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IL – 8 Serum Level Estimation in the Iraqi Myloproliferative Neoplasm Patients with and without *JAK2*-V617F Mutation

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ABSTRACT

Introduction: Myeloproliferative neoplasm (MPN) is a long-term blood disease that has an excess production of mature hematopoietic pluripotent stem cells (HPSC) in the bone marrow. The Iraqi cancer registry announced that Chronic Myeloproliferative disorders in male has 0.62% incidence rate and 0.36, in female Chronic Myeloproliferative disorders 45case 0.31% and incidence rate 0.24. The *JAK2-V617F* mutation is approximately 70% presented in the myeloproliferative neoplasm cases. This is a somatic mutation that concern changing of the amino acid valine to phenylalanine at codon region 617 (that's why it's called *JAK2-V617F*) presented in the pseudo kinase domain. *JAK2-V617F* mutation is 50% to 70% in Essential Thrombocythemia (ET), 40% to 50% in Primary Myelofibrosis (PMF), and 95% in Polycythemia Vera (PV). IL-8 elevated level in MPNs has a major part in the presence of symptoms.

Materials and Methods: Total of (60) patients screened by cohort prospective study of having MPN who are patients presented to the National Center of Hematology / AlMustansiriyah university, Depending on *JAK2-V617F* mutation we classified the patients into 3 groups: *JAK2-V617F* positive (N: 40), *JAK2-V617F* negative (N: 20) and control group. Blood sample (5) ml was obtained from each individual in each group, by venipuncture using disposable syringes for IL-8 serum estimation.

Results: A clear indication of significant differences is seen between IL-8 serum level in *JAK2-V617F* negative group and control group ($P < 0.05$). Also, a significant difference occurred among IL-8 serum level in *JAK2*-V617F positive samples and IL-8 serum level of *JAK2*-V617F negative samples.

Conclusion: IL-8 serum level in all MPN patients is high as a part of chronic inflammation, But, IL-8 serum level of *JAK2*-V617F negative group is higher than the *JAK2*-V617F positive group due to many reasons like the source of IL8 is not related to the *JAK2-V617F* mutation.

Keywords: Interleukin 8 serum level, MPN, *JAK2-V617F* negative, *JAK2-V617F* positive.

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INTRODUCTION

Myeloproliferative neoplasms (MPNs) are long-term blood diseases that have an excess production of mature hematopoietic pluripotent stem cells (HPSC) in the bone marrow. In MPNs, there are unusual increases in the output of a specific cell kind. So, MPN includes an incorrect equilibrium in the output of various hematocytes kinds, also unusual output of any given blood cell kind (1). An American hematologist named William Dameshek modeled in 1951 the myeloproliferative disorders, then after that renamed by the World Health Organization (WHO) to Myeloproliferative Neoplasms (MPNs). There are four classic types of myeloproliferative neoplasms: three Philadelphia chromosome negative which are, Essential Thrombocythemia (ET), Primary Myelofibrosis (PMF), and Polycythemia Vera (PV) and one Philadelphia chromosome positive which is Chronic myeloid leukemia (CML) (2).

In US, It is less common in females with 1.4 new cases per 100,000 versus 2.4 new cases per 100,000 in males. The median age of death is about 77 years old while 67.6% of patients have a 5 years survival rate (3). Iraqi cancer registry declared that Chronic Myeloproliferative disorders in male has 0.62% incidence rate and 0.36, in female Chronic Myeloproliferative disorders 45case 0.31% and incidence rate 0.24(4).

Janus kinase (JAK) 2 is a kinase enzyme and a signal transducer , that can add a phosphate group (phosphorylation) to STAT3,5 in the JAK-STAT pathway, which ends with the expression of several hematopoietic growth factor genes, and its mutation causing the development of MPNs. (5) The *JAK2-V617F* mutation occur in about seventy percent of MPNs. This is a somatic mutation that concern changing of amino acid valine to phenylalanine at codon region 617 (*JAK2-V617F*) presented in the pseudo kinase domain. *JAK2-V617F* mutation is about 50% to 70% in ET, 40% to 50% in PMF and 95% in with PV. (6).

