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PLATELET GLYCOPROTEIN IIB POLYMORPHISM AND PLATELET INDICES IN SUDANESE PATIENTS WITH SICKLE CELL ANEMIA

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ABSTRACT

Background: Glycoprotein II b it is a receptor on platelets for fibrinogen and von will brand factor, and play vital role on platelet activation, aggregation and adhesion to sub endothelial .glycoprotein found in chromosome 17. Furthermore, any defect in this Glycoprotein II b might be a cause for many thrombotic disease has been reviewed in literature. **Objective:** The study aimed to investigate the frequency of the Glycoprotein II b polymorphism, platelet count and platelet indices in Sudanese patients with sickle cell anemia. **Material and Methods:** 2.5 ml of EDTA anticoagulated veinous blood was taken from 44 patients with Sickle cell anemia have been referring to Omdurman hospital, during May 2015-July 2015.Then, platelets count and platelets indices were performed by full automated hematological analyzer (Sysmex –KX21N, Japan). Genomic DNA extracted from whole blood samples by using DNA extraction kit (Intron–Korea).The platelet Glycoprotein II b polymorphism at position 843 (Lsolleucine/Serine) by using RFLP-PCR. **Result:** This study is a case control study, the most genotype frequency for patients were (Ile, Ser) 22(50%) followed by (Ile, Ile) 17(38.6%) and (Ser, Ser) 5(11.4%); 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%).There were statistical significant between case and control for genotypes (Ile, Ile); (OR: 2.522 ; CI: (1.069-5.950), P.V:0.03), and (Ile, ser) (OR:0.375 ; CI:(0.154-0.912), P.V:0.029); but genotype (ser ,ser) showed statistical insignificant for both case and control (OR:1.0 ; CI:(0.268-3.731), P.V:1.0).There were no association between platelets indices (PDW, MPV and P-LCR) and platelets count with genotypes (P value :0.372, 0.758 , 0.146 and 0.414) respectively. The allele frequency for control was isolleusine :0.75 , serine :0.25 .While the allele frequency for the patients was isolleusine :0.64 , serine: 0.36 .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) and for patients group(X^2=0.28 , df=1 and p.v: >0.05). **Conclusion:** The platelet glycoprotein IIB genotypes (Ile,Ile) and (Ile,Ser) might be consider the risk of sickle cell anemia complication.

KEYWORDS: Sickle cell anemia, Platelets Polymorphism IIB, platelet indices.

INTRODUCTION

Sickle cell disease (SCD) is an inherited chronic haemolyticanaemia whose clinical manifestations arise from the tendency of the haemoglobin (HbS or sickle haemoglobin) to polymerize and deform red blood cells into the characteristic sickle shape. This property is due to a single nucleotide change in the β - globin gene leading to substitution of valine for glutamic acid at position 6 of the β- g lobin chain (β6 glu→ val or β s).[1] The allele responsible for sickle-cell anaemia can be found on the short arm of chromosome 11, more specifically 11p15.Sickle-cell disease is inherited in the autosomal recessive pattern.[2]

Several processes contribute to development of vaso–occlusion SCD. Vaso - occlusion is initiated by adhesion of young deformable red cells to the vascular endothelium, and is followed by trapping of rigid irreversibly sickle cells. Adhesion occurs in the post - capillary venules and is promoted by leucocytosis, platelet activation and inflammatory cytokines.[3]

An area that has received increasing attention in recent years has been the role of platelet glycoprotein polymorphisms in the predisposition to thrombotic disease. A number of polymorphisms that occur in platelet glycoprotein have been examined, though in most cases their relationship to thrombosis remains uncertain.[4] The most abundant platelet surface receptor is the platelet glycoprotein (GP) IIb/IIIa, which binds to fibrinogen and VonWillebrand factor.[5] This plays a central role in platelet aggregation and adhesion to subendothelial tissues, which is an essential first step in thrombus formation The gene encoding the platelet glycoprotein IIb is located on chromosome 17, lying within a 260-kb fragment in the region 17q21.Several

point mutations in the genes that encode GpIIb and GpIIIa result in disorders of platelet binding. Human platelet antigen-3 (*HPA-3*) (Baka/ Bakb) is a common polymorphism of platelet GpIIb, resulting from a thymine (T) to guanine (G) base change coding for an lsolleucine-to-serine substitution at position 843 of the GpIIb heavy chain.[6,7] Platelet activation plays a momentous role for the initiation of acute coronary syndromes. Platelet indices are potentially useful markers for the early diagnosis of thromboembolic diseases. An increase in both mean platelet volume (MPV) and platelet distribution width (PDW) due to platelet activation, resulting from platelet swelling and pseudopodia formation was hypothesized. Platelet size has been shown to correlate with their function. Large platelets are considered metabolically and enzymatically more reactive than smaller ones.[8-9] Significantly raised of mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-LCR) in patients with AMI and unstable angina.[10]

