sequence 5'-3'	Product length
Exon 14	JAK2-forward	5'-GCTACATCCATCTACCTCAG-3'	500 bps
	JAK2-reverse	5'-CTGACACCTAGCTGTGATCC-3'	

3.5.2Primer Dilution

The primer stock solution was prepared by adding 300 µl of nuclease free water to the lypolphylezed primer vial, and mixed well by vortex for 5 seconds. To prepare 10 pmol/ µl primer. Using p10 micropipette, 10 µl of the primer stock solution was added to 90 µl of nuclease free water. The mixture was added to a sterilized Eppendrof tube and stored in -20C^0 (Masser *et al*., 2015).

3.5.3 Primer Optimization

The melting temperatures were analyzed depending on PCR Annealing Tempreture Tool Calculate primers by using (https://www.thermofisher.com/tools/tmcalculator) and the applied by performing optimization of the primer to detect the suitable annealing temperature for each primer by using Gradient Polymerase Chain Reaction (PCR) (Thermal Cycler- Biorad- USA) as revealed in table (3.5) for setting temperature. Then, we conducted gel electrophoresis to present target bands.

Table 3-5: Primer optimizations procedure

NO	Step	Temperature(C^0)						Time	Cycles
1	Initial denaturation	94						5min	1
2	Denaturation	94						30sec	35x
3	Annealing	54	56	58	60	62	64	30sec	
4	Extension	72						30sec	
5	Final extension	72						5min	1

3.6 PCR Amplification

3.6.1 Reaction Components

The PCR reaction components used in the present study are summarized in table (3.6)

Table 3-6: PCR reaction component

Component	Concentration	Volume
Master mix	2x	20 µl
Forward primer	10 pmol	1 µl
Reverse primer	10 pmol	1 µl
Nuclease free water	----	8 µl
DNA template	200 ng/ µl	10 µl

3.6.2 PCR Condition

Table 3-7: PCR conditions of exon 14 of JAK2 gene in present study.

NO	Step	Temperature (°C)	Time	Cycles
1	Initial denaturation	94	5min	1
2	Denaturation	94	30sec	35x
3	Annealing	58	30sec	
4	Extension	72	30sec	
5	Final extension	72	5min	1

3.7 Gel Electrophoresis

In a glass beaker, 0.5g of agarose powder was added to 50ml of Tris/Borate/EDTA(TBE) 1X. Before it solidified, 25 µl of ethidium bromide solution was added to the agarose powder suspension, which is stirred in a microwave until the mixture became homogenous. The solution was gently shaken such that the agarose solution and the ethidium bromide solution were homogenous. The comb was positioned 0.5 cm above the plate so that

when agarose is added, a full well is produced. The agarose solution was carefully poured onto the prepared tray and allowed to harden at room temperature for at least 20 minutes. On the electrophoresis equipment, the tray was positioned. The gel was loaded with TBE 1X buffer, and the comb was carefully extracted. The first well is stocked with 3 µl of marker (3kb DNA ladder). Each well contained 10µl of PCR product. The electrophoresis device was linked to an 85-volt power source and the gel ran for one hour. After a certain amount of time, the power supply was turned off, gel was visualized under ultraviolet (UV) light, and photographed.

3.7.1 10X Tris/Borate/EDTA (TBE) Buffer Preparation

To prepare 500ml of 10x TBE buffer. 250 ml of 20XTBE buffer was mixed with 250ml distilled water in a 1L conical flask. The solution was then stored in cabinet at room temperature until use.

3.7.1.1 1X Tris/Borate/EDTA (TBE) Buffer Preparation

Fifty ml of 20x TBE buffer was poured into a 1L conical flask. The flask was filled up with water until it reached 1000ml. The solution was then stored in cabinet at room temperature until use.

3.7.1.2 Preparation of Agarose GEL

One percent of agarose gel was prepared by dissolving 0.8 gm agarose in 80 ml of 1X TBE buffer. The mixture was dissolved in microwave until it became clear. The mixture was then cooled down at room temperature and 20µl of ethidium bromide was added and mixed gently. The agarose gel was poured into the gel tray until it completely solidified.

3.7.1.3 Loading PCR Amplification Products on to the Gel and Electrophorese

With the aid of a 10µl micropipette, 3µl of each PCR product was added carefully into the agarose wells. Furthermore, 3µl of 3kb ladder was added into the first well. After that, the gel was covered with 1xTBE buffer, and the lid of the electrophoresis tank was closed and the power supply was connected and set up on 80 volts for 60 minutes. The gel was red in UV-trans illuminator and photographed. (Huang *et al*., 2010) .

Note:

The same amounts of agarose and TBE buffer were employed to construct the gel for the estimate of extracted genomic material utilized in the examination of PCR products. Every sample of the isolated genomic material consisted of 4 l, which was incorporated well with 1 l of loading dye using a 10 l micropipette on parafilm paper. Then, slowly, the liquid poured into its predetermined gel void (Ghatak *et al.,* 2013).

3.8 DNA Sequencing

3.8.1 Sanger Sequencing

Purified PCR products for exon 14 of the JAK2 gene were sequenced utilizing the Sanger technique using forward primer on a 3130 Genetic Analyzer (Applied Biosystems, Hitachi High-Technologys, Tokyo, Japan) at the (IMMUNOGENE-CENTER, Erbil-Kurdistan region-Iraq). In order to identify previously unseen mutations, the sequencing findings were evaluated using several programs as Finch TV, BioEdit, and Molecular Evolutionary Genetics Analysis. The mutation was given a name based on its reference sequence in GenBank (transcript ID= NM-004972.3). The names and citations of the sequence variations follow the conventions established by the Human Genome Variation Society (HGVS;http://www.hgvs.org). Table (3.8) lists the many components involved in a sequencing reaction.

Table 3-8: Reaction components and volume used in Sanger sequencing.

Components	**Volume**
DNA(Purified PCR product)	3 µl
Primer	1 µl
Big dye	0.5 µl
DDH_2O	0.5 µl
Total volume	4 µl

3.9 Statistical Analysis

The statistical analysis made use of IBM SPSS Statistics version 26, and descriptive statistics used for all demographic questions such as group, age, gender, ABO, Narghile, Cigarette, Alcohol, Fatigue, Headache, JAK2V617F, and EPO group. Kolmogorov-Smirnov (K-S) normality test examines if variables are normally distributed. Additionally the connection between independent factors like JAK2V617F (positive and negative) and dependent variables like Age, WBC, RBC, HGB, HCT, and PLT were examined using an independent sample t-test. The “p” value <0.05 was considered significant statistically.

Chapter Four: Results and Discussions

4.RESULTS AND DISCUSSIONS

4.Result

4.1 Socio-demographic characteristic of the study population

In the present study, the majority of the patients have secondary polycythemia (82.7%) compared to those who have polycythemia vera (17.3%). Most of the participants are 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 is 38 years.

4.2 Independent Sample T -Test

The t test for independent samples compares the means of two variables (Blbas *et al.*, 2020). The connection between independent variables like JAK2V617F (positive and negative) and dependent variables like Age, WBC, RBC, HGB, HCT, and PLT was examined using an independent sample t-test.

Table 4-1 shows a statistical significant difference between the mean positive and negative of JAK2V617F with each of the HGB, HCT, PLT, and Age individually because of the reason that their p-values are below the α=0.05 significant limit. In addition, the average of each of the HGB and HCT for negative JAK2V617F (17.812, and 52.164) is 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) is lower than the average of positive JAK2V617F (332.533 and 54.333) respectively. In addition, there is no statistical significant difference between the mean positive and negative of JAK2V617F with each of the WBC and RBC individually because their p-values are above the α=0.05 threshold for significance.

Table 4-1: Independent Sample T Test between the mean of 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(cell/cmm)	Positive	3	6.721	3.474	1.233	0.224
	Negative	47	8.694	2.648		
RBC(million/mm3)	Positive	2	6.528	0.598	1.242	0.22
	Negative	48	6.035	0.548		
HGB(g/dl)	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(cell/cmm)	Positive	2	**332.533**	82.777	2.518	**0.015**
	Negative	47	228.830	56.343		
Age (Years)	Positive	3	**54.333**	9.074	2.41	**0.020**
	Negative	49	37.041	12.171		

4.3 Purity and concentration of extracted DNA

The results of nano drop spectrophotometry are summarized in table 4-2. According to nanodrop measurements of the genomic DNA extracted from blood, the mean concentration of the samples were 39.14 ng/ µl, and average purity of 1.75.

