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Detection of Beta Fibrinogen Gene 455G/A Polymorphism in Sudanese with Type 2 Diabetic Hypertensive patients

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Abstract:

Background: *Diabetes mellitus and hypertension are complexes diseases result from inter reaction of different of predisposing factors.*

Aim: *to evaluate the role of Beta Fibrinogen Gene 455G/A polymorphism in Sudanese patients with type 2 diabetic hypertensive patients.*

Methods: *A total of 300 Sudanese patients with type 2 diabetic hypertensive were evaluated. FGB -455 G/A genotypes were determined by polymerase chain reaction with restrictive enzyme Hae III.*

Results: *The frequency of the A allele in Sudanese with type 2 diabetic hypertension patients was 62.3% and G allele was 32.7 %. However, the frequency of the -455G/A polymorphism was 14% .The A A allele more prevalent among case and control groups.*

Conclusion: *455 G/A allele present in Sudanese patients with type 2 diabetic hypertensive but not in control group and may affected in some demographic data.*

Key words: Type 2 diabetic hypertension, Beta Fibrinogen Gene 455G/A polymorphism, PCR-RFLP.

INTRODUCTION:

Diabetes mellitus (DM) is metabolic disorders characterized by chronic hyperglycemia due to disturbance of carbohydrates, fats and protein metabolism associated with absolute or relative deficiencies in insulin or insulin action or both [(1)].Over 170 million people worldwide and about 1.9-7.0% of African population were affected [(2)].

Diabetes has three main types, type 1 diabetes mellitus, which is called (Insulin Dependent Diabetes Mellitus).Type 2 diabetes mellitus, which is called (Non Insulin Dependent Diabetes Mellitus) and gestational diabetes which is classified as type two diabetes mellitus [(3)]. The long term affects and complications of diabetes include progressive development of retinopathy, nephropathy and neuropathy with micro vascular and macro vascular diseases. Macro vascular disorder such as atherosclerosis are recognized as major causes of mortality in the diabetic population ,and are implicated in circulatory disturbances that are seen in diabetes. The circulatory disturbance in platelets count and activity coagulopathy, fibrinolytic aberration, haemorrlogical factors and change in endothelial metabolism. Many studies have shown that DM cause hypercoagulable state[(4)]. The prevalence of DM in the Sudan, as in many other low-income countries, is increasing to epidemic proportions, leading to the emergence of a public health problem of major socio-economic impact. Type 2 DM in Sudan is common among the adult population of northern Sudan and prevalence of type 1 DM was estimated at 0.1 % among school children7-14 years of age [(5)].Hypertension is sustained high blood pressure (≥140/90mmHg)[(6)]. When hypertension coexists with overt diabetes, which is commonly

does, the risk for cardiovascular disease, including nephropathy, is raised two fold. Improved control of blood pressure in diabetic patients has been shown to be effective in reducing the risk of cardiovascular complications [(7)]. Polymorphisms of the beta-fibrinogen gene including the beta-455 Guanine/Adenine polymorphism, which is especially involved in the rate-limiting steps of the formation of the beta -chain have been shown to be closely related to elevation of the plasma fibrinogen level. Several studies have suggested that the -455 Guanine/Adenine polymorphism is associated with an elevated plasma fibrinogen concentration [(8)]. Beta-fibrinogen 455 G/A polymorphism is a gene mutation that may lead to alterations in the activity of fibrinogen. Previous study revealed the increased fibrinogen activity in the presence of homozygote Adenine /Adenine allele [(9)].Homozygote of the A allele of the fibrinogen beta -455 -G>A gene polymorphism, which is caused by a Guanine -to-Adenine substitution at position -455 in the 5, promoter region of the fibrinogen beta gene, show higher plasma fibrinogen levels than subjects with the GG genotype. Elevated plasma fibrinogen concentrations have been associated with increased plasma viscosity and platelet aggregability and, thus, may contribute to vascular disease [(10)]. The risk of venous thromboembolism appears to be elevated in both type 1 and type 2 diabetic patients [(11)]. Increased thrombin generation and higher concentration of procoagulant cell-derived circulating micro particles in patients with type 2 diabetes suggest that hyper coagulability may play an important pathogenic role in the increased frequency of venous thromboembolism[(12)]. Diabetic foot gangrene results from peripheral arterial disease with or without foot sepsis. It is associated with excess mortality, although venous thrombosis has been reported in association with gangrene [(13)]. Evidence also links haemostatic variables to the future risk of myocardial infarction and stroke. So far, a variety of markers of a procoagulatory tendency e.g. elevated fibrinogen, coagulation

factor VII,von Willebrand factor, platelet hyper aggregation, and plasma levels of D-dimer[14] .

