The Association Between Pregnancy Serum TNF-α Level and Postpartum Insulin Resistance in Pregnant Women With Gestational Diabetes Mellitus

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Abstract- Insulin resistance in gestational diabetes increases maternal and fetal complications. Tumor necrosis factor-alpha (TNF-α) is an inflammatory factor associated with insulin resistance. The aim of this study was to determine the association between pregnancy serum TNF-α level and postpartum insulin resistance in patients with gestational diabetes mellitus. 50 pregnant women, including 25 cases of gestational diabetes and 25 healthy pregnant women, were evaluated. First, during the third trimester of pregnancy, serum TNF-α level of all cases were measured. Two months after delivery, based on the obtained results from insulin levels and a 2-hour glucose tolerance test, HOMA-IR and HOMA-B were calculated, and the association between serum TNF-α level and insulin resistance was determined. Data were analyzed using independent t-test, Mann-Whitney, and chi-square test in SPSS software. The mean serum level of TNF-α in women with gestational diabetes mellitus was higher than healthy subjects, but there was no significant difference between the two groups. The serum level of insulin, HOMA-IR and HOMA-B indices in women with gestational diabetes mellitus were higher than healthy subjects, with a significant difference in all of the measures between two groups (P=0.0001). There was no significant correlation between TNF-α and HOMA-IR levels in insulin-resistant mothers two months after delivery (r=−0.33). Also, no significant correlation was detected between pregnancy TNF-α levels and HOMA-B index. Despite the higher serum levels of insulin, HOMA-IR, and HOMA-B in the diabetic group, the serum TNF-α level did not show any correlation with insulin resistance after delivery.

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Keywords: Tumor necrosis factor-alpha (TNF-α); Gestational diabetes; Insulin resistance

Introduction

Gestational diabetes is one of the most common endocrine disorders in the female population, which has shown an increasing incidence in recent years, especially in Iran (1). Based on previous studies, the prevalence of gestational diabetes mellitus (GDM) is an average of 17.8% and varies from 9.3% to 25.5% depending on race, geographic locations, and environmental risk factors (2). According to the American Diabetes Association (ADA) criteria, GDM refers to degrees of glucose intolerance that is diagnosed for the first time during pregnancy, but the level of hyperglycemia is not compatible with overt diabetes. The development of hyperglycemia during pregnancy is mainly due to increased insulin resistance (3). Insulin resistance Increases in 2-10% of women during pregnancy and is associated with increased risk of complications at the time of pregnancy as well as with a higher risk of future maternal diabetes mellitus by 40% (4). The severity of insulin resistance increases with the progression of gestational age, which is higher in the third trimester than in the first and second one (5).

TNF-α is an inflammatory cytokine that is produced by type 1 helper lymphocyte cells (TH-1 cells), and its level is higher in subjects with insulin resistance (6). The normal serum TNF-α level varies widely across different populations regarding age, genotype, and specific physiological status (7). According to previous studies, due to changes in the immune system's function during pregnancy, the serum TNF-α level increases routinely in normal pregnant women, and it is higher in women with gestational diabetes mellitus (8,9).

In a study about the correlation of longitudinal

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changes in insulin sensitivity during pregnancy with changes in placental hormones, insulin resistance was increased during late pregnancy in all women with and without gestational diabetes mellitus. ((Placental hormones, including TNF-alpha, leptin, cortisol, and reproductive hormones (human chorionic gonadotropin, estradiol, progesterone, human placental lactogen, and prolactin), were increased in late pregnancy (P<0.001). TNF-alpha was inversely correlated with insulin sensitivity (r=-0.69, P<0.006) during late pregnancy. Furthermore, among all of the hormonal changes measured in this study, the change in TNF-alpha from pregravid to late pregnancy was the only significant predictor of the change in insulin sensitivity (r =-0.60, P<0.02))). Based on these obtained data, TNFα was included in a new paradigm to explain insulin resistance in pregnancy (8).

