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CALR, JAK2 Exon 14, and JAK2 Exon 12 mutations profiles in patients with Myeloproliferative Neoplasms

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Myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are clonal hematological diseases involving abnormal myeloid stem cell proliferation. Understanding genetic mutations driving diseases promotes effective disease monitoring and prognosis. In this study, mutation profiles of the Calreticulin (*CALR*) gene and exons 12 and 14 within the Janus kinase 2 (*JAK2 12* and *14*) gene were investigated in Sudanese MPN patients. The subject were 140 Sudanese MPN patients; 70 patients were diagnosed with PV, 58 with ET, and 12 with PMF. Using TaqMan® Mutation Detection Assay followed by Sanger sequencing to confirm the results for *JAK2* exons 14 (*V617F)* mutation and allele-specific PCR (AS-PCR) followed by Sanger sequencing for the four common mutations on exon *12* and *CLAR* mutation. *CALR* frameshift mutations were found in 8 (5.7%) patients with ET (i.e., 6 patients with type 1 mutation and 2 patients type 2) but not observed in PV or PMF patients. Mutation in *JAK2 exon 14* were found in 70 (50.0%) patients (68.6% PV, 32.8% ET, and 50.0% PMF), while *JAK2 exon 12* mutation were found in 6 (4.3%) PV patients. Patients with *CALR* mutations showed significant higher platelet levels compared to patients with *JAK2* mutations (*P*=0.018). Patients with PV who exhibited *JAK2 exon 12* mutations had significant high Hb levels and WBCs counts (*P*=0.002, *P*=0.001 respectively) but significant lower platelet counts (*P*=0.038) compared to wildtype. This study shows that the significance of *CALR* mutations appears to take issue among MPN subtypes.

Keywords: Myeloproliferative neoplasms; Somatic mutation; JAK2; Calreticulin

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are diseases of myeloid pluripotential cells characterized by abnormal myeloid cell proliferation and fibrosis

of bone marrow and related to peripheral somatic cell abnormalities. The WHO provides criteria for the diagnostic of the subsequent MPNs subgroups: polycythemia vera (PV); essential thrombocythemia (ET); primary myelofibrosis (PMF); BCR-ABL1–positive; chronic myelogenous leukemia; chronic neutrophilic leukemia; chronic eosinophilic leukemia, not otherwise specified; mastocytosis; and MPN-unclassifiable(Swerdlow 2008). Common MPNs molecular listed within the WHO diagnostic criteria include mutations within the Janus kinase 2 (JAK2) gene and also the myeloproliferative leukemia (MPL) gene and include the mutation in JAK2 exon 14 (V617F), a mutation in exon 10 of MPL (codon W515), and a mutation in *JAK2* exon 12(Jones, Kreil et al. 2005, Pikman, Lee et al. 2006, Scott, Tong et al. 2007, Swerdlow 2008).

Novel *CALR* mutation gene in exon 9 was discovered in recent studies using next-generation sequencing (NGS) in patients with *JAK2* or *MPL* nonmutated PMF and ET(Klampfl, Gisslinger et al. 2013, Nangalia, Massie et al. 2013). In our studies, the *CALR* frameshift mutations were found in 20-25% of patients with ET and PMF but not found in patients with PV, and mostly involved insertions and deletions in DNA exon 9. The two most frequent *CALR* mutations are referred to as type 1 and type 2. The type 1 (L367fs*46) *CALR* mutation is caused by 52 (bp) deletion and located in about 50% of *CALR* mutated patients, while type 2 (K385fs*47) *CALR* mutations result from 5 bp TTGTC insertion and account for roughly 30% of CALR mutated patients(Klampfl, Gisslinger et al. 2013, Nangalia, Massie et al. 2013, Cazzola and Kralovics 2014, Tefferi, Lasho et al. 2014, Tefferi, Wassie et al. 2014). CALR mutations are related to lower Hb levels in ET patients, lower WBCs counts, higher platelet counts, and a comparatively lower thrombotic risk(Cazzola and Kralovics 2014, Rotunno, Mannarelli et al. 2014, Rumi, Pietra et al. 2014).