Table 4-2: Purity and concentration of the DNA samples.

Samples	Concentration (ng/ µl)	Purity (A260-280)
1	6.4	1.84
2	66.5	1.75
3	12.5	1.81
4	85.5	1.74
5	22	1.66
6	83	1.72
7	43	1.79
8	30.5	1.7
9	13.5	1.8
10	28.5	1.77

4.4 Genomic DNA analysis

The extracted DNA samples were analyzed using agarose gel electrophoresis as shown in figure 4.1. DNA bands were present in all samples wells, varying in sizes and brightness indicating different concentration.

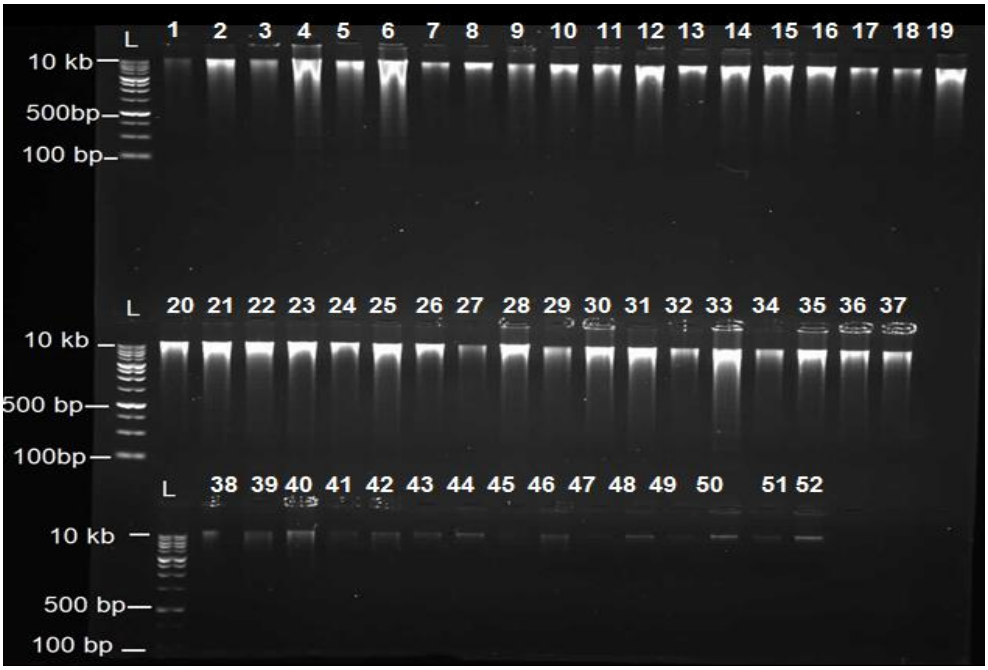


Figure 4-1: Agarose gel electrophoresis for genomic DNA extracted from blood sample. Lane (L) contains DNA ladder of 10 kbp, lane 1 to 52 contains DNA samples. The electrophoresis separation was performed on 1% agarose and ran at 80v for one hour.

4.5 Molecular Identification JAK2V617F(Exon14)

In the present study, Sanger sequencing for the exon 14 of JAK2 gene and PCR product (500 bps). The Janus Kinase2 V617F mutation was detected as heterozygous mutant forms were found in 3 (5.8%) of the total cases of polycythemic patients, and all other samples are negative to Janus Kinase2 V617F . A 500 bp fragment from exon 14 of the Janus kinase2 gene among polycythemia patients were successfully amplified with the precise primers as shown in (Figure 4-2).

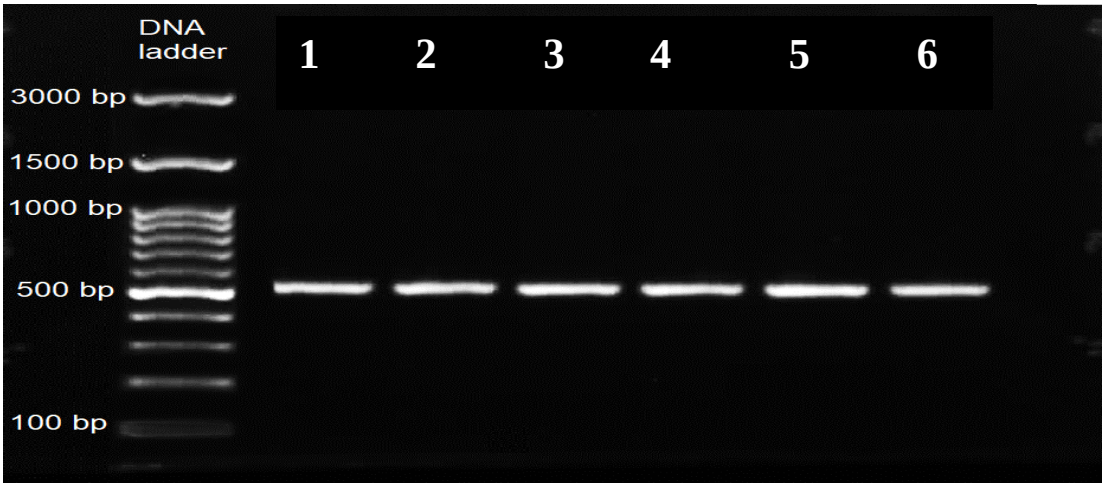
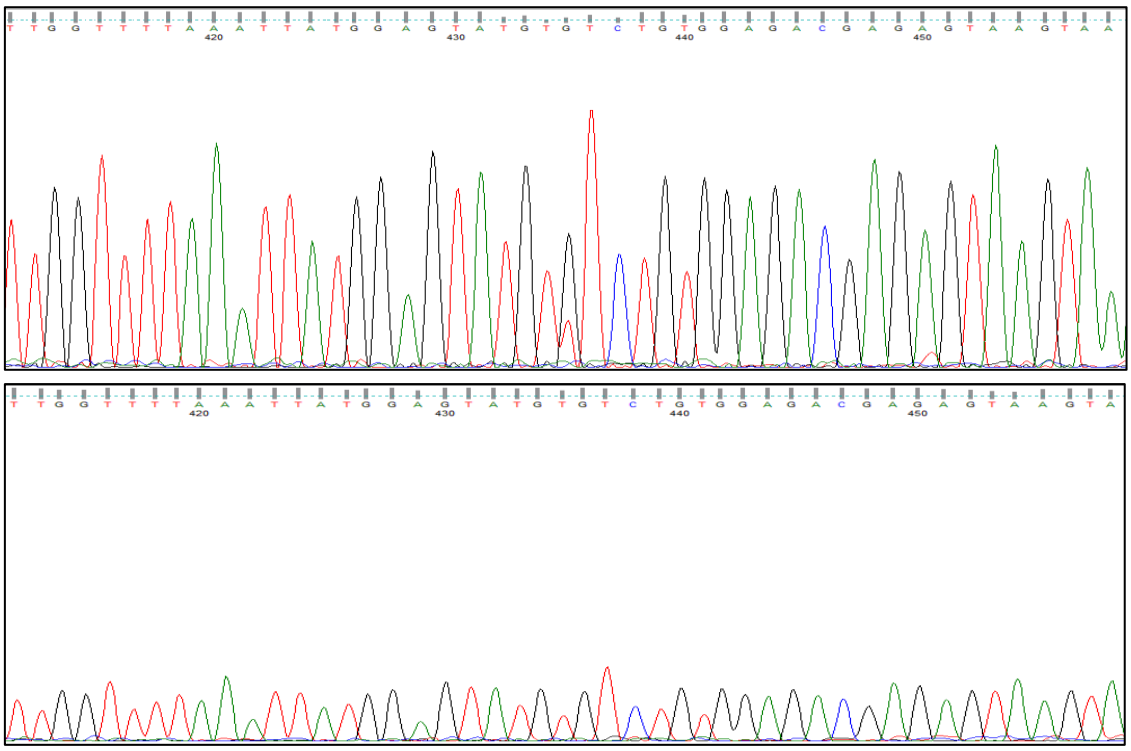


Figure 4-2: PCR results matching to a 500 (bp) fragment of the Janus kinase 2 gene were analyzed on an agarose gel. On the left, DNA markers are displayed (Bp). Lines 1 through 6 represent the PCR results of the amplified DNA from 6 separate samples of individuals with polycythemia.

