Effect of the sTREM1 with inflammatory markers (CRP, IL-β1 and PCT), Cortisol hormone, Body max index and glucose intolerance in pregnant women with gestational diabetes mellitus

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Abstract
Gestational diabetes mellitus (GDM), is defining as the glucose intolerance of varying degrees of severity with onset or first recognition during pregnancy. The aim of this study was to check the changes and compare serum sTREM1, and inflammatory markers C-reactive protein (CRP), Interleukin-β1 (IL-β1) and Procalcitonin (PCT), Cortisol hormone, Body max index (BMI) and lipid profile cholesterol (TC), triglyceride (TG), very low density lipoprotein (VLDL), Low density lipoprotein (LDL) and height density lipoprotein (HDL) and insulin resistance between the GDM patients and control subjects and assess the correlation between sTREM1 and these markers, and assess the correlation between BMI above markers and FBS, HOMA-IR and HOMA-β. Our results indicate that circular levels of sTREM1 are significantly up-regulated in pregnant women with GDM or glucose intolerance compared with healthy controls. Moreover, there is a positive correlation between inflammatory markers, BMI, insulin, CRP, PCT, Cortisol hormone, IL-β1 and oral glucose tolerance test (2hOGTT) in pregnant women. After parameters adjustment, the higher levels of sRTEM1 were associated with increased risk of glucose intolerance or GDM in pregnant women. And the present study has reported that pro-inflammatory markers are closely related to glucose intolerance and gestational diabetes mellitus (GDM) in pregnant women. However, there is a lack of concordance in the profile of inflammatory cytokines in maternal serum in glucose intolerance or GDM pregnancies. Therefore, we continued to investigate possible correlation between inflammatory markers, glucose intolerance, and GDM in women. The sRTEM1 may serve as potential biomarkers for evaluating potential glucose intolerance or GDM risk in women with pregnancy.

Keywords: Gestational diabetes mellitus; biomarkers; pathophysiology; inflammatory markers, pregnancy, gestational diabetes mellitus

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Introduction
Gestational diabetes mellitus (GDM) accounts for approximately 6% of pregnancies in world and is becoming increasingly prevalent worldwide [1], which is attributed to obesity prevalence and advancing maternal age [2]. Moreover, disturbance of the endocrine system in pregnancy may strongly associate with GDM, especially increased secretion of placental hormones leads to increase in insulin resistance throughout the second trimester [3].

GDM can resolve in about 90% of women after pregnancy [4]. Notably, women with a history of GDM and gestational glucose intolerance are predisposed to postpartum obesity and type 2 diabetes mellitus (T2DM) [5]. It is known that GDM confers a strong risk for pregnancy complications, including gestational hypertension, fetal macrosomia, and meogitocia [6, 7]. GDM can also lead to dyslipidaemia, which is an incentive to accelerate local and systemic inflammation [8, 9]. Insulin impedance plays an essential functional role in the GDM pathogenesis. In spite of comprehensive research, the basic mechanisms of insulin impedance are not completely comprehended [10]. Insulin impedance in gestation is traditionally observed to raise placental hormones as well as maternal adiposity.
with diabetogenic effect \[11\]. Although the subsidiary mechanisms are not completely realized, the basic fulfillments have concentrated on diverse modern prospective moderators of pregnancy insulin impedance reckonings \[12\].

In recent years, there is mounting evidence that chronic, low-grade inflammatory response is more frequently associated with GDM \[13\]. Several studies demonstrate serum levels of pro-inflammatory cytokines and C-reactive protein (CRP) is up-regulated and positively associated with GDM risk or insulin resistance in pregnancy \[14-16\]. At the second trimester of pregnancy, women with GDM have higher interleukin-1β (IL-1β) compared to women without GDM \[17\]. Lack of concordance of circulating inflammatory cytokines in maternal serum in GDM pregnancies is the most outstanding problem, which limits the clinical application of inflammatory biomarkers for GDM monitoring.

