Tumor Necrosis Factor and Calpain 10 Polymorphism impacts in Women with GDM

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ABSTRACT

Gestational Diabetes mellitus (GDM) results from an imbalance between insulin resistance and insulin secretion capacity during pregnancy. Tumor necrosis factor-alpha (TNF-α) is an inflammatory cytokine that is proposed to be involved in the pathogenesis of insulin resistance, and (CAPN10) has been detected as a susceptibility gene insulin resistance. The purpose of the present study was to recognize the impacts of the Tumor Necrosis Factor-alpha (-308 G/A) and CAPN10(-44 C/T, rs2975760) polymorphism on the development of GDM in the Saudi pregnant women population. We performed a case-control study and genotyped single nucleotide polymorphism of CAPN10(-44 C/T) gene and Tumor Necrosis Factor-alpha (-308 G/A) in 201 from the Western Saudi Arabia participants from Jeddah city including 101 patients with GDM and 100 normal (non-GDM). GDM and healthy subjects were genotyped with the PCR- RFLP technique. Heterozygocity for the gene polymorphisms did not occur more often in GDM women compared with non-GDM (TNF-alpha(-308 G/A): 41% versus 38%, $p > 0.05$; CAPN10(-44 C/T): 20% versus 17%; $p > 0.05$). Moreover, there was no outstanding difference between GDM and non GDM concerning homozygocity for (TNF alpha(-308 G/A) 5% versus 10%, $p > 0.05$); and CAPN10(-44 C/T) (2% versus 1%, $p > 0.05$). Contrary to the results of some other researchers, gene polymorphisms do not seem to be important in our population for the development of GDM. Tumor Necrosis Factor-alpha (-308 G/A) and CAPN10 (-44 C/T) genes are dependent on danger agents and might play roles in the risk of GDM.

Key Words: *Gestational Diabetes Mellitus (GDM), TNF-alpha, Calpain-10 (44C/T), Saudi women*

eIJPPR 2020; 10(6):15-26

HOW TO CITE THIS ARTICLE: Safaa Y. Qusti, Maha Balgoon, Amal M. Almalki, Aljohara Alghamdi, Rewaa Fadil Alturkistani, Raghad Baljoon (2020). "Tumor Necrosis Factor and Calpain 10 Polymorphism impacts in Women with GDM", International Journal of Pharmaceutical and Phytopharmacological Research, 10(6), pp.142-148.

INTRODUCTION

Gestational Diabetes Mellitus (GDM) is characterized as irregular glucose tolerance which is first detected or diagnosed during pregnancy [1-4]. Gestational Diabetes Mellitus (GDM) develops when beta cells of islets of Langerhans are unable to compensate for elevated insulin resistance during pregnancy [5, 6]. Moreover, reduced insulin secretion, as well as a history of GDM, have been indicated to predict possible type 2 diabetes [6]. Women with GDM have a 40–60 % chance of developing diabetes mellitus over the 5–10 years after pregnancy [7]. The prevalence of GDM differs from 1-20% and is increasing worldwide, parallel to the increment in the prevalence of type 2 diabetes mellitus (T2DM), and obesity. The figures are rising in developing as well as developed countries and diabetes has involved both urban and rural zones of the world. Its incidence is more commonly seen in Arab countries especially the GGC countries including the Kingdom of Saudi Arabia. Saudi Arabia has the highest prevalence (32.8%) of T2DM. We predict that the occurrence of T2DM will increase from 32.8% in 2015 to 45.36% in 2030 [8].

In a recent large-scale multinational prospective study, increased maternal glucose concentration during pregnancy was highly associated with increased neonatal birth weight, primary cesarean delivery, neonatal hypoglycemia, and increased placental C-peptide levels [9]. Gestational Diabetes Mellitus (GDM) is also reported to recur at a frequency of 45% in subsequent pregnancies [10]. Besides, the offspring of GDM women are also at risk of developing obesity and T2DM [11]. Furthermore,

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 04 July 2020; **Revised:** 26 November 2020; **Accepted:** 03 December 2020

the recent recommendation from the International Association of Diabetes and Pregnancy Study Group has lowered the diagnostic threshold of GDM and is expected to increase the incidence of GDM [12].

