EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 11
01-03
Year: 2016

THE FREQUENCY OF PLATELET RECEPTOR GP IIB POLYMORPHISM IN SUDANESE PATIENTS WITH CHRONIC KIDNEY FAILURE

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Article Received on 23/08/2016 **Article Revised on 12/09/2016** **Article Accepted on 02/10/2016**

ABSTRACT

Background Glycoprotein II b is a receptor on platelets for fibrinogen and von will brand factor and aids in the platelet activation, aggregation and adhesion to sub endothelial .Glycoprotein IIb gene found in chromosome 17 polymorphism in this gene reported to be associated with many disorder**.Objective** The purpose of this study was to determine the frequency of the GP IIb polymorphism in patients with chronic kidney failure in Sudan. **Materials and Methods** A total of 41 chronic kidney failure patients and 44 apparently healthy control subjects were evaluated to detect the frequency of GB IIb polymorphism. Platelets parameters were performed by an automated cell analyzer. The G.P IIB polymorphism was detected using RFLP-PCR. **Results** The wild genotype of GP IIb was found in 5 patients out of 34 and 27 of the control subjects; the hetero genotype was detected in 4 of patients and 12 of control subjects where as the homozygous genotype was detected among 32 patients and only among 5 of control and the difference was statistically significant (P.value 0.06) Table (1).In addition the GPIIb genotypes were not related to the platelet count and indices findings in study patients (Table 2). **Conclusion:** There was a significant association between the patients genotype and control and the homozygous genotype detected in the chronic kidney failure.

KEYWORDS: Chronic kidney failure, Platelet, Polymorphism, G.PIIb

INTRODUCTION

Chronic kidney failure, describes the gradual loss of kidney function. Your kidneys filter wastes and excess fluids from your blood, which are then excreted in your urine.[1] When chronic kidney disease reaches an advanced stage, dangerous levels of fluid, electrolytes and wastes can build up in your body.

The three most common causes of CKD are Diabetic mellitus, Hypertension Glomerulonephritis.[2]

Other conditions that affect the CKD are polycystic kidney disease. Vesicoureteral reflux .recurrent kidney infection .interstitial nephritis.[3]

GPIIb receptor known as an integrin αIIb found on platelets. It is a receptor for fibrinogen and von will brand factor and aids in platelets activation, aggregation and adhesion to Sub endothelial. A platelets aggregation is done via calcium-dependent association. GPIIb found on chromosome 17 lying with in a 260Kb fragment in the region 17q21 to22 with GPIIb to GPIIIa. Several point mutations in the genes that encode GPIIb result in disorders of platelet binding .Human platelet antigen -3 (HPA-3) (Bak\Bak) is common polymorphism of platelet GPIIb , 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.[4] 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.[5] GP II b receptor is a target of several drugs including a bciximab, eptifibatide, tirofiban.[6] Pathology Defects in glycoprotein IIb cause Glanzmann's thrombasthenia. Auto antibodies against IIb can be produced in immune thrombocytopenic purpura.[7] Medicine Glycoprotein II b/IIIa inhibitors can be used to prevent blood clots in an effort to decrease the risk of heart attack or stroke.[8] GPII b subunits contain a common amino acid dimorphism.

Our objective is to detect the frequency of GP 2b polymorphism in Sudanese patients with Chronic kidney failure and specifically to study coexistence of GP and Chronic kidney failure to correlate the relation of this polymorphism to these patients platelet count and indices since Chronic kidney failure is the most prevalent disease among them .Prevalent studies reported that an association between the presence of GP IIb polymorphism and Chronic kidney failure. However this study showed differences in the occurrence and frequency of this relationship.

MATERIAL AND METHODS

Thirty Four samples of EDTA anticoagulated venous blood collected from patients with type1 diabetes mellitus referring to Khartoum hospital and a 44 control subjects were recruited to participate in this case control study study in the period from Febroary to May 2015. Platelets count and indices were done from 2.5ml of anti coagulated blood samples by full automated hematological analyzer (sysmex –KX21N, Japan) as soon as possible after collection.