Understanding the genetic mutations driving MPNs will help to improve disease monitoring and promote a more accurate disease prognosis. In this study *CALR*, *JAK2* exon14, and *JAK2* exon 12 mutation profiles were investigated in three different MPN subtypes in Sudanese patients with PV, ET, or PMF. Correlations were also conducted between mutation patterns and clinical, hematological, and prognostic observations.

MATERIALS AND METHODS

Patients

Patients (N=140) diagnosed with MPNs and treated at Radioisotopes Centre Khartoum (RICK) in Khartoum State, Sudan, were included during this study. Inclusion criteria for this study were the availability of peripheral blood (PB) samples collected at the diagnosing or come back, not to mention symptom aggregation once a follow-up amount. Myeloproliferative neoplasm diagnosis was according to the 2008 WHO classification criteria(Swerdlow 2008) and resulted in 140 patients being diagnosed with the following MPN subtypes: 70 (50.0%) with PV, 58 (41.4%) with ET, and 12 (8.6%) with PMF. Laboratory and clinical info were collected for every patient and enclosed date of diagnosis, age, sex, ethnicity, Hb level, WBCs levels, platelet count, and presence or absence of splenomegaly(Thiele, Kvasnicka et al. 2005, Gangat, Caramazza et al. 2011, Shaffer, McGowan-Jordan et al. 2013).

Ethics approval

This study complied with the Declaration of Helsinki and was reviewed and approved by the University of Khartoum College of Medical Laboratory Sciences. All PB samples were collected following informed consent.

DNA extraction and mutations detection

Genetic DNA was extracted from patient PB samples by using QIAGEN kits (according to the manufacturer's instructions). The quantitative method assessed the quality of extracted DNA using a 2000c spectrophotometer (NanoDrop Technologies, Wilmington, DE). Purity was measured by calculating the absorbance ratio (A_{260}/A_{280}). Pure DNA should have an A_{260}/A_{280} ratio of 1.7-1.9.

The TaqMan® Mutation Detection Assay and Sanger sequencing assay were used to detect and confirm the *JAK2* exon 14 mutation. Allele-specific polymerase chain reaction (AS-PCR) and Sanger sequencing assay were used to detect and confirm the four common mutations in *JAK2* exon 12, and the *CALR* mutation. The following primers were used for PCR amplification of *CALR, JAK2* Exon 14 and *JAK2* Exon12: *CALR* forward prime, 5′-CAT TCATCCTCCAGGTCAAG-3′; *CALR* reverse prime, 5′-AGGGGAACAAAACCAAAATC-3′; JAK2 Exon 14 (V617F) forward prime, 5'-CTCCTCTTTGGAGCAATTCA-3'; JAK2 Exon 14 (V617F) reverse prime 5'-GAGAACTTGGGAGTTGCGATA-3'; *JAK2* Exon 12 forward prime, 5'-

CTCCTCTTTGGAGCAATTCA-3'; JAK2 Exon 12 reverse prime, 5′-GAGAACTTGGGAGTTGCGATA-3′; K539L, 5′-CATATGAACCAAATGGTGTTTTCACTT-3′; N542-E543del, 5′-CAAATGGTGTTTCACAAAATCAGAGATT-3′, F537-K539delinsL, 5′-CATATGAACCAAATGGTGTTAATC-3′; and H538QK539L, 5′-CATATGAACCAAATGGTGTTTTCAATT-3′.

Polymerase chain reactions were made of the following: 1 µL genomic DNA template, 2.5 µL 10x PCR buffer (50 µL PCR buffer, 1.5 µL $MgCl_2$), 0.5 µL 10 mM dNTPs, 0.5 µL forward primer (200 ng/mL), 0.5 µL reverse primer (200 ng/mL), 0.1 µL Taq DNA polymerase (plantium taq), and 19.9 µL deionized distilled water. Total PCR volume was 25 µL. The PCR was performed using the following protocol: an initial 5 min. denaturation at 94°C, 35 cycles of 94°C for 30 sec., 58°C to 64°C for 30 sec., and 72°C for 60 sec., finally 7 min. Extension at 72°C. The products of PCR were purified and sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and ABI 3731 XL full automatic sequencer (Applied Biosystems) using the above primers. The amplified, 453 bp, 280 bp, 212 bp, and 537 bp fragments corresponded to desired amplification of *JAK2* exon 12, and *CALR* exons 9, respectively.

