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MOLECULAR DETECTION OF *CYP2C19*^{*2} GENE POLYMORPHISM AMONG SUDANESE PATIENTS WITH TYPE 2 DIABETUS MELLITUS IN KHARTOUM STATE

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ABSTRACT

Background: Diabetes mellitus is a global pandemic disease it is complicated by vascular occlusion. Studies indicated that in these patients platelet indices showed platelet increased activity as well as that the polymorphism of the cyp2c19*2 gene contributes to the state of DM thrombophilia. **Aim:** This study was aimed to detect cyp2c19*2 gene polymorphism in type2 diabetic Sudanese patients. **Methodology:** A total of 148 diabetic Sudanese patients were enrolled in this study. 2.5ml of peripheral blood using PCR analysis for *cyp2c19*2* polymorphism for genotyping and 2.5 ml peripheral blood in EDTA for complete blood count in particular platelet indices. **Results:** The freuency of cyp2c19*2 for (AA, GA, GG) was (52%, 41%, 5%), (36%, 55%, 8%) respectively for controlled and uncontrolled patients. In association of age with genotype show statistical significance P. value <0.05 the correlation of genotype with gender showed statistical significance P. value (0.01,.009) respectively between patients groups. In Correlation of genotype with platelet count, MPV, PDW and P-LCR is statistically insignificant. **Conclusion:** This study concluded the frequency of GA, GG genotype was higher in the uncontrolled group, and AA genotype is higher in the controlled group. Patient's age and gender was affected by the genotype.

KEYWORDS: *CYP2C19*^{*2} gene Polymorphism -type 2 diabetus Mellitus-sudan.

INTRODUCTION

Diabetes is a global public health condition that is increasing in prevalence and is associated with an increased risk of micro-and macrovascular problems. (Dindar *et al,* 2013). Eighty percent of patients with diabetes mellitus die a thrombotic death. Seventy-five percent of these deaths are due to cardiovascular complications, and the remainder is due to cerebrovascular events and peripheral vascular complications (Feher *et al,* 2009). Vascular endothelium, the primary defense against thrombosis, is abnormal in diabetes. Endothelial abnormalities undoubtedly play a role in the enhanced activation of platelets and clotting factors seen in diabetes (De Morais, *et al* 1994)... In high blood glucose levels, the increased production of glycoproteins results in non-enzymatic glycation of these proteins on the surface of the platelets, reducing the membrane fluidity of the platelets and leading to hyperactive platelets. (Kakouros *et al* 2011). Plasma glucose content also directly stimulates the arachidonic acid pathway, resulting in increased production of thromboxane A2, a strong platelet activator that contributes to platelet hyperactivity in diabetes patients. (Jaman *et al*, 2017). Theories suggested that the less responsive to antiplatelet drugs in diabetes patients could be due to the effect of the activation enzyme produced by the Cytochrome P450 Family 2 Subfamily C Member 19 (CYP2C19) gene (Martinez-Marignac *et al 2007)*. CYP2C19 gene is a member of the cytochrome P450 gene family produced, enzymes involved in the formation and metabolism of various molecules and chemicals within including a drug called clopidogrel. Clopidogrel is an antiplatelet drug, which prevents platelets from sticking and aggregating together and forming blood clots *(*Cetinkunar *et al*, 2016).

In this study, CYP2C19*2 genotype was investigated among Sudanese diabetic type 2 patients in Khartoum state using PCR technique.

METHODOLOGYS

This is an analytical cross- sectional study, conducted at Omdurman Military hospital, Khartoum, Sudan, in the period from September 2016 to July 2021. In which, diabetic patients were diagnosed based on results of Hb A1c test, 74 controlled diabetic patients HbA1c <6.5 and 74 uncontrolled diabetic patients HbA1c >6.5 were enrolled. As exclusion criteria patients with liver disease,

