Evaluation of Clinical , Laboratory and Genetic Parameters in Patients with Primary Myelofibrosis in Nineveh Province

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ABSTRACT

Background : Primary myelofibrosis is a Philadelphia-negative myeloproliferative neoplasm(MPN) with predominant proliferation of megakaryocytes and granulocytes in the bone marrow characterized by initial proliferative phase, followed by a reactive deposition of fibrous connective tissue in the bone marrow.

Objectives : To evaluate the clinical and laboratory data in primary myellofibrosis and to correlate the genetic disorders with clinical and other laboratory parameters.

Setting : This case series study conducted at Ibn-Sina Teaching Hospital/Outpatients Hematology Department from November 2019 to April 2020.

Patients and method : Total fifteen patients with primary myelofibrosis included in this case series (retrospective and prospective) study. The records of old cases were reviewed from patients for clinical history, clinical examination, previous blood counts, bone marrow study and genetic study. For new cases clinical history and physical examinations were noted, ten mls of venous blood were aspirated from patients (new cases) by clean venipuncture and delivered into sterile EDTA and gel tubes for doing complete blood pictures, genetic study (*JAK2*) and biochemical tests. Data were collected and analyzed by using SPSS software version 24.

Result : The males were (60%) of patients and females were (40%) with male to female ratio was 1.5:1, their age ranged from 43-75 years with a mean of 59.3 years. The most common complains were fatigue (86.7%), fullness in the abdomen (73.3%) and constitutional symptoms (66.7-80%). All patients(100%) had splenomegaly at presentation. The total mean Hb was 8.2 g/dl with a range of 4.7-12.5 g/dl. Leukocytosis had been observed in (40%) of patients, while thrombocytosis was found in (26.6%) of patients. Compared in hematological parameters in PMF patients with *JAK2* Positive and *JAK2* Negative, it was found that MPV significantly increased in *JAK2* positive patients with p-value 0.03 .

Conclusion : old age groups (55-64 years) were commonly affected by primary myelofibrosis and males were predominance over females. The most common complains were fatigue and constitutional symptoms, while splenomegaly was the most common clinical sign. The mean platelets volume (MPV) significantly increased in *JAK2* positive patients with p-value 0.03.

Keywords : primary myelofibrosis , clinical , laboratory parameters , JAK2 .

تقييم المعايير السريرية ، المختبرية والجينية لدى المرضى المصابين بتليف نخاع العظم الأولي في محافظة نينوى

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الخلاصة

خلفية البحث : التليف النقوي الاولي (تليف نخاع العظم) هو ورم تكاثري نقوي سلبي فيلادلفيا مع تكاثر سائد للخلايا العملاقة والخلايا المحببة في نخاع العظم حيث تتميز بمرحلة تكاثرية أولية ، يتبعها ترسب تفاعلي للنسيج الضام الليفي في نخاع العظم.

الاهداف : لتقييم البيانات السريرية والمختبرية في تليف النخاع الأولي ، كذلك لربط الاضطرابات الوراثية بالمعايير السريرية والمختبرية الأخرى.

