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Association between Transcription Factor 7-like 2 (TCF7L2) rs7903146 gene polymorphism and Lipid profile among Diabetic Patients Refer to *Abuagla* Diabetic Center Gezira State, Wed Medani

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Abstract: *Type 2 diabetes mellitus (T2DM) is the most prevalent component of metabolic syndrome.. TCF7L2 plays a master role in regulating insulin biosynthesis, secretion, and processing .The main aim to associate of the rs7903146 single nucleotide polymorphism at the Transcription Factor 7-like 2 (TCF7L2) locus with type 2 diabetic patients. This is laboratory-based case control study conducted at Aboagalla Diabetic Center from 2017 to 2021. One hundred twenty participants, seventy T2DM patients as case and fifty as Non-Diabetic group were enrolled in this study. Five ml of blood were collected from each participant into (EDTA and heparin) anticoagulant tube to obtain plasma , lipid profile were measured by automated chemical analyzer (Cobse C311 Roche) instrument also anthropometric (body weight, body height, and waist circumference were measured using the scale then DNA was extracted from whole blood using intron extraction kit and genotype of rs7903146 was carried out by a GeneAmp® PCR System 9700 (Applied Biosystems) thermo cycler .Genotype frequencies were examined for deviations from their corresponding Hardy-Weinberg equilibrium using SNP STAT software. SPSS software, version 22 was used for the rest of statistical analyses .Analysis of the distribution of the TCF7L2 rs7903146 genotype revealed that the GA genotype was more frequent in T2DM group (47.0%) than in Non-Diabetic (30%) (OR = 0.46, 95% confidence interval (CI = 0.21-0.99,p= 0.045). The T allele was more frequent in diabetic patients (48%) than control (41%). there is no statistically significant difference between the three genotypes regarding any of studied parameters except WC in the patient group was showed significant differences (p=0.035). It is concluded that the heterozygous genotypes GA, frequency of A alleles, dominant and over-dominant models of the rs7903146 of TCF7L2 gene are associated with the susceptibility of T2DM in the Sudanese population. We recommended studying all other SNPs of theTCF7L2 gene in our population which can potentially reveal the presence of other links with the susceptibility of developing diabetes and their complications.*

Keywords: Transcription Factor 7-like 2, polymorphism, Lipid profile, Diabetes mellitus**.**

Introduction

Diabetes is a disease that is defined by a chronic state of hyperglycemia. The most common form representing 90% of cases is type 2 diabetes mellitus (T2DM), also known as non-insulin dependent diabetes mellitus (Elhourch, S *et al*, 2021). The prevalence of diabetes mellitus is developing rapidly worldwide and is reaching epidemic proportions. the worldwide incidence of diabetes among adults is anticipated to be 6.4%, affecting 285 million people in 2010 and is expected to increase to 7.7% affecting 439 million humans by 2030 (Kolhar and Priyanka ,2017). It is a syndrome of impaired carbohydrate, lipid and protein metabolism resulting from either lack of insulin secretion or reduced sensitivity of the tissues to insulin (Hinge *et al*, 2019). In Sudan, the national prevalence of diabetes in adults is 7.7% and is expected to reach 10.8% in 2035 (Ali,R *et al*,2021). Diabetes is more prevalent in men than women (Ni *et al*, 2012). In Africa, diabetes mellitus is estimated to affect around 14 million individuals and this is expected to rise to about 28 million by 2030. The rise has been attributed to lacking of physical activity, high carbohydrate intake and ageing populations. Importantly, there is an increase in the prevalence in obesity an important risk factor for T2DM in Africa (Noor *et al*, 2015). Sudan has a very high prevalence of diabetes for instance the prevalence of diabetes in the eastern part of Sudan was estimated to be 20.8%%. The number of people with prediabetes in Sudan is expected to increase from 8.2% in 2017 to 9.4% in 2045 as part of the estimation of prediabetes in the Middle East (Saleh *et a*l, 2021). Lipids are frequently defined as naturally occurring compounds that are insoluble in water but soluble in nonpolar solvents. Amphipathic lipids form plasma membranes in which cells can maintain all biological events in an intracellular environment and respond to the changes of extracellular environment. In all living cells, lipids are required to maintain cellular structure, provide energy and are involved in cell signaling. One of the major consequences of diabetes is altered lipid metabolism which may leads to hyperlipidemia and dyslipidemia. Dyslipidemia is one of the most important causes of atherosclerosis which may leads to various type of cardiovascular disease It had been predicted that the fatty tissues was solely related to insulin resistance syndrome and T2DM. Duration of disease is one of the major consequences which lead to various secondary complications (Thagele *et al*, 2018). Genome-wide association studies (GWAS) have identified over 70 loci associated with T2DM including Transcription factor 7-like 2 (TCF7L2) gene.4 TCF7L2 is located on chromosome 10q25.3.5 TCF7L2 plays a master role in regulating insulin biosynthesis, secretion, and processing Moreover, TCF7L2 through the Wnt signaling pathway is essential for proliferation of the pancreatic epithelium and islet proliferation. Recently, TCF7L2 was found to protect pancreatic cells against interleukin-1 and interferon induced cell apoptosis, stimulates cell proliferation and mediates glucose stimulated insulin secretion(Mandour, I *et al*, 2018).This gene and its many intronic variants have been reported to have a strong