kidney, and hemopoietic system diseases, and patients who did not use other drugs that could affect platelet function within 1 month. In addition, to further avoid the interaction of drugs, the patient did not take other drugs, except for clopidogrel and atorvastatin, a total of two and a half ml of venous blood sample were collected from all participants in Ethylene diaminetetraacetic acid (EDTA). The DNA was extracted using a Genomic DNA Extraction kit (Intron). The extracted DNA was then analyzed for purity and yield via absorbance spectroscopy (A260/A280) using the NanoDrop ND 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and split into 2 aliquots. Control materials consisting of DNA with the known genotypes for CYP2C19, namely for the *1, *2, *3, the quality of DNA was determined by agarose gel electrophoresis. The DNA was quantified using a spectrophotometer. The amplification condition consists of initial denaturation at 94ºC for 5 minutes; then 34 cycles [each consists of denaturation at 94ºC for 45 second, annealing at 62ºC for 40 second, and extension at 72ºC for 50 second], and a final extension at 72ºC for 7 minutes PCR was amplified using primers pair for flanking region of CYP2C19*2 with forwarding primers.5′-AAATTGTTTCCAA TCATTTAGCT-3´ and reverse primers 5′-TAAAGTCCCGAGGGTTGTTG -3´. The strategy for the design of allele-specific primers followed that off. Krishna Kattel, Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE 68198 USA. Agarose gel electrophoresis PCR products of CYP2C19 were run in 1.5% agarose gel dissolved in 1x TBE and was visualized with a gel documentation system. Digestion of cyp2c19*2 PCR products was digested with the Sma1 enzyme. In the CYP2C19 * 1 (wild-type) allele the restriction enzymes Sma I splice the 321 bp DNA fragments into 212 bp, 109 bp.

Patient's data were collected using a structured interview questionnaire and analysed by statistical package for social science (SPSS), version 21. The qualitative data were presented as frequency and percentage. Quantitative data were presented as Mean±SD. Association between qualitative variable was tested by Chi-square (X2) and Fisher's exact tests. Multivariate logistic regression analysis was used for the examination of interaction between the polymorphism and thrombophilia risk factors. The allele frequency and Hardy Weinberg Equilibrium (HWE) were calculated using the conventional formulas.

The study was approved by the scientific research committee, faculty of medical laboratory sciences, Karary University Khartoum, Sudan and informed consent was obtained from each participant before sample collection. Patients' data was kept confidentially and only used for the purpose of the study.

4. RESULTS

A total of 148 diabetic Sudanese patients were enrolled in this study. *The* Socio-Demographic data show the mean age of the uncontrolled' group was ranged from 40 to 90 years (53±12.86) and males were (34%) and females were (40%). The mean age of the controlled group was ranged from (40 to 90) years (53±13.1), males were (33 %) and females were (41 %) as shown in table 1 Clinical characteristics of participants show the Frequency of uncontrolled patients, (52%) on aspirin therapy, (58%) were obese, (62%) had a familial history of diabetes, (55%) were hypertensive, (65%) had poor dietary control (12 %) had history of diabetes complications, for controlled patients (58%) of patients on aspirin therapy, (20%) were obese, (56%) had a family history of diabetes, (25.3%) were hypertensive (7%) were poor diet control, (10%) had a history of diabetes complications as shown in table 2. For *C*yp2c19 gene polymorphism length 321 bp, the results of PCR amplification yielded two amplicons, 212pb, 109 bp was done on gel electrophoresis as shown in the table (3-4-5-6-7). Figure 1.

Table 1: The Socio- Demographic characteristics of the Participants.

Characteristic	Uncontrolled	Controlled
N	74	74
Age	53.6 ± 12.8	53.4 ± 13.1
Sex (male: female)	34:40	33:41

Table 2: Clinical characteristics of participants.

Characteristic	Uncontrolled	Controlled
Hypertension :no hypertension	55: 19	25: 50
Family history of DM: no family history of DM	62: 12	56: 18
Complications: no complication	12:62	10:64
Diet control :non diet control	9: 65	68: 6
Aspirin intake(yes: no)	52: 22	58:1 6
Obese: non obese	58: 1 6	20: 54

Table .3: showed the prevalence of patients groups genotypes.