Several types of researchhave studied the relationship between serum levels of insulin resistance in females with GDM and TNF-α, but reports are incompatible or even conflicting, especially across different ethnic groups.Historically, placental hormones, especially human placental lactogen, have been considered as primary mediators of insulin resistance during pregnancy leading to GDM. However, more recently, tumor necrosis factor-alpha (TNF-α), an inflammatory cytokine, hasbeen involved in the regulation of maternal metabolism and gestational insulin resistance [13-15].

Expression of the calpain 10 gene: The *CAPN10(-44 C/T)*was identified by Horikawa [16] in Mexican Americans, a linkage scan detected polymorphisms consistent with altered CAPN10 expression. The highest expression of CAPN10 mRNA is in the heart followed by the pancreas, the brain, the liver, and the kidney [17]. CAPN10 is involved in the control of glucose homeostasis through its functions in pancreatic β-islet cells, the liver, skeletal muscles, and adipocytes. CAPN10 is located on chromosome2q37.3 and consists of 15 31 kb exons encoding 672 amino acid intracellular protease. Several cases – control and interaction studies have revealed that polymorphisms in CAPN10 are associated with the development of T2DM and insulin resistance, especially in obese patients with early onset of disease [18-20]. The non-lysosomal cysteine protease CAPN10 calcium-dependent protease is involved in the reorganization of the actin cytoskeleton necessary for both insulin exocytosis in pancreatic beta cells and insulin-stimulated translocation of GLUT4 to the plasma membrane in adipocytes [21]. Our objective was to investigate whether such candidate genes previously found to be linked with T2DM, also offer a specific genetic predisposition to GDM in Western Saudi Arabia. Therefore, this study sought to answer the relationship between GDM, with *Tumor Necrosis Factor-alpha (-308 G/A) and CAPN10 (-44C/T)* gene in the Saudi women and assess its risk along with the clinical factors.

MATERIALS AND METHODS

Sample Collection

The study included 101 women with GDM who were treated in Al-Mesadiah hospital, Jeddah, and 100 normal (non-GDM) pregnant females with regular blood glucose. The conditions for the inclusion of the study included a diagnosis of GDM, a maternal evaluation in our hospital, and an acceptance to take part in the study.Each participant was provided with a questionnaire included: name, age, weight, height, blood pressure, pregnancy age, number of children, number of pregnancies, and miscarriage. Body mass index (BMI) was calculated at sampling time. All women signed informed consent.

Molecular Scrutiny

Three ml (3ml) of blood venous was drawn from all subjects normal (non-GDM) and GDM. Genomic DNA was extracted using a ReliaPrep TM Blood gDNA Miniprep system (A5082-250 Prep) kit as recommended by the manufacturer (Promega, USA). The concentration and purity of each DNA sample were estimated by using NanoDropTM 2000/2000c. NanoDrop spectrophotometer. DNA was stored at –80°C. The genotyping of *TNF-α* -308 G/A, and *CAPN10(-44 C/T)*polymorphism was completed by Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR–RFLP). The *TNF-α* -308 G/A forward and reverse primer pairs used for the PCR assay were 5'- AGG CAA TAG GTT TTG AGG GCC AT -3' and 5'- TCC TCC CTG CTC CGA TTC -3' respectively (PCR-SSP kit, Heidelberg University, Heidelberg, Germany). The primer sequence of *CAPN10(-44 C/T)* gene which made by (macrogen-Korea)were: (forward sequence) 5′GATGTGGGCATCCATAGCTT-′3(reverse sequence) 5′ TGATCCCATGGTCTGTAGCA-3′. The digested PCR products were electrophoresed on 3% of gel agarose including ethidium bromide.

Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS version 22). Descriptive data were given as Mean ± Standard Deviation (SD). The association between groups among genotype was assessed using a T-test if the data were normally distributed and a nonparametric Mann-Whitney test if the data were not normally distributed. Chi-square tests were applied to test the association between categorical variables. Also, an odd ratio with 95% CI was calculated from the logistic regression model to determine the risk factors for the disease. Moreover, the Hardy-Weinberg equilibrium test was determined to test the difference in allele frequencies.

RESULTS

In the current study, 201 participants including 100 normal(non-GDM) and 101 pregnant samples with GDM were involved. The mean and standard deviation (SD) were calculated for numerical variables and frequencies for categorical variables.