Statistical analysis

We applied the Fisher exact test for categorical variables and the Mann-Whitney U test for continuous variables for comparing different patient groups. All statistical analysis were carried out with SPSS software version 20.0 (SPSS, Chicago, IL), *P* values < 0.05 were considered significant.

RESULTS

Clinical characteristics of patients

The clinical characteristics of all patients are summarized in Table 1. All patients were Sudanese with a median age of 48 years (32-63 years). Of the 140 patients, 76 (54.3%) were male and 64 (45.7%) were female. Leukocyte, erythrocyte, hemoglobin, hematocrit, and platelet counts varied among the different disease subtypes.

CALR and JAK2 mutations

CALR mutations were determined in 8 (5.7%) patients with ET (Table 2). *JAK2* exon 14 mutation were found in 70 (50.0%) patients, while *JAK2* exon 12 mutation were detected in 6 (4.3%) patients with PV. Fifty-six (40.0%) patients were negative for all three mutations. Amongst patients without *JAK2* exon 14 or *JAK2* exon 12 mutations (n=64), *CALR* frameshift mutations were found in 8 (13.8%) ET patients. In the patients exhibiting *CALR* frameshift mutations, 6 (4.3%) patients have type 1 mutations (L367fs*46), and 2 (1.4%) patients have type 2 mutations (K385fs*47).

In ET patients, *CALR* mutations were found in 8 (13.8%) patients. *JAK2* exon 14 mutations were determined in 68.6%, 32.8%, and 50.0% of patients with PV, ET, and PMF, respectively. No patients with PV or PMF displayed *CALR* mutations. However, *JAK2* exon 12 mutations were detected in 8.6% of PV patients. *CALR* frameshift mutations were not found in PV or PMF subtypes. In ET patients, *CALR* mutations were significantly associated with erythrocytes (*P*=0.014), hemoglobin (*P*=0.023), and hematocrit (*P*=0.010). No significant associations were observed between *CALR* frameshift mutations and leukocyte or platelet counts in patients with ET.

Mutations in *JAK2* exon 14 were significantly associated with erythrocyte count (*P* = 0.039), hemoglobin (*P* = 0.014), hematocrit (*P* = 0.015), and platelet count (*P* = 0.025). No significant associations were observed between *JAK2* exon 14 and leukocyte count. *JAK2* exon 12 Mutations were not detected in patients with ET or PMF subtypes. In PV patients, mutated *JAK2* exon 12 were significantly associated with erythrocyte count (P=0.020). No significant were found between *JAK2* exon 12 mutations and leukocyte or platelet count, hemoglobin, or hematocrit in PV subtype patients.

In ET patients, *CALR* mutations were significantly associated with high platelet counts when compared to *JAK2* mutation patients (*P* = 0.018). Leukocyte counts were also increased in *CALR* frameshift mutation patients compared to *JAK2* mutation patients; however, the variation was not statistically significant between these groups (*P* = 0.91) (Table 3). Conversely, hemoglobin levels were high in ET patients with *JAK2* mutations compared to *CALR* frameshift mutation patients; however, this difference was insignificant between these groups (*P*=0.85) (Table 3). Finally, a higher proportion of ET subgroup patients who had *CALR* frameshift mutations were female (75.0%) compared to female patients with *JAK2* mutations (43.1%), again; however, no insignificance statistical was

observed between these groups (*P*=0.22).

Table 1: Laboratory Characteristics of 140 Patients with MPNs.

