Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 12, pp.63302-63309, December, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

STUDY OF JAK2 V617F MUTATION, SERUM B12 LEVEL AND INTERLEUKIN – 23 LEVEL IN POLYCYTHEMIA VERA

[1,*]Manal Ali Abdulsahib Al-Rubaye and [2]Abeer Anwer Ahmed

[1]National Center of Hematology, University of Mustansiriyah, Baghdad, Iraq
[2]Department of Pathology, College of Medicine, University of Mustansiriyah, Baghdad, Iraq

ARTICLE INFO

Article History:
Received 06th September, 2017
Received in revised form
23rd October, 2017
Accepted 09th November, 2017
Published online 31st December, 2017

Key words:
Polycythemia Vera,
JAK2V617F mutation,
Vitamin B12,
Interleukin – 23,
Cobalamin.

**Corresponding author:*
Abeer Anwer Ahmed
Department of Pathology, College of Medicine, University of Mustansiriyah, Baghdad, Iraq.

ABSTRACT

Introduction: Myeloproliferative neoplasms (MPNs) including Polycythemia Vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are clonal hematopoietic stem cell disorders which originate from a single stem cell. Usually one cell line predominates in a given disorder. Over the last 12 years, several somatic mutations have been described in MPN classification like: JAK2, CALR and MPL, but JAK2 is the most frequent, with frequencies of approximately 98% in PV, 50% to 60% in ET, and 55% to 65% in PMF.
It is believed that cytokines participate in the activation of JAK2V617F, one of them IL-23 that acts as a pro-inflammatory mediator and can induce chronic inflammation and its plasma level increased in MPN patients.
High serum cobalamin is frequently observed in malignant blood diseases and MPDs. An elevated level of plasma cobalamin is found in 30 to 50% of patients with Polycythemia Vera (PV).
Objectives: the study aimed to:

1. Estimate JAK2 V617F mutation gene PV in Iraqi patients.
2. Measure the levels of IL-23 and B12 in the PV cases with JAK2V617F +ve mutation.

Patients and Methods:
Thirty patients were diagnosed as polycythaemia according to their clinical and laboratory findings like: CBC, and bone marrow biopsy (for some patients), liver and renal function test, abdominal U/S some with CT abdomen and JAK2 V617F mutation.
Then levels of B12 and IL-23 were measured and correlated with CBC results.
Results and Discussion:
The mean age of PV patients was (52.0 ± 12.5) with a range of (22-71 yrs), there was a PV case in a 22 years old male which was considered as a rare condition also male to female ratio of (1:1) is unusual. Splenomegaly and hepatomegaly present in 36.7% and 13.3% of PV patients respectively.
The mean Hb was (17.2 ±1.7 g/dl), while the mean WBC count was (12.3±3.9 x10^9/L) and the platelet count was (446.0±285.2).
Plasma levels of IL-23 were significantly increased in all patients with JAK2 V617F +ve mutation PV with mean (70.00±72.18 pg/mL) and range (8.5-259.0) and there was a significant positive correlation between IL-23 level and the total WBC count.
B12 serum level was high in 16 of total 30 cases, with mean serum level (766.54±583.95pg/mL) .There was no Correlation between B12 level and Hb level, Platelet count and WBC count.
Also there was no correlation between IL-23 and B12.
Conclusions:

1. Study of JAK2V617F mutation is an important test in MPN patients, particularly in those who suspected to have PV.
2. The diagnosis of PV was based on clinical, haematological and genetic tests. While B.M. biopsy was not performed for the studied cases, it is recommended by WHO as a major criterion to establish a diagnosis of PV. (WHO criteria for PV diagnosis, 2016)
3. High levels of IL-23 in the sera of all PV patients that positively correlate with WBC count.
4. Cobalamin levels was elevated in 53.3% of PV.

Citation: Manal Ali Abdulsahib Al-Rubaye and Abeer Anwer Ahmed, 2017. "Study of JAK2 V617F mutation, serum B12 level and interleukin – 23 level in polycythemia vera", *International Journal of Current Research*, 9, (12), 63302-63309.

INTRODUCTION

Polycythaemia

Is defined as an increase in the hemoglobin concentration above the upper limit of normal for the patient's age and sex. Distinction should be made between primary, secondary, and apparent polycythemias.

Polycythemia Vera (PV)

It is an acquired myeloproliferative neoplasm arising from malignant transformation of hematopoietic stem cell. It is associated with mutations of erythropoietin receptor gene that lead to autonomous erythropoietin-independent proliferation of red cell progenitors (Nayak *et al*., 2012). It is characterized by increased, uncontrolled marrow production of red cells, granulocytes and platelets (panmyelosis). This leads to

erythrocytosis (polycythemia) and or granulocytosis and thrombocytosis in the peripheral blood. But erythrocytosis is responsible for most of the clinical symptoms (Nayak *et al*., 2012). It has been found that mutation in tyrosine kinase JAK2V617F occurs in 95% of cases and a mutation in another exon of gene (exon 12) occurs in another 4% of cases of PV. The JAK2 mutation also occurs in a significant proportion of patients with primary myelofibrosisand essential thrombocythaemia (Kawthalkar, 2013). Erythropoietin production is reduced in PV and abnormal erythroid stem cells require very small amounts of erythropoietin for their differentiation. The neoplastic clone suppresses normal hematopoietic stem cells as well as erythropoietin production. (Kawthalkar, 2013) Table (1) lists 2016 WHO criteria for diagnosis of Polycythemia Vera.

