Original Article

Frequency of Myeloproliferative Leukaemia Virus (*MPL)* W515L Mutation among Sudanese Patients with Polycythemia Vera

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Abstract:

Background: Myeloproliferative leukemia virus (MPL) plays an important role in the development of megakaryocytes and platelets as well as the self-renewal of haematopoietic stem cells. Recently, numerous MPL mutations have been identified in MPN and reported by researchers. These mutations alter the normal regulatory mechanisms and lead to autonomous activation or signaling deficiencies. The present study aimed to detect the frequency of MPL (W515L)mutation gene among Sudanese patients with polycythemia vera (PV).It may lead to new diagnostic criteria or therapy design.

Materials and methods: This was a descriptive cross sectional study in which a total of 37 patients with polycythemia vera were enrolled in this study, DNA was extracted from patients' blood samples by using salting out method, and analyzed for the detection of MPL(W515L) mutation by using allele specific polymerase chain reaction (ASPCR).Laboratory results and patient's data were analyzed using statistical packing for social sciences program(SPSS).

Results:A total of 37 Sudanese patients attended military hospitals that have been diagnosed with polycythemia vera were enrolled in this study. 36(97.3%) of them were males, one (2.7%) females; age ranged between 23-74 years

The present study showed that 10(27.0%) patients out of 37 were positive for MPL (W515L) mutation, and all of them were males.

Conclusion: This study concluded that the frequency of MPL W515L mutation in Sudanese patients with polycythemia vera is 27%.

Key words: Polycythaemia Vera, MPL W515L.

Introduction:

Polycythemia vera (PV) (also known as ,erythremia ,primary polycythemia and polycythemia rubra vera)is a myeloproliferative disorder due to a clonal proliferation of the Haemopoietic Stem Cell (HSC),characterized by increased production of red cells, granulocytes, and platelets [1,2].First reported in the medical literature in 1892. The term "myeloproliferative disorder" (MPD) was first used to describe polycythemia vera and related disorders in 1951. In 2008, the World Health Organization reclassified MPDs to "myeloproliferative neoplasms" (MPNs) to reflect the consensus that these diseases are blood cancers (neoplasms) [3].With an incidence of at last 2 per 100,000 person-years[4], and the disease was more common in men than women[5]with risk for transformation to acute myeloid leukemia, myelofibrosis and few cases transform to myelodysplastic syndrome, myeloma,or CLL[6]. A variety of symptoms can occur in individuals with polycythemia vera including nonspecific symptoms such as headaches, fatigue, weakness, dizziness or itchy skin; an enlarged spleen (splenomegaly); a variety of gastrointestinal issues; and the risk of blood clot formation, which may prevent blood flow to vital organs. More than 90 percent of individuals with polycythemia vera have a mutation of the JAK2 gene. The exact role that this mutation plays in the development of polycythemia Vera is not yet known.[3]

Myeloproliferative neoplasms (MPNs) originate from genetically transformed hematopoietic stem cells that retain the capacity for multilineage differentiation and effective myelopoiesis. Beginning in early 2005, a number of novel mutations involving Janus kinase 2 (JAK2), Myeloproliferative Leukaemia Virus (MPL), TET oncogene family member 2 (TET2), Additional Sex Combs-Like 1 (ASXL1), Casitas B-lineage lymphoma proto-oncogene (CBL), Isocitrate dehydrogenase (IDH) and IKAROS family zinc finger 1 (IKZF1) have been described in BCR-ABL1-negative MPNs. However, none of these mutations were MPN specific [7].

The Myeloproliferative leukaemia virus, also known as thrombopoietin receptor, CD110, thrombopoietin receptor precursor, C-MPL. It is aproto-oncogene located on chromosome 1 p34 plays an important role in the development of megakaryocytes and platelets as well as the self-renewal of haematopoietic stem

cells[8]. MPLW515L was first described in 2006 amongst JAK2V617F negative PMF patients and is the most frequent MPN associated MPL mutation ,resulting from a G to T transition at nucleotide 1544 on exon 10, causing a tryptophan to leucine substitution at codon 515[7].

Material and Methods:

Patients and Samples

It was a descriptive cross sectional study, in which 37 Sudanese patients attended military hospitals who have been diagnosed with PV according to World Health Organization (WHO) diagnostic criteria (2008) were enrolled in this study 36(97.3%) of them were males, one(2.7%)female; age ranged between 23-74 years. Three milliliter of venous blood sample was collected in Ethylene diamine tetra acetic acid (EDTA) container to investigate the MPL (W515L) mutation.

DNA Extraction

Genomic DNA was extracted from the EDTA blood sample by using DNA extraction KIT (GFI-1 BLOOD DNA EXTRACTION, Malaysia) and stored at -20 ºC until analysis.

MPLW515L Mutation Analysis

MPLW515L mutation was detected using ASPCR according to the protocol of Jianxiang Chi and Chryso. PCR mixture 20 µL as follows: 3 µL of DNA template, 0.5 µL from each primer (Table 1) and 15.5 µL of D.W with master mix (premix - Interon). The reaction conditions were 95ºC for 10 minutes, followed by 40 cycles at 95ºC for 30 seconds, 65ºC for 30 seconds, 72ºC for 30 seconds and final 72ºC extension for 10minutes. Five µl of the PCR product and 50 bp DNA ladder was electrophoresed on 2% Agarose gel, stained with ethedium bromide. TBE buffer was used as a running buffer. The presence of the 124-base-pair (bp) band indicated that the sample was positive for the MPLW515l mutation ,whereas absence of the band indicates that sample was negative for the mutation.

