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Forkhead Box O1 genetic variants and predisposition to gestational diabetes mellitus in Sudanese women

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Abstract

Forkhead box class O1 (FOXO1) is intimately linked.to Gestational Diabetes Mellitus (GDM). However, the association between genetic variations and susceptibility to GDM has not been explored. We aimed to detect the association of the common polymorphisms of FOXO1 gene and predisposition to GDM. In this study, a total of 193 pregnant Sudanese women aged (18-39 years) were classified into two main groups: 94 Normal pregnant women and 99 pregnant women diagnosed with GDM. The genotypes of two selected SNPs rs17446614 and rs2701858 in FOXO1 gene were determined by PCR-CTPP with proper primer designing and PCR conditions. Insulin homeostasis model assessment of insulin resistance (HOMA IR) and lipid profile parameters were also examined. We observed that differences in genotype frequencies of rs17446614, rs2701858 in FOXO1 for GDM *vs* normally pregnant women (NP) were statistically significant, particularly in the dominant model considering the major genotypes as a reference. Furthermore, we detected common haplotypes, ^{A}rs17446614 ^{C}rs2701858 (*OR*=1.89, 95%*CI*: 1.11-3.20**)** and ^{G}rs17446614 ^{T}rs2701858 (OR=2**.**13, 95%*CI*: 1.14 -3.95) within FOXO1gene showed significant difference. There was no association between genotype and biochemical parameters related to GDM. In conclusion, genetic variant rs17446614 and variant rs2701858 in FOXO1 gene may contribute to the risk of GDM in Sudanese women.

Keywords: Gestational Diabetes Mellitus, FOXO1, Sudanese Women

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Introduction

One of the most common pregnancy-related endocrinopathies is gestational diabetes mellitus (GDM), which is a specific form of glucose intolerance or hyperglycemia, up to 25% of pregnancies worldwide are affected by GDM [1]. Mounting evidence has shown that The pathogenesis of GDM has been linked to a number of cellular and functional disorders, including tissue insulin resistance and cell dysfunction [2], Insulin resistance during pregnancy is triggered by a genetic predisposition to impaired pancreatic islet-cell function, the results include an increase in the fetus's accessibility to glucose and impaired glucose metabolism, which negatively impacts the health of both mother and fetus [1-3]. A close relation between FOXO1 and the pathogenesis of GDM has been proposed [4].

FOXO1 is a multifunctional protein that regulates several processes, including proliferation, apoptosis, senescence, differentiation, stress resistance, autophagy, and metabolism [5]. The insulin receptor substrate 2 (IRS-2) proteins' tyrosine phosphorylation can be controlled by the high expression of the FOXO1 genes in insulin-resistant cells, which can improve insulin sensitivity and insulin signal transduction [6], resulting in an increase in the production of hepatic glucose and gluconeogenesis in the liver; it also causes -cell dysfunction by reducing -cell proliferation and compensatory capacity in islet [5, 7, 8].

Many particles, genes, and epigenetic processes that are known to contribute to the development of type 2 diabetes are being looked into as potential risk factors for GDM [1]. FOXO1gene has been documented to be involved in the susceptibility to type 2 diabetes in Germans [9] and Chinese population with complications-diabetic nephropathy [10]. Additional research demonstrated that FOXO1 SNPs were related to women's lifespan [11], two were found to be associated with carotid atherosclerosis [12]. The SNPs of FOXO1 gene are targeted by miR137, which has been implicated in lower hepatocellular carcinoma [13]. Nevertheless, the relationship of FOXO1genetic polymorphism and GDM has not yet been investigated Sudanese women.

In this study, we examined two single nucleotide polymorphisms within the FOXO1 gene and their haplotypes as predisposing factors to GDM.

Patients and methods

Study Subjects

One hundred and ninety-three pregnant women aged 18-39 years were recruited from the Wed Medani Teaching Hospital of Obstetrics and Gynecology, Gezira State, central Sudan. The Ethics Committee of the Faculty of Medicine University of Gezira approved the study, and informed consent was obtained from all women who participated in the study. All women

were between 24 and 28 weeks of gestation. Ninety-nine subjects were diagnosed with GDM conforming with the criteria of the WHO endorsing those of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) [14]. A total of 94 women who had Normal Glucose Tolerance (NP). None of the studied women were previously medically complicated with type 1 or 2 diabetes, history of hypertension, hyperthyroidism, hypothyroidism, polycystic ovary syndrome, inflammatory diseases, or treated with hormones or drugs that may modulate glucose or lipids metabolism.