Interleukin 8 (IL-8) is a chemokine released from macrophages , different cell types like epithelia , alveolar smooth muscle cells and endothelia(7). Endothelium store interleukin 8 in sacs, the Weibel-Palade bodies (WPBs) (8). CXCL8 gene is the source of IL-8 protein structure (9). Interlukine-8 is firstly released as a source peptide of about 99 amino acids that subject to split to make multiple vigorous IL-8 isoforms. There are multiple receptors presented on the surface membrane that can bind this interleukin; repeatedly thoughtful kinds are the G protein-coupled serpentine receptors CXCR1 and CXCR2(10).

Interleukin-8, recognized as a neutrophil chemotactic factor, has 2 essential tasks. IL-8 creates chemotaxis in the needed cells, fundamentally neutrophils and anther granulocytes, making them go to the position of infection. IL-8 activates phagocytosis immediately after reaching the target cells. Interleukin 8 as well recognized as a powerful originator of making new blood vessels. In targeted cells, interleukin 8 initiates a set of physiological reactions needed for phagocytosis and immigration, like the respiratory burst, exocytosis (e.g. histamine release), and rise in intracellular Ca2+, (9).

Interleukin 8 manifests a remarkable function within MPNs. As a strong chemo-attractant, it is presented within other tumors to initiate angiogenesis, promote leukocyte

chemotaxis/activation, and activate cellular proliferation. A new research showed that IL-8 to be connected with increased standards of circulating progenerators of MPN and the appearance of the major symptoms(11). Here in this clinical research, there will be an estimation of Interleukin-8 serum level in Iraqi MPNs patients that suffer or not suffer from *JAK2-V617F* mutation.

MATERIALS & METHODS

Sample collection

Total of (60) patients screened by cohort prospective study of having MPN who are patients presented to the National Center of Hematological diseases researches and therapy / Al-Mustansiriyah University, Patients were given their consent verbally, the patients age were between 30 to 72 years old , 35 patients were males and 25 were females, the MPNs diagnosis including (PV, ET, PMF) depended on; abdominal ultrasound, complete blood picture, blood film, biochemical, molecular (*JAK2*-V617F mutation), and bone marrow aspirate & biopsy investigations. Depending on the *JAK2-V617F* mutation, classification of the patients were done which divided into 3 groups: *JAK2-V617F* negative (N: 20), *JAK2-V617F* positive (N: 40) and control group (N: 10). Blood sample (5) ml was obtained from each individual in each group, by venipuncture using disposable syringes for IL-8 serum estimation.

Detection of IL-8 levels by ELISA

ELISA kit (Ray Bio) was applied by using the manual of instructions. In short, the microtiter plate was previously covered with anti-Interlukin-8 antibody (Ab) then samples and standards were poured into the suitable wells of the microtiter plates. A biotin-conjugated Ab prepared specifically for Interleukin-8 and avidin conjugated to Horseradish peroxidase (HRP) was poured to every well. After incubation, 3, 3', 5, 5' tetramethyl-benzidine (TMB) substrate solution put in all wells. Specifically the wells that hold Interleukin-8 biotin-conjugated antibody avidin complex showed dye alteration. The enzyme-substrate reaction was stopped by adding (according to the manual), 3 M sulphuric acid solution then dye alteration estimated by the spectrophotometer (ASYS, Australia) at a wavelength (450 nm ± 2 nm). Finally, IL-8 concentration was estimated by matching the optical density of each sample to the standard curve.

Data Analysis

We applied SPSS and used descriptive statistics in addition to differences tests using the t test, and the relationships were studied through correlation coefficient.

RESULTS

Demographic Data

The age was ranged (30 - 72) years, with 35 men and 25 women, diagnosed as MPN patients including (PV, ET, PMF) as in table 1.

Table 1: Demographic Data

Total No. of MPN cases	Age range	Sex		No. of *JAK2 V617F* positive group	No. of *JAK2 V617F* negative group	No. of the control group
		M	F			
60	30 – 72	35	25	40	20	10

Descriptive Statistics of the Interleukin-8 serum level in all MPN and control samples

In Table 2 the mean of Interleukin-8 serum level that belongs to all MPN samples was (93.5500±48.10826) while the mean of the control samples was (87.4000±12.86857). Obviously, the dispersion data of Interleukin-8 serum level in all MPN samples seen higher than control samples.

Table 2: Descriptive statistics of Interleukin-8 serum level in all MPN samples and the control group.