correlation with type-2 diabetes mellitus, impaired insulin secretion, synthesis, processing and modulation. While polymorphism of TCF7L2 is closely associated with the increased risk of diabetes mellitus, its precise role in obesity remains unclear (Mohammed, A.K *et al*, 2012). Blood glucose levels are maintained by the balance between glucose uptake by peripheral tissues and glucose secretion by the liver. Thus gluconeogenesis from liver plays a critical role in maintaining glucose homeostasis. Studies suggested TCF7L2 might be a potential negative regulator of gluconeogenesis in the liver. Norton et al (Norton, L *et al*, 2011) demonstrated that interfering in Wnt signaling by silencing TCF7L2 led to increased basal levels of hepatic glucose production in a rat hepatic cell line, associated with the over-expression of genes that encodes key rate-limiting gluconeogenic enzymes including 8 fructose-1-6-bisphosphatase, phosphoenolpyruvate carboxykinase 1 and glucose 6- phosphatase, in line with this finding, Oh et al (Oh, K.J et al 2012).

Materials and Methods

Study design: This is descriptive cross sectional case control study.

Study area and duration: This study was conducted in Sudan, Gezira state in Wed Medani Abuagla Diabetic Center from 2017 to 2021.

Study Population and Sample Size: One hundred twenty participants, seventy T2DM patients as case and fifty as controls were enrolled in this study

Inclusion criteria: Patients with T2DM both sex their age between 35 – 60 years old (case) and normal people (control) agree to participate in the study.

Exclusion criteria: Type 2 diabetes mellitus patients with: pancreatic disease and/or on treatment drugs – Malignancies **-** Immune diseases **-** Pregnancy **–** Hyperthyroidism **-** Hepatic disease and renal disease

Ethical consideration: This study was approved by ethical committee of faculty of medical laboratory sciences and permission obtained from the local health authorities of Gezira state and informed consent obtained from the study subject

Tools of data collection: The data was collected by structural questionnaire.

Methods: The patients were diagnosed according to American Diabetes Association criteria for diabetes. All patients and control subjects were subjected to thorough medical evaluation including determination of age, gender, anthropometric measures [weight, height, CW and BMI]. Biochemical tests including: fasting blood glucose, HbA1c, total cholesterol, HDL, LDL and triglycerides. Detection of *TCF7L2* polymorphisms (rs 7930146,) was done by PCR.

Anthropometric:

- Body mass index was calculated by using the following formula:

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2}$$

Sample collection: Five ml of blood were collected from each patient and divided as follows: two ml of blood in EDTA vacutainer tubes for genomic DNA study and measuring HbA1c, One ml of blood was withdrawn in fluoride tube for measuring fasting blood glucose and two ml of blood were withdrawn in Heparin tube for analyzed lipid profiles. Clinical chemistry analysis was done by Cobas C311 (Roche diagnostics, Germany).

Biochemical assays: All biochemical assays were measured use cobas c311 Roche.

DNA Extraction: Genomic DNA was extracted from whole blood using G-DEX™ Total DNA Extraction Mini Kit – iNtRON as the following: DNA concentration was measured by UV absorption at 260 and 280 nm.