Results

Measurements

Anthropometric measurements including the calculation of body mass index (BMI) was defined to assess overweight and obesity. Following an overnight fast, a 75g OGTT was conducted. Peripheral blood samples were taken at baseline, one and two hours after oral glucose administration. A glucose oxidase method was used to measure plasma glucose (mg/L), and a chemiluminescent immunoassay was used to measure insulin. The Tinder enzymatic method was used to measure the levels of total cholesterol (TC in mg/L), Triacylglycerols (TG in mg/L), High density lipoprotein (HDL-C in mg/dl), and Low-density lipoprotein (LDL-C in mg/dl). Insulin resistance was evaluated with the homeostasis model assessment (HOMA-IR) method, which uses fasting glucose and insulin level utilizing the equation [HOMA-IR (mg/L/µU/L) = (fasting Glucose mg/L× fasting Insulin µU/L)/405]. All measures were summarized as mean± standard mean error (SME).

SNP Selection and Genotyping

Genotypes and allele frequencies of FOXO1 SNPs for Sudanese population are not available on the data base yet, therefor, selection of the SNPs analyzed in this study was conducted based on the sub Saharan population according to SNP database of National Center for Bio-technology Information (NCBI) (https://www.ncbi.nlm.nih.gov/snp/), we nominated two intronic SNPs rs17446614 and rs2701858 FOXO1 gene with minor allele frequency MAF> 0.1. Using commercial spin-column procedure (innu PREP DNA Mini Kit (analytikjena AG Germany) for extracted Genomic DNA from whole blood, then DNA analyzed by electrophoresis in 0.8% agarose gels stained with ethidium bromide and visualized in a Gel. DNA was quantitated spectrophotometric ally on the Nano Drop spectrophotometer. The two foxo1 htSNPs were genotyped by polymerase chain reaction with Confronting Two-Pair Primers (CTPP-PCR) using primers newly designed (Macrogen, Seoul, Korea)was done by using the thermal cycler(TC-SQ; BOECO- Germany)are shown

on Table 4.Quality and specificity of PCR products was detected by running electrophoresis using 2% agrose gel followed by ethidium bromide staining

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 20 for Windows was used to conduct the statistical analysis. Descriptive statistics are showed as mean ± standard mean error (SME). T-test was used to detect differences of selected characteristics and genotype frequencies of the SNPs between the GDM cases and controls for continuous variables if they are statistically different. P value less than 0.05 was considered statistically significant. For the analysis of the relationship between genotypes and quantitative traits, categorical variables, one-way ANOVA was used. To estimate the connection between genotypes and GDM risk, logistic regression analyses were used to calculate odds ratios (OR) and their 95% confidence intervals (CI). The univariate logistic regression was used to calculate the crude ORs. Utilizing the SNP Stats program.
(http://bioinfo.iconcologia.net/index.php?module=Snpstats), various models of inheritance were assessed [15].

Results

Outcomes of the Clinical characteristics measurements conducted on the 99 GDM cases and 94NPcontrolswere illustrated in Table1. Results revealed that pregnancies complicated with GDM had significantly higher maternal age; however, both groups were matched for gestational age. On the other hand, there was no significant difference of the mean values in BMI between the pregnancy women complicated with GDM and NP groups. The HOMA IR was markedly elevated in GDM women compared with controls. Lipid parameters showed higher means for cholesterol, TG, and LDL-C in pregnancy complicated with GDM compared to normal NP group. The HDL levels were decreased in both groups.