The result of sequencing is shown in (Figure 4-2), including wild type, heterozygous JAK2V617F mutant at nucleotide location c.1849G>T in the 617th codon, which leads to the change of amino acid valine for phenylalanine.



(A) (B)

Figure 4-3: Shown DNA sequence chromatograms of the JAK2 gene's exon 14 mutation, c.1849GT (JAK2V617F). (A) c.1849G→T heterozygous mutant; (B) wild-type; The arrow indicates mutation locations.

4.2Descriptive Statistics

Table 4-3: Descriptive Statistics for Socio Demographic parameters in the study groups.

		N	%
Group	Secondary Polycythemia	44	**84.6%**
	Primary Polycythemia	8	15.4%
Age (Years)	19-28	12	23.1%
	29-38	17	**32.7%**
	39-48	12	23.1%
	49-58	6	11.5%
	59 and more	5	9.6%
	(Mean ± SD)	(38 ± 12.62)	
Gender	Male	49	94.2%
	Female	3	5.8%
ABO blood group	A+	12	23.1%
	A-	2	3.8%
	B+	14	26.9%
	B-	1	1.9%
	O+	18	**34.6%**
	O-	2	3.8%
	AB+	3	5.8%
Narghile	Positive	13	25.0%
	Negative	39	**75.0%**
Cigarette	Positive	29	**55.8%**
	Negative	23	44.2%
Alcohol	Positive	14	26.9%
	Negative	38	**73.1%**
Fatigue	Positive	15	28.8%
	Negative	37	**71.2%**
Pruritus	Positive	7	13.5%
	Negative	45	**86.5%**
Headache	Positive	21	40.4%
	Negative	31	**59.6%**
JAK2V617F	Positive	3	5.8%
	Negative	49	**94.2%**
EPO group	Normal	5	9.6%
	Abnormal	47	**90.4%**

Table 4-3 shows the descriptive statistics for all demographic questions such as group, age, gender, ABO, narghile, cigarette, alcohol, fatigue, headache, JAK2V617F, and EPO group from JAK2 Mutation and hormonal evaluation in Polycythemia patients. Majority of the patients had secondary polycythemia (84.6%) compared to those who have Primary polycythemia (17.3%). Most of the participants were aged between 29 and 38 years (32.7%) followed by 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. The percentage of male (94.2%) is higher than the percentage of female's participants (5.8%). Most of the patients have O+ blood group (34.6%) followed by B+ (26.9%), A+ (23.1%), AB+ (5.8%), A- (3.8%), O- (3.8%), and B- (1.9%) respectively. Next, the result of smoking cigarette for the most of the patients were positive (55.8%) but most of them had negative result of smoking hookah (75%) and alcohol consumption (73.1%). Finally, most of the patients had negative fatigue with (71.2%) negative pruritus (86.5%), and negative headache (59.6%), while most of them had negative JAK2V617F with (94.2%0 and abnormal EPO with (90.4%).

Table 4- 4: Descriptive Statistics for Age, WBC, RBC, HGB, HCT, PLT, and EPO.

Parameters	N	Mean	Std. Deviation	Minimum	Maximum
Age (Years)	52	38	12.62	19	66
WBC (cell/cmm)	52	8.96	3.29	3.16	19.30
RBC (million/mm3)	52	5.95	0.77	3.00	7.15
HGB (g/dl)	52	17.63	1.93	10.93	20.90
HCT (%)	52	52.15	5.21	31.44	63.10
PLT (cell/cmm)	52	246.77	81.20	135.00	516.00
EPO mIU/ml	52	4.01	20.78	0	146.90

Table 4- 4 illustrates the mean, standard deviation, minimum and maximum values for each of the Age, WBC, RBC, HGB, HCT, PLT, and EPO correspondingly. The mean age of patients in this study is 38 years since the lowest age and the highest age of patients is 19 and 66 years respectively.

The mean WBC is 8.96 cell/cmm since the mean of RBC and HGB are 5.95 million/mm3 and 17.63 g/dl respectively. In addition, the mean of HCT and PLT are 52.15 and 246.77 consequently.

4.3Normality Test

Before running an independent sample t-test, the assumptions of normality and homegenity of variances of the variables have to be tested. To determine if a sample of data has been taken from a population with a distribution that is normally distributed, a normality test was employed (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.

Table 4-5: Normality test for Age, WBC, RBC, HGB, HCT, and PLT.

	Kolmogorov-Smirnov	
	Statistic	p-value
Age (Years)	0.095	0.200
WBC	0.980	0.569
RBC	0.986	0.828
HGB	0.962	0.294
HCT	0.114	0.200
PLT	0.958	0.076

Table 4-5 shows the normality test for Age, WBC, RBC, HGB, HCT, and PLT. According to the normality test, the p-values of age (0.200), WBC (0.569), RBC (0.828), HGB (0.294), HCT (0.200), and PLT (0.076) are greater than the alpha value (0.05) and this indicates that all data sets are normally distributed.

Table 4- 6: Independent Sample T Test between the mean of normal and abnormal of the EPO with each of the (Age, WBC, RBC, HGB, HCT, and PLT).

EPO		N	Mean	Std. Deviation	T	p-value
WBC (Cell/cmm)	Normal	5	8.681	2.224	0.281	0.788
	Abnormal	47	8.993	3.405		
RBC (million/mm3)	Normal	5	6.281	0.675	0.965	0.339
	Abnormal	45	6.030	0.540		
HGB(g/dl)	Normal	4	18.200	0.294	0.37	0.714
	Abnormal	29	18.141	0.297		
HCT(%)	Normal	4	54.125	1.471	0.668	0.508

	Abnormal	36	53.464	1.909		
PLT(Cell/cmm)	Normal	4	195.500	41.024	1.314	0.195
	Abnormal	45	236.401	60.737		
Age (Years)	Normal	5	45.800	9.884	1.463	0.150
	Abnormal	47	37.213	12.680		

Table 4-6 shows no statistical significant difference between the mean normal and abnormal of EPO with each of the WBC, RBC, HGB, HCT, PLT, and age individually because their p-values are higher than the significant level of α=0.05.

Table 4- 7: Comparison between normal and abnormal of the EPO with each of the demographic and clinical parameters (group of Polycythemia, age group, gender, Narghile, Cigarette, Alcohol, Fatigue, and Headache).

		EPO group			
		Normal		Abnormal	
		N	%	N	%
Group	Secondary Polycythemia	4	7.7%	40	**76.9%**
	Primary Polycythemia	1	1.9%	7	13.5%
Age (Years)	19-28	0	0.0%	12	23.1%
	29-38	1	1.9%	16	**30.8%**
	39-48	2	3.8%	10	19.2%
	49-58	1	1.9%	5	9.6%
	59+	1	1.9%	4	7.7%
Gender	Male	4	7.7%	45	86.5%
	Female	1	1.9%	2	3.8%
Narghile	Positive	0	0.0%	13	25.0%
	Negative	5	9.6%	34	**65.4%**
Cigarette	Positive	4	7.7%	25	**48.1%**
	Negative	1	1.9%	22	42.3%
Alcohol	Positive	2	3.8%	12	23.1%
	Negative	3	5.8%	35	**67.3%**
Fatigue	Positive	3	5.8%	12	23.1%
	Negative	2	3.8%	35	**67.3%**
Pruritus	Positive	1	1.9%	6	11.5%
	Negative	4	7.7%	41	**78.8%**
Headache	Positive	3	5.8%	18	34.6%
	Negative	2	3.8%	29	**55.8%**

Table 4-7 compares the EPO's normal and abnormal levels for each of the (group of Polycythemia, age groups, gender, Narghile, Cigarette, Alcohol, Fatigue, and Headache). The percentage of patients who have abnormal EPO for Secondary Polycythemia group (76.9%) is higher than Primary Polycythemia group (13.5%). Most patients aged 29 to 38 (30.8%) had abnormal EPO, followed by those aged 19 to 28 (23.1%), 39 to 48 (19.2%), 49 to 58 (9.6%), and those older than 49 (7.7%) consequently.