Clinical Characteristics of Pregnant Women

Biochemical characteristics and the phenotypic of the subjects of the study (GDM cases versus non-GDM) were

assembled, analyzed, and summarized in Table 1. Age and number of children distributions showed a significant difference between two study groups, the highest values were for females with GDM compared with the non-GDM participants and found to be statistically considerable; $P<0.01$ and $P<0.05$, respectively. BMI was high for patients based on the mean besides the number of obese people who had BMI 30 or above and had also larger frequency in a patient with a significant association with p-value < 0.0001.

Table 1. Clinical Details of GDM together with Non-GDM Involved in the Trendy

Factors	GDM (n=101)	Non – GDM (n=100)	P-Value	OR	95% CI
Age (Years)	31.8 ± 6.3	29.3 ± 4.9	P = 0.010* a	-	0.510 – 3.730
BMI (kg/m^2)	34 ± 6.5	29.4 ± 6.2	P=0.000*	-	2.677 – 6.379
FBS (mg/dl)	111.7 ±4.16	97.93 ±9.89	p<0.0001	-	-
Pregnancy Month	7.8 ± 0.78	8.8 ± 8.2	P=0.118	-	-2.435 – 0.695
Number of Children	2.2 ± 2.2	1.7 ± 1.7	P =0.033*		0.017 – 1.143
Obese (yes/no)	65/28	37/55	0.000*	-	-
Had GDM before (yes/no)	52/49	1/99	P=0.000*	99.000	13.285 – 737.733
Had Thyroid (yes/no)	15/86	9/91	P= 0.277	1.646	0.677 - 3.999
Had Miscarriage (yes/no)	10/91	8/92	0.806	1.278	0.482 - 3.384

Alleles and Genotype Frequencies

All GDM and non-GDM samples were genotyped for the *TNF-α* -308 G/A polymorphism. Allele and genotype frequencies in GDM and non-GDM groups are summarized in Table 2. Statistically in TNF genotype frequency was not found between the GDM and non-GDM subjects (p=0.598). A significant positive association with the A/A genotype was found for *TNF-α* at position -308 in our GDM and non-GDM subjects (5% *vs*. 10%, p=0.403), which considered as a protective factor. Another classification was by classifyinggenotype into allele A and allele G. The results showed no significant relationship with p-value = 0.605 > 0.05 and odd ratio calculated as 0.849 which indicates that the presence of allele A decreases the risk of getting the disease. Genotypes GA and AA were found to be associated with risk for GDM. In addition, in the dominant model (GG vs. GA + AA), the -308 G/A *TNF-α* polymorphism was found to be no significantly connected with GDM (OR = 0.923, 95% CI = 0.530 – 1.608, p = 0.777), but the association was detected for the recessive model (GG + GA vs. AA).The *CAPN10 (-44 C/T)* genotype in individuals with GDM group (n = 101) contained 78.2% TT, 20% TC, and 2% CC, and non-GDM (n = 100) consisted of 82% TT, 17% TC, and 1% CC. The total T-allele frequency in the GDM group was 178, and the C-allele frequency was 24, whereas, the normal (non-GDM) demonstrated overall T-allele and C-allele occurrences of 0.80 and 0.20, respectively; whereas the non-GDM demonstrated that total T-allele and C-allele occurrences were 181 and 19, respectively; there was no noticeable disparity between the GDM subjects and the non-GDM in either the genotype or the allel frequency. However, there was no statistically important difference between the GDM and non-GDM groups in either the genotype or the allel frequency(C *vs*. T: 0.440; 95% CI 0.412-1.471; p = 1.471).

Table 2: Evaluations of Allele and Genotype Frequencies between Women with GDM and Non-GDM

Gene	Position	Alleles/Genotypes	GDM (n=101) N (%)	Non - GDM (n=100) N (%)	p-value	Odd Ratio	95% CI
TNF-α	-308	A	51	58	0.605	0.849	0.358 – 1.311
		G	150	142			
		Mutant AA	5% n=5	10% n=10	0.403	-	-
		Heterozygote GA	41% n=41	38% n=38			
		Wild GG	54% n=54	52% n=52			
		AA	5	10	0.179	0.474	156 – 1.440

