



DETECTION OF THE PLATELETS RECEPTOR GPIIB POLYMORPHISM IN SUDANESE PATIENT WITH POLYCYTHAEMIA VERA.

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ABSTRACT

Background: Glycoprotein IIb it is a receptor on platelets for fibrinogen and von will brand factor and aid in platelet activation, aggregation and adhesion to sub endothelial .glycoprotein found in chromosome 17 and defect in it will cause many diseases. **Objective:** The purpose of this study was to investigate the frequency of the GPIIb polymorphism carried out among polycythemic patients and healthy individuals in Sudan. **Material and Methods:** 44 polycythemic patients and 44 controls were evaluated to detect the frequency of GPIIb polymorphism. Platelet parameters were performed by an automated cell analyzer. The GPIIb polymorphism was detected using RFLP-PCR method. **Result:** The wild genotype for GPIIb were detected in 17 of patients and 27 of the control; the heterozygous genotype were detected in 18 of patients and 12 of control and the homozygous genotype were detected in 9 of patients and 5 for control and the difference was not statistically significant (P. value=0.065). **Conclusion:** the allele frequency for control was: serine=0.75, isoleucine=0.25.while the allele frequency for the patients was: serine =0.41, isoleucine=0.59. However, a significant deviation from the Hardy-Weinberg equilibrium was observed in control group ($X^2=3.27$, $df=1$ and $p.v>0.05$) while for patients group was insignificant ($X^2= 0.03$, $df=2$ and $p.v =0.98$).

KEYWORDS: polycythemia vera, Platelets, Polymorphism, GPIIB.

Abbreviations

P.V P.Value

D F

CI Confidence Interval

OR Odds ratio

INTRODUCTION

Polycythemia (also known as polycythaemia or polyglobulia) is a disease state in which the proportion of blood volume that is occupied by red blood cells increases. Blood volume proportions can be measured as hematocrit level. It can be due to an increase in the number of red blood cells^[1] ("absolute polycythemia") or to a decrease in the volume of plasma ("relative polycythemia").^[2]

Absolute polycythemia is known as The overproduction of red blood cells may be due to a primary process in the bone marrow (a so-called myeloproliferative syndrome), or it may be a reaction to chronically low oxygen levels or, rarely, a malignancy. Alternatively, additional red blood cells may have been received through another process—for example, being over-transfused (either accidentally or, as blood doping, deliberately) or being the recipient twin in a pregnancy, undergoing twin-to-twin transfusion syndrome.

While Primary polycythemias are due to factors intrinsic to red cell precursors. Polycythemia Vera (PCV), polycythemia rubra Vera (PRV), or erythremia, occurs when excess red blood cells are produced as a result of an abnormality of the bone marrow.^[3] Often, excess white blood cells and platelets are also produced. Polycythemia Vera is classified as a myeloproliferative disease. Symptoms include headaches and vertigo, and signs on physical examination include an abnormally enlarged spleen and/or liver. In some cases, affected individuals may have associated conditions including high blood pressure or formation of blood clots. Transformation to acute leukemia is rare. Phlebotomy is the mainstay of treatment. A hallmark of polycythemia is an elevated hematocrit, with Hct > 55% seen in 83% of cases.^[4] A somatic (non-hereditary) mutation (V617F) in the *JAK2* gene is found in 95% of cases, though also present in other myeloproliferative disorders.^[5]

GPIIb receptor

GPIIb receptor known as an integrin α IIb, one of the most abundant platelet surface receptors ($\approx 80\,000$ per platelet).^[6] It is a receptor for fibrinogen^[7] and von willbrand factor and aids in platelets activation, aggregation and adhesion to sub endothelial.^{[8][9]} A platelets aggregation is done via calcium-dependent

association, thus facilitating the generation of thrombin^{[10][11][12]} GPIIb found on chromosome 17 lying with in a 260Kb fragment in the region 17q21 to22 with GPIIb to GPIIIa.^{[13][14]} Several point mutations in the genes that encode GPIIb result in disorders of platelet binding.^[15] Human platelet antigen -3 (HPA-3) (Bak\Bak) is common polymorphism of platelet GPIIb^[16], resulting from a thymine (T) to guanine (G) base change coding for iso leucine -to-serine substitution at position 843 of the GpIIb heavy chain,^{[14][17][18][19]} resulted in the cleavage of the 253 –bp fragment in to a 126-and 127bp fragments, where as the presence of ser was characterized by the un cleaved 253-bp fragment.^[20] GP II b receptor is a target of several drugs including a bciximab, eptifibatide, tirofiban.^[21] Pathology Defects in glycoprotein IIb cause Glanzmann's thrombasthenia^[22] Auto antibodies against II b can be produced in immune thrombocytopenic purpura^[23] Medicine Glycoprotein II b/III a inhibitors can be used to prevent blood clots in an effort to decrease the risk of heart attack or stroke.^{[24][25][26][27][28][29]} GPII b subunits contain a common amino acid dimorphism.^[30]

Our objective is to detect the frequency of GP IIb polymorphism in Sudanese patients with polycythemia vera and specifically to study coexistence of GP and polycythemia vera to correlate the relation of this polymorphism to these patients platelet count and indices since polycythemia vera is the most prevalent disease among them.