Then, the percentage of the male patients with abnormal EPO (86.5%) was larger than that of female patient (3.8%). Next, the negative nerghila and alcohol had higher rates of abnormal EPO with (65.4 and 67.3%, respectively) than their positives (25% and 23.1%). But positive cigaritte is higher rates of abnormal EPO (48.1%) than negative smokers (42.3%) as shown on (Figure4-4).

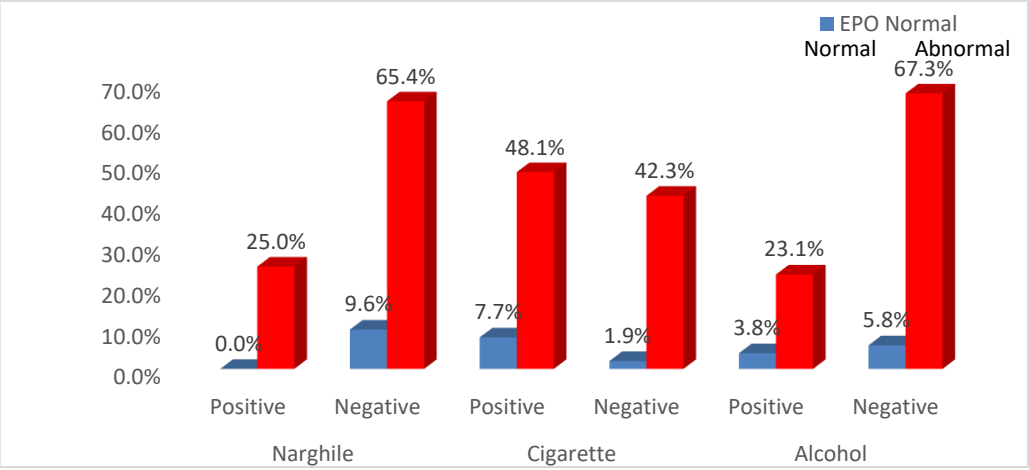


Figure 4-4: Comparison between positive and negative of narghila, cigarette, and alcohol with EPO level.

Finally, most of the patients who have negative fatigue, prurtius, and headche higher rates of abnormal EPO (67.3%, 78.8%, and 55.8%) than their positives 23.1%, 11.5%, and 34.6%) as shown on (Figure4-5).

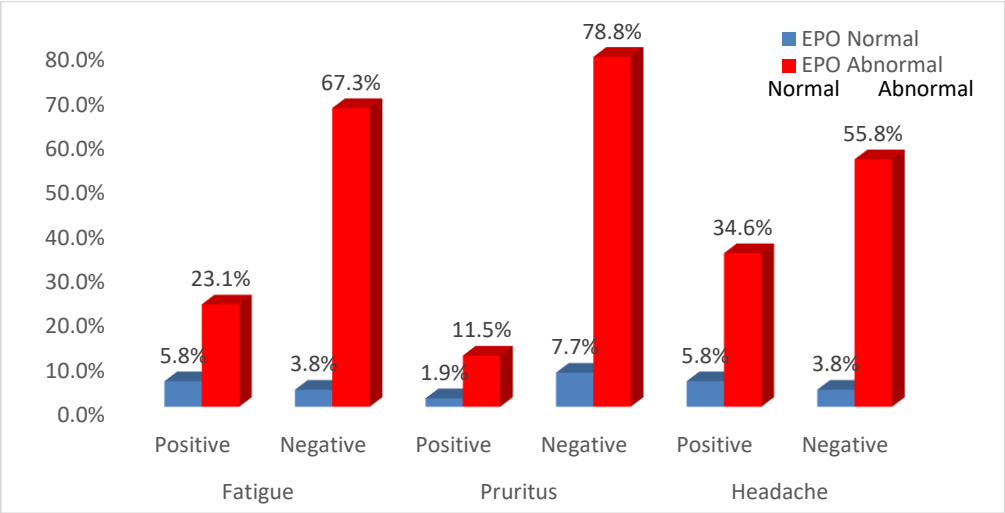


Figure 4-5: Comparison between positive and negative of fatigue, prurtius, and headche with EPO level.

Table 4-8: Comparison between normal and abnormal positivity of JAK2V617F with each of the (group of Polycythemia, age group, gender, ABO, Narghile, Cigarette, Alcohol, Fatigue, and Headache).

		JAK2V617F			
		Positive		Negative	
		N	%	N	%
Group of patients	Secondary Polycythemia	0	0.0%	44	**84.6%**
	Primary Polycythemia	3	5.8%	6	9.6%
Age (Years)	19-28	0	0.0%	12	23.1%
	29-38	0	0.0%	17	**32.7%**
	39-48	1	1.9%	11	21.2%
	49-58	1	1.9%	5	9.6%
	59+	1	1.9%	4	7.7%
Sex	male	1	1.9%	48	**92.3%**
	female	2	3.8%	1	1.9%
Narghile	Positive	0	0.0%	13	25.0%
	Negative	3	5.8%	36	**69.2%**
Cigarette	Positive	1	1.9%	28	53.8%

	Negative	2	3.8%	21	**40.4%**
Alcohol	Positive	1	1.9%	13	25.0%
	Negative	2	3.8%	36	**69.2%**
Fatigue	Positive	1	1.9%	14	26.9%
	Negative	2	3.8%	35	**67.3%**
Pruritus	Positive	1	1.9%	6	11.5%
	Negative	2	3.8%	43	**82.7%**
Headache	Positive	1	1.9%	20	38.5%
	Negative	2	3.8%	29	**55.8%**

Table 4-8 compares the JAK2V617F 's positive and negative levels for each of the group of polycythemia, age group, gender, ABO, narghile, cigarette smoking, alcohol consumption, fatigue, and headache.

The percentage of patients who have negative JAK2V617F for secondary polycythemia group (84.6%) is higher than primary polycythemia group (9.6%). Most patients aged 29 to 38 (32.7%) had negative JAK2V617F, followed by those aged 19 to 28 (23.1%), 39 to 48 (21.2%), 49 to 58 (9.6%), and those older than 49 (7.7%) consequently. Then, the percentage of the male patients with negative JAK2V617F with (92.3%) was more than female patients (1.9%).

Next, the negative nerghila, cigareete, and alcohol had higher rates of negative JAK2V617F (69.2%, 40.4%, and 69.2%) than their positives (25%, 53.8%, and 25%) respectively, as shown on (figure4-6).

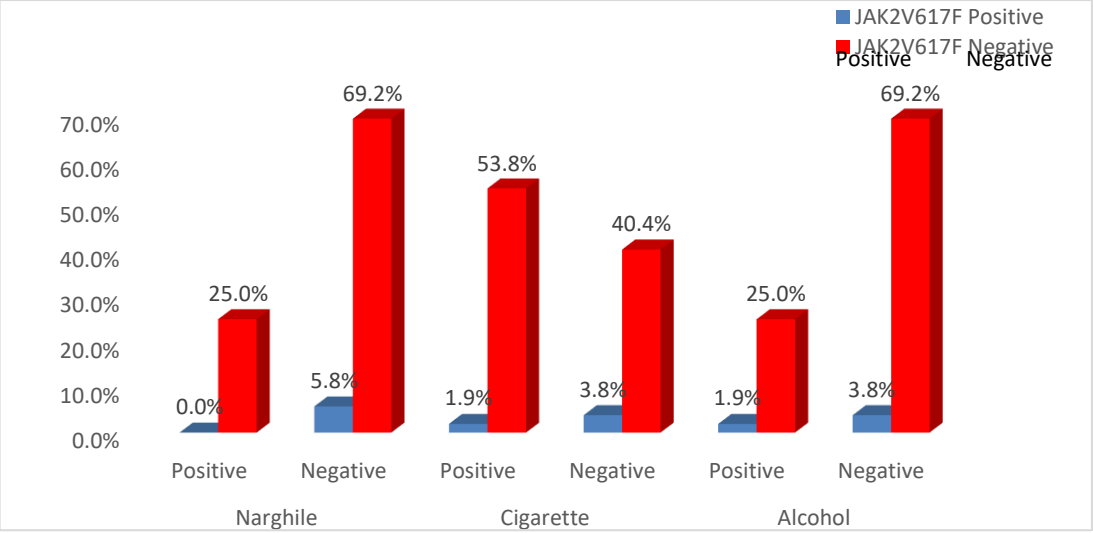


Figure 4-6: Comparison of the effects of alcohol, cigarettes, and narghila on the JAK2V617F level.