Table (1): Forward, reverse and mutation specific primer sequences

	Primer sequence
Forward primer	5'-GCCGAAGTCTGACCCTTTTT-3'
Reverse primer	5'-ACAGAGCGAACCAAGAATGCCTGTTTACA-3'
Mutant specific primer	5'GGCCTGCTGCTGCGAAGTt -3'

Statistical analysis

The results of JAK2V617F mutation,erythropoietin level and complete blood cell count (CBC) were taken from the patients records. The MPL W515L mutation gene was detected using PCR, and then analyzed all laboratory results and patients' data using statistical package for social sciences (SPSS).

This study was approved by the Faculty of Medical Laboratory Sciences: Ethical Committee, Al Neelain University, Khartoum, Sudan, and informed consent was obtained from each patient before sample collection.

Results:

A total of 37 Sudanese patients who attended military hospital and have been diagnosed with PV were enrolled in this study, 36(97.3%) of them were males, one (2.7%) female; age ranged between 23-74 years. The present study showed that 10(27.0%) patients out of 37 were positive for MPL (W515L) mutation, and all of them were males.

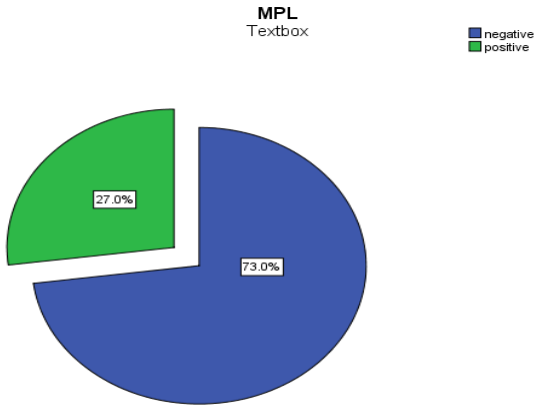

Figure (1): Frequency of MPLW515L mutation(n=37)

This study also found that, the coexistence (JAK2 V617F and MPLW515L) mutations were present in 90% of MPL W515L mutated patients with no significant differences (P. value 0.475).

The statistical analysis of this study revealed that there was no significant difference between the mean and SD of haemoglobin concentration, red blood

cells count, white blood cells count and platelets count among mutated MPL W515L patients compared to those non mutated (P. value 0.731,0.094,0.187 and 0.323) respectively. But there was a significant difference between the mean and SD of packed cell volume (PCV) and erythropoietin level (EPO) among patients mutated to MPLW515L compared to those nonmutated (P.value 0.042, 0.00) respectively.(table2).

Table (2): Correlation between MPL W515L mutation and other laboratory findings:

Variables	MPL W515L Positive Mean ±SD	MPL W515L Negative Mean ±SD	P. value
Hb g/dl	17.1± (0.85)	16.8± (0.94)	0.731
PCV%	53.0± (7.2)	50.4± (3.4)	0.042
RBCs$\times 10^{12}$/l	7.5± (2.5)	6.1± (1.74)	0.094
platelets$\times 10^{12}$/l	421.9± (130.6)	312.2± (227.8)	0.323
TWBCs$\times 10^{12}$/l	7.25± (1.68)	5.7± (1.43)	0.187
EPO Um/ml	1.21± (0.087)	1.65± (0.54)	0.00

Discussion:

The detection of molecular and cytogenetic alterations is important for the diagnosis, prognosis and classification of myeloproliferative neoplasms (MPN).Many studies showed that there was association between MPL W515L mutation and myeloproliferative neoplasm (MPN) with variation in the reports of frequency. But in Sudan there is no report about the frequency of MPL mutation among Sudanese patients with Polycythemia Vera.

This study aimed to detect the frequency of MPL W515L mutation among Sudanese patients with polycythemia vera. It was found that the mean age of mutated patients was in the fourth decade and this finding agrees with findings from a study done by Animesh Pardanani in 2011[(9)]in Mayo Clinic(U.S) which reported a mean age at the fourth decade.

The frequency of MPLW515L mutation among Sudanese patients with PV in this

study was found to be 27.0%. This finding did not agree with that reported in Mayo Clinic (U.S) <1% [9]

But this finding is lower than those among Sudanese patients. It also disagrees with Pardanani et al(2006)[10], ATefferi(2010)[7], Xi He et al in china[8], and Wei Xu et al(2008)[11] that reported the frequency was 0%.

In the present study, coexistence of JAK2V617F and MPLW515L mutations was present in 90% of MPL W515L mutated patients with no significant differences. This finding agree with that reported in Mayo Clinic [9]

In the present study, Hb, red blood cells count, PCV, leucocyte count, platelets count and EPO level were compared in polycythaemia Vera patients' positive for MPLW515L mutation and those negative for the mutation; Hb, RBCs, platelets, and leucocytes, were higher in those with the mutation than those without the mutation but the difference was not statistically significant. While PCV was similarly high in both groups. EPO level was lower in both groups and were statistically significant. These findings are consistent with the results reported by Pardanani(2011) [9] that Hb and PCV were higher and EPO level was lower in patients positive for MPL W515L mutation than unmutated one.

Conclusion:

This study concluded that the MPL W515L mutation was detected in 27% of Sudanese patients with polycythemia Vera who were enrolled in this study.

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