MATERIAL AND METHODS

44 samples of 2.5 ml of EDTA anticoagulated veinous blood collected from patients with polycythemia vera referring to Fedail hospital, during May 2015-July 2015.

Then, platelets count were performed by full automated hematological analyzer (sysmex –KX21N, Japan) Immediately. DNA extracted from whole blood samples by using Genomic DNA extraction kit (Intron –Korea), in three phases to get a pure DNA. Extracted DNA stored below -20 c until analysis.

PCR (Polymerase chain reaction): we used oligonucleotide primer forward and reverse primer as in table1; selected for (PCR) to amplification those parts of the genomic DNA.

Table (1): forward and reverse oligonucleotide primer

Forward	(5-CTC AAG GTA AGA GCT GGG TGG AAG AAA GAC-3)
Reverse	(5-CTC ACT ACG AGA ACG GGA TCC TGA AGC CTC-3)

20µL PCR mixture was performed as follow: 10µL of DNA template, 1µL from each forward and reverse primer and 8µL of D.W with master mix (premix - Interon).

The platelet GP IIb genotypes were detected by RFLP-PCR run, The thermocycling condition as follow: initial denaturation 5 minutes at 94°C, 40 cycles: denaturation in 94°C for 30 seconds, annealing in 60.5°C for 30seconds, extension in 72°C for 30 seconds and final extension 72°C for 30 seconds and final extension in 72°C for 5mintues.

The PCR products were analyzed by used 3% agarose gel with 4µL of ethidium bromide. 7µL from PCR products and 100 bp DNA ladder (Intron –Korea) were transferred on to the agarose gel and after one hour for electrophoresis the result of PCR product was 253bp for GP II b where detected by using gel documentation system (SYNGENE, JAPAN) as shown in figure1.

Restriction–enzyme digestion

The PCR products were digested by using Restriction-enzyme Fok I (Cut Smart –New England).The total 20 µL of enzyme mixture as follow 5 µL of PCR products, 2µL buffer and 0.5µL from enzyme and 12.5µL D.W. this mixture was incubated in 37 °C for 60 minutes and inactivated of enzyme reaction by 65°C for 20 minutes.10 of the digested DNA fragments were run out in to 3% agarose gel containing ethidium bromide and the result reading against DNA ladder 50 pb and identified under

UV transilluminator using gel documentation system Fragments were visualized by use of (SYNGENE, JAPAN). After digestion with Fok I, the presence of Ile at position 843 resulted in cleavage of the 253-bp fragment in to a 126 –and 127 bp fragment, where as the presence of Ser was characterized by the uncleaved 253-pb fragment against DNA lader 50 bp, genotypes were classified as (Ile, Ile) 126-127bp, (Ile, Ser) 127/250 bp and (Ser, Ser) 250/250 bp as shown in figure 2.

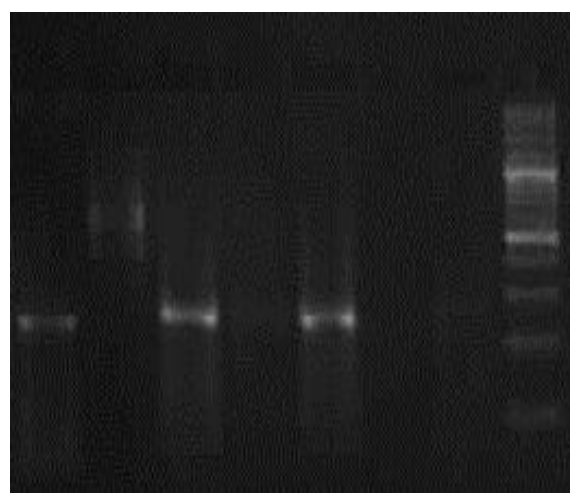


Figure (1): Platelet glycoprotein IIb at position 843 Isol-leucine/Serine and amplified fragment was 253 bp.