Finally, most of the patients who have negative fatigue, prurtius, and headche higher rates of negative JAK2V617F (67.3%, 82.7%, and 55.%) than their positives (26.9%, 11.5%, and 38.5%), as shown on (Figure4-7).

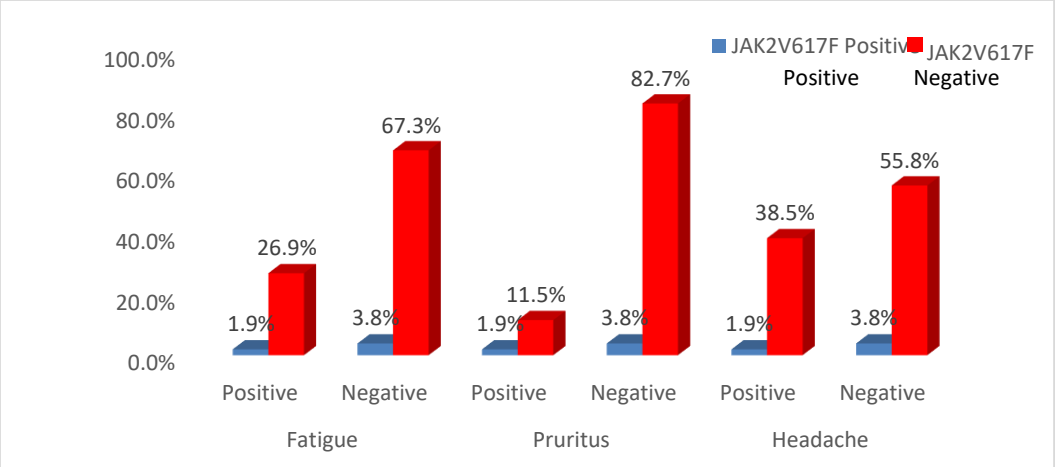


Figure 4-7 : Comparison between positive and negative of fatigue, headche, and prurtius with JAK2V617F level.

The percentage of the patients who have secondary polycythemia are smoking more nergilla and cigarette (21.2% and 51.9%) compared to primary polycythemia (3.8% and 3.8%), respectively. The percentage of the patients who have secondary polycythemia are more drinking alcohol (23.1%) compared to Polycythemia Vera (3.8%), as shown on (Figure4-8).

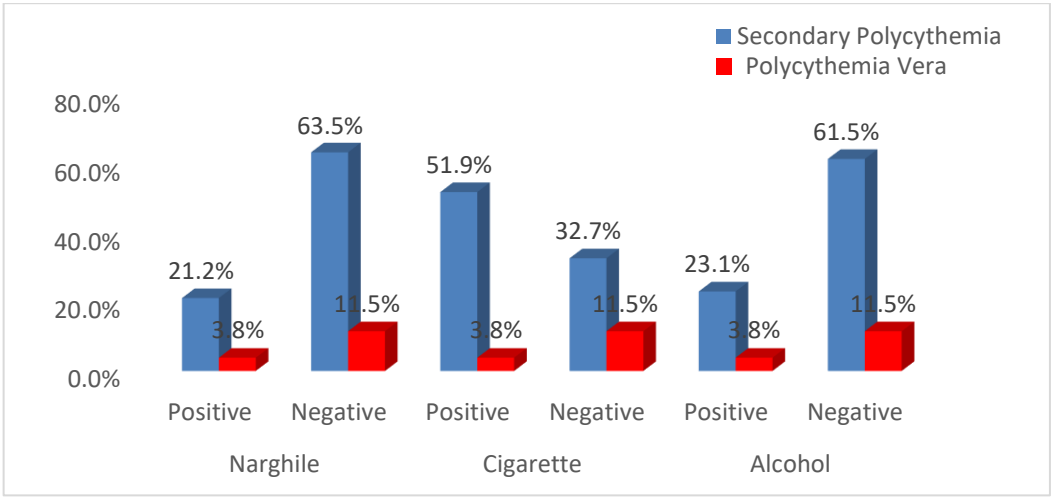


Figure4-8: Comparison between Secondary Polycythemia and Polycythemia Vera with each of the (Narghile, Cigarette, and Alcohol).

The percentage of the patients with secondary polycythemia have higher rates of positive Fatigue, Pruritus, and Headache (26.9%, 11.5%, and 36.5%) than those with primary polycythemia (1.9%, 1.9%, and 3.8%), respectively. The percentages of patients with negative secondary polycythemia and pimary polycythemia are higher than those with positive symptoms, as shown on (Figure4-9).

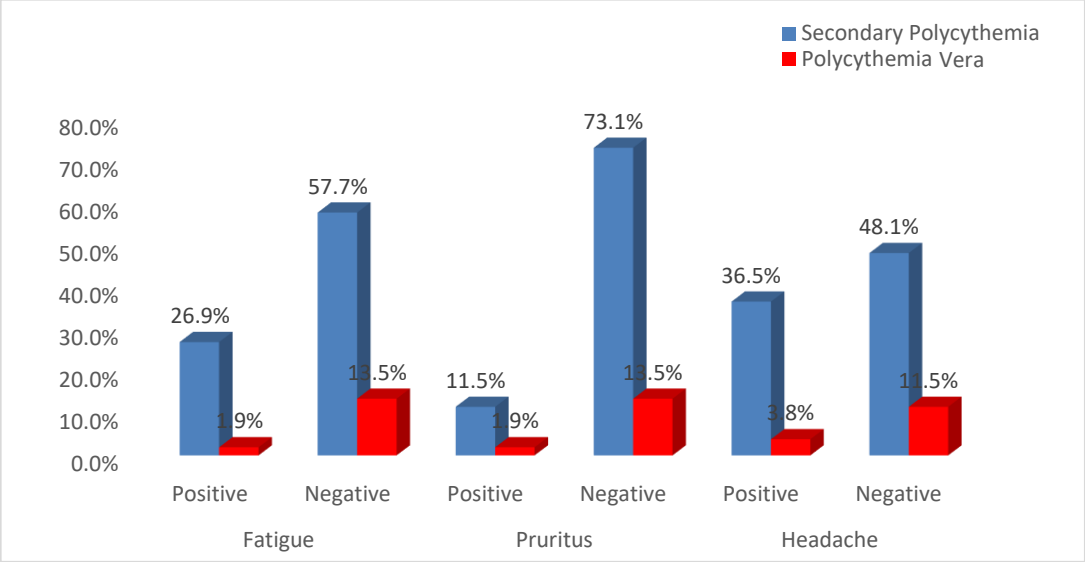


Figure 4-9: Comparison between Secondary Polycythemia and Polycythemia Vera with each of the (Fatigue, Pruritus, and Headache).

Discussion

Finding the JAK2 V617F exon (14) mutation was the primary goal of the current study in both primary and secondary polycythemia patients. In the present study, 3/52 (5.8%) of the study participants had PV with an average age of 32.7 year, our results agree with those of Srour *et al.*, (2016) where out of 44 cases of PV patients, (13.82%) were positive 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).

Regarding to gender, the majority of the study participants were male, and the PV was more frequent in males than females. The findings of this study agree with the findings of the research done by Yadav *et al.*, (2018), as he showed that out of 142 cases of polycythemic patients, the majority were male (68.18%), while females (31.82). We hypothesize that, this could be due to the fact that the gene mutation rate that is responsible for PV is more frequent in males than females.

The results of the present study show that the average of each of the HGB g/dl and HCT % for negative JAK2V617F (17.812, and 52.164) was higher than the average of the 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) was lower than the average of the 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.

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

Norashikin *et al*. explained why blood donors with a long history of giving blood tend to have elevated hemoglobin levels. Our result is in line with that of a recent local research, which linked Hb levels and donation rates significantly (Norashikin *et al.,* 2006).