Table 1. WHO criteria for PV (2016)

	Major criteria
1	Hemoglobin > 16.5 g/dl in men Or Hemoglobin > 16.0 g/dl in women Or increased red cell mass (RCM)*
2	BM biopsy showing hyper cellularity for age with tri lineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic mature megakaryocytes (differences in size)
3	Presence of JAK2V617F or JAK2exon12 mutation
	Minor criteria
1	Subnormal serum erythropoietin level

Diagnosis of PV requires meeting either all3 major criteria, or the first 2 major criteria and the minor criterion Ŧ

*… More than 25% above mean normal predicted value.
Ŧ…Criterion number 2(BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin level > 18.5 g/dl in men (hematocrit 55.5%) or >16.5 g/dl in women (hematocrit 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post –PV MF) (Arber *et al*., 2016).

JAK2 V617F gene mutation in MPN

A specific abnormality was found in 2005 when 4 independent research groups identified a novel obtained somatic mutation in the Janus kinase 2 gene (JAK2) in MPN patients. When a nucleic acid is transmutation from guanine to thymine results in conserved of valine to phenylalanine at codon 617 of the JH2. (Kralovics *et al*., 2005) Detection of JAK2 V617F exon 14 has become the first intention diagnostic test to differentiate between PV and erythrocythemic (IE) from erythrocytosis with a sensitivity of 95% and specificity of 100%. In PV type of MPN the JAK2 V617F mutation composed around 90 -95% of patients while it composed only about 50-60% of ET and MF patients. (Scott *et al*., 2007)

Interleukin -23 (IL-23)

It's a member of the IL-12 cytokine family that also includes IL-12, IL-22, IL-27 and IL-35. This cytokine family is associated with the generation of Th1 cells and the production of IL-12, stimulating innate immunity as well as the development of adaptive immunity and production of IFN. (Gee *et al*., 2009) Interleukin-23 is mainly secreted by activated macrophages and dendritic cells (DCs) located in peripheral tissues (skin, intestinal mucosa and lung). (McKenzie *et al*., 2006) Its production by DCs was controlled by prostaglandin E2, which promotes inflammatory responses. (Hayashi *et al*., 2010) It is considered that IL-23 acts as a pro-inflammatory mediator. This cytokine can induce chronic inflammation through the activation of Th17 cells and the secretion of Th17 by non-T cells. Th17 induces the production of several pro-inflammatory cytokines such as IL-17, TNFB, IL-17F, IL-6, IL-21 and IL-22. All these mediators are involved in chronic inflammatory responses in autoimmune diseases. In addition, IL-23 has a potential role with Th17 effector cytokines in coordinating responses against bacteria. Consequently, it is considered that IL-23 does not play a role in the early phase of Th17 differentiation, but rather in stabilizing and/or amplifying the Th17 phenotype. (Tang *et al*., 2011)

IL-23 plasma levels in patients with JAK2V617F +ve MPN patient

It is believed that cytokines participate in the activation of JAK2V617F; hetero-dimeric cytokine receptors can also activate JAK2V617F. Moreover, the role of cytokines is confirmed by recent reports that have described complete or major molecular remission in patients with PV after long-term immunomodulatorytreatment. (Manshouri *et al*., 2011) Inhibition of JAK2 is able to impair the expansion n of responder T helper 17 (Th17) cells that produce IL-17A. (Betts *et al*., 2011) Che Mat *et al*. suggested a positive feedback regulation of the IL-23 receptor via IL-23-mediated activation of the JAK/STAT pathway. (Che *et al*., 2010)

Vitamin B12 (Cobalamin)

Vitamin B12 is a cobalt-containing coordination compound produced by intestinal micro-organisms and found also in soil and water. Higher plants do not concentrate vitamin B 12 from the soil and so are poor sources of the substance as compared with animal tissues. (https://www.ncbi.nlm.nih.gov/mesh/68014805) Vitamin B12 only referred to cyanocobalamin, which is the first form of cobalamin that was purified. Presently the terms vitamin B12 and cobalamin are used interchangeably, although the term cobalamin is preferred. In the human body, cobalamin exists in multiple forms, of which only two are biologically active as coenzyme.

1- Methylcobalamin acts as a coenzyme with methionine synthase, which is a key enzyme in the folic acid-dependent synthesis of pyrimidines and purines.
2- Adenosylcobalamin is involved in the enzymatic degradation of fatty acids by methylmalonyl CoA mutase. In vivo the various forms of cobalamin can be converted from one to other. In addition, they can be converted into cobalamin analogues by microorganisms of the liver and gut, but most of these analogues are not biologically active. (Lee and Herbert, 1999) Intracellular conversion of vitamin B12 in to two active coenzymes: adenosylcobalamin in mitochondria and methylcobalamin in the cytoplasm. (Sobczyńska-Malefora *et al*., 2014)

Normally the liver stores a cobalamin supply of several milligrams, which is sufficient to cover the daily need for several years. Unbound cobalamin can also penetrate the tissues by means of passive diffusion. However, this

nonspecific process normally has little significance. (Festen, 1992)

Vitamin B12 (Cobalamin) level

Vitamin B12 level is actually a measurement of serum cobalamin. Measurement of vitamin B12 in serum is the most common assay used to evaluate vitamin B12 levels. The test, however, also measures both serum holohaptocorrin and serum holotranscobalamin, and as such may mask true deficiency or falsely imply a deficient state. The test is widely available at low cost and uses an automated method and competitive-binding immune chemiluminescence. (Sobczyńska-Malefora *et al*., 2014)

