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Assessment of interleukin-10 level and Janus kinase 2 V617F mutation incidence in patients with primary myelofibrosis

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Abstract:

BACKGROUND: Myelofibrosis (MF) is largely documented by an abnormal cytokine expression, which in turn could contribute to bone marrow fibrosis, angiogenesis, and constitutional symptoms. To gain additional pathogenetic insight regarding cytokine phenotype correlations in MF, this study estimated the level of interleukin-10 (IL-10) abnormality and Janus kinase (JAK2 V617F) mutation in primary MF.

OBJECTIVE: The objective of this study was to assess serum IL-10 level and it's relation with the presence of JAK2 V617F mutation in patients with MF.

MATERIALS AND METHODS: JAK2 V617F mutation detection was performed in 32 patients with MF using amplification refractory mutation screening-polymerase chain reaction. IL-10 level was estimated in 36 patients with MF using enzyme-linked immunosorbent assay technique compared to 27 healthy controls who were enrolled for comparison.

RESULTS: The study showed higher significant ($P \leq 0.002$) increase of IL-10 in patients with MF with cutoff value ≥8.9510 pg/ml and area under the curve value of 0.749 ($P < 0.001$) and also in patients with serum level of IL-10 more than 13.6 pg/ml characterized with significant lower white blood cell count, nonsignificant difference with lower hemoglobin level, normal platelet count, and smaller size splenomegaly. About 59% of the studied primary MF (PMF) patients had JAK2 positive, 63% of them were male. There was no significant correlation between IL-10 and JAK2.

CONCLUSION: JAK2 mutation and IL-10 as anti-inflammatory cytokines may play a role in the pathogenesis and hematological presentation of patients with PMF. High IL-10 level may predict good prognosis in patients with PMF.

Key words:

Interleukin-10, Janus kinase 2 V617F mutation, myelofibrosis

Primary myelofibrosis (PMF) is a clonal myeloproliferative neoplasm of the pluripotent hemopoietic stem cell, in which the proliferation of multiple cell lineages is accompanied by progressive bone marrow fibrosis, and also it is associated with anemia, thrombocytopenia, often progressive splenomegaly, hepatomegaly, and various debilitating symptoms including the constitutional symptoms of fever, weight loss, and night sweats.[1]

Molecular pathogenesis of MF is poorly understood[2] but deregulation of kinase activity has emerged as a major mechanism by which cancer cells evade normal physiologic constraints on growth and survival. Janus kinase (JAK V617F) activates several signaling pathways crucial for cellular survival and proliferation, supporting that the bone marrow histologic changes that characterize myelofibrosis (MF) are reactive and mediated by cytokines.[3]

Cytokine levels in MF have been shown to predict response to treatment with immunomodulating agents,[4] and JAK inhibitor therapy-induced downregulation of pro-inflammatory cytokines has been correlated with response in constitutional symptoms.[5] The inflammatory cytokine-suppressing activity of JAK1/JAK2 inhibition has also been demonstrated in a murine model of MPN, using another JAK1/JAK2 inhibitor (CYT387).[6]

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Interleukin-10 (IL-10) is a regulatory cytokine produced by T-cells, B-cells, keratinocytes, monocytes, macrophages, mast cells, eosinophils, and placental trophoblasts.[7] It potently downregulates the production of macrophage pro-inflammatory cytokines such as IL-1, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor, and notably tumor necrosis factor-α, through a feedback inhibition loop. However, in addition to these anti-inflammatory effects, IL-10 promotes B-cell activation, regulates immunoglobulin class switching, and maintains B-cell viability by inhibiting apoptosis.[8,9]

These observations strongly suggest the contribution of abnormal JAK-STAT signaling not only to clonal myeloproliferation but also to cytokine-driven debility.[10]

From this review, this study aimed to assess the IL-10 level in patients with PMF and compare its level in relation to the presence of JAK2 mutation and blood index counts of patients with PMF.