Triggering receptor expressed on myeloid cells -1 (TREM-1) is a biomarker discovered in 2000. It is an immunoglobulin family member that can be found on the surface of neutrophils, monocytes, macrophages and endothelial cells, and has a role in response to infection. Bouchon et al. first described how TREM-1 activates the inflammatory reaction, synthesis of inflammatory mediators, and inhibition of anti-inflammatory mediators \[18\]. TREM-1 enables the synthesis of proinflammatory cytokines via Toll-like receptor (TLR) and modulates the innate inflammatory response by enhancing the signal pathway mediated by TLR. TREM-1 is a member of the TREM family of receptors, coded by a gene on the human chromosome 6p21.1. The soluble form of TREM-1 is a soluble triggering receptor expressed on myeloid cells -1 (sTREM-1), that is delivered to the circulation after proteolytic unbinding mediated by metalloproteinase \[19-22\].

In the present study, we performed a crosssectional study to identify specific inflammatory markers associated with GDM or glucose intolerance in pregnant women by analyzing the correlation between sTREM and another inflammatory marker (CRP, PCR and IL-1β) and BMI with glucose tolerance or GDM.

**Material and methods**

A total of 90 pregnant women were enrolled from May 2019 to April 2020, who visited (Al-Zahra Hospital for Obstetrics and Gynecology) for 24 to 28 weeks gestation screening. Height and weight were measured to calculate the body mass index (BMI), SBP and maternal age for all participants. Clinical experiments were obtained with written informed consent from all patients. The study was approved by the Ethics Committee of the district of holy Karbala Health / Karbala City/ Iraq. Exclusion criteria were as follows: pre-existing diseases including T1DM, T2DM, polycystic ovarian syndrome, inflammatory bowel disease, chronic inflammatory conditions, etc.; infectious disease including hepatitis B, herpes virus, etc.; received corticosteroids treatment; renal insufficiency and endocrine diseases and smokers and multiple pregnancies. All subjects were subjected to clinical examination included anthropometric measurements and blood pressure readings. Body mass index (BMI) was calculated as body weight in kilograms divided by square of height in meters (Kg/m²).

**Measurements**

Five milliliters of venous blood were drawn from all enrolled pregnant women in second trimesters which were detected during follow up, after 12 hours fasting. The collected of blood was transferred into plane tube, left at room temperature for 10 min for clotting, then centrifuged (at 3000 g) for 10 min in order to provide serum. Sera were separated into four aliquots and stored at (-20°C) until time of analysis \[23\]. Levels of serum glucose, total cholesterol, triglyceride and highdensity lipoprotein (HDL) were determined using commercially available kits (Biolabo/ France). Insulin and PCT were measured using assay kits (Calbiotch / USA). IL-1β, CRP, Cortisol hormone and sTREM1, were measured using assay kits (Elabscience Biotechnology Inc. / USA).

**Statistical analysis**

Statistical analysis was performed using two statistical software, the Statistical Package of Social Science (SPSS ver. 21) and Graphpad Prism ver.5. Continuous variables were expressed as mean ± standard deviation (SD). Significant
differences were assessed using Paired t-test and independent t-test for variables with equal and unequal frequencies respectively. Bivariate correlations were assessed using standardized Pearson coefficients. The p values obtained of less than 0.05 and 0.01 were considered as statistically and highly statistically significant respectively [24].

Results
Demographic characteristics and physiological and biochemical parameters of subjects Using oral glucose tolerance test (OGTT) criteria, 45 (50%) had normal glucose tolerance, 45 (50%) pregnant women were diagnosed with glucose intolerance (abnormal OGTT), had overt GDM. Demographic characteristics and physiological and biochemical parameters are presented in Table-1. The results demonstrate that FBS, TG, IL-1β, total cholesterol, HDL, LDL had obvious difference among the two groups. However, maternal age, pre-pregnancy BMI, CRP, insulin, triglycerides, FBS, IL-1β, total cholesterol, HDL, LDL, 1hOGTT and 2hOGTT were highly statistically significant among the two groups. Compared with healthy controls, the age of pregnant women with glucose tolerance or GDM was older. Pre-pregnancy BMI measurement in GDM group was significantly increased compared with healthy control group. Pregnant women with GDM had higher PCT levels than the healthy control group but CRP and cortisol hormone in GDM group was significantly increased compared with healthy control group. In addition, insulin and 1hOGTT levels increased significantly in GDM group when compared with the healthy control group.

