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

ISSN 2349-8870
Volume: 3
Issue: 12
473-476
Year: 2016

PLATELET RECEPTOR GLYCOPROTEIN IIB POLYMORPHISM AND PLATELET INDICES IN SUDANESE PATIENTS WITH DIABETES MELLITUS TYPE 2

Lubna Bashir Balla Haj Ahmed and *Ibrahim Khider Ibrahim

Sudan.

***Corresponding Author: Ibrahim Khider Ibrahim**
Sudan.

Article Received on 19/10/2016 **Article Revised on 09/11/2016** **Article Accepted on 30/11/2016**

ABSTRACT

Background: Glycoprotein IIb it is a receptor on platelets for fibrinogen and von willbrand factor and aid in platelet activation, aggregation and adhesion to sub indothelial. glycoprotein gene 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 Diabetes mellitus type 2 patients in Sudan. **Material and Methods:** Forty tow Diabetes mellitus type 2 patients and 44 controls subjects were evaluated to detect the frequency of G.P IIb polymorphism. Platelet parameters were performed by an automated cell counter. The G.P IIb polymorphism was detected using RFLP-PCR method. **Result:** The wild genotype for G.P IIb were detected in 24 of patients and 27 of the control; the heterozygous genotype were found in 9of patients and 12 of control and the homozygous genotype were 9 among patients and 5in control and the difference was not statistically significant(*P.value* 0.427). **Conclusion:** The control group genotype and patients as in table (1); the allele frequency for control was: serine=0.75, iso-inlleusine=0.25. while the allele frequency for the patients was: serine =0.32, isolleusine =0.68. 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=10.7, df=2 and *p.v*0.004).

KEYWORDS: Diabetes mellitus type 2, Platelets, Polymorphism, G.PIIB.

INTRODUCTION

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period.[1]

Diabetes is due to either pancreas do not produce enough insulin or the cells of the body not responding properly to the insulin.[2]

There are three main types of diabetes mellitus. Type1 DM referred to as insulin- dependent diabetes mellitus (IDDM)[2], Type 2 DM referred to as non insulin-dependent diabetes mellitus (NIDDM), Type3 Gestational diabetes.

NIDDM is a metabolic disorder that is characterized by hyperglycemia in the context of insulin resistance and relative lack of insulin.[3]

The classic symptoms are excessive thirst, frequent urination and constant hunger. caused by a combination of lifestyle and genetic factors.[4][5]

Number of complications with which it is associated such as cardiovascular disease, including ischemic heart disease and stroke.[6]

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 within a 260Kb fragment in the region 17q21 to 22 with GPIIbtoGPIIIa.[7]

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 isoleucine-to-serine substitution at position 843 of the GpIIb heavy chain.[7] 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 uncleaved 253-bp fragment.[8] GP IIb receptor is a target of several drugs including abciximab, eptifibatide, tirofiban.[9] Pathology Defects in glycoprotein IIb cause Glanzmann's thrombasthenia. Auto antibodies against IIb can be produced in immune thrombocytopenic purpura.[10] Medicine Glycoprotein

IIb/III a inhibitors can be used to prevent blood clots in an effort to decrease the risk of heart attack or stroke.[11] GPII b subunits contain a common amino acid dimorphism.

Our objective is to detect the frequency of GPIIb polymorphism inSudanese patients with Diabetes mellitus type 2 (INDDM) and specifically to study coexistence of GP and (INDDM) to correlate the relation of this polymorphism to patients platelet count and indices. (INDDM) is the most prevalent disease among them.

MATERIAL AND METHODS

Forty four samples of EDTA anticoagulated veinous blood collected from patients with (INDDM) 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 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 oligonucletideprimer 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 thegenomic 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 IIb 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 of ethidium bromide for staining. 7 micro litters PCR products were transferred on to the agrose gel the band was in 253bp range GPII b appeare.

Restriction –enzyme digestion: Restriction-enzyme Fo kI (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, 2micro litters buffer and 0.5 micro litters Fo kI, 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 used1for the wild (aa),2 for hetero(ab) and 3for homo (bb).

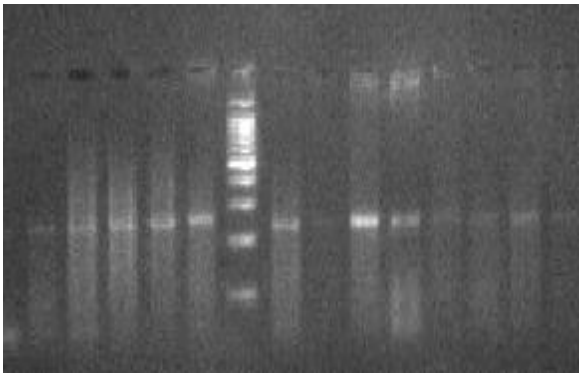

Figure1. PCR product of GpIIb DNA 253bp

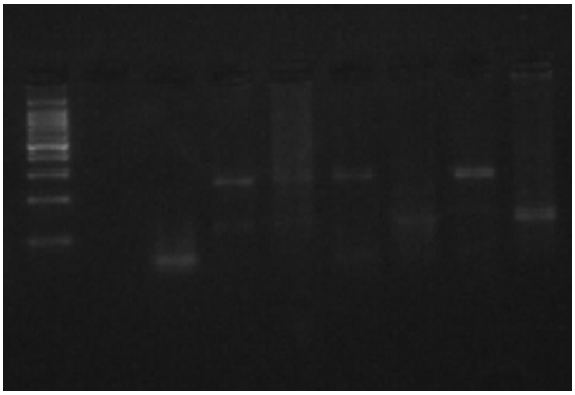

Figure2. Restriction enzyme aa (Ile, Ile)127bp, ab (Ile, Ser)127,250bp and bb (Ser, Ser)250bp

RESULT

This study was a case control study included 86partispants, 42 of them were Sudanese patients with Diabetes mellitus type 2 and 44 of them were healthy volunteers as a control group.

