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Research Article

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Tumor Necrosis Factor-alpha (-308 G/A) is a Predictor for Development of Gestational Diabetes Mellitus in Saudi Pregnant Women

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ABSTRACT

*Inflammation may play a role in the pathogenesis of gestational diabetes mellitus (GDM) and also, it is one of the most popular pregnancy complications. The goal of the research was to study the association exists among common many forms in the stimulate area (-308G/A) of the TNF-alpha gene and increased danger for become better of GDM in Saudi pregnant women population.GDM and healthy subjects were genotyped with PCR- RFLP technique. No association was noticed among the TNF-α -308 G/A in different and sensitivity to GDM disease in our Saudi cohort. There was a secondary combination among TNF-α –308 G/A polymorphism in GDM (vs. normal control) (p = 0.605), which its triviality may be caused to survivor agent. The frequency of the -308 A allele (5) and genotype classification was in Hardy-Weinberg equilibrium (GG, n=54; GA, n=41; AA, n=5).The G-308A various, detected by PCR amplification and Nco-1 digestion, determines the lowest of a restriction site finding in a single band of 107 bp (the (A) allele). The (A) allele frequencies of the -308 (G/A) TNFα polymorphism were 26% in the GDM group and 29% in the depend subjects, with no significant difference among the two groups may be due to considerable variance in allele frequencies of the polymorphism by BMI quartiles (≥25 kg/m2, for each quartile n=86) with -308G allele transporters had contains a higher BMI than A allele transporters (P=0.000**). Furthermore, considerable was found positively correlated with BMI, age, and children number. CONCLUSION: It could concluded that theTNF-α-308 G>A genotype frequency comparison among patients and control was not statistically significant in the Saudi women and also, the SNP in situation -308 (G/A) for the human. TNF- gene is a dependent danger agent or a predictor for GDM.*

Key words: *Tumor Necrosis Factor-alpha,-308TNF-α promoter, polymorphism, Saudi women.*

INTRODUCTION

The Gestational diabetes mellitus (GDM) is an impaired glucose tolerance with onset or first recognition through pregnancy and it is one of the greatest popular pregnancy complications [1]. GDM is thought to be partly refear to secretion of upregulated inflammatory cytokines from gestational tissues that is the begining of moving more quickly towards insulin resistance [2]. Recently, researchers have concentrated on many new possible moderators of insulin resistance, which has a main function in the become better of GDM, involve the cytokines [3]. Among these cytokines, extensive attention has been given to TNF-*α*. Definition of TNFα is a strong immunomodulator and pro-inflammatory cytokine and it could be having been to move their function in the pathogenesis of multiple inflammatory or autoimmune diseases. TNF-α gene in human is a single copy gene and is position of on the short arm of chromosome 6 in lock linkage with MHC genes [4]. Many researches propose that TNF-α has an anti-insulin impact by suppressing the phosphorylation of the insulin receptor and its

substrates [5]. Neutralization of circulating TNF-α by in vivo injection of soluble TNF-α receptor-immunoglobulin G chimeric protein leads to a considerable making greater in insulin sensitivity [6], and infusion of TNF-α during euglycemic hyper-insulinemic clamp prevent but not completely half of the glucose is available by muscle [7], propose that high TNFα levels may share in to become better of insulin resistance. The production of TNFα is monitoring and is affecting by a polymorphism, −G308A, and also, the TNFα gene is position within the grade III area of the main histocompatibility complex on the little arm of chromosome 6 (6p21.1–21.3) [8]. This is a well-defined biallelicbase commutation polymorphism, which involves a common various with a guanine (G) at located −308 and an unpopular different with an adenine (A) at −308. Therefore, the A allele has been safety associated with higher TNFα production and in some situation, with higher morbidity and mortality in many infectious, autoimmune, and other immune trouble [9]. TNF-*α,* on the other hand, is one of candidate molecules responsible for causing insulin resistance during late pregnancy [10]. Pregnant women with preeclampsia (hypertension with proteinuria) increase of the TNF-α concentration were noticed when compared with pregnant women with gestational hypertension (hypertension without proteinuria). This result suggests that TNF-α can be used as a marker of severity in pregnancy hypertensive syndromes [11]. Many polymorphisms and mutations have been described in the promoter and translated areas of the TNF-α gene. Therefore, ownership of a specific genetic polymorphism can be a predisposing factor for ability to some diseases as well as the detection of such genetic polymorphism could be beneficial as a sign enabled soon diagnosis [12]. The −308 G/A polymorphism is a transition mutation, with exchange of guanine for adenine in the promoter area of the TNF-α gene. The possible association between TNF-α -308 G/A (rs1800629) G/A polymorphism [13]. The aim of this study whether there is association between allelic frequency of tumor necrosis factor-alpha (TNF-alpha) in gestational diabetes mellitus (GDM) of Saudi women pregnancy and to determine if there is a specific allele of TNF-alpha associated with GDM susceptibility.