Genotype / Allele	Controlled (74)	Uncontrolled (74)
AA	39(52.7%)	27(36.5%)
GA	31(41.9%)	41(55.4%)
GG	4(5.4%)	6(8.1%)

A A, G A, GG genotype (52.7 %, 41.9 %, 5.4%) in controlled group and (36.5%, 55.4%, 8.1%) in uncontrolled group respectively.

Table 4: Showed the correlation between age and genotype.

Genotype	Age	N	Mean	P-value
AA	40-49	14	1.00	0.00
	50-59	12	2.00	
	60-69	1	3.00	
GA	60-69	20	3.00	
	70-79	16	4.00	
	80-89	4	5.00	
	>=90	1	6.00	
GG	60-69	1	3.00	
	70-79	2	4.00	
	80-89	3	5.00	

Statistically significance P .value <0.05, P .value (0.00) when compared ages groups.

Table 5: Showed the correlation of genotype with gender.

Genotype		Gender		P. value
		Male	Female	
Genotype (uncontrolled)	AA	8(23.5%)	19(47.5%)	0.010
	GA	21(61.8%)	20(50.0%)	
	GG	5(14.7%)	1(2.5%)	
Genotype (controlled)	AA	11(33.3%)	28(68.3%)	0.009
	GA	20(60.6%)	11(26.8%)	
	GG	2(6.1%)	2(4.9%)	

Statistically significance P. Value (0.010, 0.009) .respectively when compared between the patients groups**.**

Table 6: Correlation of genotype with platelet count and platelet indices (MPV, PDW, P-LCR) for uncontrolled groups.

Genotype (uncontrolled)	N	platelet count	MPV	P_LCR	PDW
AA	27	(300.07)79.17	(11.77) 1.08	(38.878) 8.98	(16.12) 3.04
GA	41	(274.36) 75.05	(11.99) 0.816	(41.06) 5.73	(16.77) 2.62
GG	6	(287.83) 79.84	(11.18) 0.614	(33.61) 4.66	(14.68) 1.68
P-value		.328	.686	.693	.816

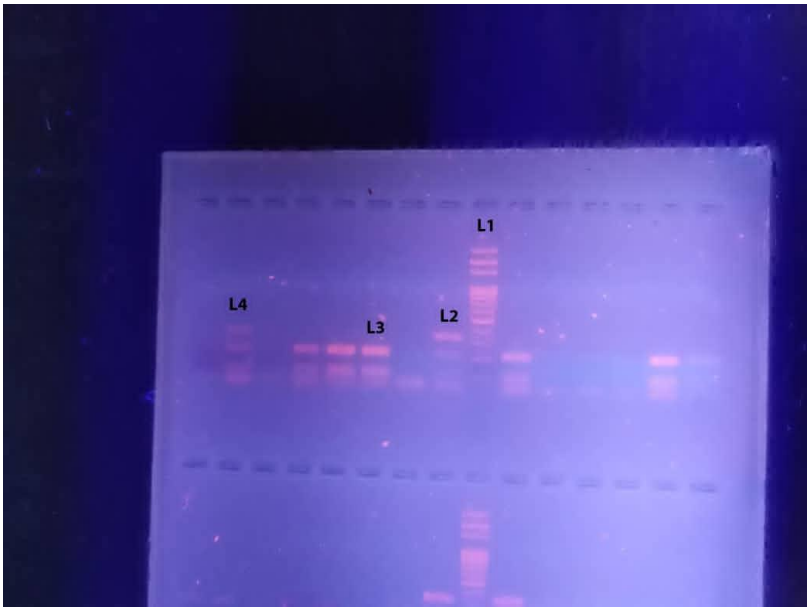
Statistically insignificant P. value > 0.05, P. value (0.32, 0.68, 0.69, 0.81) respectively.

Table 7: Showed Multinomial Logistic Regression between patients groups revealed correlation of GG vs AA genotype with thrombophilia risk factors diet control and weight.