The patient`s age range was from 35years- 75 years (mean 60.2). 13(31.0%) of patients were males and 29(69%) of patients were females.

The mean of platelet count and indicies for the patients and control showed in significant association for the platelet and MPV, while P-LCR and PDW showed significant association as in table(2)

The GpIIb genotype for patients and control group showed that 24 of patients and 27 of control presented

with wild genotype (aa);9 of patients were hetero and12 of control were hetero genotype(ab), the homozygous genotype (bb) was found 9 of patients and 5 of control, but the difference between the two group was statistically insignificant (P.value 0.427) as shown in table(1). There was in significant association between the genotype and the mean of plt count(*p.value*:0.275) and P-LCR(P.value:0.529), PDW(p.value:0.606) and MPV(P.value0.305)as in table 2.There was in significant association between the gender and the genotype (P.Value.0.606).

Table1

Genotype	Patient	control	p.value
Wild	24	27	0.427
Hetero	9	12	
Homo	9	5	

Table2

Variable	control	Patient	p.value
	Mean+SD		
PLt	261.16 ± 66.002	245.95±78.593	0.333
MPV	12.1364±2.24739	12.8810±1.87672	0.100
PLCR	17.3409±5.89025	25.6429±7.14253	0.000
PDW	8.6136±0.89484	10.1190±1.10878	0.000

DISCUSSION

G.PIIB is an important protein which response for plateletes adhesion; activation and aggregation and it is an important receptor in the platelete. Play arole for treatment response and the effect of diseases complication to the patient.

The mean of plateletes count was lower in patient than in control; the mean of MPV, PDW and P-LCR was higher in patient than in control.

The findings of the percent study reported that there is insignificant association between the gpIIb polymorphism and diabetes mellitus type 2, this finding is in agreement with Tschoepe *et al* which reported that the number of glycoprotein II b/IIIA per platelet was significantly elevavted in diabetic patients.[12]

There was a significant association between the genotype as in (Tschoepe D[1], Roesen P, Kaufmann L, Schaauseil S, Kehrel B, OstermannH, Gries FA et al. Evidance for abnormal platelet glycoprotein expression in diapetes mellitus]. Eur J C 2009; lin Invest, Apr, 1990; 20(2): 166-70.

D. Tschoepe1, B. Menart1, P. Ferber1, C. Altmann4, M. Haude4, B. Haastert3, P. Roesen2 Genetic variation of the platelet- surface integrin GPIIb-IIIa.

(PIA1/A2-SNP) shows a high association with Type 2 diabetes.

Mellitus highly significant 23 October 2002 / Revised: 27 March 2003.

Published online: 21 June 2003c Springer-Verlag 2003

REFERENCES

1. About diabetes. World Health Organization. Retrieved4 April 2014.
2. Shoback, edited by David G. Gardner, Dolores (2011). "Chapter 17". Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical. ISBN 0-07-162243-8
3. Kumar, Vinay; Fausto, Nelson; Abbas, Abul K.; Cotran, Ramzi S.; Robbins, Stanley L. Robbins and Cotran Pathologic Basis of Disease (7th ed.). Philadelphia, Pa.: Saunders, 2005; 1194–1195. ISBN 0-7216-0187-1.
4. Ripsin CM, Kang H, Urban RJ. "Management of blood glucose in type 2 diabetes mellitus". *Am Fam Physician*, January 2009; 79(1): 29–36. PMID 19145963.
5. Risérus U, Willett WC, Hu FB. "Dietary fats and prevention of type 2 diabetes". Progress in Lipid Research, January 2009; 48(1): 44–51. doi:10.1016/j.plipres.2008.10.002. PMC 2654180.PMID 19032965.
6. Risérus U, Willett WC, Hu FB. "Dietary fats and prevention of type 2 diabetes". Progress in Lipid Research, January 2009; 48(1): 44–51. doi:10.1016/j.plipres.2008.10.002. PMC 2654180.PMID 19032965.
7. Carter AM, Catto AJ, Bamford JM et al: Assocition of the platelet glycoprotein IIbHP-3polymrphism with survival after acute ischemic stroke. Stroke, 1999; 30: 2606-11.
8. Unkelbach K, Kalb R, Santoso S et al: Genomic RFLP typing of human platelet alloantigens Zw(PIA), Ko, Bak and Br(HPA-1,2,3,5).BrJ Haematol, 1995; 89: 169-76.
9. Cattanen N:Haemorrhagic stroke during anti-platelete therapy. Eur J Anaesthesiol, 2008; 25(42): 12-15.
10. McMillan R (October 2007). "The pathogenesis of chronic immune thrombocytopenic purpura". Semin. Hematol. 44(4,5): S3-S11.doi:10.1053/j.seminhematol.2007.11.002.PMID 18096470.
11. Molecular Hematology by Drew Provan MD FRCP FRCPath and John G Gribben MD DSc FRCP FRCPath, 2000, 2005.
12. Tschoepe D1, Roesen P, Kaufmann L, Schaauseil S, Kehrel B, OstermannH, Gries FA et al. Evidance for

abnormal platelet glycoprotein expression in diapetes mellitus]. Eur J C 2009; lin Invest, Apr, 1990; 20(2): 166-70.