Genotypes analysis

The genotype frequencies ofrs17446614deviated from Hardy-Weinberg Equilibrium, while those of rs2701858were under HWE. Association analysis of the rs17446614 and rs2701858 with GDM were assessed between the women with GDM and NP applying logistic regression analysis and the results were depicted in Table 2. For rs17446614,the dominant model of comparison showed that the GA and AA were more frequent women with GDM than NP group, demonstrated that pregnant women with the GA or AA having 2 times risk to develop gestational diabetes (OR= 1.81,95% CI:1.02-3.23, p= 0.043). Similarly, the minor genotype AA was represented as 27% in GDM group vs 15% in NP group, however,

the difference was within the borderline of significance (OR= 1.97 95% CI: 0.97-4.01, p= 0.055).The increased risk with the genotypes of rs17446614 was still significant in the log-additive model analysis (OR= 1.48, 95%CI: 1.04-2.12, p=0.028). The association analysis was also conducted on the intronic locus rs2701858,the frequencies of the genotypes CC, CT and TT were 40.4%, 51.5%, and 8.1%respectively in patients with GDM compared to 55.3%, 40.4%, 4.3% observed in the NP group. In dominant model CT+TT vs. CC, showed significant association with GDM (OR= 1.83 95% CI:1.03-3.23, p=0.038). Likewise, the log-additive model has showed significant association with GDM risk (OR= 1.68,95%CI:1.04-2.72, p=0.031). Nevertheless, no statistical difference was observed in recessive and codominant models between the study groups.

Analysis of variance was applied to assess the interaction between the genotypes of both SNPs and the selected parameters in women with GDM: BMI, FBG, FI, HOMAIR,TG, TC, HDL-C, and LDL-C. The impact of the genotypes on the modulation of the parameters did not reach the level of significance.

Haplotype analysis

Haplotype construction analysis for the rs17446614 and rs2701858was performed in the two groups. Carriers of ^{A}rs17446614 ^{C}rs2701858 haplotype and ^{G}rs17446614^{T}rs2701858haplotype found to elevate the risk of GDM (*AC* haplotype *OR*: 1.89, 95%CI: 1.11-3.20 p= 0.02)(GT haplotype *OR*= 2.13**,** 95%*CI*: 1.14 -3.95 *p*=0.018).

Discussion

In trophoblastic cells, it has been proven the interplay between the expression of FOXO1 gene and the tumor necrosis factor-alpha that provoke insulin resistance and involved in the pathogenesis of gestational diabetes [16]. In the context of the genetic markers of GDM, LncRNA X inactivate-specific transcript (XIST) was inversely related to miR-497-5p that target the FOXO1 gene, hence, the XIST/miR-497-5p/FOXO1 axis seems to have a potential role in control of hyperglycemia associated with pregnancy [17]. In like manner, the gene-gene interaction has been elucidated in studying the miR-142-3pand FOXO1 expression in GDM. The up regulation of miR-142-3p accompanied by sub expression of FOXO1 inhibits the pancreatic beta cells apoptosis and boost their proliferation [18]. In human pancreatic β-cell line, it has been proved that the decreased levels of vitamin D or non-functionality of vitamin D

receptors augmented the transcriptional and translational activities of FOXO1 gene resulted in increasing the oxidative stress and the process of programmed cell [19].

Regarding the observation between the GDM and the juvenile diabetes, a study on mouse model showed that the FOXO1 up regulates the expression of DNA Methyltransferase 3 Alpha which suggested the impaired glucose tolerance in offspring of mothers with GDM(20). Moreover, the significant correlation of FOXO1 with deregulation of glucose metabolism has been documented in experimental models of diabetes during pregnancy. Offspring showed activation of FOXO1 and some of the gluconeogenic enzymes [21].

We investigated the frequency of FOXO1genepolymorphisms rs17446614and rs2701858 and their haplotypes in GDM among Sudanese women. It was showed that the significant association between GDM risk and rs17446614 in both dominant genetic model and Log-additive model. In fact, the rs17446614 presented significant relations in Chinese patients with BMI, and risk for type 2 diabetes nephropathy (DN) carriers of allele A are two times more likely to have DN [22]. One explanation might be referred to an important genetic overlapping that has been evidenced between type 2 diabetes mellitus and GDM [23-24]. In agreement with our results, Wang and his colleagues reported that carriers of the rs17446614 in the recessive model have associated with sepsis risk in the Chinese population [25].

The results of single locus analysis revealed in dominant model and Log-additive model of inheritance in FOXO1 gene locus rs2701858, showed significant association with the GDM risk, although no any former study, which address the association of rs2701858 with GDM or T2DM was found yet, other SNPs of FOXO1 gene however showed such associations Additional functional research on various populations is required.