Variable	n	Mean±SD	95% (C.I.) for Mean	
			Lower Bound	Upper Bound
IL-8 serum level in all MPN samples	60	93.5500±48.10826	81.1223	105.9777
Control samples	10	87.4000±12.86857	78.1944	96.6056

Measure the differences between Interleukin-8 serum level in all MPN and the Control samples

A *t* test was used in the case of two independent samples to determine whether there was a difference between Interleukin-8 serum level in all MPN samples with control samples. Table 3 presents the results of the test where the value of *t* is 0.400 with significant level (P > 0.05). A clear indication of no significant differences was observed between Interleukin-8 serum level in all MPN samples and control samples.

Table 3: *t* test study between Interleukin-8 serum level in all MPN samples and the Control samples

Variable	Mean± SE	*t*	DF	Sig. (2-tailed)
Interleukin-8 serum level in all MPN samples and the Control samples	6.15000±15.38940	0.400	68	0.691

Descriptive Statistics of IL-8 serum level in *JAK2*-V617F positive samples and the Control samples

We can see that Table 4 is showing mean of IL-8 serum level in *JAK2*-V617F positive group was (108.3375±52.54087) while the mean of the control group was (87.4000±12.86857). Obviously, the dispersion data of IL-8 serum level in *JAK2*-V617F positive group was higher than the control group.

Table 4: Descriptive statistics of IL-8 serum level in *JAK2*-V617F positive samples and the Control samples

Variable	n	Mean±SD	95% (C.I.) for Mean	
			Lower Bound	Upper Bound
IL-8 serum level in *JAK2*-V617F positive group	40	108.3375±52.54087	91.5341	125.1409
Control group	10	87.4000±12.86857	78.1944	96.6056

Measure the differences between IL-8 serum level in *JAK2*-V617F positive samples and the Control samples

A *t* test used in case of two independent samples to determine whether there was a difference between IL-8 serum level in *JAK2*-V617F positive samples and control samples. Table 5 presents the results of the test where the value of *t* is 1.242 with significant level (P > 0.05). A clear indication was seen of no significant differences between IL-8 serum level in *JAK2*-V617F positive samples and control samples.

Table 5: t test study between IL-8 serum level in JAK2-V617F positive group and control group

Variable	Mean± SE	*t*	DF	Sig. (2-tailed)
IL-8 serum level in *JAK2-V617F* positive group and the Control group	20.93750±16.85968	1.242	48	.220

Descriptive Statistics of IL8 serum level in *JAK2*-V617F negative group and the Control

In Table 6 the mean of IL-8 serum level in *JAK2*-V617F negative samples was (63.9750±11.80597) while the mean of the control samples was (87.4000±12.86857). Obviously, the dispersion data of IL-8 serum level in *JAK2*-V617F negative samples was higher than the control samples.

Table 6: Descriptive statistics of IL-8 serum level in *JAK2*-V617F negative group and the Control group

Variable	n	Mean±SD	95% (C.I.) for Mean	
			Lower Bound	Upper Bound
IL-8 serum level in *JAK2*-V617F negative group	20	63.9750±11.80597	58.4496	69.5004
Control group	10	87.4000±12.86857	78.1944	96.6056

Measure the differences between IL-8 serum level in *JAK2*-V617F negative samples and the Control samples

A *t* test used in case of two independent samples to determine whether there was a difference between IL-8 serum level in *JAK2*-V617F negative samples and control samples. Table 7 presents the results of the test where the value of *t* is 4.391 with significant level (P < 0.05). A clear indication of significant differences is seen between Interleukin-8 serum level in JAK 2 V617F negative samples and control samples.

Table 7: *t* test study between Interleukin-8 serum level in *JAK2*-V617F negative samples and control samples

Variable	Mean± SE	*t*	DF	Sig. (2-tailed)
Interleukin-8 serum level in *JAK2-V617F* negative group and Control group	-23.42500±4.70864	-4.975	28	0.000

Measure the differences between IL-8 serum level in *JAK2*-V617F positive and negative groups

A t test was used in the case of two independent samples to determine whether there was a difference between IL-8 serum level in *JAK2*-V617F positive and negative samples. Table 8 presents the results of the test where the value of t is 3.714 with significant level (P < 0.05). It shows that a clear indication of significant differences between IL-8 serum level in *JAK2*-V617F positive samples and IL-8 serum level of *JAK2*-V617F negative samples.