SUBJECTS AND METHODS

i. Ethical aspects

This study was approved by the Ethics Committee of Directorate of Health Affairs, Jeddah, Saudi Arabia. Informed consent was obtained from all subjects.

ii. Subjects

Information was collected retrospectively in connection with 100 GDM pregnant women and 100 control women with no history of GDM. The controls had no clinical signs of the disorder. They originated from a regional population and were enrolled by random selection in this study. The inclusion criteria for normal subjects were uncomplicated pregnancy, 16–45 years old, no risk factors for DM, GDM, no glucose intolerance in pregnancy following 75-g glucose challenge screening test (GCT) and having no history of any medical disorders. The inclusion criteria for the GDM group mothers were pregnant women with risk factors for GDM, 16–45 years old, at 3rd trimester of pregnancy. GDM was diagnosed according to World Health Organization, fasting plasma glucose C7.0 mmol/l or plasma glucose after 2 h C7.8 mmol/l, (168). Some of them suffered from hypertension (n=7), hypothyroidism (n=25), and haemophilia (n=5). Almost all included pregnant women with GDM were treated with nutrition therapy except 21 cases in 3rd trimesters were treated with insulin therapy.

iii. DNA Extraction and Determination of the Genotypes

Genomic DNA was isolated from peripheral blood leukocytes, using salting out method. Cytokine typing was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany). The 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. Briefly, amplification was carried out using a thermal cycler TechneFlexigene apparatus (Rosche, Cambridge, UK). The presence or absence of PCR products was visualized by 3.5% agarose gel electrophoresis. All individuals were genotyped for polymorphic site of the cytokine gene *TNF-α* -308 G/A.

iv. Statistical Methods

Statistical analyses were performed with EPI info software. Allele, genotype, and haplotype frequencies for cytokine gene polymorphisms were calculated by direct counting. Frequencies of alleles, genotypes, and haplotypes were compared between the patient and control groups using the SPSS. The odds ratio with 95% confidence intervals was calculated. Descriptive data were given as mean ± SD. Association between groups among genotype was assessed using T test if the data were normally distributed, and by nonparametric test

Mann-Whitney test if the data were not normally distributed. Chi-square tests were applied to test the association between categorical variables.

RESULTS

Clinical and Demographic profile of patients with GDM versus Controls.

The clinical properties of the GDM women and controls are reported in Table (1). The mean of age in patients with GDM and the control group was 31.63 ± 6.4 years and 29.51 ± 5.05 years, respectively. All characteristics of the participants differed significantly between GDM and normoglycemic control ($p < 0.0001$). Patients with GDM s had significantly higher values for body mass index, hypothyroidism, GDM, and miscarriage than normoglycemic control participants ($p < 0.0001$). No significant differences were found between the GDM group and GDM-free controls.

Table 1: Sociodemographic characteristics of GDM and matched non-GDM control.