A haplotype is a group of associated SNPs that are found on the same homologous chromosome and are transmitted to offspring as a whole. Haplotype analysis has more statistical power than analysis of a single SNP since it provides more information about SNPs [26]. We found that the presence of the variants t^{A}rs17446614 ^{C}rs2701858 and^{G}rs17446614 ^{T}rs2701858, are significantly correlated with the risk of GDM patients. In our analysis, we evaluated how the genotypes of both SNPs and biochemical markers in women with GDM interacted to estimate the contribution of genotypes to BMI, age, HOMA-IR, TG, TC, HDL-C, and LDL-C, no significant associations were identified between the genotypes of both SNPs and biochemical parameters for GDM risk between cases and controls.

Furthermore, to be noted are some limitations on this study, Considering the limitations of our study, the small size sample of the study, which could be the reason why our analysis of the gene-genotype interaction failed to demonstrate any statistical significance., other limitation is

the lack of functional SNP analysis. This study demonstrated links between FOXO1 genetic polymorphisms and GDM in Sudanese women, and a base line for forthcoming studies.

Table 1.

Comparing gestational diabetes patients' clinical characteristics to those of normal pregnant women (Means ± SEM).

Parameter	**GDM (n = 99)**	**Normal (n= 94)**	***P* value**
Maternal age (year)	29.23± 0.50	27.52 ± 0.05	0.020
BMI (kg/m2)	30.01± 0.52	28.64 ± 0.56	0.076
FBG (mg\dl)	102.43± 2.75	81.69 ± 0.54	<0.000
FI (µU\ml)	9.32±0.52	8.86±0.25	0.437
HOMA IR	2.46±0.18	1.79±0.05	0.001
Cholesterol(mg\dl)	202.47± 4.18	182.40 ± 4.68	0.001
TG (mg\dl)	179.78± 6.35	155.34 ± 5.93	0.005
HDL(mg\dl)	35.20± 0.74	32.40 ± 0.75	0.008
LDL(mg\dl)	143.92± 4.36	130.98 ± 4.53	0.029

BMI: Body Mass Index, FBG: Fasting Blood Glucose, FI: Fasting Insulin, HOMA IR: Homeostasis Model Assessment of Insulin Resistance, TG: Triacylglycerol, HDL: High Density Lipoprotein, LDL: low-Density Lipoprotein

Table 2.

Genotypic Frequencies of rs17446614and rs2701858of FOXO1 gene among cases and controls

Model	SNP	Cases (%)	Controls (%)	OR(95%CI)	*P* value
rs17446614					
Codominant	GG	50 (50.5%)	61 (64.9%)	1.00	
	GA	22 (22.2%	18 (19.1%)	1.49(0.72-3.08)	0.089
	AA	27 (27.3%)	15 (16%)	2.20(1.05-4.57)	
Dominant	GG	50 (50.5%)	61 (64.9%)	1.00	
	GA+AA	49 (49.5%)	33 (35.1%)	1.81(1.02-3.23)	0.043
Recessive	GG+GA	72(72.7%)	79 (84%)	1.00	
	AA	27(27.3%)	15(16%)	1.97 (0.97-4.01)	0.055
Over dominant	GG+AA	77 (77.8%)	76 (80.8%)	1.00	
	GA	22 (22.2%)	18 (19.1%)	1.21 (0.60-2.43)	0.600
Log-additive				1.48(1.04-2.12)	0.028
rs2701858					
Codominant	CC	40 (40.4%)	52 (55.3%)	1.00	
	CT	51 (51.5%)	38 (40.4%)	1.74 (0.97-3.14)	
	TT	8 (8.1%)	4 (4.3%)	2.60 (0.73-9.25)	0.095
Dominant	CC	40 (40.4%)	52 (55.3%)	1.00	
	CT+TT	59 (59.6%)	42 (44.7%)	1.83(1.03-3.23)	0.038
Recessive	CC+CT	91 (91.9%)	90 (95.7%)	1.00	
	TT	8 (8.1%)	4 (4.3%)	3.19 (0.39-25.87)	0.270
Over dominant	CC+TT	48 (48.5%)	56 (59.6%)	1.00	
	CT	51(51.5%)	38(40.4%)	1.57(0.89-2.77)	0.120
Log-additive				1.68 (1.04-2.72)	0.031

Table 3.