المكان والزمان : اجريت دراسة سلسلة الحالات في العيادة الخارجية لمستشفى ابن سينا التعليمي/ قسم امرض الدم من شهر نوفمبر ٢٠١٩ الى أبريل ٢٠٢٠ .
طرق البحث : تم تضمين إجمالي خمسة عشر مريضًا مصابًا بالتليف النِقْوِيّ الأولي في دراسة سلسلة الحالات(اثر رجعي ومستقبلي). بالنسبة للحالات القديمة, تمت مراجعة التاريخ السريري والفحص السريري وتعداد الدم السابق ودراسة نخاع العظم والدراسة الجينية من المرضى. بالنسبة للحالات الجديدة، سجل تاريخ المرض والفحوصات السريرية, تم سحب عشرة مليليتر من الدم الوريدي للمرضى عن طريق بزل الوريد النظيف ونقلها الى انابيب EDTA وانابيب جل المعقمة لعمل صورة الدم الكاملة, الدراسة الجينية والفحوصات البيوكيميائية. تم جمع وتحليل البيانات باستخدام برنامج SPSS الاصدار ٢٤.
النتائج : كان الذكور (٦٠٪) من المرضى والإناث (٤٠٪) وكانت نسبة الذكور إلى الإناث ١.٥: ١ وأعمارهم تراوحت بين ٤٣-٧٥ سنة بمتوسط ٥٩.٣ سنة. الاعراض الأكثر شيوعاً هي التعب (٨٦.٧٪) ، امتلاء البطن (٧٣.٣٪) والأعراض البنيوية (٦٦.٧-٨٠٪). جميع المرضى (١٠٠ ٪) لديهم تضخم في الطحال عند التقديم. كان متوسط الهيموكلوبين الكلي ٨.٢ جم / ديسيلتر بنطاق ٤.٧-١٢.٥ جم / ديسيلتر. لوحظ زيادة عدد الكريات البيضاء في (٤٠٪) من المرضى ، بينما لوحظ كثرة الصفيحات في (٢٦.٦٪) من المرضى. بالمقارنة مع المعلمات الدموية في مرضى تليف النخاع الاولي مع JAK2 إيجابي و JAK2 سلبي ، وجد أن حجم الصفائح الدموية زاد بشكل ملحوظ في المرضى الايجابيين JAK2 بقيمة p 0.03.
الاستنتاج : تأثرت الفئات العمرية (٥٥-٦٤ سنة) بشكل شائع بالتليف النقوي الأولي وكان الذكور هم المسيطرون على الإناث. كانت الاعراض الأكثر شيوعًا هي التعب والأعراض البنيوية ، بينما كان تضخم الطحال هو العلامة السريرية الأكثر شيوعًا. زاد متوسط حجم الصفائح الدموية بشكل ملحوظ في المرضى الموجبين لـ JAK2 بقيمة p 0.03.

الكلمات المفتاحية : تليف النخاع الاولي ، السريرية ، المختبرية ، طفرة JAK2 .

INTRODUCTION

Myelofibrosis (MF) is one of various Philadelphia-negative MPNs, which are derived from pluripotent, hematopoietic myeloid precursors [1,2]. It is categorized by stem cell-derivative clonal myeloproliferation, atypical cytokine expression, extramedullary hematopoiesis (e.g., splenomegaly), fibrosis of the bone marrow, cytopenias and constitutional symptoms like fatigue, weight loss, night sweats, fever and pruritis [3,4]. It is most frequently categorized by *JAK2* (V617F) mutations that can be noticed in nearly 40-60% of cases[5] , whereas MPL mutations are established in nearly 5% of primary myelofibrosis patients [6,7]. Mutations of calreticulin are commonly exclusive with both *JAK2* and *MPL* mutations and are established in nearly 20-25% of all PMF patients [8]. Myelofibrosis patients with *CALR* mutations were young and presented with lower frequencies of anemia, leukocytosis, higher platelet count [9]. Bone marrow failure, thromboembolic events and transformation to acute myeloid leukemia are the main causes of morbidity and mortality, but additional symptoms secondary to hepatosplenomegaly and abnormal blood counts frequently impair quality of life. Despite the recent advances in development of the targeted therapies, allogenic hematopoietic stem cell transplantation(allo-HSCT) remains the only curative option available for myelofibrosis [10].

AIMS OF STUDY

1. To evaluate the clinical and laboratory data in primary myellofibrosis in Nineveh province.
2. To assess the value of genetic study (*JAK2*) in the diagnosis using World Health Organization (WHO) and British Committee for Standards in Haematology (BCSH) criteria
3. To correlate the genetic disorders with clinical and other laboratory parameters.
4. To evaluate the differences between the two popular classification protocols (WHO and BCSH) for primary myelofibrosis.