A systematic review of cytokine levels in gestational diabetes mellitus, including twenty-two studies with 1982 participants reporting levels of 9 cytokines (IL-1B, IL-2, IL-6, IL-10, IL-13, IL-18, IFN-γ, TGF-β, and TNF-α) revealed that TNF-α concentration was slightly higher in GDM than in control patients, although not significant (WMD=0.45, 95% CI -0.34-1.23) (9). Nonetheless, data in regard to serum TNF-α and insulin resistance during the postpartum period are scarce. This issue is important because the persistence of increased serum TNF-α may indicate the continuation of immune abnormalities and greater risk for the future development of type 2 diabetes.

Considering the initial relationship between TNF-α level and progressive insulin resistance during pregnancy (8,9), we, therefore, performed the present study. The primary aim of this study was to determine the relationship between increased serum TNF-α level and GDM in the third trimester of pregnancy, and the secondary aim was to investigate the relationship between increased serum TNF-α level with the persistence of insulin resistance in women with GDM until two months after delivery.

Materials and Methods

The patients of this case-control study were recruited among pregnant women who presented with GDM in 24 to 28 weeks of gestation, referred to Valiasr Hospital, Zanjan, Iran. Diagnosis of GDM was confirmed with 100 grams glucose tolerance test according to ADA criteria (3). Blood glucose levels after 8 hours overnight fasting, and then first, second, and third hour's blood glucose levels after taking 100 grams of standard oral glucose were measured. Based on the modified Carpenter-Coustan criteria (10), subjects who met the criteria were considered to have GDM.

In a 6 months period, we recruited 25 mothers with criteria of GDM in our study. Age-matched women with normal blood glucose levels, who had the inclusion criteria to enter the study, were considered as the control group. Subjects with a history of infectious, inflammatory, or systemic diseases, taking medications affecting serum glucose or TNF-α level such as corticosteroids over the 6-month period prior to inclusion, patients with a history of diabetes, previous GDM or those with insulin resistance syndromes were excluded from the study.

Data were collected regarding demographic characteristics including age (based on year of birth), pre-pregnancy weight (based on medical records of self-reports), weight during pregnancy and two months after delivery (in kilograms with digital scales), Height (in centimeter with standing meters), waist circumference (WC) after delivery (in centimeters with a strip meter at the standing position across the umbilical line), post-delivery hip circumference (HC) (in centimeters with a strip meter across the iliac crest line) and the BMI based on the weight in kg divided to the square of the meter (kg/m^2). Serum TNF-α in all participants was measured at the time of the first visit by the ELISA's method using Bender Med Kit (Thermo Scientific Fisher). According to manufacturer databases, serum TNF-α level between 3 to 900 ng/L considered normal in the normal population. Two months after delivery, fasting blood glucose, serum glucose level two hours after taking 75 grams of standard oral glucose solution, and serum insulin level were determined in all of the normal and diabetic subjects. Indices of insulin resistance (HOMA-IR) and beta-cell function (HOMA-B) were calculated using the following formulations:

HOMA-B=[insulin (μl/l)×20]/[FBS (mmol/l)-36]

HOMA-IR=[Insulin (μl/l)]×[FBS (mmol/l)]/22.5

According to established Iranian studies, Insulin resistance 2 months after delivery was defined as the presence of HOMA-IR values higher than 2.1 (11).

The Kolmogorov-Smirnov test was used to evaluate the distribution of variables. Values were expressed as mean±standard deviation, median (25-75 percentiles), and number (percentage), as appropriate. Serum TNF-α level and insulin resistance were compared between the case and control groups. Comparisons of variables with normal distribution were performed by independent t-test, and those with skewed distribution were compared

using the Mann-Whitney U test. Receiver operating characteristics (ROC) analysis was applied to find the sensitivity and specificity of TNF-α in discriminating between those with and without insulin resistance. The area under the ROC curve (AUC) and cut-off value were determined based on the scores closest to the value of peak sensitivity and specificity. *P* less than 0.05 were considered significant. All analysis was performed using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

The proposal of this study was approved by the Ethics Committee of Zanjan University of Medical Sciences (ZUMS).