Patients with chronic philadelphia-negative classical MPNs, who typically have increased HCT % and/or WBC cell/cmm , 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 most of the patients were smokers (55.8%), but most of them were negative for smoking Narghile (75%), and alcohol consumption (73.1%). On the contrary, a study conducted by All-Rubaie *et al.,* (2014) showed that 79 out of 94 (84.04%) blood donors smoked cigarettes, Narghile, or both, and that one of the causes of their elevated HCT was their blood donation, furthermore, 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.

Our result showed that there were no mutation in the secondary polycythemia patients. On the contrary, smokers have 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).

In the present study, the levels of RBC, HCT, or WBC were normal in patients with mutation in JAK2. On other hand, in smokers, the JAK/STAT signaling system is constantly activated, which contributes to accelerated erythropoiesis, myelopoiesis, and thrombopoiesis (Scott, 2011).

Correlation between smoking and hemoglobin range analysed in some studies, the results shown that 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 narghile , and 2% (3 out of 121) smoked both cigarettes and narghile. Additionally, among non-smokers, 2.5% and 3.5%, respectively, noted daily exposure to cigarettes and , respectively (AlQahtany *et al.,* 2020).

In the current work, the JAK2 exon 14 gene and PCR product were sequenced 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, aresearch 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). The difference in the positive rate of JAK2 mutation could be due to difference in the sample size.

According to a study conducted by All-Rubaie *et al.,* (2014), the PV patients had a mutant ratio of JAK2V617F that was much higher than that of blood donors who had the mutation, with the mean values of 5826.9 (range >1.3 - 100) and 11.1012.6 (range >1.3 - 42.6), respectively.

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 have changed since 2005, when screening for the JAK2 mutation started (Deadmond and 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 and Zrari, 2020).

Then, most of the patients have O+ blood group (34.6%) followed by B+ (26.9%), A+ (23.1%), AB+ (5.8%), A- (3.8%), O- (3.8%), and B- (1.9%) respectively. These results are similar, the first two blood groups majority to other studies that shows out of 44 cases of polycythemia vera, majority were in blood group O (40.92%), proceeded by blood group A (27.27%), blood group B (25%), least common was AB blood group (6.81%) (Yadav *et al.,* 2018).

Finally, most of the patients had negative fatigue (71.2%) negative pruritus (86.5%), and negative headache (59.6%), while most of them have negative JAK2V617F (94.2%0 and abnormal EPO (90.4%). Furthermore, smoker participants had higher levels of EPO, this could be due to the fact that the blood of the smokers has low level of oxygen, therefore, bone marrow produced higher levels of oxygen to compensate for it. Other investigations have found that the

EPO level is below normal, and its level may have a limited impact on the PV diagnosis (Lupak *et al.,* 2020). It was discovered that the EPO measurements of patients with a PV diagnosis were within the normal range. These findings indicate that a low EPO level alone has a reasonable predictive accuracy for PV but does not provide diagnostic information when the JAK2 V617F mutation status is present. This result sits in parallel to the existing research (Spivak and Silver, 2008; Barbui *et al.,* 2018). On the other hand, previous research has demonstrated that a low EPO level is highly diagnostic of PV (Messinezy *et al.,* 2002; Messinezy *et al.,* 1995; Chait *et al.,* 2005;). However, the discovery of the JAK2 axon 12 and JAK2 V617F mutation states did not prompt a reevaluation of the reliability of the previously established link (Mossuz et al., 2004).

Chapter Five: Conclusions and Recommendations

5-CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. In conclusion, our findings in this study emphasize that most of the patients had secondary polycythemia, JAK2V617F mutation in exon 14 for PV patients proved by molecular methods.
2.The study shows higher risk for male compare to female individuals. Also hemoglobin level increase with high secretion of EPO. Our negative cases have higher abnormal EPO as a result, individuals with secondary EPO elevations are due to coexisting conditions may go undiagnosed with PV.
3.The study estimated that PV cigarette smokers have higher abnormality of EPO than hookah users and alcohol consumers.

Recommendations

1.Detection of epigenetic factors such as micro RNA, DNA methylation and Chromatin remodeling.

2.Molecular genetic testing for JAK2 exon 12,13,and 15 for both secondary and primary polycythemia patients.

3.Molecular testing of CARL gene (exon9) and MPL gene for both groups.

4.Investigating larger size of samples

5.Investigating body mass index and environmental factors, such as food habits and occupation.

6. Other hematological parameters like blood film examination and iron deficiency anemia among polycythemia patients.

7. Study of family history.

8.Study of other related genes.

9.Finally, other variations require additional research. Future research can take into account non-JAK2 V617F patients with PV who may have mutations in the JAK2 exon 12 region, and environmental factors.

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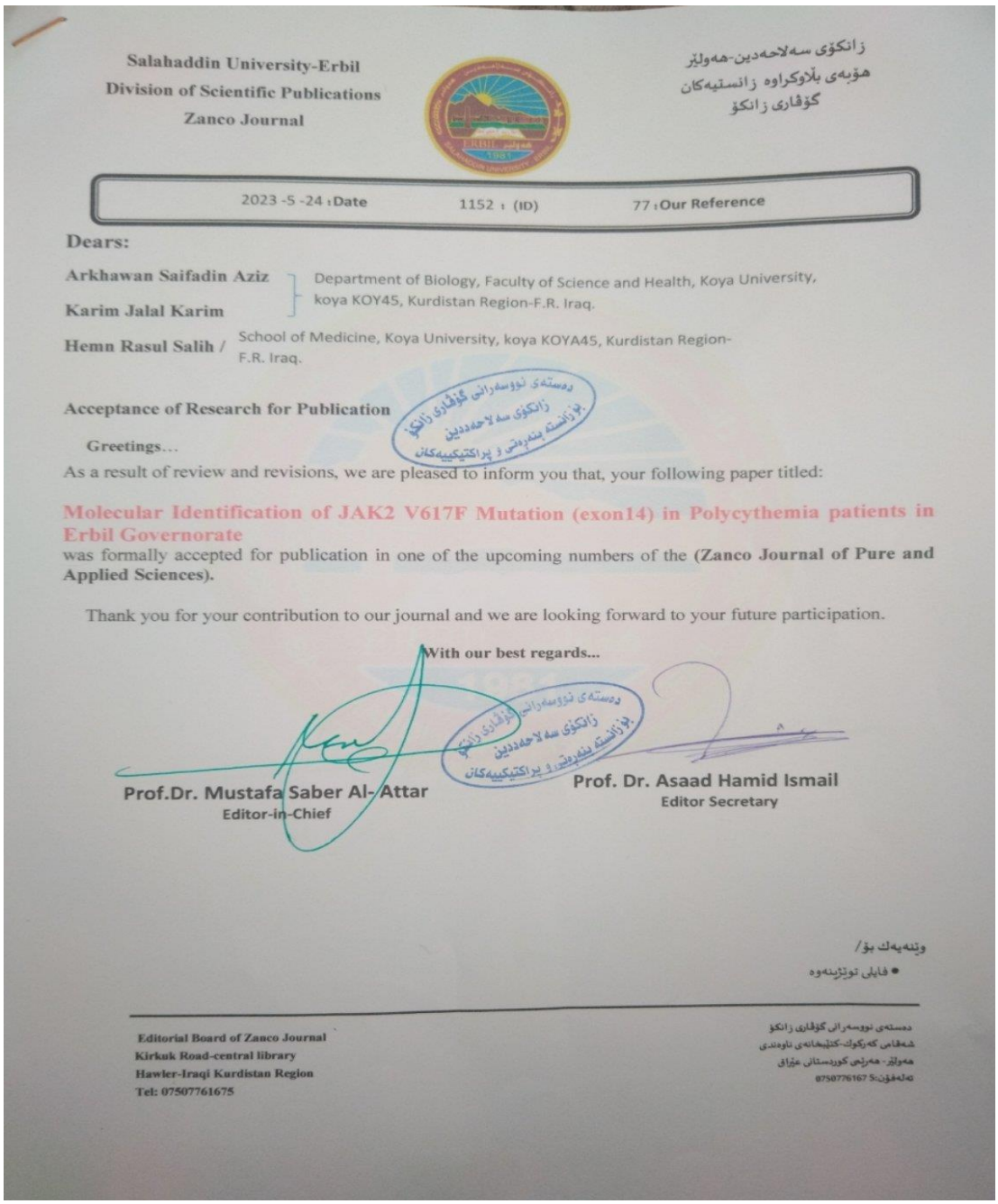
Appendices

Appendix (1): Acceptance letter for publication paper.