Variable	Mean ± SD		P value	OR	95% CI
	study	control			
Age	31.63 ± 6.405	29.51 ± 5.058	0.01**	-	0.510 – 3.730
Children number	2.25 ± 2.182	1.66 ± 1.718	0.000**	-	0.017 – 1.143
BMI	33.91 ± 6.517	29.38 ± 6.203	0.035*	-	2.677 – 6.379
Pregnancy month	7.84 ± 0.788	8.83 ± 8.211	0.133	-	-2.435 – 0.695
BMI ≥ 25	86	66	0.000**	5.646	-
BMI < 25	6	26			
Age 16 – 25	19	21	0.026*	-	-
Age 26 – 35	54	67			
Age 36 – 45	27	12			
Had GDM before (yes/no)	50/50	1/99	0.000**	99.000	13.285 – 737.733
Had hypothyroidism (yes/no)	14/86	9/91	0.268	1.646	0.677 - 3.999
Had miscarriage (yes/no)	10/90	8/92	0.621	1.278	0.482 - 3.384

*Quantitative data are presented as (mean±SD) and qualitative data presented as frequencies. P values are based on chi-square test for categorical variables. T test was done for normal continuous variables and Mann-whitney test for not normal continuous variables. OR: odd ratio calculated only for 2 by 2 categorical comparisons.

Genotyping

Restriction fragment length polymorphism was analysed. Figure (1) showed a photograph of PCR products of -308G/A gene polymorphism before using Restriction enzyme. The appearance of a single band of 107 base pair (bp) indicates the presence of PCR products.

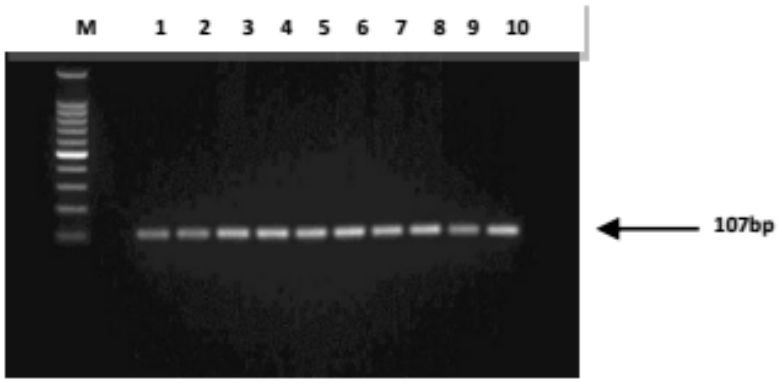

Figure 1: photograph of a 3.5% agarose gel showing PCR product of TNF-α gene (-308G/A); M: 100 bp DNA marker, lanes 1 to 10 PCR products with molecular size of 107 bp.

Figure (2) showed the genotype of -308G/A polymorphism after using NcoI restriction enzyme. The appearance of single band of 107 bp indicated the presence of allele A which lacks the restriction site for NcoI enzyme, the appearance of 2 bands of 87 and 20 bp showed the presence of allele G and having the restriction site of NcoI enzyme, while the appearance of 3 bands of 107, 87 and 20 bp showed the presence of allele G and allele A.

Alleles and genotype frequencies

All patients and control subjects were genotyped for the *TNF-α* -308 G/Apolymorphism. Allele and genotype frequencies in patients and control groups are summarized in Table (2). Statistically in TNF genotype frequency was not found between the patients and controls (p=0.598). A significant positive association with the A/A genotype was found for TNF-α at position -308 in our patients compared to controls (5%*vs*. 10%, p=0.403), which is considered a protective factor. Another classification was by classified genotype into allele A and allele G. The result showed no significant relationship with p value = 0.849 > 0.05; odd ratio was calculated as 0.849 which indicates presence of allele A decreases the risk of getting the diseased. 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 association was detected for the recessive model (GG + GA vs. AA). The relative allele frequencies of -308 G/A TNF-α promoter polymorphism for the study patients are shown in Table (3). This variant was in Hardy-Weinberg equilibrium in GDM. The results show no significant differences in allele frequencies. The results of the estimation of odd ratios for disease risk using binary logistic regression among genotypes categories was shown in Table (4). No significant impacts were calculated for genotypes' disease risk (P>0.05 for all genotypes). The largest odd ratio belongs to GA genotype calculated as 1.134, P=0.664.