High serum Cobalamin and blood disorders

High serum cobalamin is an anomaly frequently observed in malignant blood diseases and these essentially involve MPDs, including chronic myelomonocytic leukemia and primary hypereosinophilic syndrome (HES), myelodysplastic syndromes and acute leukemias, notably promyelocytic leukemia. In patients with chronic myelogeneous leukemia (CML), plasma levels of cobalamin are often significantly elevated, sometimes up to 10 times, this is probably related to an elevated production of HC by an increased number of leukocytesbecomes saturated with cobalamin. (Gimsing, 1995) In 30 to 50% of the patients with polycythemia vera (PV) an elevated level of plasma cobalamin is found but less dramatic than in CML, and the elevated plasma concentration of cobalamin is also caused by an increase of HC. The sialic acid-poor form of HC is also increased causing a strongly elevated unsaturated binding capacity for cobalamin. It is assumed that the enlarged pool of mature leukocytes is responsible for this phenomenon. Currently, an elevated level of plasma cobalamin is considered as a minor criterion for the diagnosis PV. (Gimsing *et al*., 1995)

Objectives: the study aimed to:

1. Estimate JAK2 V617F gene mutation in Iraqi patients with PV.
2. Measure the levels of IL-23 and B12 in the PV cases with JAK2V617F +ve mutation.

Subjects and methods

This is a case control study. It was approved by ethical committee of Department of Pathology, College of Medicine at University of Mustansiriyah. All participants gave their oral consent to participate in accordance with declaration of Helsinki.

Patient group: A total of 130 patients presented to national center of hematology during the period from (26 /2/2016 to 10/7/2016). They were diagnosed as MPD cases according to their CBC result, CT-abdomen or abdominal ultrasound and the bone marrow examination which was done to some of them. They were referred for JAK2 V617F mutation analysis. From 48 JAK2 +ve mutation patients, 30 cases were selected who met the diagnostic criteria of PV according to: questionnaire that included their hematological finding by CBC, smoking, previous disease, family history and physical examination including abdominal examination for palpable liver and spleen. (Liver and renal function test was done to all of them to exclude liver and renal impairment).

Control group: Fifteen females and 15 males with age ranging between 22 to 71 years represent the research cases. Control group consisted of 30 age and sex matched volunteers were also taken. CBC, ESR, C-reactive protein, Liver and renal function test was done to all of them. Our population (patient and control) were not on any medication and none of them had symptoms or laboratory signs of active infection, inflammatory disease, or kidney impairment.

Samples preparation method

Six mls of venous blood sample was taken from each individual (patients and controls) by using disposable syringes, then 2ml dispensed in a tube containing EDTA as anticoagulant and kept immediately at 4 °C for DNA extraction. The remaining 4 ml was placed in plain tube with gel (activator clotting tube) for about 30 min at room temperature (allowing the blood to clot). The blood was centrifuged at 4000 rpm for 4 min. The serum was taken and transferred into 2 tubes (one for B12 level measurement and the other for IL-23 level measurement). The 2 tubes were stored in (-80 °C) till time of examination.

PCR method

By using the WizPrepTMg DNA Mini Kit (Blood) which provides a fast (within 2 hr.) and simple method to isolate genomic DNA from patients' serum. Tetra Primer Amplification Refractory Mutation Screening Polymerase Chain Reaction (ARMS-PCR) contain 2 primer pairs to amplify wild and mutant type. These primers were diluted by adding nuclease free water according to the manufacture company's information IDT and Bulgarian. Table (2) shows sequence of primers. PCR steps are detailed in Table (3). (Kim *et al*., 2015)

Table 2. The oligonucleotide PCR tetra primers specific for the JAK2V617F gene

Primers	Nucleotide sequences (5′ ⟶ 3′)	Tm C°
Forward Outer (FO)	5' TCCTCAGAACGTTGATGGCAG 3'	54
Reverse Outer (RO)	5' ATTGCTTTC CTTTTTCACAAGAT 3'	48
Forward inner Wild Type (Fwt)	5'GCATTTGGTTTTAAATTATGGAGTA TATG 3'	52.9
Reverse inner mutant specific primer (Rmt)	5' GTTTTACTTACTCTCGTCTCCACAAA A 3'	55.1

Table 3. Polymerase chain reaction (PCR) program steps and conditions

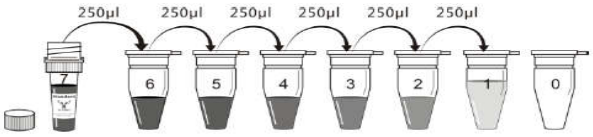
Each PCR step	PCR condition, minutes
Initial denaturation	94 °C for 5 min
2 cycle of denaturation	94 °C for 45 sec
Annealing	55 °C for 45 sec
Extension	72 °C for 45 sec
Final extension	72 °C for 10 min
Cycles number	40

The amplified PCR product was analyzed by agarose gel electrophoresis. Which were visualized using UV-Trans illuminator Scope photographs.In heterozygote three fragments

were generated two small allele specific fragments and a large control product, while two fragments in homozygote

IL- 23 level measurement

Human IL-23 ELISA Kit from CUSABIO was used. Catalog number CSB –E08461h. This assay employs the quantitative sandwich enzyme immunoassay technique. Preparation of standard was carried out according to figure 1. Assay procedure is summarized in Figure (2).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
pg/ml	400	200	100	50	25	12.5	6.25	0

Figure 1. IL-23 standard preparation

The assay procedure of IL-23 is summarized in figure 2.