Materials and Methods

Patients

This prospective multicenter study was approved by the Review Ethical Committee of hematology which included 36 Iraqi patients with PMF between January 2014 and December 2015. All patients were newly diagnosed, and patients who did not receive any specific treatment related to PMF and chronic illness were excluded from the sampling. Peripheral blood indices and spleen size were taken from patients' records at the time of sampling and diagnosis. Screening for IL-10 levels was performed in all patients, and also polymerase chain reaction (PCR) for JAK2 V617F mutation screening test was performed in 32 patients only. Another 27 samples were also taken from healthy persons as control group.

Sample collection

Five milliliter of peripheral blood was collected from patients with PMF, 2 ml was dispensed in ethylenediaminetetraacetic acid (EDTA)-containing tube as anticoagulant and kept immediately at 4°C for DNA extraction for JAK2 V617F mutation detection while 3 ml was dispensed in plain tube, and serum specimens were separated and then stored at −20°C until using in the determination of IL-10 concentrations.

Serum interleukin-10 estimation

The concentration of IL-10 in patient and control serum samples was determined by commercial quantitative sandwich enzyme-linked immunosorbent assay (Quantikine, R & D Systems, Minneapolis, USA).

Analysis of Janus kinase 2 V617F mutation

Genomic DNA was extracted from 300 µl EDTA blood using Wizard DNA Purification Kit (Promega, USA). Amplification-refractory mutation screening-PCR technique was used according to the procedure suggested by Nadali *et al*.[11] This technique depends on using four primers, 2 forward and 2 reverse primers to produce three potential PCR bands (463bp, 279bp, and 229bp). In all cases (normal and mutant), the band of size 463bp generates a control band, while the 229bp band represents the wild-type allele and the 279bp band represents the mutant allele. The PCR reaction was conducted in a thermal cycler (C 1000 thermal cycler, BIO-RAD, USA), the PCR product subjected to electrophoresis using 2% agarose gel for 45 min (10 volts/cm^2) was amplified, and then visualized using desktop gel imager (Scope 21 UV Transilluminator, China) as shown in Figure 1.

Results

The studied samples consist of 36 patients with PMF; general characteristics were shown in Table 1, the mean age of patients with PMF is 59.53 ± 10.35 years, with male:female ratio of 1.25:1 while the control group consists of 27 samples with mean age of 54.69 ± 11.04 years. The percentage of JAK2 mutation in the studied patients is shown in Figure 2, about 59% of the patients with PMF were JAK positive. The differences in the mean level of different blood indices according to JAK2 mutation result are shown in Table 2. About 63% of the patients with PMF with JAK2 positive were male and 50% of the patients were in the age between 50 and 69 years. Patients with JAK2 positive had insignificant higher white blood cell (WBC) and platelet count, and also they had significant higher hemoglobin level than patients with JAK2 negative as shown in Figure 3. There was no significant difference in spleen size between JAK2-positive and JAK2-negative patients with PMF.

The statistical analysis of IL-10 level among patient group versus control group according to JAK2 mutation is shown in Tables 3 and 4. There is a significant higher IL-10 level in

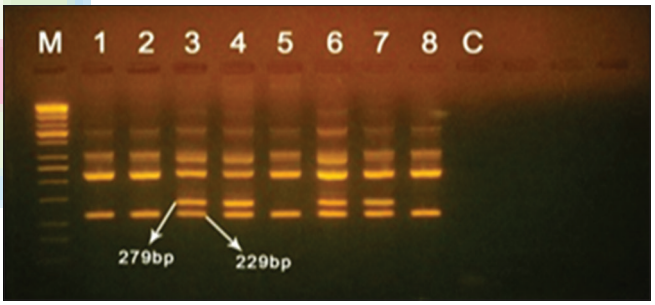

Figure 1: Amplification refractory mutation screening-polymerase chain reaction amplification results for Janus kinase 2 V617F mutation. Lane M: 100 bp DNA ladder, Lane 1, 2, 5, 8 show negative result, Lane C show negative control (water) and Lane 3, 4, 6, 7 show positive result