PATIENTS AND METHODS

A total of 15 cases who previously diagnosed or suspected to have primary myelofibrosis were enrolled from Outpatients Hematology Department of Ibn-Sina Teaching Hospital from November 2019 to April 2020. They involved 11 old cases and 4 new cases. The records of old cases were reviewed from patient for clinical notes, clinical examination, previous blood counts, bone marrow study and genetic study, when they visited the hospital for follow-up . For new cases, clinical history was taken from patients and physical examination (mainly for assessment the presence of pallor, ecchymosis and organomegaly) was done. Ten mLs of venous blood were aspirated from patients (new cases) by clean venipuncture and delivered into sterile EDTA tubes (3ml), then stored at 4◦C to be used within three days for molecular study, second EDTA tubes (2ml) for doing complete blood pictures using (NIHON KOHDEN and Hycount 3N Hycel Coulter counter)

and (5mls) in gel tubes for serum uric acid and lactate dehydrogenase (LDH) using COBAS 1-11 from Roche according to instruction of the kits.

Detection of JAK2 V617F mutation done by Amplification of DNA according to OncoReal JAK2 V617F Kit from (Ingenetix GmbH) method.

Ethical Issues: This study was approved by the Medical Research Ethics Committee, College of Medicine, University of Mosul, and Nineveh Health Directorate. Additionally, written informed consent for was obtained from patients before examination and taking blood sample.

STATISTICAL ANALYSIS

Data were collected and analyzed by using SPSS software version 24. The results were expressed as mean ± SD for quantitative variables, while qualitative variables are presented as frequency and percentages. Independent t-test was used for comparison of means. A p value < 0.05 was considered to indicate a statistically significant difference.

RESULT

The number of cases were 15 patients with bone marrow diagnosis of primary myelofibrosis. Their ages ranged from 43 to 75 years with mean age 59.3 years. They included 9 (60%) males with mean age 57.2 years (range 43-74 years) and 6 (40%) females with mean age 62.5 years (range 50-75 years). Male : Female ratio was 1.5: 1. Most patients were in their 6th and 7th decade of life at the time of diagnosis (Figure 1).

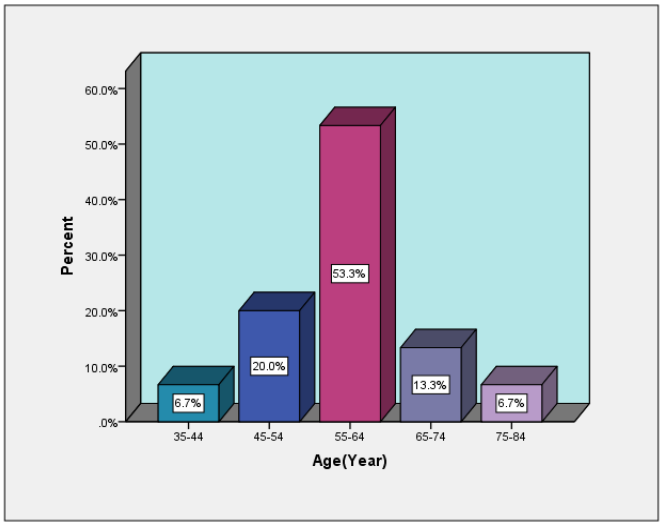

Figure (1): Age distribution of patients with primary myelofibrosis

The most common presenting symptoms were fatigue 13(86.7%), weight loss 12 (80%), anorexia 11(73.3%). night sweats 11(73.3%) dizziness 11(73.3%) and fullness in the abdomen 11(73.3%). All patients (100 %) had splenomegaly at presentation (Table 1). Bleeding was present in 5 (33.3%) patients, epistaxis was the most common one (20%).