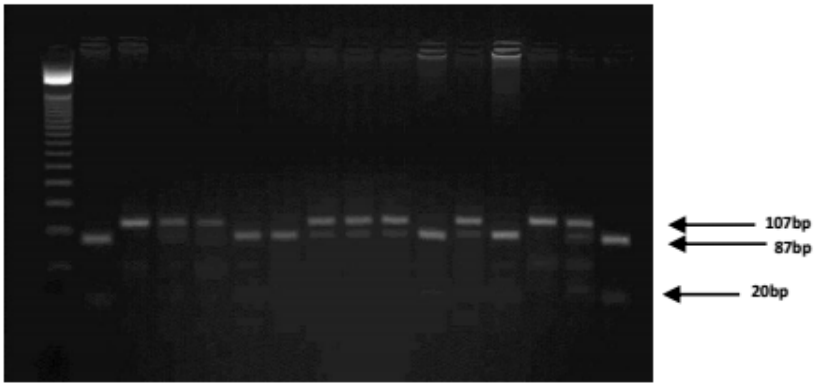

Figure 2: photograph of 3.5% agarose gel showing the digested PCR products for 308G/A polymorphism genotyping. Lane M: 50 bp DNA marker. (a) Homozygous AA genotype that produces one band of 107 bp as lanes 2 and 13. (b) Heterozygous GA genotypes that produce 3 bands of 107, 87 and 20 bp as lanes 3, 4, 7, 8, 9, 11 and 14. (c) Homozygous GG genotype that produce 2 band 87 and 20 bp as lanes 1, 5, 6, 10, 12 and 15.

Table 2: Comparisons of allele and genotype frequencies between patients with GDM and controls

Cytokine	position	Alleles/genotypes	Patient n=100 N (%)	Control n=100 N (%)	p-value	Odd ratio	95% CI
TNF-α	-308	A	26	29	0.605	0.849	0.358 – 1.311
		G	74	71			
		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 GA + GG	5 (5%) 95 (95%)	10 (10%) 90 (90%)	0.179	0.474	156 – 1.440
		GG AA+GA	54 (54%) 46 (46%)	52 (52%) 48 (48%)	0.777	0.923	0.530 – 1.608

Table 3: Comparisons of allele frequencies between patients with GDM and controls

Allele Frequency	G	A	P value
Patients	149 (74.5%)	51 (25.5%)	0.43
Control	142 (71%)	58 (29%)	0.44

P value based on Hardy- Weinberg equilibrium test was determined by using Chi-square.

Table 4: Estimate of odds ratio for disease risk using binary logistic regression among genotypes categories

Genotype	GDM	Control	P value	OR	OR 95% CI	
Mutant **AA**	5 (5%)	10 (10%)	0.188	0.474	0.156	1.440
Heterozygote **GA**	41 (41%)	38 (38%)	0.664	1.134	0.643	2.000
Wild **GG**	54 (54%)	52 (52%)	0.777	1.084	0.622	1.888

B: Logistic regression coefficient. OR: Odds ratio. CI: confidence interval.

Association of TNF-α (-308G/A) with the risk of GDM, stratified by BMI (Table 5) indicates highly significant differences between study and control groups for category GA with p value 0.003 < 0.01, and category GG with p value 0.001 < 0.01. Besides, no significant difference had indicated between study and control for AA. Since Odd ratio value was < 1, the AA genotype increases the probability of not getting a disease which is considered a protective factor.

Table 5: Association of TNF-α (-308G/A) with the risk of GDM, stratified by BMI.

Genotype	BMI (Mean ± SD)		Comparison	Mean difference 95% CI (L - U)
	Study	Control	P value	
Mutant **AA**	32.52 ± 6.55	29.30 ± 6.81	0.398	-4.742 – 11.186
Heterozygote **GA**	33.38 ± 5.75	29.05 ± 5.86	0.003	1.501 – 7.166
Wild **GG**	34.38 ± 7.03	29.26 ±6.42	0.001	2.103 – 7.400

By using binary logistic regression to estimate the odds ratio for disease risk in presence of AA, GA and GG (Table 6), a significant impact on GA and GG for BMI was recorded (P= 0.006 and P= 0.001, respectively), which concluded that increasing BMI increase the risk of having the disease by odds calculated as 1.139 and 1.111 for GA and GG, respectively. Categorically by classify BMI into obese and non-obese, a significant impact was recorded on GA and GG (P= 0.006 and P= 0.012, respectively), which concluded that being obese increase the risk of having the disease by odds calculated as 4.200 for GA and 2.838 for GG. Moreover, having GDM before increase the risk of having the disease by odds calculated as 59.160 for GG.