		Uncontrolled			Controlled		
Equation	**Variable**	**Coefficient (Mean)**	**Std. Deviation**	**p-value**	**Coefficient (MEAN)**	**Std. deviation**	**p-value**
GA vs AA	**DRUG.ASP**	0.5815	0.804114	0.3685	1.0135	0.6872	0.3685
	OBISTY	-1.3056	0.921737	0.1244	-0.7160	0.6226	0.1244
	FMILY.HI	-0.1239	0.82668	0.8561	0.1726	0.7435	0.8561
	HTN	-0.9955	0.831805	0.1502	0.8008	0.5949	0.1502
	DIET.COL	0.1292	0.902552	0.8740	-0.5105	0.6561	0.8740
GG vs AA	**DRUG.ASP**	-1.6330	1.153256	0.2195	-2.0053	1.2723	0.2195
	OBISTY	-2.9288	1.182117	**0.0361**	-9.1666	120.0293	**0.0361**
	FMILY.HI	10.0362	10.04909	0.9208	13.8898	0.6597	0.9208
	HTN	-1.0922	1.05882	0.3300	1.2035	1.2373	0.3300
	DIET.COL	-18.2422	0.0000	**0.0000**	-1.0733	1.4378	**0.0000**

P- value (0.000, 0.0361) respectively is statistically significant when compared patients groups.

- Figure. 1 showed the restriction digestion pattern of CYP2C19*2. Sma I splice the 321 bp DNA fragments into 212 bp, 109 bp agarose gel stained with ethidium bromide (EtBr) and visualized with a UV transilluminator (High-performance UV Transilluminator; UVP). The CYP2C19 variant CYP2C19*2 was genotyped by the gel-based genotyping restriction digestion pattern.



Lane 1: ladder
Lane 2: wild type genotype (*1 3*1) of CYP2C19*2 showing one band of 321 bp
Lane 3: homozygous mutant genotype (*2 3*2). Showing two bands 212 bp 109
Lane 4: Heterozygous genotype (*1 3*2) of CYP2C19*2 showing three bands 321 bp, 212 bp and 109 bp.

5. DISCUSSION

Diabetes is a growing global public health problem associated with an increased risk of microvascular and macrovascular complications. (Bertilsson *et al*, 2002) An increase in MPV, PDW, and P-LCR is a potential biomarker of an increase in platelet size and reactivity. In hyperglycemic condition, the higher blood glucose level increases the production of glycoproteins and causes non-enzymatic glycation of these proteins on the surface of the platelets, which decrease platelets membrane fluidity and causes hyperactive platelets. (Hamdy *et al*, 2002) .This study agreed with a study done in Mexico by (Hoyo *et al*, 2010) the frequency of CYP2C19 showed statistically significant P. value. (0.012) in diabetic patients. This study correlated the gene polymorphism CYP2C19 *2 with age which is statistically significant P. value <0.05 there was not published data explain this correlation. This study found out the frequency of genotype AA, GA, GG in the controlled group male: female percentages (33.3 %:68.3 %) wild type (60.6%: 26.8%) heterozygous type (6.1%:4.9 %) homozygous type showed significant statistical difference P. value (0.009). In uncontrolled group (28.5%: 47.5%) wild type (61 .8 %: 50.0 %) heterozygous type (1 4 .7%:2.5 % homoozygous type. showed significant statistical difference P. value (0.010) no published data was explaining this correlation. The correlation of genotype with platelet indices (platelets count, MPV, P-LCR, PDW) P.value (0.328, 0.686, 0.693, 0.816) respectively showed statistical insignificance. No published data was explaining this correlation.

6. CONCLUSION

This study was concluded that the frequency of GA, GG genotype was higher in the uncontrolled group than controlled group. The Genotype was affected by patient gender and Age. Platelets count, MPV, PDW, and P-LCR were not affected by the genotype. Multivariate analysis showed that the effect of the CYP2C19*2 allele was better pronounced in the presence of non-genetic risk factors such as weight and diet control.

Abbreviations: (Hb A1c) Hemoglobin A1c, (EDTA**)** Ethylene Diamine Tetra Acetate, (MPV) Mean Platelet Volume, (PDW) Platelet distribution Width, (P-LCR) Platelet Large Cell Ratio, (DM) Diabetes Mellitus.

Conflict of Interest: Nil.

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