Previous studies reported that an association between the presence of GP IIb polymorphism and (SCA) .[11,12,13]The study aimed to investigate the frequency and association of Platelet glycoprotein IIb gene polymorphisms, platelet count and Platelet indices in Sudanese patient with sickle cells anemia.

MATERIAL AND METHODS

2.5 ml of EDTA anticoagulated veinous blood was taken from 44 patients with Sickle cell anemia have been referring to Omdurman hospital, during May 2015-July 2015.Then, platelets count and platelets indices were performed by full automated hematological analyzer (sysmex –KX21N, Japan). Genomic DNA extracted from whole blood samples by using DNA extraction kit (Intron –Korea). Extracted DNA stored below -20 c until analysis.

Polymerase chain reaction (PCR)

We used oligonucletide primer forward and reverse primer as in table (1); selected for PCR to amplification those parts of the genomic DNA, platelet glycoproteinII b in chromosome 17 q21.

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 $^{3'}$)

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

The platelet glycoprotein II genotypes were detected by RFLP-PCR

The thermocycling condition as follow: initial denaturation 5 minutes at 94 ºc, 40 cycles : denaturation in 94 ºc for 30 seconds, annealing in 60.5 ºc for 30 seconds , extension in 72 ºc for 30 seconds and final extension 72 ºc for 30 seconds and final extension in 72 ºc for 5mintues .

The PCR products were analyzed by used 3% agarose gel with 4 µL of ethidium bromide. 7 µL from PCR products and100 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*) 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, 2 µL buffer and 0.5 µL from enzyme and 12.5 µL D.W. this mixture was incubated in 37 ºc for 60 minutes and inactivated of enzyme reaction by 65 ºc 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 IIe 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, genotypes were classified as (Ile, Ile), (Ile, Ser) and (Ser, Ser).

As shown in figure (2).

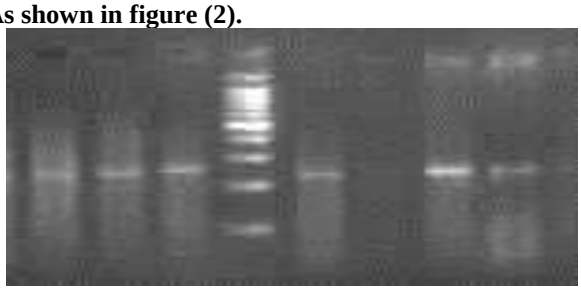

Figure: 1 Platelet glycoprotein IIb at position 843 lsolleucine/Serine and amplified fragment was 253 bp.

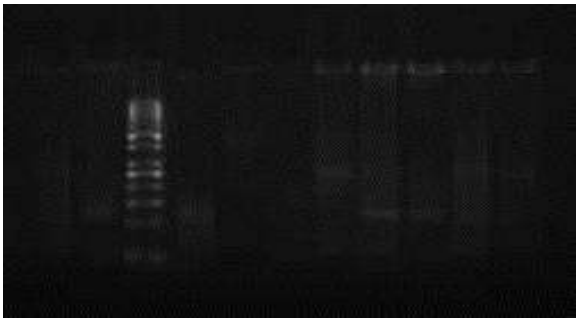

Figure: 2 Digested fragment by Restriction enzyme FokI showed 126 /127 bp(Ile, Ile), 126/253 bp (Ile, Ser) and 253/253 bp (Ser, Ser).

Data analysis

Data was analysis by using Statistical Package for Social Sciences (SPSS) version 16.The genotypes were analyzed by using Chi-square test. While, the quantitative data were analyzed by **using pearson correlation**.