At the second trimester of pregnancy, women with GDM have higher interleukin-1β (IL1β) compared to women without GDM [17]. Similarly, increased maternal serum sTREM1 may be useful to predict the development of GDM [25], intriguingly, patients with GDM have significantly sTREM1 levels than healthy pregnant women. Correlation between inflammatory markers and BMI, insulin, 1hOGTT and 2hOGTT by using the Spearman linear regression analysis, BMI, insulin, 1hOGTT and 2hOGTT was correlated with CRP.

Table-1: Demographic characteristics and physiological and biochemical parameters.
Fasting blood sugar (FBG), Triglyceride (TG), Body max index (BMI), Oral glucose tolerance test (OGTT), lipopolysaccharide binding protein (LBP), C-reactive protein (CRP), Interleukin-β1 (IL-β1), Cortisol hormone, Height density lipoprotein (HDL), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) and Procalcitonin (PCT).

As shown in Figure-1 and Table-2, there was a positive correlation between BMI and insulin (r=0.153 and P=0.036), TG (r=0.781 and P=0.031), Cholesterol (r=0.070 and P=0.569), LDL (r=0.133 and P=0.302), VLDL (r=0.045 and P=0.712), IL-β1 (r=0.103 and P=0.586), OGTT (r=0.138, P=0.286), HOMA-β (r=0.312 and P=0.093), HOMA-IR (r=0.176 and P=0.351), and sTREM1 (r=0.043 and P=0.823) and negative correlated with FBG (r= -0.002 and P= 0.975), HDL (r=-0.081 and P= 0.559 ), CRP (r=-0.04 and P= 0.832), PCT (r= -0.06 and P= 0.754) and Cortisol (r= -0.154 and P= 0.417).

Table-2: Correlation between BMI with the investigated parameters in the GDM patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>-0.002</td>
<td>0.975</td>
</tr>
<tr>
<td>OGTT (2 hr) (mg/dl)</td>
<td>0.138</td>
<td>0.286</td>
</tr>
<tr>
<td>Insulin (mg/dl)</td>
<td>0.153</td>
<td>0.036</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.176</td>
<td>0.351</td>
</tr>
<tr>
<td>HOMA-β%</td>
<td>0.312</td>
<td>0.093</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>0.781</td>
<td>0.031</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.070</td>
<td>0.569</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>-0.081</td>
<td>0.559</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>0.133</td>
<td>0.302</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>0.045</td>
<td>0.712</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>-0.04</td>
<td>0.832</td>
</tr>
<tr>
<td>PCT (mg/dl)</td>
<td>-0.06</td>
<td>0.754</td>
</tr>
<tr>
<td>IL-β</td>
<td>0.103</td>
<td>0.586</td>
</tr>
<tr>
<td>sTREM1</td>
<td>0.043</td>
<td>0.823</td>
</tr>
<tr>
<td>Cortisol</td>
<td>-0.154</td>
<td>0.417</td>
</tr>
</tbody>
</table>
Moreover, we also found that sTREM1 was significantly and positively correlated with HOMA-IR ($r=0.010$ and $P=0.958$) and CRP ($r=0.100$ and $P=0.599$) and negative correlated with PCT ($r=-0.070$ and $P=0.713$), IL-$\beta_1$ ($r=-0.095$ and $P=0.616$), Cortisol ($r=-0.095$ and $P=0.616$) and HOMA-$\beta$ ($r=-0.176$ and $P=0.353$), as shown in Figure-2 and Table-3.