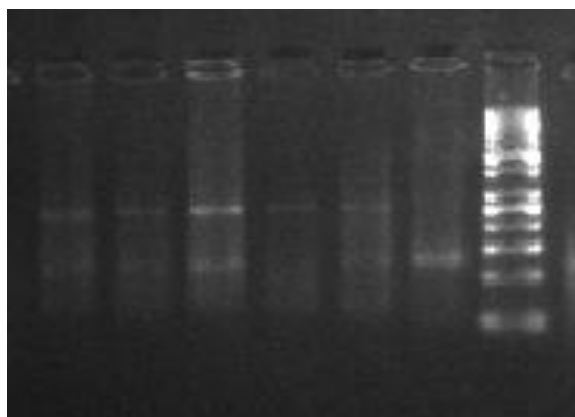


Figure (2): Digested fragment by Restriction enzyme FokI showed 126 /127 bp(Ile, Ile), 126/253 bp (Ile, Ser) and 253/253 bp (Ser, Ser).

RESULT

This Study is a case control study was include 88 sample. 44 samples were polycythemia vera patients samples. 15 (34.1%) of them were males and 29(65.9%) were females. While, 44 samples were volunteers samples as a normal control, the control group of 17 (38.6%) were male and 27 (61.4%) were female see figure 3.

The most genotype frequency for patients were (Ile, Ser) 18 (40.9%), followed by (Ile,Ile) 17 (38.6%) and (Ser, Ser) 9 (20.5%). While, the most frequency genotypes of control group were (Ile, Ile) 27(61.4%) followed by (Ile, Ser) 12 (27.3%) and (Ser, Ser) 5(11.4%) see figure 4.

There were no statistical significant association shown for both case and control for all genotypes. (Ile, Ile), OR: (1.00), CI: (0.42-2.35), P.V: (1.00), and (Ile, ser) OR: (0.69), CI: (0.29-1.60), P.V: (0.39), and (ser,ser) OR: (2.00), CI: (0.61-6.55), P.V: (0.24).

There were a significant association between patients gender and platelets count with genotypes as in table 2.

The allele frequency for control was isoleucine =0.75, serine =0.25, while the allele frequency for the patients was: isoleucine=0.59, serine=0.41 as seen in figure 5. However, a significant deviation from the Hardy-Weinberg equilibrium was observed in control group ($X^2=3.27$, $df=1$ and $P.v>0.05$) while for patients group was insignificant ($X^2=0.03$, $df=2$ and $P.v: 0.98$).

Table (2): The patient association with platelets count

Variable	Case	Control	P.value
	Mean±SD		
PLt	611.79±507.11	398.04±205.23	0.011
Gender	1.65±0.47	1.40±0.49	0.019
Genotype	1.81±0.75	1.72±0.65	-0.065