Table 1: Distribution of patients and control groups according to age and gender with the mean level of blood indices count and spleen size among patients group

	MF patients	Control	*P*
Number of patients	36	27	-
Age (mean±SD)/year	59.53±10.35	54.69±11.04	0.083[a]
Male (%)	20 (55.6)	11 (40.7)	0.311[b]
Female (%)	16 (44.6)	16 (59.3)	
Male:female ratio	20:16 (1.25:1)	11:16 (0.6:1)	
WBC count × 10^9	14.98±14.99		
Platelets count × 10^9	290.79±290.80		
Hemoglobin (g/dl)	10.24±10.25		
Spleen size (cm below intercostal margin)	22.22±22.23		

[a]*t*-test, [b]Chi-square. *P*<0.05 considered significant. SD=Standard deviation, WBC=White blood cell, MF=Myelofibrosis

Table 2: The mean level difference of blood count indices among patients group according Janus kinase 2 mutation

	JAK2 positive (%)	JAK2 negative (%)	OR (95%CI)	*P*
Male, *n* (%)	12 (63.2)	7 (36.8)	-	-
Female, *n* (%)	7 (53.8)	6 (46.2)	-	-
Age (years), *n* (%)				
<50	3 (60)	2 (40)	-	-
50-69	10 (50)	10 (50)	-	-
>70	6 (85.7)	1 (14.3)	-	-
Mean WBC count × 10^9	17.02±9.57	12.22±9.70	1.059 (0.973-1.152)	0.183
Mean PLT count × 10^9	354.28±244.55	200.92±156.43	1.004 (1-1.009)	0.077
Hb (g/dl)	11.28±2.80	9.31±1.30	1.541 (1.013-2.344)	0.043
Spleen size/cm below intercostal margin	22.11±2.88	22.69±3.63	0.942 (0.729-1.216)	0.646

P value of binary logistic regression. Data presented using mean±SD. OR=Odd ratio, CI=Confidence interval, SD=Standard deviation, WBC=White blood cell, PLT=Platelet, Hb=Hemoglobin, JAK=Janus kinase

Table 3: The mean level difference of interleukin-10 level among patients and control groups

	PMF patients	Control	*P*
IL-10 pg/ml	11.29±6.19	7.57±2.68	0.002

Two-sample *t*-test. IL=Interleukin, PMF=Primary myelofibrosis

Table 4: The mean level difference of interleukin-10 level according Janus kinase 2 among patients group

	JAK2 positive	JAK2 negative	OR (95%CI)	*P*
IL-10 pg/ml	11.81±7.44	10.59±4.47	1.033 (0.916-1.166)	0.593

Data presented using mean±SD. *P* value of binary logistic regression. OR=Odd ratio, CI=Confidence interval, SD=Standard deviation, JAK=Janus kinase

Table 5: Receiver operating characteristic of the possibility to use interleukin-10 to differentiate myelofibrosis patients from control

	AUC	*P*
IL-10 pg/ml	0.749 (0.629-0.87)	0.001

AUC=Area under the curve, IL=Interleukin

Figure 2: Percentage of Janus kinase 2 mutation among primary myelofibrosis patients

patients with PMF than those of control group, and there is no significant difference in IL-10 level between JAK2-positive and JAK2-negative groups as shown in Figure 4.

The IL-10 level had fair ability to differentiate patients from control (area under the curve [AUC] >0.7, but <0.8 which at level indicate good differentiation), the optimal cutoff point (≥8.9510) at which it will differentiate patient from control; any value above this point highly associated with MF patients with high specificity 88.9% and low sensitivity 55.6%, so we can conclude that it is specific test rather than sensitive one as in Tables 5, 6, and Figure 5 illustrate this results. The IL-10 level of ≥13.6 pg/ml in PMF showed significant lower WBC count, insignificant normal platelets count, lower hemoglobin level and smaller size splenomegaly. This difference in mean of selected measurement by ordered categories of serum IL-10 among cases with PMF illustrated in Table 7.