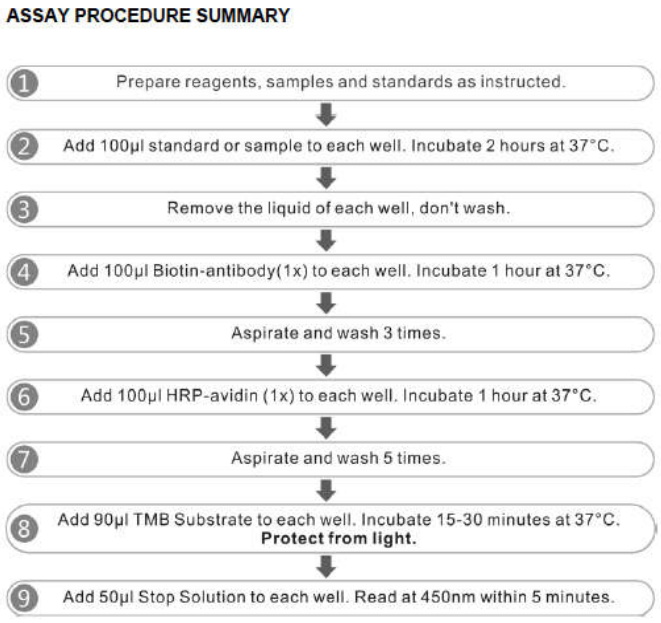


Figure 2. IL-23 assay

Results were measured by automated ELISA reader at 450 nm. A standard curve was plotted and sample readings measured accordingly. (https://www.cusabio.com/ELISA-Kit/Human-Interleukin-23IL-23-ELISA-Kit-84470.html

B12 level measurement

MAGLUMI Vitamin B12 (CLIA) kit was used. Catalog no. 130213002M.The method can be used for samples over the range of 50.0-2000.0pg/ml. The test was performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer Maglumi 800.

Principle of the test: Competitive immunoluminometric assay: Use purified VB12 antigen to label ABEI, and use a VB12 binding-protein to label FITC. Sample, Calibrator or Control with ABEI Label, FITC Label and nano magnetic microbeads coated with sheep anti-FITC are mixed thoroughly and incubated at 37□, forming antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for I time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of VB12 present in samples.

Test procedure is summarized in the following table:

Table 4. B12 assay

Amount	Substance
100µl +50µl +50µl	Sample , Calibrator Displacing reagent Buffer
2min	incubation
+100µl	ABEI label
5 min	Incubation
+20 µl	magnetic microbeads
10 min	Incubation
400µl	Wash cycle
3s	Measurement

Calculation of Results

The analyzer automatically calculates the FA concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in pg/ml. (http://www.ral-sa.com/files/sb605017.pdf)

RESULTS

Demographic Distribution

By using a Pearson chi-square test at 0.05 level, the mean age of PV patients was (52.0 ± 12.5) with a range of (22-71 yrs). While the mean age of the control group was (50.7±12.1 yrs) with a range of (29-70Yrs). With equal male to female ratio (1:1)

Table 5. Distribution of the studied groups according to age, gender and address

		JAK2V617F Positive Polycythemia		Control		P value
		No	%	No	%	
Age (years)	<40	3	10.0	6	20.0	0.758
	40---49	8	26.7	7	23.3	
	50---59	10	33.3	9	30.0	
	=>60	9	30.0	8	26.7	
	Mean±SD (Range)	52.0±12.5 (22-71)		50.7±12.1 (29-70)		0.684
Gender	Male	15	50.0	15	50.0	-
	Female	15	50.0	15	50.0	
Address	Baghdad	22	73.3	30	100	-
	Other	8	26.7	-	-	

11 patients presented with splenomegaly 2 of them have huge spleen.while Hepatomegaly found in only 4 patients. This finding was agree with world study result that mentions (Hepatomegaly is less common symptom in PV than splenomegaly which was considered as one of the diagnostic signs in suspected MPN according to WHO 2008 Diagnostic Criteria)

Table 6. Clinical findings

		JAK2V617F Positive Polycythemia	
		No	%
Splenomegaly	Yes	11	36.7
	No	19	63.3
Hepatomegaly	Yes	4	13.3
	No	26	86.7
Smoking history	Yes	4	13.3
	No	26	86.7
Itching	Yes	14	46.7
	No	16	53.3

Patients history regarding smoking and itching

In our study only 4 patients (13.3% of patients) were smokers. While itching was reported in 14 patients (46.7%) this correlated with occurrence of pruritus mainly after hot path or heavy clothes in winter (as some patient described during history taken) the itching mainly present in patient with high Hb as figure (3) below.