Comparison of genotype frequencies by clinical characteristics for the rs17446614 and rs2701858 polymorphisms in the F*OXO1*gene

Phenotype	**rs17446614 Genotype**	**N**	**Cases Mean ±SEM**	**Overall p value**	**rs2701858 genotype**	**N**	**Cases Mean ±SEM**	**Overall p*value**
BMI	GG	50	30.72±0.78	0.373	CC	40	29.90 ±0.75	0.93
	GA	22	29.5800±1.16		CT	51	30.00±0.77	
	AA	27	29.06±0.80		TT	8	28.13±1.68	
FBG (mg\dl)	GG	50	102.04±3.68	0.118	CC	40	109.15±5.98	0.132
	GA	22	111.77±7.73		CT	51	97.74±2.31	
	AA	27	95.55±3.67		TT	8	98.75±4.06	
FI (µU\ml)	GG	50	8.58±0.05	0.354	CC	40	9.29±0.69	0.716
	GA	22	10.34±1.58		CT	51	9.55±0.84	
	AA	27	9.83±1.03		TT	8	7.91±1.12	
HOMA IR	GG	50	2.21±0.17	0.207	CC	40	2.57±0.26	0.207
	GA	22	3.05±0.62		CT	51	2.44±0.29	
	AA	27	2.42±0.31		TT	8	1.95±0.31	
Cholesterol (mg\dl)	GG	50	203.58±6.09	0.907	CC	40	194.90±6.44	0.155
	GA	22	199.00±8.158		CT	51	204.98±5.49	
	AA	27	203.25±8.21		TT	8	224.37±19.51	
TG(mg\dl)	GG	50	186.58±8.79	0.268	CC	40	170.22±8.80	0.362
	GA	22	160.68±10.05		CT	51	188.60±9.87	
	AA	27	182.74±14.34		TT	8	171.25±15.68	
HDL(mg\dl)	GG	50	35.36±1.02	0.909	CC	40	33.97±1.07	0.376
	GA	22	34.59±1.58		CT	51	35.90±1.06	
	AA	27	35.40±1.49		TT	8	36.87±3.06	
LDL(mg\dl)	GG	50	146.12±6.14	0.793	CC	50	139.22±6.70	0.266
	GA	22	138.50±8.85		CT	22	144.03±5.69	
	AA	27	144.25±8.85		TT	27	166.62±21.77	

Table 4.

No	rs17446614	rs2701858	Total Freq	Control	Case	OR(95%CI)	*P* value
1	G	C	0.488	0.5819	0.3963	1.00	-
2	A	C	0.2193	0.1734	0.2654	1.89(1.11-3.20)	0.02
3	G	T	0.1908	0.1628	0.2199	2.13(1.14 3.95)	0.018
4	A	T	0.102	0.0819	0.1185	1.73(0.80 -3.75)	0.17

List of Haplotypes of FOXO1Gene and their Association with GDM

Table 5.

The information for the designed CTPP primers rs17446614and rs2701858 and PCR Product size of the FOXO1 gene

SNPS	CTPP primer set		Length (bp)	Product size (bp)	Tm
rs17446614	P f1	5AAGTGAGAGCAGTGCAGTGTG3	24		56 c^0
	P r1	5TCTTTATGATTCCACTATGTCTGA3	21	383	
	Pf2(A)	5ATGAGATAGTATGCGTGGAAGGA3	23	255	
	Pr2(G)	5TAGCTAATAGAGATTAAGGTAGGC3	24	174	
rs2701858	P f1	5CACAATATTCCATCATGACAGT3	21		53 c^0
	P r1	5TTGGCAAAGTTAGGAAACAGC 3	22	460	
	P f2(c)	5AATTAGTATATGTCCCACCTAAG 3	23	336	
	Pr2 (T)	5ACATTCAAGAAGCGTTCACTCAT 3	23	165	

ABBREVIATIONS

FOXO1: Fork Head Box O1, GDM :Gestational Diabetes Mellitus, T2DM:Type -2Diabetes Mellitus, BMI: Body Mass Index, FIRI: Fasting Insulin Resistance Index.NP: Normal Pregnancy, TC: Total Cholesterol, HDL-C: High Density Lipoprotein Cholesterol, OGTT: Oral Glucose Tolerance Test, IR: Insulin Resistance

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Ethical approval

The Ethics Committee of the Faculty of Medicine, University of Gezira, Sudan, gave its approval to the study.

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