		GA + GG	95	90			
		GG AA+GA	54 46	52 48	0.777	0.923	0.530 – 1.608
CALN10	44 (rs2975760)	C	24	19	0.44	0.889	0.412-1.471
		T	178	181			
		Mutant CC	(2%) n=2	1% n=1			
		Heterozygote TC	19.8% n=20	17% n=17	0.676	0.588	0.049- 7.067
		Wild (TT)	78.2% n=79	82% n=82	0.554	0.482	5.419-0.043
		TT vs. TC + CC	79 22	82 18	0.502	1.269	0.633-2.543
		CC vs. TT + TC	2 99	1 99	0.567	0.500	0.045-5.604

Moreover, ANOVA was used to test the differences in BMI, age, number of children, and genotype categories (Table 3). Association of TNF-α (-308G/A) with the risk of GDM, stratified by BMI, indicated highly significant differences between study and control group for category GA with *p*-value 0.003 < 0.01, and category GG with *p*-value 0.001 < 0.01. Besides, no significant difference was indicated between study and control for AA, since, Odd Ratio value < 1, the AA genotype increased the probability of not getting a disease which was considered as a protective factor. We can numerically conclude that the GDM group and BMI were higher for CC genotypes of *CAPN10(-44 C/T*, and GDM patients with TT genotype had more children).

Table 3: Association of TNF-α (-308G/A) and CAPN10 (-44 C/T) with the Risk of GDM, Stratified by BMI.

	Genotype	**BMI (Mean ± SD)**		**Comparison**
		GDM	**Non - GDM**	***P*-value**
TNF-α (-308G/A)	Mutant (AA)	32.52 ± 6.55	29.30 ± 6.81	0.398
	Heterozygote (GA)	33.38 ± 5.75	29.05 ± 5.86	0.003
	Wild (GG)	34.38 ± 7.03	29.26 ±6.42	0.001
CAPN10(-44 C/T)	Mutant (CC)	37.1 ± 2.5	-	-
	Heterozygote (TC)	33.2 ± 7	32 ± 6.8	0.696
	Wild (TT)	34.1 ± 6.5	28.8 ± 6	0.262

By using binary logistic regression, the odds ratio for disease risk in the presence of AA, GA, and GG of TNF-α (-308G/A) was estimated.

Table 4. A significant impact on GA and GG for BMI; P= 0.006 and P= 0.001 res. which concluded that increasing BMI increased the risk of having the disease by odds calculated as 1.139 and 1.111 for GA and GG, respectively. Categorically by classifying BMI into obese and non-obese, a significant impact was conducted on GA and GG; P= 0.006 and P= 0.012, respectively, which concluded that being obese increased the risk of having the disease by odds calculated as 4.200 for GA and 2.838 for GG. Moreover, having GDM before increasing the risk of having the disease by odds calculated as 59.160 for GG.

There are no binary logistic results of *CAPN10(-44 C/T)* for CC genotype because the sample has only 3 observations of it. We conducted a significant impact on TT for BMI; P=0.026 so P < 0.05 which states that high BMI will increase the risk of having the disease by odds calculated as 1.116. For people who had Thyroid, no significant impact was conducted but we can observe from the odd ratio that having thyroid and TC genotype increases the risk of having GDM by odds 2.429 beside lower risk for TT genotype by odds 1.388.

Table 4. An estimate of Odds Ratio for Disease Risk Using Binary Logistic Regression.

		Factor	*B*	*P-value*	*OR*	*OR 95% CI*	
	Wild GG	*Age*	0.053	0.117	1.054	.987	1.125
		BMI	0.106	0.001**	1.111	1.042	1.185
		Obesity	1.043	0.012*	2.838	1.259	6.398

TNF-α (-308G/A)		*Thyroid*	4.080	0.000**	59.160	7.615	459.594
	GA	*Age*	0.047	0.255	1.048	.967	1.137
		BMI	0.131	0.006**	1.139	1.038	1.251
		Obesity	1.435	0.006**	4.200	1.509	11.687
		Thyroid	21.662	0.998	-	-	-
	AA	*Age*	0.507	0.128	1.661	.865	3.190
		BMI	0.077	0.373	1.080	.912	1.279
		Obesity	1.253	0.274	3.500	.372	32.971
		Thyroid	23.505	0.999	-	-	-
CAPN10 (-44 C/T)	*Wild TT*	*Age*	0.040	0.387	1.041	0.951	1.139
		BMI	0.110	0.026*	1.116	1.013	1.230
		Obesity	0.150	0.800	1.162	0.363	3.725
		Thyroid	0.328	0.588	1.388	0.424	4.550
	TC	*Age*	0.159	0.155	1.172	0.942	1.458
		BMI	0.019	0.858	1.019	0.829	1.253
		Obesity	-0.258	0.864	0.772	0.040	14.909
		Thyroid	0.887	0.516	2.429	0.166	35.427