Variable	Total MPN (n = 140)	PV (n= 70)	ET (n = 58)	PMF (n = 12)
Male/female, male%	76/64 (54.3%)	38/32 (54.3%)	33/25 (56.9%)	5/7 (41.7%)
Age, y (mean±SD)	47.65±7.97	46.63±7.11	48.66±8.51	48.75±10.06
Leukocytes, $\times10^9$/L (mean±SD)	32.31±45.59	21.43±29.52	39.61±56.77	60.58±46.76
Erythrocyte, $\times10^9$/L (mean±SD)	5.73±1.63	6.78±1.13	4.66±1.20	4.77±1.94
Hemoglobin, g/dL (mean±SD)	13.33±4.01	16.29±3.09	10.48±2.36	9.82±1.64
Hematocrit, % (mean±SD)	42.74±11.11	50.92±7.95	34.91±7.24	32.88±6.00
Platelets, $\times10^9$/L (mean±SD)	757.47±656.96	413.74±467.58	1260.66±573.94	330.50±299.33

Table 2: Number of Diseases with Mutations.

Mutation	No. (%) of Cases			
	Total MPN (n = 140)	PV (n= 70)	ET (n = 58)	PMF (n = 12)
JAK2				
***JAK2*, exon 14 (V617F)**	70 (50.0%)	48 (68.6%)	19 (32.8%)	6 (50.0%)
***JAK2*, exon 12**	6 (4.3%)	6 (8.6%)	0	0
***CALR* frameshift mutations**	8 (5.7%)	0	8 (13.8%)	0
Type 1 mutation	6 (4.3%)	0	6 (10.3%)	0
Type 2 mutation	2 (1.4%)	0	2 (3.4%)	0
Triple negative	56 (40.0%)	16 (22.8%)	31 (53.4%)	6 (50.0%)

Table 3: Laboratory Characteristics of 58 Patients with ET Stratified According to Mutation Profiles.

Variable	Total ET (n = 58)	JAK2 exon14 Mutated (n = 19)	CALR Mutated (n = 8)	Triple Neg.ative (n = 55)	P-Value (JAK2 exon 14 vs. CLAR)
Male/female, male%	33/15 (56.9%)	13/6 (68.4%)	2/6 (25%)	31/24 (56.4%)	0.22
Age, y (mean±SD)	48.66±8.51	47.63±7.96	51.50±2.77	47.58±9.81	0.266
Leukocytes, $\times10^9$/L (mean±SD)	10.48±2.36	69.62±51.11	32.44±24.35	24.61±49.41	0.909
Erythrocyte, $\times10^9$/L (mean±SD)	4.66±1.20	4.60±1.04	4.48±1.26	5.41±1.61	0.428
Hemoglobin, g/dL (mean±SD)	39.61±56.77	11.05±1.87	9.85±2.11	11.57±3.42	0.851
Hematocrit, % (mean±SD)	34.91±7.24	37.01±6.12	33.98±7.58	37.87±10.27	0.420
Platelets, $\times10^9$/L (mean±SD)	1260±573.94	1172.11±596.84	1884.50±636.615	878.85±624.14	0.018

Table 4: Laboratory Characteristics of 70 Patients with PV According to Their Mutation Profiles.

Variable	Total PV (n = 70)	JAK2 exon 14 Mutated (n = 48)	JAK2 exon 12 Mutated (n = 6)	Triple Negative (n = 16)	P-Value (JAK2 exon14 vs. JAK2 exon 12)
Male/female, male%	38/32 (54.3%)	27/21 (56.26%)	2/4 (25%)	8/8 (50.0%)	0.148
Age, y (mean±SD)	46.63±7.10	46.25±5.99	47.33±2.58	47.50±10.61	0.001
Leukocytes, $\times10^9$/L (mean±SD)	21.43±29.52	15.94±22.21	88.77±0.00	12.64±21.69	0.001
Erythrocyte, $\times10^9$/L (mean±SD)	6.78±1.13	6.83±1.30	6.40±0.00	6.78±0.71	0.002
Hemoglobin, g/dL (mean±SD)	16.29±3.10	16.15±2.92	20.7 0±0.00	15.08±2.81	0.002
Hematocrit, % (mean±SD)	50.91±7.95	50.58±7.89	59.20±0.00	48.83±7.93	0.009
Platelets, $\times10^9$/L (mean±SD)	413.74±467.584	480.48±547.798	175.00±0.00	303.06±123.866	0.038

Table 5: Comparison of Laboratory Characteristics in Patients With Type 1 and Type 2 CALR Mutations.