Table 6: Estimate of odds ratio for disease risk using binary logistic regression in presence of AA, GA and GG.

Genotype	Factor	B	P value	OR	OR 95% CI	
Mutant 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
	GDM	23.505	0.999	-	-	-
Heterozygote 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
	GDM	21.662	0.998	-	-	-
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
	GDM	4.080	0.000**	59.160	7.615	459.594

B: Logistic regression coefficient. OR: Odds ratio. CI: confidence interval

DISCUSSION

Gestational diabetes mellitus (GDM) affects both maternal and fetal health. Therefore, the definition of gestational diabetes mellitus is carbohydrate unwillingness diagnosed for the first period through pregnancy and is increasing worldwide. It influences approximately 1 to 20% of all pregnancies worldwide as well as its

prevalence among saudi women is about 4 to 14% of all pregnancies [14]. Both environmental risk factors and genetic background contribute to the development of GDM. TNF-α increase with pregnancy development and the primary production source of this cytokine appears to be the placenta [15]. Increased TNF-α can exacerbate insulin resistance, which is normal in pregnancy; this favors the development of GDM [16]. Various studies have evaluated the concentrations of circulating TNF-α in pregnant woman with GDM; however, the results remain controversial. Some studies observed increased TNF-α in the blood of mothers who developed GDM [17]. However, other studies did not confirm such findings [18]. The functionality of SNPs with respect to gene expression is an important subject in the studies of association to diseases [19] The vital role of this cytokine in the arrangement of inflammatory and immune responses are of the clinical interest, and may be caused by the relationship between the presence of SNP in the TNF-α and their plasma levels [20]. In summary, TNF-α is related to obesity, glucose intolerance, type-2 diabetes mellitus, and GDM, and same result was revealed in our study as it is positively correlated with body mass index (BMI) [21]. SNP in the human TNF-α may help to achieve insulin resistance and leading to type 2 diabetes mellitus as there is proof to propose that TNF-α can prevent insulin signaling [22] and therefore impair insulin secretion. In pregnancy, TNF-α production in maternal adipose tissue is enhanced by the placental production of TNF-α, which makes it an important factor in the pathogenesis of insulin resistance and GDM. This challenges the traditional theory that reproductive hormones alone reduce insulin sensitivity during pregnancy [23]. Although this has not been completely defined, TNF-α seems to be the marker of "demetabolism" and maternal glycemic control in pregnancies that are complicated by type-2 diabetes mellitus and GDM. In the near future diagnosis and administration of diabetes in pregnancy are highly vital in decreasing its results. To date, there are no present accepted reference experimentals for GDM and the diagnosis is generally established based on the utility of the oral glucose tolerance (OGTT) test, and the accuracy of OGTT test in the diagnosis of GDM remains highly doubtful. For this reason, the tests that would help present physicians to identify possible subjects early in pregnancy or even before idea happen could be investigated. This would help the clinical management of this condition. Various studies have evaluated the concentrations of circulating TNF-α in pregnant woman with GDM; however, the results remain controversial. Some studies observed increased TNF-α in the blood of mothers who developed GDM [24]. However, other studies did not confirm such findings [25]. Several association studies have discussed the importance of clarifying the relationship between genetic polymorphisms and the development of GDM.