B: Logistic Regression Coefficient. OR: Odds Ratio. CI: Confidence Interval

DISCUSSION

Controlling the blood glucose levels in GDM utilizes diet, medications, and lifestyle changes. Early recognition and control of GDM correct the health of both the fetus and the pregnant woman [22, 23]. Therefore, early detection and avoidance of GDM may also benefit from the identification of susceptible individuals.

During pregnancy, the development of TNF-α in maternal adipose tissue is increased by the placental development of TNF-α, which makes it an important factor in the pathogenesis of insulin resistance and GDM. This contradicts the conventional view that sexual hormones alone decrease resistance to insulin during pregnancy [24]. Several studies have evaluated the concentrations of circulating TNF-α in a pregnant female with GDM; however, the results remain ambiguous. Some studies have shown an improvement in TNF-α in the blood of mothers who developed GDM [25]. The functionality of SNPs concerning gene expression is an important subject in the studies of association withdiseases. Both environmental risk factors and genetic background contribute to the development of GDM. TNF-α rise with pregnancy production and the main production source of this cytokine appears to be the placenta [26]. Increased TNF-α can worsen insulin resistance, which is common in pregnancy; this promotes the production of GDM [27]. Some researchers have been demonstrated an improvement in TNF-α in the blood of mothers who have developed GDM [25]. However, those observations have still not been verified by other studies [28]. The functionality of SNPs to gene expression is an important subject in the studies of association withdiseases [29]. The vital role of this cytokine in the arrangement of inflammatory and immune responses are of clinical interest and may be caused by the correlation between the existence of SNP in the TNF α and their plasma levels [30]. In summary, TNF-α is linked to obesity; α is related to obesity as the study results wereconfirmed in Table 3, glucose intolerance, T2DM, and GDM, and the same notice in our results is associated with body mass index (BMI) [31]. SNP in the human TNF α may help achieve insulin resistance after that driving to T2DM may be due to the proof to propose that TNF α can prevent insulin signaling, and therefore, impair insulin secretion. The results of our study showed no important difference between the GDM group and non-GDM C regarding the TNF-alpha gene SNPs genotypes or alleles distribution. This is in agreement with Montazeri *et al*. (2010) who genotyped the G to A exchange at position -308 of the TNF-alpha gene and found no difference in allele frequency between the two groups.Meanwhile, some reports supposed that allele A is the highest commodities for sale version, in opposite allele G also was noticed as the highest producer likewise. In addition, it is observed that the polymorphism of TNF-α gene at local-308 G/A is relationship with transcriptional activation [32]. It was caused these to differ striking finding, the question of whether TNF-α gene is included or not in the pathogenesis of a change state in glucose metabolism still remains to be answered. From the results it could be showed that there is no association among the TNF-α -308 G/A mutation and fasting plasma insulin that is proposing no connection