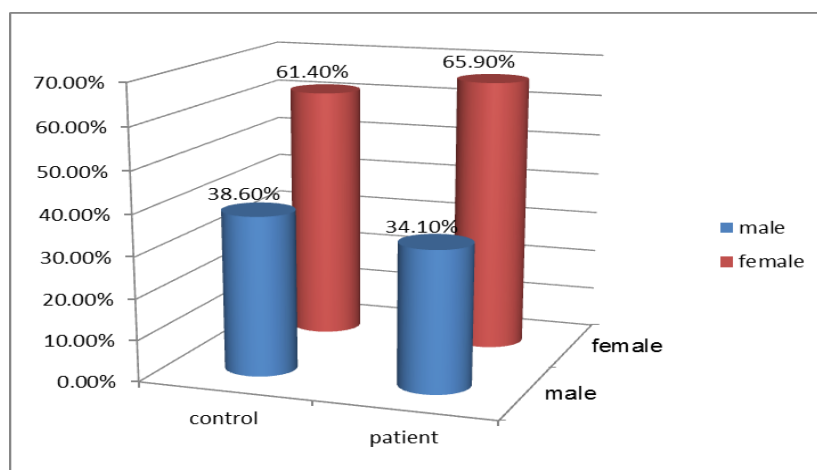


Figure (3): Distribution of study population according to gender %

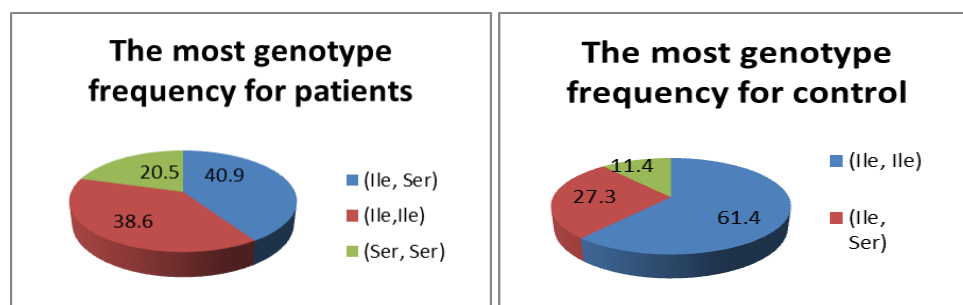


Figure (4): The most genotype frequency for patients

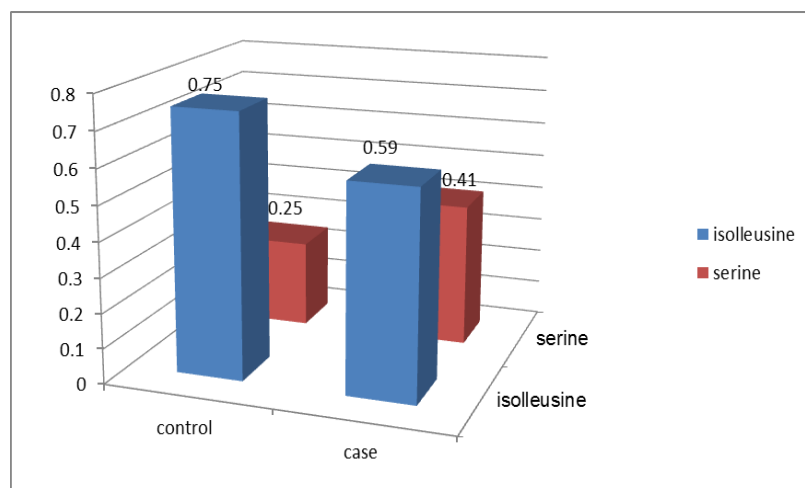


Figure (5): The allele frequency for control and patient

DISCUSSION

The base pair substitution results in an amino acid polymorphism at residue 843 of the mature heavy chain q21. In study done By Suzanne Lyman, *et al*^[31] showed that The Bak^a form of GPIIb encodes an isoleucine at this position, whereas the Bak^b allele contains a serine. The substitution of a G for a T at this position resulted in an isoleucine * serine polymorphism at amino acid 843 of the GPIIb heavy chain. while study made by Asier Jayo, *et al*^[32] the analysis showed that HPA-3 (Ile⁸⁴³Ser) is a common polymorphism of platelet GPIIb, And homozygosis for the HPA-3 polymorphism that results from a T to G mutation in exon 26 changing Ile⁸⁴³ to Ser. However, In the present study we aimed to investigate the frequency and association of Platelet glycoprotein IIb polymorphisms and Platelet count in Sudanese patients diagnosed with polycythemia vera And the result showed that The heterozygous genotype (Ile,Ser) is higher in the patients than the control group which agree with the studies mentioned above, while the wild genotype (Ile,Ile) is lower in patient than control group and homozygous genotype (Ser,Ser) is the lowest in both groups which disagree with study done by Hao Duan *et al*^[33] founded that The HPA-3 (Ser,Ser) genotype in patients was significantly higher than in control subjects ($P=0.008$), The HPA-3 (Ser,Ser) genotype was a stronger risk factor for ischemic stroke among males ($P=0.032$) than females ($P=0.184$). but agree with study done by Park S *et al*^[34] that detect The sub group analysis of younger males (≤ 55 years) showed that the patient who carry HPA-3 (ser,ser) had a lower risk for MI compared to the patient carrying HPA-3 (Ile,ser) or HPA-3 (Ile,Ile). When concerning gender in our study The highest frequency of this polymorphism was found in female more than male which disagree with the same study mentioned above that done by Hao Duan *et al*^[33] although there was insignificant difference in the GPIIb genotype distributions of patients and healthy control subjects in our study which agree with study done by Angela M. Carter *et al*^[35] suggests that there was insignificant difference in the HPA-3 genotype distributions of patients and healthy control subjects but

still disagree with Hao Duan *et al*^[33] study that indicates among study participants <60 years, there was a significant difference in the HPA-3 genotype distributions of patients. In our study There were a close association between patients gender and platelets count with these genotypes representing a risk factor with patient diagnosed with polycythemia vera which agree with the same study mentioned above that done by Hao Duan *et al*^[33]. There was insignificant association in the allele frequency in our study and the most allele frequency was isoleucine =0.59 which agree with study done by Angela M. Carter *et al*^[35] that found The genotype distributions of patients with ischemic stroke and healthy control subjects did not differ significantly from Hardy-Weinberg equilibrium, The HPA-3 polymorphism was not in linkage disequilibrium with the PI^A polymorphism ($D'=8\%$; $P=0.2$). Finally There were studies reported that an association between the presence of GPIIb polymorphism and Myeloproliferative Diseases but provided limited information on the functional implications of the changes in membrane GPIIb in polycythemia vera.^[36] However this study showed differences in the occurrence and frequency of this relationship. This variation in results may be due to sample size, genetic factor and different geographical area.

CONCLUSION

Conclusively platelet glycoprotein IIb polymorphism may related to polycythemia vera and patient gender and platelet count influence the increasing risk in some way while genotypes appears to be independent genetic risk factors because it was not a useful marker for genetic risk of this polymorphism.

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