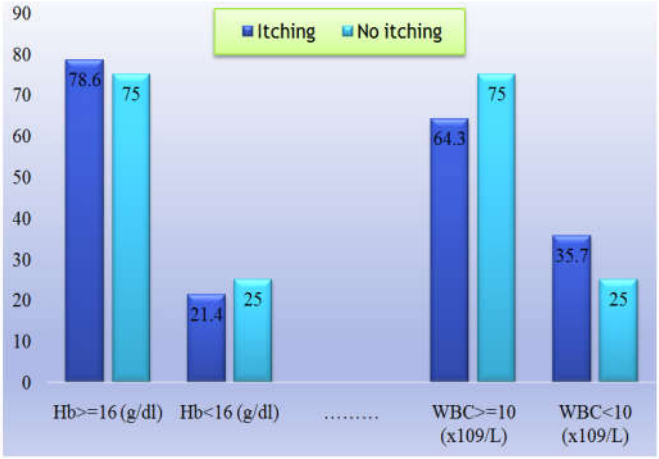


Figure 3. Incidence of itching in relation to Hb conc. And WBC count

Peripheral blood findings (Hb level, WBC, Platelet count) of PV patients groups and healthy control group

There were significant differences between two independent means using Students-t-test at 0.05 levels". "($P < 0.0001$) in (Hb level, WBC, Platelet count) betweenthe two groups of patients and control**.**

Table 7. Hematological parameters in PV cases and control group

	JAK2V617F Positive Polycythemia	Control	P value
Hemoglobin (g/dl)	17.2±1.7 (16.0-21.1)	13.5±1.7 (10.7-15.6)	0.0001 *
WBC (x109/L)	12.3±3.9 (6.5-24.5)	7.7±2.2 (4.0-11.7)	0.0001 *
Platelets (x109/L)	446.0±285.2 (123.0-1364.0)	230.4±90.0 (103.0-469.0)	0.0001 *
ESR (mm/hour)	8.8±3.4 (2.0-15.0)	8.2±3.0 (2.0-13.0)	0.494

*Significant difference between two independent means using Students-t-test at 0.05 level.

JAK2 V617F Gene Mutation detection

In this study, JAK2V617F mutation in peripheral blood of MPN was demonstrated in figure (2.3). The internal control band in all cases is represented by a 463 bp fragment, whereas the JAK2 V617F mutant is represented by a 279 bp fragment, in the presence of wild type JAK2, specific primer is represented by a 229-bp fragment. This technique also allows to distinguish between homozygous and heterozygous individuals with the JAK2 V617F mutation. All of 30 cases suspected as PV according to their hematological finding were positive for JAK2 V617F mutation (7 cases had homozygous type were as 23 with heterozygous) as the electrophoresis picture by using 0.5 % agarose gel show in fig below.

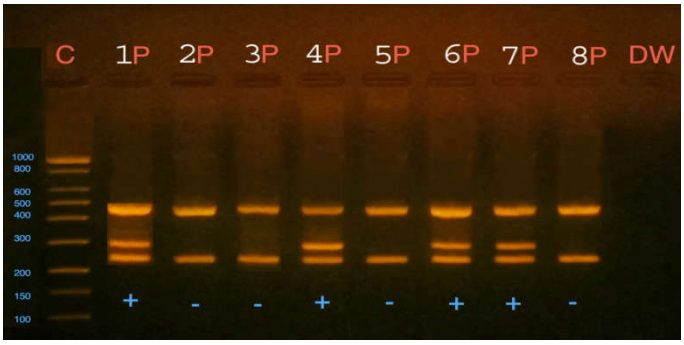


Figure 4. Gel electrophoresis of PCR for JAK2 V617F mutation

(C=Control) band in 463 bp, amplified DNA in a 229 bp fragment, whereas the JAK2 V617F mutant in a 279 bp fragment, 100 bp ladder.(P=Patients samples) 1P,4P,6P:show positive results (heterozygous at 279,229 bp).2P,3P,5P: show negative results .7P: show positive control. 8P: show negative healthy control while (DW=distilled water).

Plasma level of IL-23

Plasma levels of IL-23 were significantly increased in all patients with PV. IL-23 mean was (70.00±72.18 pg/mL) with range (8.5-259.0) in patients in comparison to mean of control group (9.33±7.13 pg/mL) with range (2.3-34.1) with p value <0.0001.

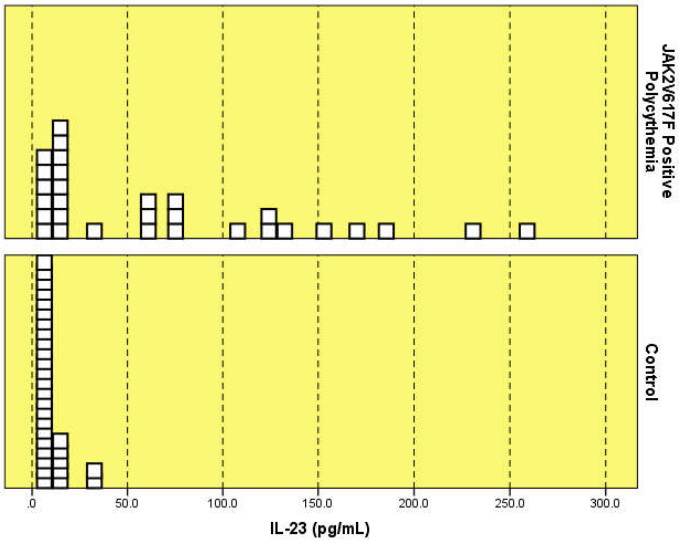


Figure 5. Serum IL-23 levels in Polycythemia Vera patients and controls

The relation between serumIL-23 and CBC data show a significant positive correlation between IL-23 level and the total WBC count. On the other hand, there is no correlation between IL-23 level with Hb level, Platelet count andB12 level as shown in table (8) below.