It was determined that the increased A/A allele is associated with increased cardiovascular events and increased prevalence of lacunar infarct in brain [15]. This study to detect beta fibrinogen gene 455G/A polymorphism in Sudanese with type 2 diabetic hypertensive patients.

MATERIALS AND METHODS:

Subjects:

This was a descriptive study conducted on Sudanese with type 2 diabetic hypertensive patients and were done in Khartoum teaching hospital in Khartoum state during the time period from January 2014 to April 2016**.** The Demographic data (Number, age, gender, duration of disease, type of treatment, dosage of therapy and presence or absence of other diseases) were collected through questionnaire. The study was included (300) of Sudanese type 2 diabetic hypertensive patients and (100) non diabetic non hypertensive individual as controls. Diabetic patients without hypertension or with type 1 Diabetes Mellitus were e excluded. The objectives of the study were explained at the beginning to all individual under study. Written consent was obtained from each participant in the study. An interview with subjects was conducted to obtain the clinical data, questionnaire including informative data (Number, age, gender, duration of disease, type of treatment, and presence or absence of other diseases.)

Genotype determination:-

DNA extraction: Blood will be collected in tubes containing EDTA at baseline, centrifuge to collect leukocytes and store at -80 until use. DNA was extracted using the Vivantis DNA blood kits (Malasia) according to manufacture instructions.

Genotyping:

Beta fibrinogen 455 G/A gene poly morphism (rs1800790) was detected using PCR-RFLP method. As a negative control, PCR mix without DNA sample was used to ensure contamination free P C R product. The polymerase chain reaction (PCR) primers for DNA -fragments in the promoter region of the fibrinogen gene -455 G/A polymorphism were forward, 5-AGGGTCTTTCTGATGTGT-3 and the reverse 5-AAGTTAGGGCACTCCTCA-3. PCR amplification was performed with a 50-µL reaction volume containing 1 µL of DNA template, 1 µL of each of the sense and antisense primers, 4 µL of deoxynucleotide triphosphate, 5 µL of MgCl2, and 0.5 µL of AmpliTaq GoldTM polymerase. The amplification conditions were as follows :an initial denaturing step at 96 C^0 for 7 min, followed by 35 amplification cycles of denaturation at 94 Cfor 30 sec, annealing at 55 C for 30 sec, and extension at 72 C^0 for 30 sec, and a final extension step of 72C^0 for 10 min. PCR products were electrophoresed on a 2% agarose gel, and the amplified genomic DNA fragments were extracted from the agarose gel After 45 µL of amplified PCR products was mixed with 5 U of restrictive enzyme Hae III and buffer, they were allowed to react at 37C^0 for one hour. The final product was electrophoresed on a 1.2% gel containing Ethedium Bromide, and its genotypes were analyzed using a UV transilluminator.

Restrictions Enzyme digestion:

The PCR products were digested with *Hae*III restriction endonuclease (Vivantis Company, Malaysia) and subjected to electrophoresis on a 1.2% agarose gel. Bands were visualized with ethidium bromide staining under ultraviolet light. The PCR product of -455A allele was not cleaved by *Hae*III generating a 336-bp band, whereas the PCR product of -455G allele was cleaved by the enzyme generating 215 and 121-bp fragments [2] (Fig. 2). The G/A heterozygote generate three bands: 336, 215, and 121-bp bands.