Discussion

The mean age of the Iraqi PMF patients in this study was 59.5 ± 10.35 (41–80) years, with slight male predominance as male:female ratio (1.25:1). More than half of the studied PMF patients were JAK2 positive, of these patients group, about two-third were male gender and more than 80% of them with age more than 50 years old, give us an idea that JAK2 mutation has an essential role but not the only one causing MF and this result same as other studies where they used sensitive methods and demonstrate the presence of JAK2 mutation in PMF over 50% of cases.[12,13]

The JAK2 is critically involved in cell growth, survival, development and differentiation of hematopoietic, it's provide the principal signaling pathway for a variety hematopoietic cytokines and growth factors that depend on signal transmission by cytoplasmic NRTKs[14] also Geissler *et al.*,[15] study showed an increased autonomous CFU-GM growth is an *in vitro* characteristic of MF which may reflect aberrant hematopoiesis *in vivo*. In this study, mean level of IL-10 in PMF patients was significant higher than the control group (11.29 ± 6.19 vs. 7.57 ± 2.68 pg/ml consequently) (*P* = 0.002), without significant differences between JAK2 positive and JAK2 negative patients. This result was same that found by Tefferi *et al.* and Vaidya *et al.*[10,16] studies where IL-10 was significant higher in PMF studied group. Patients with JAK2 mutation positive are less likely to have anemia than those without JAK2 mutation, which may be due to uncontrolled release of hormone or cytokines like erythropoietin (EPO) caused by abnormal continuous activation JAK2 receptors, in contrast to WBC count, platelets count and spleen size where the study showed no significant difference with JAK2 mutation.

The level of IL-10 in PMF patients at the 0.749 pg/ml (0.629–0.87) was highly significant AUC value (*P* = 0.001) with the cutoff level of ≥8.95 has specificity 88.9% and sensitivity 55.6%. It's seems patients with PMF and IL-10 level of more

Table 6: Optimal cut-off value of various variables that can differentiate between patients and controls using receiver operating characteristic test

Cut-off IL-10 pg/ml	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV at pretest probability (%)		NPV at pretest probability (%)
				50%	90%	10%
≥8.9510	55.6	88.9	69.9	83.4	97.83	94.7

Value more than the cut-off indicate more probability of MF patients. PPV=Positive predictive value, NPV=Negative predictive value, IL=Interleukin, MF=Myelofibrosis

Table 7: The difference in mean and median of selected measurements by ordered categories of serum interleukin-10 among primary myelofibrosis patients

	Serum IL-10 (pg/ml) among cases-quartiles			*P*
	First (lowest) quartile (≤7.2)	Average (interquartile range) 7.3-13.5	Fourth (highest) quartile (13.6+)	
WBC count				
Range	4.8-39.5	1.83-28.64	4.33-19.54	0.037
Mean±SD	21.6±13.1	13.3±7.9	12.3±5.1	
Platelets count				
Range	118-633	43-717	69-772	0.41 (NS)
Mean±SD	355.1±160.6	257.6±194.6	274.1±234.8	
Hb (g/dl)				
Range	9.6-15.3	7.7-16.3	6.4-14.5	0.07 (NS)
Mean±SD	11.8±2.2	9.8±2.2	9.7±2.8	
Spleen size				
Range	20-28	17-28	16-27.3	0.14 (NS)
Mean±SD	24.2±3	21.8±3	21.5±4.1	

SD=Standard deviation, WBC=White blood cell, NS=Not significant, Hb=Hemoglobin

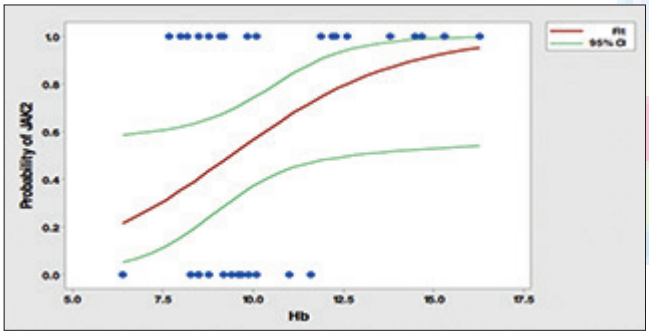

Figure 3: Probability plot of the relationship between hemoglobin and Janus kinase 2