Table (1): Clinical presentation and observation of patients with PMF

Clinical presentation		Sex		Total (n=15)
		Male (n=9)	Female (n=6)	
Fatigue	No.	7	6	13
	%	46.7%	40.0%	86.7%
Dyspnea	No.	6	4	10
	%	40.0%	26.7%	66.7%
Dizziness	No.	5	6	11
	%	33.3%	40.0%	73.3%
Blurred vision	No.	2	4	6
	%	13.3%	26.7%	40.0%
Burning pain after hot bath	No.	4	3	7
	%	26.7%	20.0%	46.7%
Thrombosis	No.	0	1	1
	%	0.0%	6.7%	6.7%
Bleeding	No.	3	2	5
	%	20.0%	13.3%	33.3%
Pruritus	No.	3	1	4
	%	20.0%	6.7%	26.7%
bone pain	No.	5	4	9
	%	33.3%	26.7%	60.0%
fullness in the abdomen	No.	6	5	11
	%	40.0%	33.3%	73.3%
Left hypochondrial pain	No.	1	2	3
	%	6.7%	13.3%	20.0%
Fever	No.	5	5	10
	%	33.3%	33.3%	66.7%
night sweats	No.	6	5	11
	%	40.0%	33.3%	73.3%
Weight Loss	No.	7	5	12
	%	46.7%	33.3%	80.0%
Anorexia	No.	6	5	11
	%	40.0%	33.3%	73.3%
Pallor	No.	6	5	11
	%	40.0%	33.3%	73.3%
Splenomegaly	No.	9	6	15
	%	60.0%	40.0%	100.0%
Hepatomegaly	No.	4	2	6
	%	26.7%	13.3%	40.0%

The total mean Hb was 8.2 g/dl with a range of 4.7-12.5 g/dl. The most common type of anemia was normochromic normocytic. Nucleated red blood cells presented in peripheral blood of 9 (60%) patients. Leukocytosis had been observed in 6 (40%) patients, while immature cells were found in peripheral blood of 8 (53.3%) patients, they mostly were myelocytes. Thrombocytosis was found in 4 (26.6%) patients, three (75%) of them were positive for *JAK2* mutation, while thrombocytopenia was found in 7 (46.6%) patients, two (28.6%) of them were positive for *JAK2* mutation.

The total mean, range and SD of serum LDH level were 276, 156-546, 156 U/L respectively, six (40%) patients had elevated LDH. The total mean, range and SD of serum uric acid were 6.5,4.2-8.6, 2.1 mg/dl respectively, it increased in three (20%) of cases (Table 2).

Table (2): Hematological parameters for patients with PMF

Hematological Parameters	Sex								
	Male (n=9)			Female (n=6)			Total (n=15)		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
Hb g/dl	7.6	5.4 – 9	1.2	9.2	4.7 – 12.5	2.7	8.3	4.7 – 12.5	2.1
HCT %	24.2	17.3 – 28.5	3.6	29.9	16 – 41	8.4	26.5	16 – 41	6.4
RBC 10^6/Ml	2.8	1.6 – 3.4	.5	3.7	1.5 – 5.7	1.4	3.2	1.5 – 5.7	1
RDWC %	19.4	15.8 – 23.1	2.7	20.2	10 – 38.2	9.6	19.7	10 – 38.2	6.1
MCV Mm^3	88	76.5 – 110	9.6	79.7	65 – 87.3	10.9	84.7	65 – 110	10.6
MCH Pg	28.8	22.8 – 34.2	3.9	26.7	19.4 – 33.1	5.8	28	19.4 – 34.2	4.6
MCHC g/dl	64.8	29.8 – 331.2	99.9	31.5	29.4 – 34.6	1.8	51.5	29.4 – 331.2	77.4
WBC 10^9/L	21.2	.5 – 75	28.1	11.5	5 – 20	7.2	17.3	.5 – 75	22.3
Neutrophil %	51.8	35 – 70	12.3	56	42 – 80	13.5	53.5	35 – 80	12.5
Lymphocyte %	28.4	10 – 50	11.7	29.3	12 – 44	11.2	28.8	10 – 50	11.1
Monocyte %	7.8	1 – 23	6.7	4	.0 – 8	2.7	6.3	.0 – 23	5.6
Basophil %	.9	.0 – 8	2.7	3.5	.0 – 11	4.2	1.9	.0 – 11	3.5
Eosinophil %	1.3	.0 -8	2.6	.5	.0 – 1	.5	1	.0 – 8	2.1
Myelocyte %	9.6	3 – 1	5.8	7.3	2 – 14	5.7	8.6	2 – 16	5.5
Metamyelocyte%	2	1 - 3	1.4	5	5 -5	.0	3	1 – 5	2
Blast%	7	2 - 17	6.3	3	2 – 4	1.4	5.9	2 – 17	5.5
PLT 10^9/L	466.8	10 - 1848	590.9	244.2	100 – 528	176	377.7	10 – 1848	472.6
MPV Mm^3	8.4	7– 9.4	.8	8.3	7.4 – 9.3	.8	8.4	7 – 9.4	.7
PDW %	12.5	8 – 19.3	3.8	12.7	10 – 18.5	3.5	12.5	8 – 19.3	3.6