Table 8. Correlation between IL-23 and hematological parameters

Hematological parameter		IL-23 level in JAK2V617F Positive Polycythemia patients (n=30)
Hemoglobin (g/dl)	r	-0.025
	P	0.894
WBC (x10^9/L)	r	0.367*
	P	0.046
Platelets (x10^9/L)	r	0.037
	P	0.848
B12 (pg/mL)	r	0.133
	P	0.483

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

B12 level result data

It shows a significant difference between PV +ve JAK2V617F patients and control group.The mean of B12 in PV patients were (766.54±583.95 pg/mL) with rang (240.8-2000.0 pg/mL) while in control group the mean was (301.67± 120.29 pg/mL) with rang (164.3-624.0 pg/mL) as in Figure (6) below.

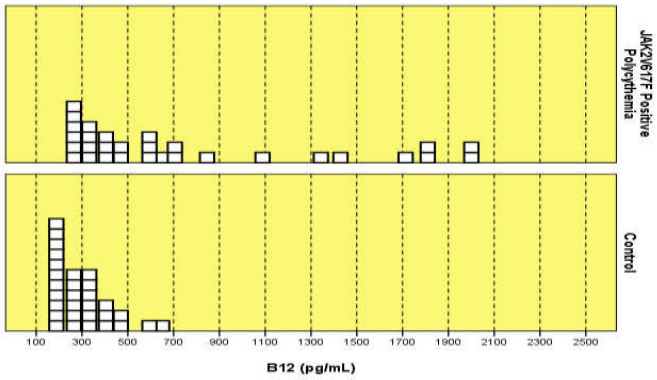


Figure 6. Serum B12 levels in Polycythemia Vera patients and controls

Table 9. Correlation between B12 level and hematological parameters in Polycythemia vera cases

Hematological parameter		B12level in JAK2V617F Positive Polycythemia patients (n=30)
Hemoglobin (g/dl)	r	-0.072
	P	0.706
WBC (x10^9/L)	r	0.068
	P	0.720
Platelets (x10^9/L)	r	-0.161
	P	0.396
IL-23 (pg/mL)	r	0.133
	P	0.483

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

B12 serum level was above normal in 16 patients (53.5%)

Table (9) represents the correlation of B12 level with Hb, WBC and platelet in JAK2V617F Positive Polycythemia patients, there was no correlation between B12 level and Hb level , platelet count, WBC count and IL-23 level, however a very high serum B12 level (more than 1300 pg/ml) were reported in seven patients of PV with higher Hb level.

DISCUSSION

As shown in table (5) it is clear that PV can occur at any age, but the peak incidence is in the 5th to 7th decade of life. "Twenty percent of patients were below the age of 40 years old and there was a PV case in a 22 years old male which was considered as a rare condition, 63% of cases were between 5th to 7th decades of life. Similar findings were reported by other study. (New Drugs Control Symptoms of Myeloproliferative Disorders and Improve Quality of Life for Patients) There was equal male to female ratio (1:1), and this is unusual finding as the disease known to affect males more than females in a (1.8:1) ratio. But this result comparable to areserhstudy on 405 cases of PV which found that the 51% of patients were females, while the males were 49% of all cases. The PV manifestations occurred at a younger age in women compared to men. Therefore, identified gender is important as a potential host modifier, also the gender influenced the JAK2 V617F allele burden within the disease phenotypes and the magnitude of change in the JAK2 V617F allele burden through the course of the disease duration, with women having lower mutational burdens than men.

Splenomegaly

It is considered as one of the diagnostic signs in suspected MPN according to WHO 2008 Diagnostic CriteriaIt was found that 36.7% of patients had splenomegaly as shown in the table (6) comparable to Seon Young Kim study in which he 35%of his PV patient was presented with splenomegaly (Kim *et al*., 2015).

Hepatomegaly

A small percentage of PV patient presented with enlarged liver (13.3%). Hepatomegaly considered as a less common symptom in PV. The liver enlargement may occur because of liver involvement by JAK2 positive maturing hematopoietic cell, also a massive hepatomegaly is reported by the "American Association for the Study of Liver Diseases" on 2010 in a case of 69 years old lady with PV.

Smoking

In our study the total percentage of patients smoking history was 13.3%.It doesn't have a direct effect on disease development or involve in mutation of JAK2 occurrence but it has a big stamp on the patient general health specifically the vascular complication which represent the venous thrombosis in JAK2+ve PV.

Itching

Itching was reported in 46.7% of PV patient as shown in table (6) and Figure (3). In general, the pruritus is a common symptom in MPN cases particularly PV with +ve JAK2 burden correlated with occurrence of pruritus mainly after hot path or heavy clothes in winter as some patient described during history taken. Pruritus in PV patients was reported in other study and it is a common symptom in patients with Philadelphia chromosome negative myeloproliferative disorders. The pathophysiology of MPD-associated pruritus is

unclear. However, Pieri *et al*. (2009) found that basophil count was increased in patients with JAK2V617F positive myeloproliferative neoplasms, and was correlated with the V617F burden. Moreover, ex vivo experiments revealed that pre-treatment with a JAK2 inhibitor reduced PV basophil activation.

Hematological Determinations and Peripheral blood findings

Hemoglobin of all cases was above the normal range for corresponding age and sex with a range between (16-21.1 g/dl) with mean of (17.2 ±1.7 g/dl) (in spite of doing venesection several times for some cases). This finding is matching with WHO criteria for PV as first major criteria emphasize that a PV patient should have Hb more than (16.5 g/dl) in male and more than (16.0 g/dl) in female. There wasa significant difference in WBC count between patient group and control group with mean of (12.3±3.9) while the mean of control group was (7.7±2.2) with p value 0.0001**.** The study also show that the platelet count presented to have a mean of (446.0±285.2) while the mean of control group was (230.4±90.0). The hematological finding was high in general because the PV cause panmylosis.