RESULT

A total of 88 samples were enrolled in the study 44 patients samples with sickle cell anemia, their ages were range from 9 month-15 years (mean 5.4818) and 44 samples as normal control group, (mean 17.54) . 26 (59.1%) of patients were males and 18(40.9%) of patients were females. While, the control group of 17 (38.6%) were male and 27 (61.4%) were female. This study is a case control study, the most genotype frequency for patients were (Ile, Ser) 22(50%) followed by (Ile, Ile) 17(38.6%) and (Ser, Ser) 5(11.4%); 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%).There were statistical significant between case and control for genotypes (Ile, Ile); (OR: 2.522; CI: (1.069-5.950), *P.V*:0.03), and (Ile, ser) (OR:0.375 ; CI:(0.154-0.912), P.V:0.029); but genotype (ser ,ser) showed statistical insignificant for both case and control (OR:1.0 ; CI:(0.268-3.731), *P.V:1.0*). There were no association between platelets indices (PDW, MPV and P-LCR) and platelets count with genotypes (table 2) The allele frequency for control was isolleusine =0.75 , serine =0.25 , while the allele frequency for the patients was: isolleusine =0.64 , serine =0.36 .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) and for patients group(X^2=0.28 , df=1 and P.v: >0.05).

Table2

Variable	Case	Control	p.value
	Mean±SD		
PLt	398.05±205.234	261.16±66.002	.414**
MPV	8.5909±1.07786	12.1364±2.24739	-.758**
PLCR	15.9227±5.91470	17.3409±5.89025	-.146
PDW	10.4636±1.85448	8.6136±0.89484	-.372**
Genotypes	1.5±6.99	1.73±0.663	.167

DISCUSSION

Platelet glycoprotein receptor IIB (GPIIb) is an important protein which response for platelets adhesion, activation and aggregation. And also, it is play role for treatment response and the effect of diseases complication to the patients. GP IIb found on chromosome 17 laying with in a 260Kb fragment in the region 17q21. Platelet glycoprotein receptor IIb or Human platelet antigen-3 (*HPA-3*) is a common polymorphism of platelet glycoprotein, resulting from a thymine (T) to guanine (G) base change coding for an isoleucine-to-serine substitution at position 843 of the GpIIb heavy chain.[15] Our study aimed to detect the frequency of GP IIb polymorphism, platelet count and platelet indices in Sudanese patients with Sickle cell anemia (SCA). Many studies showed that, the relation between platelet glycoprotein polymorphism II b and thrombotic disorder. Duan *et al.* they are found that ,the platelet glycoprotein IIb Ile/Ser gene polymorphism is associated with ischemic stroke among young and meddle aged adults <60 years specially male.[14] Also, study done by Park *et al.* they are HPA-3 polymorphism was associate with MI in Korean individuals younger than 56 of age. Our results showed that, the genotype (Ile, Ser) was the most frequent genotype for patients and (Ile, Ile) for control and those genotype were affected in the platelets glycoprotein II b receptor.[16] In contrast, study done by Al-Subaie *et al.* found that the association of HPA polymorphisms with SCA VOC, of which HPA-3 can, appears to be independent genetic risk factor for SCA VOC.[23] That due to different ethnic group. Carter. *et al.* found that no significant difference in the genotype distribution of patients and controls. [17]Reiner *et al.*(Ser, Ser) more prevalent in ischemic stroke cases than control, high in subgroup of women with hypertension and D.M. Also, he found that the association with risk of ischemic stroke for glycoprotein IIb Ile/Ser^{843} polymorphism.[24] Platelet indices are potentially useful markers for the early diagnosis of thromboembolic diseases. Vagdatli *et al* found that the MPV and PDW are simple platelets indices, which increase during platelet activation. PDW is a more specific marker of platelet activation, since it does not increase during simple platelet swelling.[20] Our study showed that, there were no statistical significant of platelets count and platelet indcies with sickle cell disease ,but Renata P *et* al reported a significant association in platelet count between case and control; case(375) and control(293) *p.v* (<0.01).[21] In the study done by, Mohan JS, *et al.* found that patients with SCD have various abnormalities in their platelets regardless of genotype: there are more numerous platelets, which are smaller an, contain less P selctin per cell, but have ahigher concentration of granules than those of HbAA subject. This differentses may mark and /or promote the prothrombtic state in SCD.[20] Celik *et al.* found that the MPV was significantly higher in patient with cerebrovascular events. Also MPV values increased with increasing incidence of the crisis[21]

Our result showed that the most frequent allele in patients and control was isoleucein. Study done by Chiras.T *et al* found HPA-3 as isoleucein0.62 and serin

0.38.[22] And Carlsson *et al* also found the most allele frequency was isoleucein (0.61) and serin was 0.39.[23]

CONCLUSION

The platelet glycoprotein IIB genotypes (Ile,Ile) and (Ile,Ser) might be consider as risk of sickle cell anemia complication.

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