(HB: Hemoglobin; HCT: Hematocrit; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBC: White blood cell ; PLT: Platelets; MPV: Mean platelets volume; PDW: Platelet distribution width; SD: standard deviation).

Compared in hematological parameters in PMF patients with *JAK2* Positive and *JAK2* Negative, it was found that MPV significantly increased in *JAK2* positive patients with p-value 0.03 (Table 3).

Table (3): Comparison in hematological parameters in PMF patients between *JAK2* Positive and *JAK2* Negative

Hematological parameters	*JAK2*	Mean	SD	p-value
Hb g/dl	positive	8.7	2.1	0.41
	negative	7.9	2.1	
HCT %	positive	28	6.7	0.42
	negative	25.2	6.3	
RBC 10^6/Ml	positive	3.6	1.1	0.20
	negative	2.9	1	
RDWC %	positive	21.8	7.6	0.22
	negative	17.9	4.1	
RDWS Mm^3	positive	54.9	22.4	0.45
	negative	47.7	13.3	
MCV Mm^3	positive	81.2	8.5	0.25
	negative	87.7	11.9	
MCH Pg	positive	26.6	4.2	0.30
	negative	29.2	5	
WBC 10^9/L	positive	23	25.8	0.37
	negative	12.4	19	
Neutrophil %	positive	57	13	0.32
	negative	50.4	12	
Lymphocyte %	positive	23.7	9.3	0.09
	negative	33.3	11.2	
PLT 10^9/L	positive	520.9	619.7	0.28
	negative	252.5	281.3	
MPV Mm^3	positive	8.8	.3	0.03
	negative	8	.8	

Among patients with documented primary myelofibrosis, there were 7(46.7%) patients who were positive for *JAK2* mutation, four (57.1%) were males and 3(42.9%) were females, while 8 (53.3%) patients were negative for the same mutation, five (62.5%) males and three (37.5%) females.

Bone marrow examination results were the following:

Five (33.3%) patients were in prefibrotic stage showed hypercellular marrow with myeloid hyperplasia and megakaryocytic proliferation with atypia and mild-moderate interstitial fibrosis. Ten (66.6%) patients were in fibrotic stage showed hypocellular marrow with decreased erythropoiesis and megakaryocytic atypia (large dysplastic, dense clustering, aberrant nucleus/cytoplasmic ratio, hyperchromatic irregular folded nuclei) and diffuse interstitial fibrosis.

By applying WHO criteria for diagnosis of PMF, it was found that *JAK2* confirmed the diagnosis of PMF in 7 cases, while eight cases satisfied the criteria although they were negative (Figure 2).