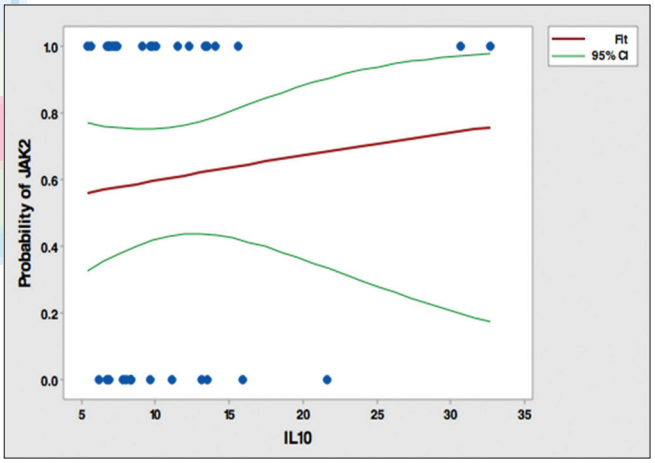

Figure 4: Probability plot of the relationship between interleukin-10 and Janus kinase 2 mutation

than 13.6 pg/ml had significant lower WBC count with lower hemoglobin level, normal platelets count and smaller size splenomegaly although they were not statistically significant. Because of IL-10 is a cytokine synthesis inhibiting action, so we speculated that IL-10 may inhibit pathological overproduction of myeloid cells in MF by suppression of autonomous myelopoiesis. As Vainchenker *et al.* said, JAK1 and JAK2 are involved in interferon-γ signaling and physically associate with receptors for type II cytokines such as IL-6, IL-10, IL-11, IL-19, IL-20 and IL-22; JAK2 is activated by hormone like cytokines such as growth hormone, prolactin, EPO, thrombopoietin, as well as those involved in hematopoietic cell development including IL-3 and granulocyte macrophage colony stimulating factor.[17] Studies with Janus-associated kinase 1 (JAK1)/JAK2 inhibitor drugs have demonstrated drug induced downregulation of pro-inflammatory cytokines which was accompanied by improvement in constitutional symptoms.[5] These observations suggest the presence of a cytokine signature in PMF that might provide additional pathogenetic insight, affect prognosis and serve as a laboratory tool for predicting and monitoring treatment response.[10]

Conclusion

JAK2 mutation and IL-10 may play a role in pathogenesis and hematological presentation of PMF patients. IL-10 as anti-inflammatory cytokine regardless presence of JAK2 mutation, may support the idea of IL-10 inhibition of apoptosis through its regulatory effect of immunoglobulin by promotes B cell activation which may supporting other studies.

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Nil

Conflicts of interest
There are no conflicts of interest.

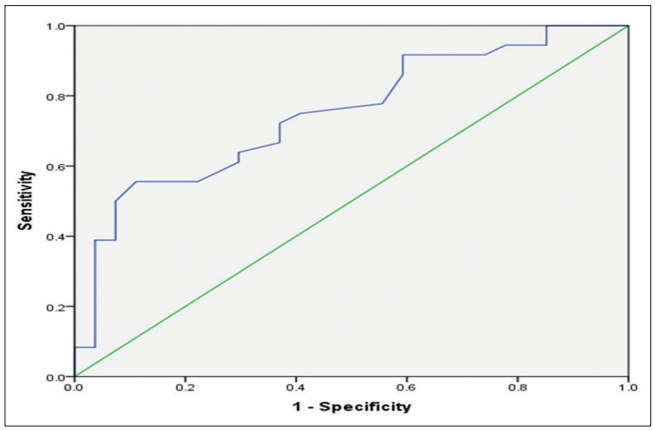

Figure 5: Receiver operating characteristic of interleukin-10 to differentiate myelofibrosis patients from control

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