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

ISSN 2349-8870
Volume: 3
Issue: 9
62-64
Year: 2016

THE FREQUENCY OF PLATELET RECEPTOR GP IIB POLYMORPHISM IN SUDANESE PATIENTS WITH HYPERTENSION

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Article Received on 02/07/2016 **Article Revised on 23/07/2016** **Article Accepted on 14/08/2016**

ABSTRACT
Background: Glycoprotein II b it is a receptor on platelets for fibrinogen and von will brand factor and aid in platelet activation, aggregation and adhesion to sub indothelial .glycoprotein found in chromosome 17 and defect in it will cause many disease. **Objective:** The purpose of this study was to investigate the frequency of the GP IIb polymorphism in hypertension patients in sudan. **Material and Methods:** Forty three hypertension patients and forty four controls were participated to detect the frequency of G.P IIB polymorphism. Platelet count and indices were performed by an automated cell analyzer. The G.P IIB polymorphism was detected using RFIP-PCR. **Results:** The wild genotype for G.P IIB were detected in 17 of patients and 27 of the control; the heterozygous genotype were 22of patients and 12 of control and the homozygous genotype were 5 for patients and 5 for control and the difference was not statistically significant(P.value 0.496). **Conclusion:** The control group genotype and patients as in table(1); the allele frequency for control was: a=0.75 ,b=0.25.while the allele frequency for the patients was: a=0.67 ,b=0.33. However, a significant deviation from the Hardy- Weinberg equilibrium was observed in control group(X2=3.27, df=1 and p.v>0.05) and for patients group(X2=9.52 ,df=1 and p.v=0.001.

KEYWORDS: Hypertension, Platelets, Polymorphism, G.PIIB.

INTRODUCTION
Arterial Hypertension (HTN) or high blood pressure is a chronic medical condition in which the blood pressure in the arteries is elevated more than 100–140 mmHg systolic (top reading) and 60–90 mmHg diastolic (bottom reading); this equals the maximum and minimum pressure, respectively. Hypertension puts strain on the heart, possibly leading to hypertensive heart disease and coronary artery disease[1,2,3]. and major risk factor for stroke[4].

GPIIb receptor
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[5]. 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[6]. GP II b receptor is a target of several drugs including a bciximab, eptifibatide, tirofiban[7]. Pathology Defects in glycoprotein IIb cause Glanzmann's thrombasthenia. Auto antibodies against II b can be produced in immune thrombocytopenic purpura[8]. 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[9]. GPII b subunits contain a common amino acid dimorphism. Our objective is to detect the frequency of g p 2b polymorphism in Sudanese patients with hypertension and specifically to study coexistence of g p and hyper tension to correlate the relation of this polymorphism to these patients platelet count and indices since hypertension is the most prevalent disease among them. Prevalent studies reported that an association between the presence of gpIIb polymorphism and hypertension. However this study showed differences in the occurrence and frequency of this relationship.

MATERIAL AND METHODS
Forty three samples of EDTA anticoaglated veinous blood collected from patients with hypertension referring to Omdurman hospital, during May 2015-July 2015.

Immediate platelets count and indices were done from 2.5ml of anti coagulated blood samples by full automated

hematological analyzer (sysmex –KX21N,Japan). 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.

DNA extracted from blood using Genomic DNA extraction kit (Intron –Korea), inthree phases to get a pure DNA. Exterated 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 three methods including conventional technique used 3% agarose gel with 4 micro litter ethidium bromide staining. 7 micro litters PCR products were transferred on to the agrose gel the band was in 253bp range GP II b amplified.

Restriction –enzyme digestion: Restriction-enzyme Fo k 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 Fo k 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 Fo k 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).

RESULTS

This was a case control study included 87 partispants, 43 of them were Sudanese patients with hypertension and 44 healthy volunteers were included in the study as control group.

The patients age were range from 35-75 years (mean 53.5116).15 (34.9%) of patients were males and 28(65.1%) of patients were females.

The GP IIb genotype for patients and control group were 24 of patients and 27 of control with wild genotype; 10 of patients were heterozygous and12 of control were hetero, the homozygous genotype was detected 9 of patients and 5for control group, but the difference was not statistically significant(*P.Value*:0.496) as shown in table(1). There was no significant association between the genotype and the mean of plt count(*P.value*:0.514) ;P-LCR (*P.value*:0.479) ; PDW(*P.value*: 0.161) and MPV(*P.value*: 0.133) as in table 2. There was no significant association between the gender and the genotype (*P.Value*: 0.148).

Table 1

Genotype	Patient	control	*P.value*
Wild	24	27	0.496s
Hetero	10	12	
Homo	9	5	

Table 2

	Control	Patient	*P.value*
PLt(mean)	261.16	253.19	0.514
MPV(mean)	12.1364	12.9767	0.133
PLCR(mean)	17.3409	25.7907	0.479
PDW(mean)	8.6136	10.2326	0.116

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 high in control(253.19) than in patient(261.16); the mean of MPV was high in patients(12.9767) and low in control(12.1364); the mean of PDW was lower in control(17.3409) than the patient group(25.7907) while the P-LCR was higher in patient group(17.34) than the patients(15.92). The wild genotype is higher in the patients and the control group, while the homozygous genotype is lower in patient and control group.

The ser843 GPIIb may increased the risk for MI in young women as found in: Alexander P.Reiner1,2,Stephen M.Aramaki,Bruce M. Psaty and David S.Siscovick
Article firest published on line:20 DEC 2001.[10]

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