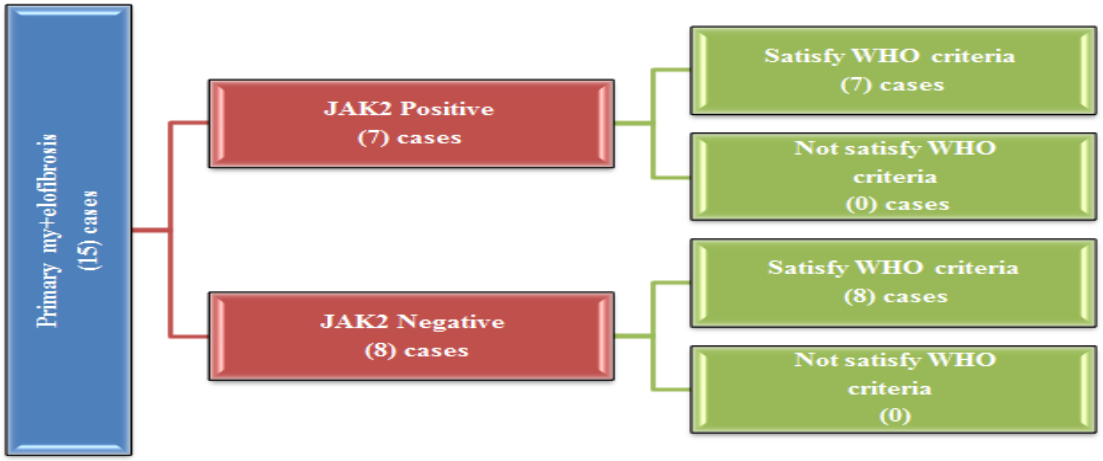

Figure (2): Applicability of revised WHO criteria 2016 for diagnosis of Primary myelofibrosis

Applying BCSH criteria for diagnosis of PMF, it was found that *JAK2* confirmed the diagnosis of PMF in 7 cases, while eight cases of *JAK2* negative cases satisfied the criteria although they were negative (Figure 3).

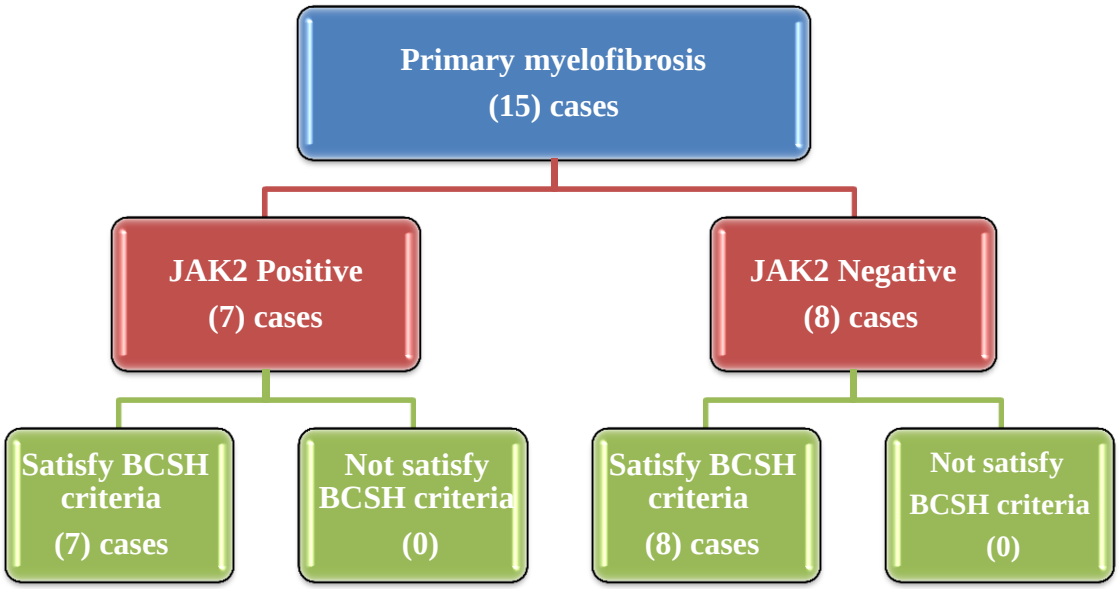

Figure (3): Applicability of BCSH criteria for diagnosis of Primary myelofibrosis

DISCUSSION

In the current study, the mean age of myelofibrosis patients was 59.3 years. In previous multi-country, Italian and Brazilian studies reported mean age 59.6, 63.5 and 64.7 years respectively that is consistent with this study [11-13]. In Ha et al. (2012), study in Korea, the mean age was 67.2 years [14]. Mazzotta et al. (2006) study revealed that ages of myelofibrosis patients were ranging between (48-77 years) and that is in agreement with present study, in which ages ranged (43-75 years) [15]. However, a wide range of ages (34-81 years and 28-89 years) were reported by Porto-Soares et al. (2020) and Harrison et al. (2017) [11,13].