PCR allele specific- and Genotyping: The PCR based allele specific method was employed for genotyping of the rs7903146 polymorphism in all PCR reactions; all amplification reactions were carried out on a GeneAmp® PCR System 9700 (Applied Biosystems) thermo cycler. Specific PCR reactions were conducted in a final volume of 15 µl containing 2X Green GoTaq® Flexi Buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1.25 U of GoTaq DNA Polymerase (Promega) and 0.5 mg of genomic DNA was used. PCR primers used are;

Forward (allele specific) primer:

TCF7L2rs7903146-146G (5´-GAGAGCTAAGCACTTTTTAGAgAC -3´)

TCF7L2rs7903146-146A (5´GAGAGCTAAGCACTTTTTAGAgAT-3´)

And backward (common) primer:

TCF7L2rs7903146-146CP (5´GCTTCTCAGTCACACAGGCCT-3) .Wild type allele is (G) and Mutant allele is (A).

Internal control primer:

Hb-1: CAACTTCATCCACgTTCACC

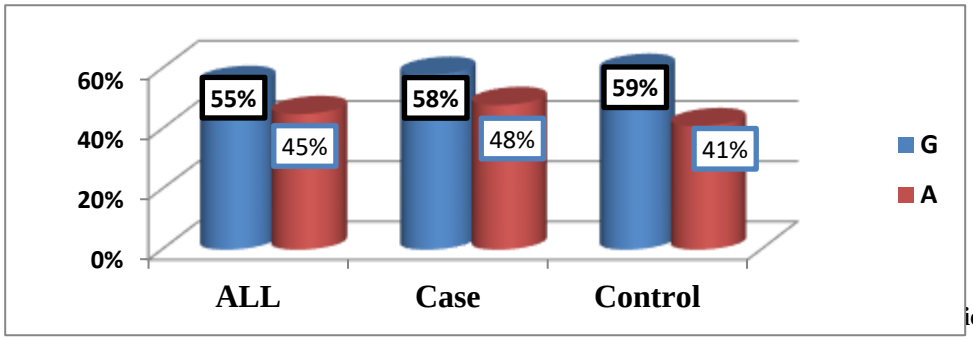
Hb-2: gAAgAgCCAAggACAggTAC

Internal controls1 and 2 primers wear 268bp and used to ensure the verification and quality of the method. We designed an allele-specific PCR reaction for genotyping the rs7903146 SNP. For this reaction we designed two doubly mismatched reverse primers with mismatches in their last 3' nucleotide and at the third nucleotide from the 3' end such that allow to specifically amplify one of the two variants (G/T) of the rs7903146 polymorphism. Briefly, for each sample, two PCR reactions were run in parallel, combining a common forward primer (CFw) and one of the two reverse primers in order to detect the presence of the polymorphic base (Rev G and Rev T). The observation of a 168-bp band in PCR reaction G or T indicated the presence of the respective allele.

PCR conditions were as follows: 3 minutes at 94˚C followed by 40 cycles of 45 s at 94°C, 30 s at 54°C, 45 s at 72°C and final extension of 72˚C for 2 min .the resulting PCR products were visualized by 2% a garose gel electrophoresis after ethidium bromide staining7µL(40%).

Statistical analysis: Genotype frequencies were examined for deviations from their corresponding Hardy-Weinberg equilibrium using SNP STAT software. SPSS software, version 22 was used for the rest of statistical analyses. Mean age, sex, BMI, WC and duration of DM of cases and controls, fasting plasma glucose, HbA1c and lipid profiles were compared by ANOVA test **.**Demographic data use frequency percentage

Results:



ic group

The allele frequency showed that G allele (58%) in T2DM cases and in controls (59%). The model analysis was studied to detect the association of TCF7L2 genetic polymorphism. For SNP rs7903146, association of rs7903146 polymorphism was found with T2DM in the different genetic models. In the co-dominant model, the heterozygous AG contributed 47% occurrence of T2DM (OR = 0.39; 95% CI =0.16-0.93; p =0.10) and in the dominant model (AG/AA) imparted 71% risk (unadjusted OR =0.49, 95% CI =0.23—1.06; p = 0.07) for the disease development. (Table 4.1.3.1).