Variable	Total (n = 8)	Type 1 (n = 6)	Type 2 (n = 2)	P-Value
Male/female, male%	2/6 (25%)	2/4 (16.6%)	0/2 (0%)	*0.107*
Age, y (mean±SD)	51.50±2.78	52.00±3.09	50.00±0.00	*0.580*
Leukocytes, ×109/L (mean±SD)	32.44±24.35	32.28±28.81	32.91±0.00	*0.931*
Erythrocyte, ×109/L (mean±SD)	4.48±1.26	3.89±0.73	6.27±0.00	*0.044*
Hemoglobin, g/dL (mean±SD)	9.85±2.07	9.47±2.31	11.00±0.00	*0.530*
Hematocrit, % (mean±SD)	33.98±7.58	31.13±6.45	42.50±0.00	*0.146*
Platelets, ×109/L (mean±SD)	1884.50±636.615	2117.00±554.91	1187.00±0.00	***0.000***

JAK2 Exon 12 mutations and correlations with hematological characteristics

In PV subgroup *JAK2* exon 12 mutated patients, Hb levels and leukocyte level were significantly higher (P = 0.0020 and P = 0.0010 respectively); however, platelet levels were significantly lower (P = 0.038) (Table 4) when compared to patients with CALR mutations.

The comparison of laboratory and hematological characteristics in patients with Type 1 & Type 2 CALR mutations

CALR type 1 frameshift mutations were detected in 6 ET subgroup patients, while *CALR* type 2 frameshift mutations were detected in 2 ET subgroup patients; however, no statistically significant variations were determined between the mutation groups in ET subtypes (Table 5). A lower level of erythrocytes, with slight significance (P=0.044) (Table 5), was observed in *CALR* mutated type 2 patients compared to mutated type 1 patients.

DISCUSSION

In this study, frequent (13.8%) *CALR* mutation was determined in ET patients without mutations in *JAK2* exons 12 or 14, but not in PV or PMF patients. Those results are in line with past studies(Klampfl, Gisslinger et al. 2013, Nangalia, Massie et al. 2013, Fu, Xuan et al. 2014, Tefferi, Lasho et al. 2014). Past reports showed that FLT3 mutation in Korean patients with acute myeloid leukemia showed a significantly low mutation compared to Western and Asian patients(Yoo, Park et al. 2006, Bang, Ahn et al. 2008). The

results reported in this study, and by a past study involving Chinese ET subgroup patients, incontestable no critical distinction in frequency of *CALR* mutation when compared to previous Western studies(Fu, Xuan et al. 2014). Therefore, ethnicity might not significantly influence the occurrence of *CALR* mutations. Furthermore, the results from this study discovered that *CALR* mutations were not observed in PV or PMF patient's subgroups. However, this study was limited by a low number of patients.

Hematological and clinical characteristics of *CALR* mutated ET subgroup patients were compared with *JAK2* exon 14 mutation patients with revealing that patient with *CALR* mutations showed significantly high levels of platelets when compared to with *JAK2* with mutation patients (*P*=0.018). Also, leukocyte count were increased in *CALR* frameshift mutated patients compared to *JAK2* mutation patients, however, no significant variation among these groups (*P* = 0.910). Lower hemoglobin levels were observed in *CALR* frameshift mutated patients compared to *JAK2* mutated patients, however no statistical significance was noted among these groups (*P* = 0.850).