Table 8: *t* test study between IL-8 serum level in *JAK2*-V617F positive group and IL-8 serum level of *JAK2*-V617F negative group.

Variable	Mean± SE	*t*	DF	Sig. (2-tailed)
IL-8 serum level in *JAK2*-V617F positive group and IL-8 serum level of *JAK2*- V617F negative group	44.36250±11.94326	3.714	58	0.000

DISCUSSION

Fundamentally MPNs contain Essential Thrombocythemia (ET), Primary Myelofibrosis (PMF) and Polycythemia Vera (PV). MPN is an Onco-inflammatory disorder. Obviously there is a strong connection between the chronicity and the MPN pathogenesis. Multiple researches presented cytokine agendas in MPN patients. Anther researches utilized animal models or cell lines to know the role of interleukins in the pathology of MPNs(12). since the finding of the JAK2-V617F mutation as an MPN disease indication in 2005,So many progresses happened in understanding MPN pathogenesis and treatment (13,14). Shortly, different genetic mutations were discovered in MPNs like calreticulin (CALR) and myeloproliferative leukemia protein (MPL) and (15–17).

Scientists suggested that chronicity manifests a crucial function in MPNs development, it is lately submitted by a lot of researches which presented the important function of the inflammation in the start and advancement of MPNs; that's why MPNs are an Onco-inflammatory cancers (18–23). Clinical surveys concentrated on inflammatory biochemical molecules like cytokines which estimated in bone marrow plasma or blood serum from MPNs patients (24).

In our study results, the interleukin 8 serum level of all MPN cases were elevated comparing to the control group, which corresponded with other studies that showed this elevation in interleukin 8 represent a major action within MPNs(25). Others explained that a part of the inflammation is clonal that MPNs clonal cells output increased level of inflammatory interleukins (interleukin-8, IL-9, IL-6, OSM, CCL3 (MIP-1α) and TNF- α (26).

Prolonged genetic researches and murine models failed to complete demonstration most of the chronic blood tumors like MPN. This manifested genetic abnormalities, in spite they are important, they may be not enough for a myeloid or lymphoid origin malignancy to propagate. Plenty of awareness now focused to bone marrow microenvironment with interleukin output, the human microbiome, tumorigenic contagious microorganisms, and the host's immune action(27). The inflammatory condition with Interleukins profile of a MPNs patients are predicted to be differ depending on the existence of MPN subtype , JAK-2, CALR, or MPL mutation, personal genetic background and the ultimate etiology of inflammation preceding MPN-driving mutation (28).

In our study a significant differences seen between IL-8 serum level in JAK2- V617F negative samples and control samples which supported by many studies which explained that the prime inflammatory interleukins are released separately of MPN-linked mutations with manifestation that JAK2-V617F might be delayed incident in MPN progression are stable assuming that long-term activation of myelopoiesis (by inflammatory process) can forego procuration of mutations in JAK-2 (CALR and MPL?) gene(s) in subtypes of MPNs cases(29).Also some researchers said that in MPNs there are absence of engagement between the *JAK2-V617F* load and the serum or blood estimations to those interleukins. Actually, so likely that it is only a part of those interleukins is guided by JAK2-V617F such as interleukin 8, which plentiful released by other than hematopoietic (nonclonal and nonmutated) cells(26,30,31).

Several scientific cohorts have focused on the presence of inflammatory interleukins in *JAK2-V617F* mutated cells or in murine *JAK2-V617F* steering MPNs examples. Yet, some studies understood that, in vitro *JAK2-V617F* may rise output of interleukin 8, interleukin 6, OSM, interleukin 9, CCL4, TNF- α, and CCL3(32–34) and that explain out reported data about the presence of a significant difference between IL-8 serum level in JAK2-V617F positive group and IL-8 serum level of JAK2-V617F negative group which is clearly presented in our study.

CONCLUSIONS

IL-8 serum level in all MPN patients is high as a part of chronic inflammation, But, IL-8 serum level of *JAK2*-V617F negative group is higher than the *JAK2*-V617F positive group due to many reasons like the source of IL8 is not related to the *JAK2*-V617F mutation.

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