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Karim Jalal Karim
Department of Biology, Faculty of Science and Health, Koya University, koya KOY45, Kurdistan Region-F.R. Iraq.

Hemn Rasul Salih / School of Medicine, Koya University, koya KOYA45, Kurdistan Region-F.R. Iraq.

Acceptance of Research for Publication

Greetings…

As a result of review and revisions, we are pleased to inform you that, your following paper titled:

Molecular Identification of JAK2 V617F Mutation (exon14) in Polycythemia patients in Erbil Governorate

was formally accepted for publication in one of the upcoming numbers of the **(Zanco Journal of Pure and Applied Sciences).**

Thank you for your contribution to our journal and we are looking forward to your future participation.

With our best regards...

Prof.Dr. Mustafa Saber Al- Attar
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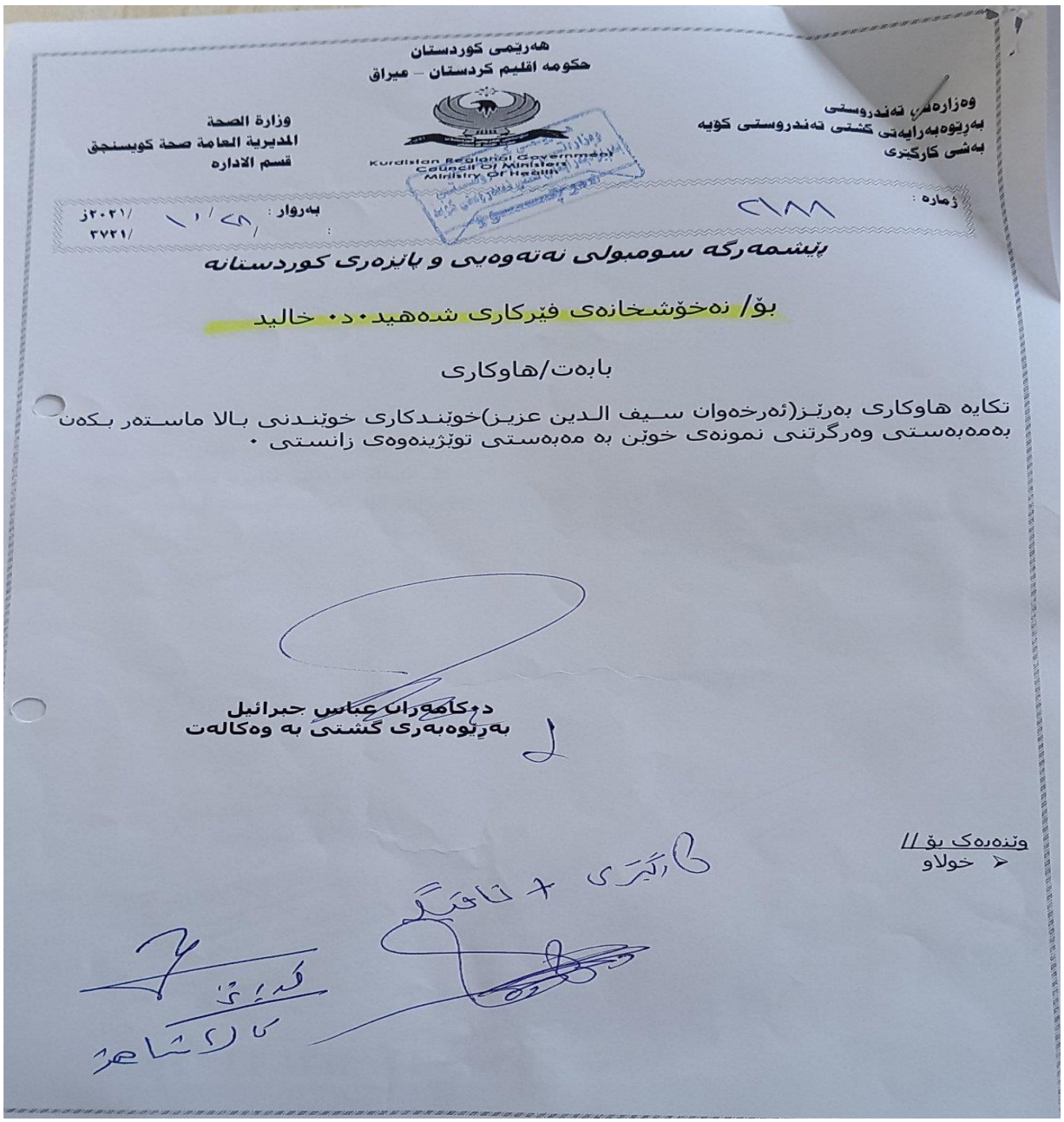
Appendix (2): Questionnaire form.

City:	Week:	Name of the /Hospital :
Samples no.		Sample collection date:
Patient name		

Age					
Sex					
Family history of polycythemia					
Narghile smoking					
Smoking					
Alcohol consumption					
Headache					
Pruritus					
Fatigue					
Hematological parameter	RBC count:	Hb conc.:	HCT:	PLT count:	Total WBC count:
EPO:					
Blood Group					

Appendix (3):



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بەروار : ٢٠٢١/١٠/٢٨
٣٧٢١/

پێشمەرگە سومبولی نەتەوەیی و پایزەری کوردستانە

بۆ/ نەخۆشخانەی فێرکاری شەهید د. خالید

بابەت/هاوکاری

تکایە هاوکاری بەڕێز(ئەرخەوان سیف الدین عزیز)خوێندکاری خوێندنی بالا ماستەر بکەن بەمەبەستی وەرگرتنی نمونەی خوێن بە مەبەستی توێژینەوەی زانستی .

دڵکامەران عباس جبرائیل
بەڕێوەبەری گشتی بە وەکالەت

وێنەیەک بۆ //
➢ خولاو

Appendix (5):

حكومة اقليم كوردستان/ العراق
وزارة الصحة
المديرية العامة لصحة اربيل
قسم الامور الفنية

حکومەتی هەرێمی کوردستان / عێراق
وەزارەتی تەندروستی
بەڕێوەبەرایەتی گشتی تەندروستی هەولێر
بەشی کاروباری هونەری

Kurdistan Region Government/ Iraq
Ministry of Health
General Directorate of Health – Erbil

العدد:
التاريخ: / ١١ / 2021

ژمارە:
ڕێکەوت: / / 2720 ی کوردی

بۆ/ نەخۆشخانەی (ئانە کەلی وە بەڕێوەبەرایەتی بانکی خوێن)

بابەت/ئاسانکاری

ئاماژە بەنووسراوی زانکۆی کۆیە// کۆلێژی فاکلتی زانست وتەندروستی ژمارە ((١٣٣٣/٩/٧)لە(٢٠٢١/١٠/٢٦))

ئاسانکاری بکرێت بۆ خاتوو (ئەرخەوان سیف الدین عزیز)قوتابی خویندنی باڵا/ماستەر لە بەشی بایۆلۆجی بۆ سالی خویندنی(٢٠٢١-٢٠٢٢) بۆ وەرگرتنی نمونەی خوێن بەمەبەستی توێژینەوەی زانستی بەمەرجێك نابێ مەوادی تاقیگە بەکار بهێنێت.

لەگەڵ ڕیزماندا ....

دکتۆر
دلۆڤان محمدفاتح جلال
بەڕێوەبەری گشتی
٢٠٢١/١١/

وێنەیەك بۆ/
- نووسینگەی بەڕێز بەڕێوەبەری گشتی // بۆ زانین لە گەڵ ڕێزماندا..
- بەشی کاروباری هونەری // هۆبەی تاقیگەکان...
- زانکۆی کۆیە//کۆلێژی فاکلتی زانست وتەندروستی// بۆزانین لەگەڵ ڕیزماندا...