Results

25 patients with GDM and 25 controls were recruited for analysis. The mean age of patients and controls was 30.2±4.9 years and 27±3.4 years, respectively (Table 1). In the course of study, the mean levels of the waist and hip circumferences, age, and BMI were higher in the gestational diabetes group than healthy subjects. 36% of the individuals in the GDM group and 24% of the control group had a positive family history of diabetes. 36% of the patient and 16% of the healthy women had a history of one or more abortions, but none of these differences between the two groups were significant.

Table 1. Demographic and biochemical characteristics of patients with GDM and pregnant women without GDM

Variables		GDM Group	Control Group	*P*
Number of patients (n)		25	25	-
Age (year)		30.2± 4.5	27.0± 3.4	0.007
Familial history of diabetes (%)		36	24	0.360
Previous abortion (%)		36	16	0.110
Prior parity (%)		80	60	0.380
BMI (kg/m^2)	**Pregestational**	26.3± 3.8	24.1± 2.6	0.018
	Postpartum	28.1± 3.8	25.7± 2.6	0.011
WC (cm)	**Pregestational**	NA	NA	-
	Postpartum	128± 4.0	125± 2.0	0.006
HC (cm)	**Pregestational**	NA	NA	-
	Postpartum	112.0± 4.0	107± 0.58	0.006
Serum FBS (mg/dL)	**Pregestational**	NA	NA	-
	Postpartum	99.0± 2.9	95.7± 1.7	0.611
Serum TNF-α level in pregnancy (ng/L)		231±127 194 (178-222)*	227±111 192 (133-310)*	0.900
Postpartum insulin (mIU/mL)		16.96±7 16.5 (14-21)*	6.22±2.8 6.1 (4−7.9)*	0.0001
Postpartum HOMA-IR		4.5±2.45 4.9 (2.7-5.8)*	0.84±1.49 1.4 (1-1.75)*	0.0001
Postpartum HOMA-B		5.79±3.7 4.5 (3.7-6.9)*	2.24±1.30 1.9 (1.15-3.0)*	0.0001

BMI: Body mass index, FBS: Fasting blood sugar, HC: Hip circumference, NA: Not assessed, WC: Waist circumference, Asterisks show median (ranges) values

Although the mean serum level of TNF-α in women with gestational diabetes mellitus was higher than healthy subjects, its level was within the normal range in both two groups. The serum level of insulin, HOMA-IR index, and HOMA-B index in the gestational diabetes group were significantly higher than healthy subjects. All of the participants were categorized into two groups based on the HOMA-IR index: Normal individuals with a HOMA-IR index of less than 2.1 and the insulin-resistant group with a HOMA-IR ≥2.1 (11). Based on this categorization, 80% of women with GDM and only 19% of healthy subjects had insulin resistance. No significant relationship was present between the serum TNF-α level and different levels of HOMA-IR (Table 2). About the correlation between TNF-α and HOMA-IR (r=−0.33, *P*=0.819) or HOMA-B (r=0.123, *P*=0395), we found no significant difference between the two groups. There was no significant correlation between the TNF-α and HOMA-IR (r=-0.061, *P*=0.771) or HOMA-B (r=129.9, *P*=0.9953) indices in gestational diabetic mothers too.

Table 2. Comparison of TNF-α level with different levels of HOMA-IR

Group/ Variable	HOMA-IR <2.1		HOMA-IR ≥2.1		*P*
	Mean±SD	Median (25-75%)	Mean±SD	Median (25-75%)	
TNF-α (ng/l)	232±126	192 (156-306)	224±112	199 (176-232)	0.812

No significant correlation was detected between the TNF-α level and BMI or age (P>0.05). ROC curve was plotted based on sensitivity against 1-specificity for TNF-α in comparison with insulin resistance. AUC was 0.510 (95% CI: 0.346-0.673). An optimal cut-off value of 193 for TNF-α was determined based on the scores closest to the value of peak sensitivity (0.542) and specificity (0.538) (Figure 1).