In the present study, males were predominate with M:F ratio of 1.5:1, which corresponds to studies done by (Ha et al., 2012; Mazzotta et al., 2006; Harrison et al., 2017) [14,15,11] ; whereas, females were slightly predominate in Porto-Soares et al. (2020) study with M:F ratio 1:1.04 [13].

The most common complaint among myelofibrosis patients was fatigue (86.7%), followed by dizziness (73.3%) and abdominal discomfort (73.3%), in addition to constitutional symptoms (weight loss 80%, night sweats 73.3%, anorexia73.3% and fever 66.7%). These findings agree with a previous study done by (Emanuel et al., 2012) who mentioned that fatigue 96%, abdominal discomfort 66%, and constitutional symptoms (fever 22%, night sweats 52% and weight loss 62%), were the most common presenting features [12]. Another study that is consistent with the current study which revealed that fatigue 54% and abdominal discomfort 30% were the commonest complaints [11].

Regarding vascular complication, bleeding was more common (33.3%) than thrombosis (6.7%), this is inconsistent with Emanuel et al. (2012) study who reported that thrombosis (14%) was more common than bleeding (7.1%) among the enrolled MF patients [12]. Other studies revealed higher percentage of thrombosis (33%) and (17.7%) [11,13].

In the present study, pallor, splenomegaly and hepatomegaly were common clinical signs among MF patients and that agree with the results of a previous study which reported splenomegaly 100% and hepatomegaly 77.7% [15]. In (Harrison et al., 2017) study, hepatosplenomegaly reported in 64.7% of patients, this difference in percentage may be attributed to the late presentation of patients [11].

The common findings in hematological parameters that observed in the current study were anemia (86.6%), leukocytosis (40%), leucopenia (20%), thrombocytosis (26.6%) and thrombocytopenia (46.6%).

A previous multi-country study of 293 cases with myelofibrosis done by Emanuel et al 2012, reported anemia in 49%, leucopenia in 9.8% and thrombocytopenia in 26% of patients which were lower percentage than results of the present study. This may be attributed to the small sample size of present study [12].

Yap et al. (2018) and Harrison et al. (2017) revealed that mean Hb, WBCs count and PLTs count were (9.8, 9 g/dl), (21.8, 22.3 x10^9/L) and (324.4, 350.7 x10^9/L) respectively, that agree with results of this study [11,16]. The (Porto-Soares et al., 2020) study reported mean, range of Hb(12.5, 5.2-20 g/dl), WBCs count (12.2, 1.9-56.2x10^9/L) and PLTs count (688, 49-1674 x10^9) which are inconsistent with the findings of this study [13].

Although serum LDH level elevated in 40% of our patients, but its mean was within normal range (276 U/L), that disagrees with a study which reported very high mean of serum LDH (781U/L). This can be attributed to the differences in method assay [13].

The frequency of *JAK2* mutation among patients with PMF was 46.7%, and it was in agreement with studies from Morocco, China, and India that reported *JAK2* mutation frequencies 46.2%, 40% and 52% respectively [17-19]. However, the results from others were 15.4% in Iran, 23.4% in Turkey and 33.3% in Algeria [20-22].

All *JAK2* positive and *JAK2* negative myelofibrosis patients followed the WHO (2016) and BCSH (2015) criteria so no further evaluation was needed for these patients.

CONCLUSION

The most common age groups affected by primary myelofibrosis were old age (55-64 years) and males were commonly affected than females. Fatigue, constitutional symptoms and fullness in the abdomen were the most common complains, while pallor and splenomegaly were the most common clinical signs. The total mean Hb was of moderate anemia, MPV significantly increased in *JAK2* positive patients with p-value 0.03. All myelofibrosis patients diagnosed by the two protocols (WHO and BCSH), so there is no difference between the two protocols in the diagnosis.

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