among TNF-α -308 G/A mutation and GDM in our people. In contrast, the study results of Jafar T.*et al*. revealed that AA genotype and A allele were remarkablyhigher in the infantile nystagmus syndrome (INS) group than controls [33]. Several reports haveindicatedthe association of polymorphism with the Type 1 diabetes mellitus disease, TIDM [34], pro-inflammatory cytokines are increased in patients at the beginning of diabetes. Meanwhile, the concentrations of circulating TNF-α moderate did not correlate with metabolic abnormalities in vivo in human being with various degrees of overweight and insulin resistance [35]. From the results, it could be demonstrated that the AA genotype or subjects carrying an A allele of G-308A did not have an altered risk of metabolic syndrome in our population. The polymorphism of the TNF-α gene is correlated with transcriptional activation at position -308G/A and its operation has been described [36]. Whilst some reports werefound that the allele A is the highest various producer [37], in obverse allele G also it could be detectedas the highest producer, likewise [38]. Meanwhile from the results, it could be observed that an increase in the frequency of allele A between patients was relative to normal controls and also, did not explain any considerable association among TNF-α -308 G/A polymorphic genotypes/alleles with GDM. Since our data, the Odd Ratio value < 1, the AA genotype increased the probability of not getting a disease which is considered a protective factor. The present study is the first to evaluate the distribution of *Tumor Necrosis Factor-alpha (-308 G/A)* and *CAPN10 (-44C/T)*polymorphism in GDM women from Western Saudi Arabia. The *(CAPN10)* plays an important role in regulating the blood glucose concentrations and biochemical changes in the *CAPN10 (-44 C/T)* protein encoded by this gene affect the increase in blood glucose [39, 40]. Gene polymorphisms are essential indicators of genetic changes and genetic variations within humans [40]. Previous researches have indicatedthat gene polymorphisms of CAPN10 are correlated with T2DM in China, and the waist-hip ratio and body mass index of patients with the GG genotype of SNP43 were relatively high-level [41]. Other experiments have shown that the frequency of distribution of single nucleotide polymorphisms (SNPs) of CAPN10 differs across various cultures and ethnicities, so the detection of the same genotype change cannot be utilizedto determine the sensitivity of Europeans and Asians to GDM. The association between CAPN10 polymorphisms and GDM threats remains unclear, although some reports have also included CAPN10 variants and GDM [42, 43]. *CAPN10* is a gene-based cysteine protease on chromosome 2q37.It is commonly found in various tissues, including the pancreatic islets [44]. Calpain inhibitors have been found to improve insulin secretion by speeding insulin granule exocytosis in mouse pancreatic islets [45]. In addition, the CAPN10 isoform, a Ca2 + sensor, has recently been shown to cause exocytosis in pancreatic beta cells [46]. Genetic correlationstudies and functional analyses have linked CAPN10 to diabetes. Four main polymorphisms of CAPN10 have been associated with diabetes: SNP-43 (rs3792267), SNP-44 (rs2975760), SNP-63 (rs5030952), and InDel-19 (rs3842570) [46]. A general opinion on the suitable analytical procedures and thresholds for the analysis of GDM remains ambiguous [47]. During pregnancy, women face elevated adiposity and enhanced insulin resistance. Insulin resistance that occurs for the period of pregnancy is partially described by the improved development of human estrogen, lactogen, and prolactin. Women with GDM are thought to have decreased β-cell insulin secretion activity, whichis parallel to women with T2DM [48]. There was no statistically significant variation between the GDM and the control groups in either the genotype or allel frequency. The findings do not indicate any interaction of the *CAPN10(-44 C/T)*polymorphism with GDM relative to their controls in our study. From the results, it could be demonstrated that the CC genotype, or subjects carrying an C allele of *CAPN10(-44 C/T)*did not have an altered risk of metabolic syndrome in our population. Since our data, the Odd Ratio value < 1, the CC genotype increases the probability of not getting a disease which is considered a protective factor.Our outcomes were similar to those of the [49] study. This research was done in GDM and non-GDM Scandinavian females from Sweden.But In contrast, Asian Indians carrying the CAPN10 have a susceptibility to GDM and T2DM [50]. Demirci *et al*,. CAPN10(-44 C/T) genotype was found to be significantly frequent in T2DM patients with respect to the control group ($p < 0.01$) [51]. While the review evaluations of the meta-analysis also suggested that the SNP-44 was associated with elevated danger of GDM in recessive models. However, only two studies were included in this SNP, published by Leipold *et al*. [52] in 2004 and by Wu and Yang [53] in 2009, another study with this subject by Luo et al. [54] is only published in Chinese, so this result had limited significance. More experiments are needed to evaluate the relationship between *CAPN10(-44 C/T)*polymorphism and GDM risk. People's difference may also provided to the conflicting results among previous experiments [40]. Although CAPN10 genetic variant distribution in the GDM population doesn't meet the Hardy- Weinberg-Equilibrium, variation in the CAPN10 gene has been found to be not correlated with GDM in populations with other ethnic backgrounds [52, 55]. Consistent with the results in the insignificant study performed by Leipold *et al*. for SNP43, they did not detected any important variation in allele or genotype frequencies between GDM and controls [52]. However, they reported correlation with SNP-63 as well as a haplotype mixture of SNP-43, 19, and

63 (121/221) [52], but no information were available on the degree of linkage disequilibrium between these SNPs. , In Scandinavians,the SNP-63 was seen to be in near partnership imbalance with SNP-43 and SNP-44 [56]. In light of the potential physiological link between T2DM and GDM, two studies studiedthe relationship between variants within the CAPN10 gene and GDM. Shaat *et al.,*first observed that the minor allele frequency of both SNPs (SNP-43 and SNP-44) was similar in a sample of 588 Scandinavian women with GDM and in 1189 control subjects [49]. A further larger scale study should be conducted to confirm the relationship between *CAPN10* gene polymorphism and GDM patients to elucidate the underlying mechanism explaining the effect of genetic polymorphism on diabetes and metabolic derangements.