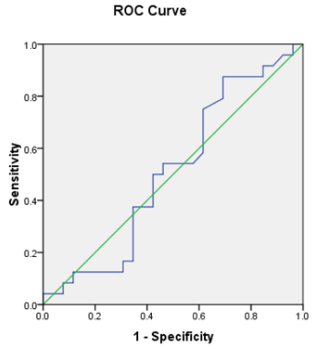

Figure 1. The sensitivity and specificity of TNF-α in discriminating between those with and without insulin resistance. (ROC Curve)

Discussion

This case-control study was conducted to determine the level of TNF-α in the third trimester of pregnancy and its association with insulin resistance in gestational diabetic women two months after delivery, compared to healthy pregnant women. The mean TNF-α level in the two groups did not show any significant difference, and there was no relationship between serum levels of TNF-α in pregnancy and different degrees of postpartum insulin resistance.

Of course, it should be noted that the definition of the normal range for the level of TNF-α in pregnant women was considered similar to that of non-pregnant women with a wide range of serum levels. There was no information regarding the definition of the normal level of TNF-α for the different trimesters during pregnancy.

Recent studies suggest that maternal BMI from an early stage of pregnancy, regardless of serum cytokines level, is associated with insulin resistance as a major independent factor (12). Winkler and associates showed that serum TNF-α level in healthy pregnant women was higher than non-pregnant subjects and also higher in the third trimester compared to the first trimester. They found that a significant correlation between serum TNF-α and insulin levels with BMI is predictable in diabetic and non-diabetic pregnant women. This association refers to the probable relationship between the increase in serum TNF-α levels following the advancement of the gestational month and weight gain, changes in the levels of hormones secretion from the placenta, and finally, the level of insulin resistance during pregnancy (13).

In our study, TNF-α level was only measured in the third trimester, and despite the higher BMI of individuals with gestational diabetes than healthy subjects, there was no significant correlation between TNF-α level and BMI of mothers. Although serum TNF-α level in women with GDM was higher than healthy pregnant women, all of the participants had a serum level within a normal range. This suggests that there may be other causative factors other than the patient's weight, such as the level of hormonal factors secreted from the placenta and or genetic factors affect the insulin resistance degree, which can increase the TNF-α level in pregnant diabetic patients. In addition, the normal range for the TNF-α level is very wide, and this may be due to a few studies on the normal level of TNF-α in pregnant and non-pregnant healthy women. It may be necessary to define new normal levels of this cytokine, especially in certain instances such as pregnancy.

Another study that investigated the relationship between serum levels of adiponectin, leptin, CRP, and TNF-α with insulin sensitivity in pregnancy; showed that serum; TNF-α level is significantly higher in patients with gestational diabetes mellitus and have a reverse relationship with insulin secretion during pregnancy. The level of leptin and CRP in the pregnancy phase was higher in comparison with the post-delivery period, but the level of adiponectin was not significantly different in pregnancy and post-delivery phase (14).

Our study also showed that regardless of the presence or absence of gestational diabetes, TNF-α level had a mild reversal association with the HOMA-IR index, based on the level of insulin resistance during pregnancy; however, this relationship was only close to

the meaningful border of statistical significance. Therefore, the index of insulin resistance seems to be a more significant indicator of blood glucose level relative to the level of TNF-α.

In another study, the association of serum TNF-α level with insulin resistance during normal pregnancy was studied. The serum TNF-α level was significantly higher in the third trimester than in the first and second trimester. There was also a significant positive correlation between TNF-α and BMI in pregnant women, but no clear correlation was found between insulin resistance and TNF-α level in normal pregnancy (15).

In our study, there was a significant correlation between insulin resistance and BMI and the age of the affected individuals, so the higher BMI and the age of the patients, the greater chance of gestational diabetes. There was no correlation between TNF-α levels and insulin resistance two months after delivery.

Perhaps, if we had access to the trimester-specific TNF-α referral range in the normal pregnancy and considered a higher sample size of the patients, we could obtain better results.

Despite the lack of a meaningful statistical association between the levels of serum TNF-α and the degrees of post-delivery insulin resistance in this study, we suggest future studies with higher sample size and complementary trimester-specific serum TNF-α level.

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