Table 1: Association of rs7903146 SNP of TCF7L2 gene according to the model of inheritance in Type 2diabetic patients and Non-Diabetic group

rs7903146 association with response STATUS (n=120, crude analysis)					
Model	**Genotype**	**Diabetic**	**Non- Diabetic**	**OR (95% CI)**	**P-value**
Co dominant	**G/G**	**20 (28.6%) 2**	**22 (44%)**	**1.00**	**0.10**
	A/G	**33 (47.1%)**	**15 (30%)**	**0.39 (0.16-0.93)**	
	A/A	**17 (24.3%)**	**13 (26 %)**	**0.68 (0.26-1.77)**	
Dominant	**G/G**	**20 (28.6%)**	**22 (44%)**	**1.00**	**0.07**
	A/G-A/A	**50 (71.4%)**	**28 (56%**	**0.49 (0.23-1.06)**	
Recessive	**G/G-A/G**	**53 (75.7**	**37 (74%)**	**1.00**	**0.81**
	A/A	**17 (24.3%)**	**13 (26%)**	**1.11 (0.48-2.58)**	
Over dominant	**G/G-A/A**	**37 (52.9%)**	**35 (70%)**	**1.00**	**0.05**
	A/G	**33 (47.1%)**	**15 (30%)**	**0.46 (0.21-0.99)**	

As showed in (table 2) there is no statistically significant difference between the rs7903146 three genotypes and lipid profile among cases and control group

Table 2: Comparison between TCF7L2 rs17903146 genotypes among diabetic patients group and Non-Diabetic group according to anthropometric and Biochemical parameters.

Parameters	**Cases**			**P.Value**	**Control**			**P.Value**
	A/A	**A/G**	**G/G**		**A/A**	**A/G**	**G/G**	
TCH	**161.59±42.90**	**179.44±36.45**	**167.16±31.89**	**0.225**	**167.00±20.54**	**167.07±22.03**	**173.18±20.65**	**0.595**
TG	**112.24±40.53**	**127.65±74.83**	**120.16±53.29**	**0.703**	**75.92±8.66**	**84.47±15.66**	**79.73±9.65**	**0.157**
HDL	**36.98±4.67**	**39.32±19.03**	**36.13±5.87**	**0.692**	**39.77±4.48**	**41.67±4.37**	**39.96±4.82**	**0.458**

LDL	**104.65±35.22**	**117.56±34.04**	**113.16±36.32**	**0.465**	**120.46±23.99**	**117.80±26.23**	**125.00±24.56**	**0.678**

Discussion

The aim of this study was to investigate the association between the TCF7L2 rs7903146 gene and T2DM among study groups. The A allele frequency of rs7903146 gene polymorphism was observed in about was 48% among the cases while it was about 41% in control group. This finding is consistent with the previous reports done by Sarah Elhourch and other from Moroccan (Elhourch S *et al*, 2021) reported that A allele was 38% in the control subjects and 45% in T2DM. Other study done by Taha Hameed and other in the population of Khyber Pakhtunkhwa of Pakistan reported that A allele was 47% and35% in case and control respectively. Regarding to allele frequency of rs7903146 gene polymorphism A allele was common allele frequency among diabetic cases this finding agree with previous study done in Moroccan and Pakistan(Elhourch, S *et al* ,2021;Hameed, T , *al et* ,2021). G allele was common allele frequencies among study population by the other hand A allele frequencies was increase in case this finding agree with study done in Sudanese diabetic retinopathy patients (Shawki, H.A *et al* ,2020). Regarding to A allele was common allele frequency (48%) among study population, this finding agree with Studies have shown that frequencies of the A allele are highest in Africa, moderate in Asia, and lowest in North-America and Europe (Guinan KJ, *et al, 2017*). Different studies yielded controversial results in different ethnic groups. the A allele was protective against diabetes in North Indian population and Cameroon population (Verma S *et al* 2016 ; Guewo-Fokeng M *et al* ,2015) . In Sudanese and a Turkish population the A allele at rs7903146 was strongly associated with T2DM risk (Ibrahim AT *et al* , 2016 ; Demirsoy IH *et al* ,2016). In addition, there was no association between rs 7903146 variant and T2DM in Euro-Brazilian individuals. GA genotype was significant association with D.M ,this finding agree with study done in Cameroon (Guinan KJ , *et al* ,2017).Furthermore, This finding is consistent with those of other studies in diverse ethnic populations(Groves CJ *et al* ,2006; Berhouma R *et al* ,2012) .

When compared duration, lipid profile with the rs7903146 Genotype. We found no statistically significant association with these factors. Similar results were found in study conducted in the Moroccan population (Elhourch, S *et al*, 2021). In contrast to our result, GA + AA genotypes of rs7903146 were significantly associated with lower levels of total cholesterol in Iranian Kurdish ethnic population (Shokouhi *et al*, 2014)

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