We categorized the studies according to their designs into two groupsconcerning BMI matched and BMI not matched between control and GDM groups. We found that *TNF-α -308 G/A,G* allele remained significantly elevated in GDM patients compared to their BMI. Thisdata suggests that maternal weight in GDM seems to have a significantrole in modifying disease levels. Our results show increases in the risks associated with GDM in carriers of the *G* allele, and it is correlated with body mass index (BMI) factor, age, the number of children had GDM before, had hypothyrodism, and had a miscarriage. The sitting research was award further proof that the TNF-α gene moves a function insensitively to GDM. Moreover, the lowest frequent A allele 5% of the G-308A polymorphism was stated to be correlated with a decreased risk of GDM. Therefore, the haplotype G-G was associated with a high risk of GDM, whereas the haplotype A-A was observed to be protective. Considering the results it could be demonstrated that the AA genotype or subjects carrying an A allele of G-308A did not have an altered risk of metabolic syndrome in our population. Meanwhile from the results, it could be observed that an increase in the frequency of allele A between patients relative to normal controls and also, did not explain any considerable association among *TNF-α -308 G/A* polymorphic genotypes/alleles with GDM. Since in our data, the Odd Ratio value < 1, the AA genotype increases the probability of not getting a disease which is considered as a protective factor.

In estimating the controversial results aforementioned, such an increased frequency of allele A in GDM as an all can be explained in double ways. The first way either allele A is the highest producer and the second way the allele A is the lowest producer various and may be caused TNF-α can move a double function, both promoter and dampener in becoming better than GDM. In truth, some researchers have concluded that the occurrence of the mutant allele i.e. the A-allele at place −308 in the organizer of the human TNF-α gene is very unusual in Asians [57], the reason for this rarity is unclear. Our results found that the frequencies of SNP at location −308 in the promoter area of the human TNF α gene. In our people, the frequency of this allele between the control and GDM subjects wasA 26% and G 74%, while the genotype frequency between the control and GDM considerable were AA 5%, GA 41%, and GG 54%. Other findings observed that the G/G genotype of the TNF-α –308G/A polymorphism was increased in insulin levels and insulin resistance in women with GDM and that the AG haplotypeare a genetic danger agent for GDM in our research about people. High and low maternal body mass index (BMI) plays a role in the majority of the complications of pregnancy, so pregnancy in abnormal women should be considered as a high-risk pregnancy [58]. According to an unpublished under review systematic study by the authors in 45 studies, the relation between GDM and BMI has been investigated in 33 of them and this relation has been assessed in BMI categories and in the remaining studies BMI has been entered as a continuous variable in the models and its linear association has been considered while this assumption might not be correct by default,and, therefore, it is explainable to consider BMI at the beginning of pregnancy as an alarm for GDM as well. [59]. Our result of *CAPN10(-44 C/T)* showed that there was a significant difference between the study and control group in BMI with (*P*-value =0.000). *CAPN10(-44 C/T)* increases the risks associated with GDM in carriers of the T allele, and it is positively correlated with the body mass index (BMI) factor. We conducted a significant impact on TT for BMI; P=0.026 so P < 0.05 which states that high BMI will increase the risk of having the disease by odds calculated as 1.116. Demirci et *al,*. (BMI) was found to be significantly high in TC genotype with type 2 diabetic patients (p < 0.05). *CAPN10(-44 C/T)* allele frequency was found to be lower in type 2 diabetic patients compared with the controls (p < 0.01). They had also found that there is a statistically significant difference between BMI and weight with SNP-44 genotypes, especially in male Turkish T2DM patients. Taken together, the CAPN10 gene SNP-44 TC genotype in Turkish T2DM patients may be a risk factor for obesity development, especially in males [51]. Asian peoplehave a higher occurrenceof diabetes than other ethnicities. Possible explanations for this racial difference include the following. First, centripetal obesity diabetes is an independent predictive factor [60], and abdominal and visceral fat accumulates to a greater degree in Asian individuals than in European and American individuals with the same waist circumference. Second, although body weights are lower in Asian populations than in European and American populations, there is a higher incidence of insulin resistance in Asian populations [61].