In the present paper we studied the association between TNF-alpha gene polymorphisms (*TNF-α* -308 G/A) and the susceptibility for developing GDM in Saudi pregnant Women in 100 Saudi GDM patients and 100 healthy controls. Many association investigations had contained, with inconsistent results. Fernandez-Real et al. [26] showed the in vitro G-308A mutation in the excitation area of TNF-α as more powerful transcriptional activator than the wild-type TNF-α and also, there could be significant association among the G-308A and insulin sensitivity [27], and it was proposed that a greater transcriptional activation would be resulted in more intense TNF-α concentrations followed by the lowest insulin sensitivity [28]. 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 [29]. Laboratory research on cultured cells observed that the TNF-α perhaps exert its anti-insulin impact by preventing the phosphorylation of the insulin receptor and its substrates [30]. Adipose tissue is associated with insulin resistance in transgenic animals' overexpression of TNF-α mRNA. The results of our study showed no significant difference between the patient group and controls 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 commodity for sale version, in opposite allele G also was noticed as the highest producer likewise. Also, it is observed that the polymorphism of TNF-α gene at local-308 G/A is related with transcriptional activation [31]. These different striking findings raised the question of whether TNF-α gene is included or not in the pathogenesis of a change state in glucose metabolism, which still remains to be answered. From the results it could be concluded that there is no association among TNF-α -308 G/A mutation and GDM in our people. In contrast, Jafar *et al*. concluded in their study that AA genotype and A allele were significantly higher in the infantile nystagmus syndrome (INS) group than controls [32]. Several reports had shown the association of polymorphism with the Type 1 diabetes mellitus disease, TIDM [33], and pro-inflammatory cytokines are increased in patients at the beginning of diabetes. A considerably higher TNF-α -308 G/A, A/A genotypes in Indian patients with T1DM were reported [34]. Das et al [35] observed higher TNF-α -308 A allele in patients

with diabetes in the Hungarian people and also, a considerable association of TNF-α -308 A allele and G/A genotype with T1DM in North Indians [36]. TNF-α -308 G/A was not an important genetic agent for sensitively to T1DM, and the associations of the TNF gene are caused a Linkage disequilibrium (LD) among TNF and DR3-DQB 1*0201 haplotype communicate in Chinese and Caucasian populations [37]. The association lost its significance may be due to appearance of various association of TNF-α -308 G/A with T1DM, but after regulat the finding for LD with DRB1-DQB1 and B18-DR3 haplotypes. Boraska et al., [38] showed that there was mot powerful evidence of association between TNF promoter polymorphism with TIDM, but they were studied the relation of TNF gene promoter polymorphism TNF-α -308 G/A with TIDM in a case–control study from South Croatia. Whilst there was not any association between TNF polymorphism with type 1 diabetes sensitivity in Korea. Haplotype TNF-α -308 G/A was also noticed many overwhelmingly in patients with TIDM than in controls. SNP TNF-α -308 G/A was reported to be more frequent in patients with TIDM [39]. Whilst the adiponectin gene polymorphism was studied in many researches, each estimates various polymorphisms. There were better differences in each of the researches with consideration to the selection criteria (ethnicity, GD criteria, BMI categorization). Moreover, even during all these researches, an association among genetic variants and GDM was onserved, and these results should be possessed with caution and cannot be generalized [40]. Specific technical questions on the SNPs, as well as carry out statistical analyses. Our results showed that there was a consistent increase in frequency of allele G among patients at each category relative to health controls. Meanwhile, we did not explain any significant association among TNF-α-308 G/A polymorphic genotypes/alleles with GDM group. Moreover, the distribution of the polymorphic alleles between GDM subjects without diabetic was compared and the difference was found to be non-significant.

Many of the studies included in the meta-analysis found that patients with GDM had significantly higher TNF-*α* and leptin concentrations and lower adiponectin concentrations than control women. The differences remained statistically significant after adjusting for BMI in some studies, but not all. Moreover, some studies found a significant positive correlation between BMI values and levels of TNF-*α* and leptin and an inverse correlation between BMI and adiponectin levels in GDM [41].

We categorized the studies according to their designs into two classes with respect to BMI matched and BMI not matched between controls and GDM groups. We found that *G* allele remained significantly elevated in GDM patients compared to their BMI. These data suggest that maternal weight in GDM seems to have important role in modifying disease levels. Our results show increases in the risks associated with GDM in carriers of the *G* allele, and it is positively correlated with body mass index (BMI) factor, age, number of children, if they had GDM before, had hypothyrodism, and had miscarriage; table (1). The sitting research provided further proof that the TNF-α gene have a function in sensitively to GDM. Moreover, the lowest frequent A allele (5%) of the G-308A polymorphism was reported to be associated 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.

