EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 2/ May 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

The Frequency of Platelet Receptor GP IIb Polymorphism in Sudanese Patients with Diabetes Mellitus Type 1

OMAYMA ALTAYB MAHGOUB
IBRAHIM KHIDR IBRAHIM

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 type1 diabetes mellitus in Sudan.*

Materials and Methods: *A total of 34 type1 Diabetes mellitus patients and 44 apparently healthy control subjects were evaluated to detect the frequency of GP IIb polymorphism. Platelets parameters were performed by an automated cell analyzer. The GP IIb polymorphism was detected using RFLP-PCR.*

Results: *The wild genotype for G.P IIb was found in 3 patients out of 34 and 27 of the control subjects; the hetero genotype was detected in 10 of patients and 12 of control subjects whereas the homozygous genotype was detected among 21 patients and only among 5 of control and the difference was statistically significant(P.value 0.000).*

Conclusion: *There were significant association between the patients genotype and control and the homozygous genotype detected in the type1 diabetes patient.*

Key words: Type1 Diabetes Mellitus, Platelet, Polymorphism, GPIIb

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] Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Serious long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers and damage to the eyes.[2] Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma.[3] Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced.[4] .type1 results from the body's failure to produce enough insulin This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown.[2] is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin.[5]

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.[6] resulted in the cleavage of the 253 –bp fragment in to a 126-and 127bp fragments, whereas the presence of ser was characterized by the un cleaved 253-bp fragment.[7] GP II b receptor is a target of several drugs including a bciximab, eptifibatide, tirofiban.[8] Pathology Defects in glycoprotein IIb cause Glanzmann's thrombasthenia. Auto antibodies against IIb can be produced in immune thrombocytopenic purpura.[9] 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.[10] GPII b subunits contain a common amino acid dimorphism.

Our objective is to investigate the frequency of GP 2b polymorphism in Sudanese patients with type1 Diabates Mellitus and specifically to study coexistence of GP and Diabetes Mellitus type1 to correlate the relation of this polymorphism to these patients platelet count and indices. In Sudan there is no published data regarding the frequency of this polymorphism. So we conduct this study to clarify the occurrence of this polymorphism among Sudanese type 1 Diabetes mellitus patients.

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 in the period from February 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 agarose gel the band was in 253bp range for amplified 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, whereas 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.

RESULT

This was a case control study included 78 participants, 34 of them were Sudanese patients with Type1 Diabetes Mellitus and 44 healthy volunteers were included in the study as control group.The patients age were range from 9 years to 45 years (mean 22.0000).24 (70.6%) of patients were males and 10(29.4%) of patients were females.

The wild genotype for GP IIb was found in 3 patients out of 34 and 27 of the control subjects; the hetero genotype was detected in 10 of patients and 12 of control subjects where as the homozygous genotype was detected among 21 patients and only among 5 of control and the difference was statistically significant(P.value 0.000) Table(1).In addition the GPIIb genotypes were not related to the platelet count and indices findings in diabetic patients (Table 2).

Table (1)

Genotype	Patient N (%)	Control N (%)	P.value
Wild	3	27	0.000
Hetero	10	12	
Homo	21	5	

Table (2)

Variable	Control	Patient	P.value
Mean Platelet count	261.16	291.21	0.47
Mean MPV	12.14	9.9471	0.62
Mean PLCR	17.35	24.58	0.40
Mean PDW	8.61	12.43	0.79

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 including G.P IIb/IIIa.

The mean of platelets was high in patient(291.21) than in control(261.16) ;the mean of MPV was low in patients(9.9471) and high in control(12.14); the mean of PDW was lower in control(8.61) than the patient group(12.43) while the P-LCR was high in patient group(24.58) than the control group(17.35).

The wild genotype is low in the patients and high in the control group, while the homozygous genotype is high in patient and low in the control group.

This is the first study to report the prevalence of this Polymorphism in Sudanese Patients with diabetes mellitus type 1

In our study there was no significant association to the platelet count and indices findings in diabetic patient

The homozygous genotype was detected more commonly among patients than among control subjects, this finding is in agreement with, and in consistent with finding of polymorphism.

CONCLUSION:

There were significant association between the patients genotype and control and the homozygous genotype detected in the type1 diabetes patient.

REFERENCES

1- Betes Federation. 17 March 2006.
2- "About diabetes". World Health Organization. Retrieved 4 April 2014.
3- "Diabetes Fact sheet N°312". WHO. October 2013. Retrieved 25 March 2014.
4- Kitabchi, AE; Umpierrez, GE; Miles, JM; Fisher, JN (Jul 2009). "Hyperglycemic crises in adult patients with diabetes.". Diabetes Care 32 (7): 1335–43. doi:10.2337/dc09-9032. PMC 2699725. PMID 19564476. .
5 - RSSDI textbook of diabetes mellitus. (Rev. 2nd ed.). New Delhi: Jaypee Brothers Medical Publishers. 2012. p. 235. ISBN 9789350254899.
6.Carter AM, Catto AJ,Bamford JM et al:Associtionof the platelet glycoprotein IIbHP-3polymrphism with survival after acute ischemic stroke .Stroke,1999;30:2606-11
7-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
8-Cattanen N:Haemorrhagic stroke during anti-platelete therapy.Eur J Anaesthesiol,2008,25(Suppl.42):12-15
9-McMillan R (October 2007). "The pathogenesis of chronic immune thrombocytopenic purpura".Semin.Hematol.44(4 suppl 5):S3S11.doi:10.1053/j.seminhematol.2007.11.002.PMID18096470.

10-Molecular Hematology by Drew Provan MD FRCP FRCPath and John G Gribben MD DSc FRCP FRCPath 2000, 2005.