**Table-3:** Correlation between sTREM1 with the investigated parameters in the GDM patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$r$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>0.233</td>
<td>0.216</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>0.043</td>
<td>0.823</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.010</td>
<td>0.958</td>
</tr>
<tr>
<td>HOMA- $\beta$</td>
<td>-0.176</td>
<td>0.353</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>0.028</td>
<td>0.762</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.064</td>
<td>0.561</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>0.007</td>
<td>0.965</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>-0.127</td>
<td>0.296</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>0.042</td>
<td>0.703</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.100</td>
<td>0.599</td>
</tr>
<tr>
<td>PCT (mg/dl)</td>
<td>-0.070</td>
<td>0.713</td>
</tr>
<tr>
<td>IL-$\beta_1$</td>
<td>-0.095</td>
<td>0.616</td>
</tr>
<tr>
<td>Cortisol</td>
<td>-0.095</td>
<td>0.616</td>
</tr>
</tbody>
</table>
Discussion
In the present study, we found that sTREM1 is the most important inflammatory biomarker associated with increased risk of glucose intolerance and GDM in the late second trimester. Our results are similar to previous studies showing elevated levels of sTREM1 in pregnant women with GDM [26, 27]. In addition, we report up-regulation of CRP and PCT in pregnant women with glucose intolerance and GDM. Whereas the difference in IL-1β levels remains highly significant in women with GDM after adjusting for glucose, insulin and BMI values [26]. These findings suggest that deregulated circulating proinflammatory cytokines may be involved in the incidence of glucose intolerance and GDM in pregnant women. Although GDM can spontaneously disappear after delivery, it is strongly linked to postpartum complications in both the newborn infants and puerperal [1].

Cumulative evidence has proved that GDM is a high risk factor for abnormal glucose tolerance, obesity and T2DM in postpartum women [2, 28, 29]. In recent years, there has been increased interest to the development of GDM. Unlike an acute pro-inflammatory response, chronic and low-grade inflammatory response is usually accompanied with obesity, which is the leading factor of GDM [30]. In our study, elevated circulating levels of CRP had been observed in pregnant women with glucose intolerance and GDM. Our study and our conclusions have confirmed that the levels of IL-1β are associated with GDM.

Age and BMI are associated with the homeostasis of endocrine metabolic system, such as glucolipid metabolism and blood pressure [31]. Moreover, elevated inflammatory response is observed with increases in both age and BMI [32, 33]. So we hypothesized that elevated inflammatory response may be associated with glucose metabolic disorders. Our data suggest that maternal age and pre-pregnancy BMI have significant associations with GDM. We also found that BMI was significantly and positively correlated with sTREM1, this pro-inflammatory marker also positively correlated with the up-regulation of insulin and OGTT. Therefore, the current findings provide strong evidence that BMI may play a role in determining the levels of inflammatory markers and contribute to the development of GDM.

Our findings demonstrated taken together, that the circular levels of sTREM1 were significantly up-regulated in pregnant women with GDM or glucose intolerance. Higher inflammatory markers were associated with greater risk of GDM at 24 to 28 weeks gestation. However, further studies will be focused on larger sample sizes, which are...
needed to verify our current conclusions. Furthermore, the levels of inflammatory markers may be under dynamic state across the pregnancy cycle. Therefore, multi-time measurement of inflammatory markers will be elaborated the correlation of inflammation and GDM.

There were significant differences among the two groups of patients concerning routine laboratory parameters. CRP is an important diagnostic marker of inflammatory states, including infections, which was confirmed in our study also, since the level of CRP did differ among groups. In our study, PCT was the highest in GDM patients but it did not differ significantly among important marker in differential diagnosis of GDM and control groups. These results are probably the result of the smaller sample of patients in our study, compared to other studies. Cortisol is one of the important stress hormones. Cortisol could increase hepatic glucose production, aggravate β cell function, and decrease insulin secretion, all of which could lead to hyperglycemia. The same trend was observed in cortisol. Compared with the Control group, the content of cortisol in the GDM group was increased, showing no significant difference between the two groups and these results were disagreement with our results.

Conclusions
The present study demonstrates the correlation between the sTREM-1 level and various physiological and pathophysiological states in GDM women. The present study illustrated the effect of sTREM-1 on blood glucose concentration and insulin levels and performed HOMA-IR and HOMA-β to check the degree of insulin resistance and beta cell dysfunction and other parameters. Our results indicate that sTREM-1 is a reliable marker in the diagnosis of GDM in the light of the new definition, neither in the differential diagnosis of GDM. Also, sTREM-1 did prove itself to be a significant biomarker for differentiation of patients with GDM who had insulin resistance from the pregnant women. The significance of sTREM-1 as a diagnostic and prognostic marker in GDM is to be defined and determined in future research.

Declaration of interest: The authors have no conflict of interest to declare.

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