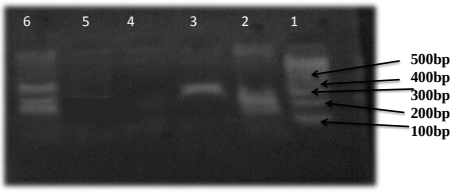
The data of this study will be analyzed by SPSS computer program version 16.0 software to calculate means, standard deviation and probability value (P.value) of parameters of fibrinogen level, D-dimer and fibrin degradation product for case and control and numbers. Independent T. test will be used to analysed data and then results. Age, duration of disease, and dosage of therapy will be classified into three groups by One Way ANOVA. Qui square I will be used to place frequencies of each alleles to each group.

RESULTS:-

Patients' characteristics

A total of 400 participants were included; the number of female patients was significantly higher than male patients. However, female sex predominated in the control group. The mean age was 55 years. Age of patients was significantly higher compared with the other control groups. All risk factors for patients were significantly when compared with controls (Table 1).

Restriction enzyme HaeIII digest P.C.R product to three alleles-455 A/A , 455G/G and 455G/A .



Lane 1 marker ladder 100, 200,300,400, 500 basepairs; lane 2 G/G genotype (215, 121bp); lane 3 , A/A genotype(336bp); lane 6, G/A genotype(336,215 ,121 b p); lane 4 and 5 negative control.

Figure (1)

Table 1: Risk factors detected in the case and control groups

Risk Factors	Case			control			*P* value
Age							
Range (Years)	(38-55)	(56-72)	(73-90)	(38-55)	(56-72)	(73-90)	< 0.00
Number	129	163	8	91	9	1	
Sex [N (%)]							
Male	92 (32)			40 (10)			> 0.08
Female	208(52)			60(15)			
Hypertension [N (%)]							
Essential	296(74)			0			< 0.02
Secondary	4(1)			2			
Retinopathy [N (%)]	112(28)			5			< 0.00
Nephropathy[N (%)]	12(3)			3			< 0.01
Neuropathy [N (%)]	40(10)			0			< 0.04
Others Diseases [N (%)]							
Athero scorlosis	4 (1)			0			< 0.00
Thyroid	4(1)			0			< 0.05

Case: Sudanese with type 2 diabetic hypertensive patients. $P < 0.05$, significant, $P > 0.05$, insignificant. Control group; Sudanese non diabetic non hypertensive individual.

Table 2: Genotypic distribution of fibrinogen -455G/A polymorphism in different studied groups

Genotype Frequencies	Case (N=300) [*N* (%)]	Control (N=100) [*N* (%)]	*P*value
A/A	187 (47)	63(15)	0.00
G/G	71(18)	37(9)	0.02
G/A	42(10)	0(0)	0.01

A: Adinine; G:Guadinine

Table 3: Allele frequencies of fibrinogen -455G/A polymorphism in different studied groups

Allele Frequencies	Case (N=300) [*N* (%)]	Control (N=100) [*N* (%)]	*P* value
A	416(69)	127(64)	0.00
G	73(36)	184(30)	0.00

DISCUSSION:-

Present study used PCR–RFLP to evaluate the role of -455G/A fibrinogen polymorphism (rs1800790) in the occurrence of type 2 diabetic hypertensive Sudanese patients referred to

Khartoum Teaching hospital in Khartoum State were compared with controls. As expected, the classic risk factors for type 2diabetic hypertensive were significantly more common in the study group. Similar to other studied populations, Sudanese patients showed more prevalence of the AA genotype followed by the GG, and the least common was GA genotype [2]. This is in agreement with the study by Lam KS, *et al.* [16] , who did found difference between control individuals and patients, AA genotype frequency were found in diabetic patients with heart disease ($p < 0.05$ and $p < 0.005$, respectively vs unaffected patients). However, in our study, AA genotype and allele A frequency were more common among the case and control groups, raising the question about the protective role of the A allele previously .This is in agreement with the study by Kessler C, *et al.* [17], he was found homozygosity for the A allele was more common in patients with CVD resulting from large-vessel disease. These data demonstrate that the AA genotype of the beta-fibrinogen G/A-455 polymorphism occur significantly more frequently in patients with CVD resulting from stenosis of large, brain-supplying vessels.

CONCLUSION:

The AA genotype and A allele of fibrinogen -455 gene polymorphism may confer some protection against cardiac disease, whereas GG and GA genotype may be less common Sudanese with type 2 diabetic hypertensive patients. However, further studies on larger scale and multicenter are needed.

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