JAK2 V617F Mutation Gene detection

JAK2 V617F mutation has been included as an essential component (major criteria) in the 2008 WHO diagnostic criteria for PV, ET and PMF. The JAK2 V617F mutation in MPN can be determined by several methods. The precise method was isolate DNA from whole blood leukocytes and used PCR-direct sequencing. In this study, JAK2V617F mutation in peripheral blood of MPN was demonstrated in figure (4). The internal control band in all cases is represented by a 463 bp fragment, whereas the JAK2 V617F mutant is represented by a 279 bp fragment, in the presence of wild type JAK2, specific primer is represented by a 229-bp fragment. The technique allow distinguishes between homozygous and heterozygous individuals with the JAK2 V617F mutation and it was played a key role in acting as a dependable screening assay for the presence or absence of the mutation in individuals with MPN. All of 30 cases suspected as PV according to their hematological finding were positive JAK2 V617F mutation. Finding of this study is in agreement with Tefferi (2012) & Amy *et al.* who found JAK2 V617F mutation is present (<90%) in PV patients. (Tefferi, 2012; Amy V. Jones *et al*., 2005) The study results show that 7 cases had homozygous type were as 23 had heterozygous JAK2 V617F mutation as the electrophoresis picture by using 0.5 % agarose gel show in fig (4).

IL-23 result data

Plasma level of IL-23 were significantly increased in all patients with PV in comparison to controls: 70.00±72.18 pg/mL with range of 8.5-259.0 vs 9.33±7.13 pg/mL and range 2.3-34.1 with a p value <0.0001" as in figure (5). Similar findings was reported in other studies. These results were comparable with many researches that measured the levels of ILs and studied the relations between many ILs and MPN likeHus *et al*. (2011) who found increased frequencies of Th17 cells secreted IL-23 in patients with MPDs disorders. Moreover Gee *et al*. (2009) suggested a positive feedback regulation of the IL-23 receptor via IL-23-mediated activation of the JAK/STAT pathway.Endogenous expression of IL-23 has been reported to promote tumor incidence and growth, and IL-23/IL-23R pathway is a potential route to facilitate the malignant progression of cancers.

The relation betweenIL-23 result data and peripheral blood findings

Table (8) shows the correlation of IL-23 with Hb, WBC and platelet in JAK2V617F Positive Polycythemia patients. There was a significant positive Correlation between IL-23 level and the total WBC count. While there is no correlation between IL-23 level and both Hb level, Platelet count and B12 level.

B12 level result data

B12 serum level was high in 16 of total 30 PV patients included in this study, similarly many researchers reported that an elevated level of plasma cobalamin is found in 30 to 50% of the patients with polycythemia vera. An elevated serum cobalamin level in myeloid proliferations is primarily linked to the release of HCs by tumor granulocytes and their precursors. It has been suggested that the concentration of apo-HC has prognostic value.

The relation between B12 result data and CBC

This is shown in table (9). This table represents the correlation of B12 level with Hb, WBC and platelet in JAK2V617F Positive Polycythemia patients, there was no correlation between B12 level and Hb level, Platelet count, WBC count and IL-23 level, however a very high serum B12 level (more than 1300 pg/ml) were reported in seven patients of PV with higher Hb level. A study by Chiche (2008) *et al* also found a statistically significant association between vitamin B12 levels >1275 pg/ml and the existence of a malignant blood disease, hence suggesting an in depth etiological search for a possible blood disease when plasma levels of vitamin B12 are particularly elevated.

Conclusion

1. Study of JAK2V617F mutation is an important test in MPN patients, particularly in those who suspected to have PV.
2. The diagnosis of PV was based on clinical, haematological and genetic tests. While B.M. biopsy was not performed for the studied cases, it is recommended by WHO as a major criterion to establish a diagnosis of PV (WHO criteria for PV diagnosis, 2016).
3. High levels of IL-23 in the sera of all PV patients that positively correlate with WBC count.
4. Cobalamin levels were elevated in 53.3% of PV.

Recommendations

1. Measure the cytokines levels in bone marrow aspirates sample for more reliable results.
2. Use B.M. aspirate cells cultures to study the effect of adding exogenous IL-23 on the expansion of JAK2 mutations positive cells.
3. Study the correlation between IL-23 level and disease progression, response to treatment and final outcome

would clarify the relationship between IL-23 and Myeloproliferative neoplasms.

4. Measurement of B12 level is of diagnostic value in discriminating polycythemia vera from other MPNs.

REFERENCES

Amy V. Jones, Sebastian Kreil, Katerina Zoi, Katherine Waghorn, Claire Curtis, Lingyan Zhang, Joannah Score, Rachel Seear, Andrew J. Chase, Francis H. Grand and Helen White, 2005. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood First Edition Paper, May 26, 2005; DOI 10.1182/blood-2005-03-1320.

Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, *et al*. 2016. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. BLOOD, 127 (20):2391- 2405

Betts BC, Abdel-Wahab O, Curran SA, St Angelo ET, Koppikar P, Heller G, Levine RL, Young JW. 2011. Janus kinase-2 inhibition induces durable tolerance to alloantigen by human dendritic cell-stimulated T cells yet preserves immunity to recall antigen. Blood, 118(19):5330-9. doi: 10.1182/blood-2011-06-363408. Epub 2011 Sep 13.