أقليم كوردستان
اربيل-تقاطع البارزاني الخالد

Kurdistan Region
Erbil-Barzani Namr Q.
E-Mail: info@dohhawler.org
Website: www.dohhawler.org

هەرێمی کوردستان
هەولێر-چوارڕیانی بارزانی نەمر
نورمال : 2225757 -2230404-2230401 (066)

الخلاصة

كثرة الحمر الحقيقية (PV)هو مرض تكاثر نقوي مزمن نسيلي يسبب تكاثر السلائف النخاعية المستقلة عن السيتوكين ويؤثر بشكل أساسي على كبار السن. هذا يؤثر في المقام الأول على سلالة الكريات الحمر ويسبب عددا كبيرا بشكل غير طبيعي من كريات الدم الحمراء المنتشرة. كما لوحظت أعداد متزايدة من الخلايا المحببة والصفائح الدموية المنتشرة في كثير من الحالات.

كان الغرض من هذه الدراسة هو الكشف عن طفرات JAK2 في المرضى الذين يعانون من كثرة الحمر الأولية والثانوية. بالإضافة إلى ذلك ، تم اختبار مستويات هرمون الإريثروبويتين (EPO)في مرضى كثرة الحمر جنبا إلى جنب مع المعلمات الدموية الأخرى التي تمت دراستها في ٥٢ مريضا بكثرة الحمر (٨ مع PV و ٤٤ متبرعا بالدم). تم أخذ عينات من المرضى الذين يترددون على مستشفى ناناكالي، مديرية بنك الدم في أربيل ، ومستشفى الشهيد الدكتور خالد في كويا في إقليم كردستان العراق.

أظهر تسلسل سانجر لنتائج تفاعل البوليميراز المتسلسل JAK2 exon 14 و (٥٠٠ bp)أن ٣ (٥٫٨٪) من المرضى متعددي الحمر لديهم طفرات Janus Kinase2 V617F غير متجانسة الزيجوت ، بينما كانت جميع العينات الأخرى سلبية. كان متوسط عمر المشاركين في الدراسة ٣٨ عاما ، وكان المشاركون الذكور (٩٤٫٢٪) أعلى من الإناث (٥٫٨٪). كانت النسبة المئوية للمرضى الذين لديهم EPO غير طبيعي لمجموعة كثرة الحمر الثانوية (٧٥٪) أعلى من مجموعة كثرة الحمر (١٥٫٤٪). لوحظ مستوى غير طبيعي من EPO في المرضى الذين تتراوح أعمارهم بين ٢٩ و ٣٨ (٣٠٫٨ ٪). كان متوسط كل من الهيموجلوبين (HGB)والهيماتوكريت JAK2V617F (HCT) السلبية أعلى من JAK2V617F الإيجابي ، في حين كان متوسط كل من PLT وعمر JAK2V617F السلبية أقل من JAK2V617F الإيجابي. علاوة على ذلك ، كان للنيرغيلا السلبية والسجائر والكحول معدلات أعلى من JAK2V617F السلبية من إيجابياتها.

أخيرا ، فإن معظم المرضى الذين يعانون من التعب السلبي ، والحكة ، والرأس لديهم معدلات أعلى من JAK2V617F السلبية مقارنة ب JAK2V617F الإيجابية.

جمهورية العراق الفيدرالي
حكومة إقليم كوردستان
وزارة التعليم العالي و البحث العلمي
جامعة كويه

تحديد الطفرة في مورثة Janus Kinase2 وتقييم بعض قياس الدم في مرض بۆليسايثيميا – محافظه اربيل

رسالة مقدمة الى فاكلتي العلوم والصحة في جامعة كويه
وهي جزء من متطلبات نيل شهادة ماجستير علوم .(MSC) في البايلوجي

من قبل
ئه رخه وان سيف الدين عزيز

حاصلة على شهادة البكالوريوس علوم فى قسم البايلوجي / جامعة كويه, ٢٠١٥

باشراف
أ. م. د. كريم جلال كريم
م. د. هيمن رسول صالح

٢٠٢٣

پوختە

پۆلیسایتیمیا ڤێرا (PV) نەخۆشییەکی درێژخایەنی کلۆنالّی مایلۆپرۆلیفێراتیڤە کە دەبێتە هۆی زیادبوونی سەربەخۆی ساینتاکینەکانی پێشەکییەکانی مایلۆید و بە شێوەیەکی سەرەکی کاریگەری لەسەر کەسانی بەسالّاچوو هەیە. ئەمەش بە پلەی یەکەم کاریگەری لەسەر ڕەچەلّەکی خانە سوورەکان دەبێت و دەبێتە هۆی ژمارەیەکی زۆری نائاسایی لە خانە سوورەکانی سووڕانەوە. هەروەها زیادبوونی ژمارەی گرانولۆسایت و پلاکتەکانی سووڕاو لە زۆر حالّەتدا بەدیکرا.

ئامانجی ئەم توێژینەوەیە دیاریکردنی گۆڕانکارییەکانی JAK2 بوو لە نەخۆشانی تووشبوو بە پۆلیسایتیمیا سەرەتایی و لاوەکی. سەرەڕای ئەوە، ئاستی ئێریترۆپۆئیتین (EPO) لە نەخۆشانی پۆلیسایتیمیا لەگەڵ پارامێتەرەکانی تری خوێن لە ٥٢ نەخۆشی پۆلیسایتیمیا (٨ لەگەڵ PV و ٤٤ خوێنبەخش) لێکۆڵینەوەیان لەسەر کرا. نمونەی ئەو نەخۆشانەی لە نەخۆشخانەی نانەکەلی و بەڕێوبەرایەتی بانکی خوێنی هەولێر و نەخۆشخانەی دکتۆر خالید شەهید لە کۆیە لە هەرێمی کوردستان وەرگیراون.

ڕێکخستنی سەنگەر بۆ ئەنجامەکانی JAK2 ئێگزۆن ١٤ و (٥٠٠ bp) PCR دەریخست کە (٥,٨%)٣ لە نەخۆشە پۆلیسیتمییەکان گۆڕانکاری نایەکسانی Janus Kinase2 V617F یان هەبووە، لە کاتێکدا هەموو نمونەکانی تر نێگەتیڤ بوون.تێکڕای تەمەنی بەشداربووانی توێژینەوەکە ٣٨ ساڵ بووە، و بەشداربووانی نێر (٩٤,٢%) زیاتر بوون لە بەشداربووانی مێینە (٥,٨%).ڕێژەی ئەو نەخۆشانەی کە EPOی نائاساییان هەبوو بۆ گروپی پۆلیسایتیمیا لاوەکی (٧٥%) زیاتر بوو لە گروپی پۆلیسایتیمیا (١٥,٤%).ئاستێکی نائاسایی EPO لە نەخۆشەکان کە لە نێوان تەمەنی ٢٩ و ٣٨ (٣٠,٨%)، مامناوەندی هەردوو هیمۆگلۆبین (HGB) و هیماتۆکریت (HCT)ی JAK2V617F نێگەتیڤ زیاتر بوو لە پۆزەتیڤ JAK2V617F، لە کاتێکدا مامناوەندی هەردوو PLT و تەمەنی JAK2V617F نێگەتیڤ بوو کەمترە لە JAK2V617F پۆزەتیڤەکان.

لە کۆتاییدا، زۆربەی ئەو نەخۆشانەی کە ماندوێتی نەرێنی، خوران و سەرئێشەیان هەبووە ڕێژەی نێگەتیڤی JAK2V617Fیان بەرزتر بووە بە بەراورد بە پۆزەتیڤی JAK2V617F.

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کۆماری فیدراڵی عێراق
حکومەتی هەرێمی کوردستان
وەزارەتی خوێندنی باڵا و توێژینەوەی زانستی
زانکۆی کۆیە

دیاریکردنی بازدان لە بۆهێڵی Janus Kinase2 و هەڵسەنگاندنی هەندێك لە پێوەرە کانی خوێن لە نەخۆشانی پۆلیسایثیمیا - پارێزگای هەولێر

ماستەرنامەیەکە پێشکەشی
فاکەلتی زانست و تەندروستی کراوە لە زانکۆی کۆیە وەك بەشێك لە پێداویستی
بەدەستهێنانی بڕوانامە ی ماستەر لە زانستی بایۆلۆجی

لە لایەن
ئە رخە وان سیف الدین عزیز

بەکالۆریوسی وەرگرتوە لە بایۆلۆجی
لە فاکەلتی زانست و تەندروستی/ زانکۆی کۆیە/ لە ساڵی ٢٠١٥

بە سەرپەرشتی

پ. ی. د. کریم جلال کریم
د. هیمن رسول صالح

٢٠٢٣