The early study reports that mutated *CALR* patients related to an early age, high platelet levels, low risk of thrombosis, low DIPSS score for PMF, and less leucocytosis were less probably to be transfusion-dependent(Tefferi, Lasho et al. 2014). In ET patients, *CALR* mutation associated with low counts of WBCs, low Hb levels, and high platelet levels were in line with past studies' investigation(Rotunno, Mannarelli et al. 2014, Rumi, Pietra et al. 2014). Past reports showed that *CALR* mutated patients are predominantly younger and male (Rotunno, Mannarelli et al. 2014, Rumi, Pietra et al. 2014). The findings presented here, males also predominated this cluster, but no variation in age was observed between *JAK2* and *CALR* mutated patients.

Calreticulin is a Ca^{+2} binding macromolecule primarily located to the endoplasmic reticulum and located on theb19p13.2. Calreticulin macromolecule has 3 domains: carboxyl-terminal C domain, amino-terminal N domain, and central proline-rich P domain(Tefferi, Lasho et al. 2014). The Calreticulin function involves Ca^{2+} in the coagulation process, disposal of misfolded proteins, immune responses, and cell adhesion(Gold, Eggleton et al. 2010). Most mutations of *CALR* include insertions and deletions within the C domain that lead to mutation and are classified into type 1 & type 2. Past reports showed high platelet levels, and low Hb and WBCs levels in ET patients who had type 1 and type 2 mutations than mutated *JAK2* patients.

Other variables associated with type 1 and 2 *CALR* mutations also include being male and of a younger age(Tefferi, Wassie et al. 2014). Platelet levels are also high in type 2 mutated patients compared to type 1 mutated patients(Tefferi, Wassie et al. 2014). Comparisons of PMF patients with mutated type 2 *JAK2* and *CALR* patients showed many similarities than variations. In contrast, comparisons between type 1 and type 2 *CALR* mutations unconcealed the latter related to higher DIPSS, increase WBCs, increased blood percentages, and reduced survival(Tefferi, Lasho et al. 2014). In this study, type 2 *CALR* mutations patients had slightly high platelet counts and a slightly significantly lower erythrocyte level (*P*=0.044), however the patient cohort in this study was too little for a complete analysis. Subsequently, a further prospective multicenter study ought to be a lot of analysis, and assess the significance of prognostic in Sudanese MPN patients.

These studies were exploring *JAK2* exon 12 mutations, Scott *etal*. noted that even though WBCs and platelet levels were inside the normal ranges, some *JAK2* (V617F) negative patients who had myeloproliferative syndrome, characterized by segregated erythrocytosis and lower serum erythropoietin, displayed mutation in *JAK2* exon 12(Scott, Tong et al. 2007). Mutations in *JAK2* exon 12 are settled within the residue region that stretches from 543 to 547 bp (i.e. adjacent to the beginning of the pseudo-kinase domain) with limited impact on other JAK family members (Butcher, Hahn et al. 2008, Pietra, Li et al. 2008). Previous research describes the foremost frequent *JAK2* exon 12 mutation as: N542E543del comprising 23% of the cluster group, E543-D544del creating 11%, F537-K539delinsL and K539L creating 10%, and R541E543delinsK comprising 8% of those mutations(Scott, Tong et al. 2007). Other mutations were reported as a 2 bp substitution and 4 other duplications (Butcher, Hahn et al. 2008, Pietra, Li et al. 2008).

Scott et al. also noted that the activation of JAK-STAT signaling and cytokine independent hypersensitive proliferation in erythropoietin receptor expressing cell lines are primarily caused by exon 12 mutant-alleles. Moreover, JAK2 K539L induces PV phenotypes during a murine implant model(Scott, Tong et al. 2007). Mutations in *JAK2*

exon 12 were also found in negative *JAK2 V617F* PV (5%) and occur only in those with erythrocytosis with no connected thrombocytosis leukocytosis, as opposed to *JAK2 V617F*(Scott, Tong et al. 2007). Research by Scott *et al*. also revealed *JAK2* exon 12 mutation patients at a significantly younger age than those with *JAK2 V617F* mutations. Overall, data showed differences in clinical phenotypes were related to entirely different mutations, while exon 12 mutation primarily impacted erythropoiesis.