Third, mitochondrial dysfunction is involved in the development of central obesity and insulin resistance [62]. More common mitochondrial DNA 16 189 variants have also been shown to contribute to the onset of T2DM in East Asia but not in European populations. Hence, although diabetes is a global problem, more attention should be paid to diabetes in Asia [63]. Neither having thyroid nor miscarriage had showed a significant relationship with having the disease and this may be due to thesamplingsize. Accordingly, the odd ratio is not significant, but it may explain what the situation will be when the sample size becomes larger and the relationship becomes significant. Therefore, the odds ratios for hypothyroidism and miscarriage were calculated as 1.646 and 1.278, respectively which enhancethe dangerof getting the disease.Research from Iran found that the frequency of GDM increased in women who had a previous abortion, stillbirth, history of macrosomia, and a prior history of GDM. Pre-pregnancy BMI was studied as apredicting development of GDM [64]. A deficiency of their research is that T2DM females before pregnancy were not included as a study group in their GDM study. The occurenceof cesarean sections was drastically higher than that of regular spontaneous vaginal delivery in patients with GDM compared with the non-GDM subjects [65]. On the other hand, our patients who have agreed with hypothyroidism increases the possibilityof getting the disease. Our demographic data might be attributed to that hypothyroidism leads to the difficulty to lose weight and thus increase the BMI, which has a strong relationship to getting the GDM. Thyroid hormones play an important role in glucose metabolism. T_3 (triiodothyronine),is the biologically active hormone that is mainlyresponsible for glucose metabolic activity [66], and 80% of circulating T_3 is converted peripherally via deiodinase activity and the mono-deiodination of T_4 [67]. Some investigationshave concludedthat there is a reciprocal relationship between (free T_4) FT_4/BMI and Log_{10} FT_4/BMI and that there is a direct relationship between maternal free T_3 (FT_3) levels or $Log_{10}FT_3$ and BMI in pregnant women [68]. Specifically, when the level of FT_4 decreased, the level of FT_3 and BMI correspondingly increased, and obesity increased the chances of developing GDM. Furthermore, another study found that the ratio between FT_3/FT_4 levels and BMI increased when the level of FT_4 was low in euthyroid pregnancies, suggesting an increase in peripheral deiodinase activity [69]. Studies have also shown that the peripheral rate of transformation of T_4 to T_3 rises with excessive energy intake, indicating that the activity of peripheral deiodinase is affected by taking energy. All of the above findings show that a low FT_4 level is correlated with the incidence of diabetes [70]. The study of the Kashyap et al. on GDM discovered that the proportions of mutant-type homozygotes of two classic SNPs (rs2975760 and rs3792267) of CAPN10 were smaller than those of wild-type homozygotes. Although in the research group the number of mutations was higher than in the control group, there was no meaningful correlation between the improvement in SNP polymorphisms and GDM.Though there were more heterozygotes mutant, If the physiological disorders influenced the heterozygote of the mutant-type might be raised. Thus, pregnant women with mutant genotypes of CAPN10 may be classified as having an elevated risk of GDM, and the effect of base mutations should be acknowledged. Previous studies have suggested that the base mutations can reduce the function of the encoded non-lysosomal cysteine proteases and the capacity to degrade blood glucose [71]. Some research cases came up with conclusions based on relatively large pooled sample sizes. For others, the sample size was too small. Therefore, regarding SNPs, a systematic meta analysis is necessary. Their association with GDM risk warrants further evaluations as more evidence becomes available.

CONCLUSION

We suggest that the CAPN10 polymorphism studied in this analysis is of little importance in predicting the occurrence and diagnosis of GDM. Functional experiments are also required to assess the exact functions of these variants and pathways.

We conclude that the *TNF-α (-308G/A) and CAPN10(-44 C/T)* gene polymorphism may be accepted as a risk factor in the development of GDM and the factors of elevated BMI, age, and children number in GDM patients in a Saudi population. Further studies are needed to determine whether there are correlations between these two polymorphisms.

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