DNA extracted from blood 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 oligonucletide primer forward (5-CTC AAG GTA AGA GCT GGG TGG AAG AAA GAC-3) and reverse primer (5-CTC ACT ACG AGA ACG GGA TCC TGA AGC CTC-3) selected for (PCR) to amplification those parts of the genomic DNA.

PCR was performed on 10 micro litter of DNA template ,1 micro litter from forward and reverse primer respectively and 8 micro litter of D.W in total volume 20 micro litter master mix (premix --interon) we detected GP II b by PCR run : denaturation 5 minutes at 96c temperature, anylining 40 cycles :initiation in 96 c for 30 seconds ,extension in 60.5 c for 30 seconds and final extension 72 c for 30 seconds at last final extension in 72c for 5mintues.

Analysis of the PCR products: In order to analyze the PCR products 3% agarose gel with 4 micro litter ethidium bromide staining was done. 7 micro litters PCR products were transferred on to the agrose gel the band was in 253bp range for amplifie GP IIb.

Restriction –enzyme digestion: Restriction-enzyme Fok I (cut smart –New England) digestion of the PCR products were performed under condition recommended by the manufacturers. We took 5 micro litters of PCR products with 12.5 D.W, 2Mm buffer and 0.5 micro litters Fok I, (cut smart –New England) ,mixed well then incubated in 37c for 1hr ,and inactivated the reaction in 65 c for 20 minutes, 10 of the digested DNA fragments were then run out in to 3% agarose gel containing ethidium bromide 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 aa (Ile, Ile), ab (Ile, Ser) and bb (Ser, Ser); and we used 1for the wild (aa),2 for hetero(ab) and 3for homo (bb).

This study was approved by ethical committee of the faculty of medical laboratory sciences, Al Neelain University, and informed consent was obtained from each participant before sample collection

RESULTS

This was a case control study included 85 participants, 41 of them were Sudanese patients with chronic kidney disease and 44 healthy volunteers as control group.

The duration of time the patients had been on hemodialysis ranged between 3–5 years .and their age were range from 25 - 87 years (mean 55. 9756), 25 (61. 0%) of patients were males and 16 (39. 0%) of patients were females.

The wild genotype of GP IIb was found in 5 patients out of 34 and 27 of the control subjects; the hetero genotype was detected in 4 of patients and 12 of control subjects where as the homozygous genotype was detected among 32 patients and only among 5 of control and the difference was statistically significant (P.value 0.06) Table (1).In addition the GPIIb genotypes were not related to the platelet count and indices findings in study patients (Table 2).

Table1

Genotype	Patient (%)	Control (%)	P.value
Wild	5	27	0.061
Hetero	4	12	
Homo	32	5	

Table2

Variable	control	Patient	P.value
PLt	261.16	234.12	0. 556
MPV	12.1364	0.19451	0. 783
PCT	17.3409	16.0074	0. 520
PDW	8.6136	7. 6439	0. 250

DISCUSSION

platelets play an important role in the pathogenesis of thromboembolic disease, and the possibility of inherited platelets risk factor for acute thrombosis is intriguing and especially important in assess adhesion; activation and aging clinical risk and in prophylactic and therapeutic interventions. platelets thrombosis by several platelets membranes receptor complexes ,e.g G.P IIb/IIIa.

The mean of platelets was low in patient (234.1915) than in patient (261.16); the mean of MPV was low in patients(7.6439) and high in control (12.14); the mean of PDW was low in control(8.61) than the patient group(16.0073). The wild genotype is low in the patients and the control group, while the homozygous genotype is high in patient and control group.

In our study there was no significant association of the platelet count and indices finding in patients with chronic kidney disease to be discussed with other; The PIA2 allele frequency among the hemodialysis patients was significantly higher than that in the control group[9]; and

GPIa gene alone or in combination with the PlA2 allele had no major effect on premature myocardial infarction risk.[10] however in other study carried out in Taiwan, it was found that this polymorphism was absent in their population.[11]

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