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.

From our information this is the first study evaluating the role of the TNF-α -308 G/A with GDM in a Saudi population. The polymorphism of TNF-α gene is correlated with transcriptional activation at position -308G/A and its operation has been described [42]. Whilst some reports found that the allele A is the highest producer various [43], in obverse allele G also it could be noticed as the highest producer likewise [44]. Meanwhile based on the results it could be seen that there was an increase in 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. According to our data, by the Odd Ratio value < 1, the AA genotype increases the probability of not getting a disease which is considered a protective factor.

In estimate to controversial results aforementioned obviously such increased frequency of allele A in GDM as an all can be explain in double ways. The first way, either allele A is the highest producer and the second way the allele A is the lowest producer, and may cause TNF-α has a double function, both promoter and dampener to become better of GDM. In fact, some researches had revealed the frequency of the mutant allele i.e. the A-allele at position −308 in the promoter of the human TNF-α gene is very unusually in Asians [45]; the reason for this rarity is unclear. Our results found 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 were A 26% and G 74%, while the genotype frequency between the control and GDM were AA 5%, GA 41% and GG 54%. Others findings revealed that the G/G genotype of the TNF-α –308G/A polymorphism increased insulin levels

and insulin resistance in women with GDM and that the AG haplotypes are risk factors for GDM. Klara Rosta et al. (2017) reported that TNF α does not appear to provide something great for insulin resistance, and there was not any association among TNF-α and insulin sensitivity in either GDM or control subjects [46]. Winkler et al. (2002) suggest that circulating TNF α should not be carefully as endocrine cytokine. In fact, it may act in a paracrine or autocrine manner and it may not alter the action of insulin [47]. Kubaszek et al. [48] investigated TNF-α and IL-6 polymorphisms and established that TNF-α -308 A-allele was associated with an incresed danger of type 2 diabetes compared with the TNF-α -308 G. In situation of type 2 diabetes, high levels of cytokines have been shown as risk agents. The TNF-α -308 A-allele of TNF-α, TNF-α -308 G/A polymorphism is a predictor for the transformation from IGT (impaired glucose tolerance) to type 2 diabetes.

From our results it could be noticed the G-308 A mutation of the TNFα gene is likely to have better role in the expansion of GDM, in this Saudi population and it is a dependent risk factor. In the fact TNF-α may have disagree keep safe effects in expansion of GDM; and a linkage among TNF-α and GDM could be noticed with its delayed complications. These findings have been oserved in many other researches in different people and that changes in TNFα are the consequence and not the essential reasons of the metabolic abnormalities reported in insulin-resistance and it's associated metabolic and clinical trouble. Small numbers, lack of homogeneity, uncertainty in clinical definitions of patients/controls, and other interference may provide something to lying negative finding. For instance, the sample size is obviously under strong to reveal an association with an OR minimal than 2, which may be the expected level of association in multigenic complex type, while it could be found in present investigate has 90% to be able to reveal an association with an odds rate (OR) of 3 or more. Some determinations must be considered when interpreting the findings of our research.

For instance, in order to decrease the number of changes, patients could be classified according to the period of GDM at the begining of each a difficulty that completely remove the effect of such a major environmental agent and places patients in phenotypically more homogeneous sub-groups, which in transformation need for an increase number of patients to reach statistical power. Moreover, a multivariate type like diabetic complications can be produced or be affected by various corresponding contributions of genetic vs environmental factors. Additionally, some investigations have shown associations among GDM and opposite pregnancy outcomes. However, pregnancy outcomes were not collected in our research cohort. Finely, participants in this study were pregnant women living in Jeddah, and the finding may not be generalized. Repeating this study and collecting higher number of samples will improve the roboustness of the study. The correlation between the TNFα promoter genotypes and the risk of developing GDM remains controversial due to the many discrepancies between the different studies available, the results of these studies suggest the need for further investigation.

CONCLUSION

The goal of this investigation was carried to explore the possible association of TNF α SNP in GDM in a local people in Saudi. We could establish manyresons–effect relationship.

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