Che Mat NF1, Zhang X, Guzzo C. and Gee K. 2011. Interleukin-23-induced interleukin- 23 receptorinpathway in human CD4 T cells. *J Interferon Cytokine Res*., (4):363-71. doi: 10.1089/jir.2010.0083. Epub 2010 Dec 7.

Chiche L, Jean R, Romain F, Roux F, Thomas G, Canavese S, *et al*. 2008. Implications cliniques de la de´couverted' unehypervi- tamine´mie B12 en me´decine interne. *Rev Med Interne*, 29:187–94.

Festen, HPM. 1992. Fysiologie en pathofysiologie van de intrinsic factorsecretie en de cobalamine (vitamine B12)-absorptie. *Ned TijdschrGeneesk*, 136:1851–6.

Gee K, Guzzo C, Che Mat NF, Ma W. and Kumar A. 2009. The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. Inflam. Allergy-Drug Targets, 8, 40-52.

Gimsing P, Petersen CO. and Hippe E. 1995. The cobalamin and cobalamin binding proteins in plasma related to the clinical condition in chronic myelogenous leukemia. *Leukemia*, 9:1604–9.

Gimsing, P. 1995. The cobalamin forms and analogues in plasma and my- eloid cells during the course of chronic myelogenous leukemia related to the clinical condition. *Br J Haematol*., 89:812–9.

Hayashi F, Yanagawa Y, Onoe K. and Iwabuchi K. 2010. Dendritic cell differentiation with pros- taglandin E results in selective attenuation of the extracellular signal-related kinase pathway and decreased interleukin-23 production. *Immunology*, 131:67–76.

http://www.ral-sa.com/files/sb605017.pdf

https://www.cusabio.com/ELISA-Kit/Human-Interleukin-23 IL-23-ELISA-Kit-84470.html

https://www.ncbi.nlm.nih.gov/mesh/68014805

Hus, I., J. Tabarkiewicz, M. Lewandowska, M. Wasiak, P. Wdowiak, M. Kusz, M. Legiec, A. Dmoszynska and J. Rolinski, 2011. Evaluation of monocyte-derived dendritic cells T regulatory and Th17 cells in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors, Folia Histochem. *Cytobiol*., 49, 153–160.

Kawthalkar, SM. 2013. Essentials of Hematology. Second edition, New Delhi. Jaypee brothers. P 289

Kim SY, Im K, Park SN, Kwon J, Kim JA, MD and Lee DS. 2015. CALR, JAK2, and MPL Mutation Profiles in Patients with four Different Subtypes of Myeloproliferative Neoplasms Primary Myelofibrosis, Essential Thrombocythemia, Polycythemia Vera, and Myeloproliferative Neoplasm, Unclassifiable. *Am J Clin Pathol*., 143: 635-644 DOI:10.1309/AJCPUAAC16LIWZ MM

Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR *et al*. 2005. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *New England Journal of Medicine*, 352:1779-90.

Lee GR. and Herbert VD. 1999. Nutritional factors in the production and function of erythrocytes. In: In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, editors. Wintrobe's Clinical Hematology. Vol I. Baltimore: Williams and Wilkins, p. p. 228–267.

Manshouri T, Estrov Z, Quintás-Cardama A, Burger J, Zhang Y, Livun A. *et al*. 2011. Bone marrow stroma-secreted cytokines protect JAK2 (V617F)-mutated cells from the effects of a JAK2 inhibitor. *Cancer Res*., 71(11):3831-40. doi: 10.1158/0008-5472.CAN-10-4002. Epub 2011 Apr 21.

McKenzie BS, Kastelein RA. and Cua DJ. 2006. Understanding the IL-23/IL-17 immune pathway. *Trends Immunol*., 27:17–23.

Nayak R, Rai S. and Gupta A. 2012. Essentials in Hematology and Clinical Pathology. First Edition, New Delhi. Jaypee Brothers. P.133

New Drugs Control Symptoms of Myeloproliferative Disorders and Improve Quality of Life for Patients. The University of Texas MD Anderson Cancer Center, OncoLog, Vol. 56, No. 1.

Pieri L, Bogani C, Guglielmelli P, Zingariello M, Rana RA, Bartalucci N, Bosi A. and Vannucchi AM. 2009. The JAK2V617 mutation induces constitutive activation and agonist hypersensitivity in basophils from patients with polycythemia vera. *Haematologica*, 94(11):1537-45. doi: 10.3324/haematol.2009.007047. Epub 2009 Jul 16.

Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR. *et al*. 2007. JAK2 exon 12 mutations in poly-cythemia vera and idiopathic ery- throcytosis. *N Engl J Med*., 356: 459-468.

Sobczyńska-Malefora A, Gorska R, Pelisser M, Ruwona P, Witchlow B. and Harrington, D. 2014. An audit of holotranscobalamin ("Active" B12) and methylmalonic acid assays for the assessment of vitamin B12 status: application in a mixed patient population. *ClinBiochem*., 47:82-6.

Tang C, Chen S, Qian H. and Huanh W. 2011. IL-23: as a drug for autoimmune inflammatory diseases. *Immunology*, doi: 10.1111/j.1365-2567.2011.03522.x.

Tefferi A. Myeloproliferative neoplasms (2012). The John M. Bennett 80th birthday anniversary lecture. *Leukemia Research*, 36:1481-1489.

****