Current study detected exon 12 mutations in patients negative and positive for *JAK2 V617F* by focusing on deletions in four alleles using allele-specific PCR (AS-PCR). The assay was done on a complete of 140 patients investigated previously for *JAK2 V617F* mutation. Multiplex PCR was deemed appropriate for this study as a result of it will amplify a region using over one combined of primers, saving effort and time among the laboratory while not compromising assay utility. In the current study, *JAK2* exon 12 mutation were located in 4.3% of all patients with MPNs. In previous reports by Scott et al.(Scott, Tong et al. 2007) *JAK2* exon 12 mutation were only found in PV patients, not in ET or PMF patients. In this study, 6 (8.6%) out of 70 PV patients carried *JAK2* exon 12 mutations consistent with previous studies. Furthermore, Scott *et al.* also noted that although WBCs and platelet levels are among usual ranges, some negative *JAK2 V617F* exon 14 patients with MPNs exhibiting segregated erythrocytosis and low serum erythropoietin have mutations in exon 12 of the *JAK2* gene(Scott, Tong et al. 2007). In agreement with these reports, the current study reveals that the presence of *JAK2* exon 12 mutations significantly correlates with high hemoglobin (P=0.001) and hematocrit (P = 0.001) in PV negative V617F exon 14 patients.

The former absence of *JAK2* exon 12 mutation might result from patients being misdiagnosed with thrombocythemia related to thrombocytosis, causing exon 12 mutation to be usually related to erythrocytosis and lower of erythropoietin level(Butcher, Hahn et al. 2008, Pietra, Li et al. 2008). Several studies have also reported the presence of exon 12 mutation in a very tiny fraction of blood cells that may be lost throughout PCR, particularly if the mutation is found in a very tiny clonal size(Scott, Tong et al. 2007). Using associate degree assay with increased analytical sensitivity may improve detection of the mutation. However, the variation in exon 12 mutation makes it tough to find any of those mutations, especially when using assays like allele-specific (AS-PCR) that are limited to detecting only known mutations(Scott, Tong et al. 2007). Therefore, different mutations may be found but not detected because of the restriction of this assay.

Some studies report association of *JAK2* exon 12 mutations with certain ethnic groups suggesting that exon 12 mutations may be rare in Sudanese patients(Butcher, Hahn et al. 2008). There is no specific clinical phenotype related to exon 12, making it harder to know the pathological role of this mutation. Additional investigations into the mutation patterns of *JAK2* exon 12 in relation to clinical characterization, and using sensitive detection methods, would possibly increase our understanding of this disorder. In this study, clinical and hematologic characteristics of *JAK2* exon 12 mutation patients were compared with carrying *JAK2* exon 14 (V617F) mutation patients revealing that the PV patients with JAK2 exon 12 mutations had high Hb levels and WBCs levels, and significantly low platelet levels (P=0.038).

CONCLUSION

In conclusion, mutation frequencies were observed in Sudanese MPN patients that were consistent with previous reports. Of all the MPN subtypes, the *CALR* mutation was found more frequently in ET patients; therefore clinical significance of *CALR* mutations appears to vary among MPN subtypes and requires more research to further investigate this mutation in the MPNs stages. The *JAK2* exon 14 (V617F) mutation was detected in half of the Sudanese MPN patients accounting for the vast majority of PV patients, and close to half of ET and PMF patients. Conversely, *JAK2* exon 12 mutations were detected only in Sudanese MPN patients with PV and not in other MPN subtypes..

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Elrashed B. Yasin has designed, performed, and drafted the manuscript and did critical editing

of the study. Zainab Jawdat Alalla and Heyam Abdulsamad Abdulqayoom have supported and assisted in sample collection and analysis with statistics. Raed Alserihi, Hanadi Talal Ahmedah, Heba Alkhatabi, Raed Felimban, and Hossam H. Tayeb have supervised this manuscript writing and preparation.

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