Original Article
Correlation between inflammatory markers (hs-CRP, TNF-α, IL-1β, IL-6, IL-18), glucose intolerance, and gestational diabetes mellitus in pregnant women

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Abstract: Background and aim: Several studies have reported that pro-inflammatory cytokines 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 Chinese women.

Methods: Using glucose challenge test (GCT) and oral glucose tolerance test (OGTT) criteria, 102 pregnant participants were stratified into three groups: the healthy control group (n = 32), the glucose intolerance group (abnormal GCT and normal OGTT; n = 41), and the GDM group (abnormal GCT and abnormal OGTT; n = 29). Circulating inflammatory cytokines were measured in 24 to 28 weeks pregnant women. The correlation coefficients between inflammatory cytokines and BMI, HbA1c, insulin, or 1hGCT were calculated using the Spearman linear regression analysis.

Results: Our results indicate that circular levels of hs-CRP, IL-6, and IL-18 are significantly up-regulated in pregnant women with GDM or glucose intolerance compared with healthy controls. Moreover, there is a positive correlation between inflammatory cytokines, BMI, HbA1c, insulin, or 1hGCT were calculated using the Spearman linear regression analysis. Results: Our results indicate that circular levels of hs-CRP, IL-6, and IL-18 are significantly up-regulated in pregnant women with GDM or glucose intolerance compared with healthy controls. Moreover, there is a positive correlation between inflammatory cytokines, BMI, HbA1c, insulin, or 1hGCT in pregnant women. After parameters adjustment, the higher levels of hs-CRP and IL-18 were associated with increased risk of glucose intolerance or GDM in pregnant women. Conclusion: Hs-CRP and IL-18 may serve as potential biomarkers for evaluating potential glucose intolerance or GDM risk in Chinese women with pregnancy.

Keywords: Inflammatory cytokines, glucose intolerance, gestational diabetes mellitus

Introduction

Gestational diabetes mellitus (GDM) accounts for approximately 6% of pregnancies in China 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 third 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 meconitocia [6, 7]. GDM can also lead to dyslipidaemia, which is an incen-
women with GDM have higher interleukin-1\(\beta\) (IL-1\(\beta\)) compared to women without GDM [17]. Similarly, increased maternal serum IL-6 may be useful to predict the development of GDM [18]. Intriguingly, patients with GDM have significantly higher IL-18 levels than healthy pregnant women, however, IL-18 has no significant difference after adjusting for glucose, insulin and BMI values [19, 20]. 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.

In the present study, we performed a cross-sectional study to identify specific inflammatory markers associated with GDM or glucose intolerance in pregnant women by analyzing the correlation between inflammatory markers (hs-CRP, TNF-\(\alpha\), IL-1\(\beta\), IL-6, IL-18) and glucose tolerance or GDM.

Material and methods

Participants

A total of 102 pregnant Chinese women were enrolled from January 2015 to January 2017 in the Affiliated Heping Hospital of Changzhi Medical College (Changzhi, China) for 24 to 28 weeks gestation screening. Height and weight were measured to calculate the body mass index (BMI) for all participants. In addition, maternal age, SDP and DBP were recorded. Clinical experiments were obtained with written informed consent from all patients. The study was approved by the Ethics Committee of the Affiliated Heping Hospital of Changzhi Medical College (Changzhi, China).

Exclusion criteria were as follows: (1) pre-existing diseases including T1DM, T2DM, polycystic ovarian syndrome, inflammatory bowel disease, chronic inflammatory conditions, etc.; (2) infectious disease including hepatitis B, herpes virus, etc.; (3) received corticosteroids treatment; (4) renal insufficiency and endocrine diseases; (5) smokers and multiple pregnancy.

10 ml of blood sample from pregnant women were collected with ethylenediaminetetraacetic acid (EDTA)-containing tubes (Becton, Dickinson and Company) after 1-hour glucose challenge test (1HGT) and 3-hour oral glucose tolerance test (3HOGTT), as described previously [1, 3, 12].

Table 1. Demographic characteristics and physiological and biochemical parameters of subjects

<table>
<thead>
<tr>
<th></th>
<th>Healthy control ((n = 32))</th>
<th>Glucose intolerance ((n = 41))</th>
<th>GDM ((n = 29))</th>
<th>(p)-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (Years)</td>
<td>26.9 ± 3.0</td>
<td>29.4 ± 3.4**</td>
<td>29.9 ± 3.8**</td>
<td>0.002</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m(^2))</td>
<td>24.1 ± 3.0</td>
<td>25.8 ± 3.4</td>
<td>28.1 ± 3.2***; **</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>113.6 ± 9.0</td>
<td>114.1 ± 9.6</td>
<td>114.4 ± 7.9</td>
<td>0.884</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>66.6 ± 5.8</td>
<td>65.4 ± 5.3</td>
<td>66.2 ± 5.3</td>
<td>0.649</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>2.35 ± 0.63</td>
<td>4.58 ± 1.19***</td>
<td>5.11 ± 1.49***</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/mL)</td>
<td>6.04 ± 1.19</td>
<td>6.55 ± 1.99</td>
<td>6.56 ± 1.17</td>
<td>0.102</td>
</tr>
<tr>
<td>IL-1(\beta) (pg/mL)</td>
<td>2.01 ± 0.69</td>
<td>1.90 ± 0.64</td>
<td>1.86 ± 0.66</td>
<td>0.611</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>2.96 ± 0.70</td>
<td>5.43 ± 1.50***</td>
<td>5.10 ± 1.20***</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-18 (pg/mL)</td>
<td>22.4 ± 5.1</td>
<td>45.2 ± 8.5***</td>
<td>52.2 ± 10.3***; ##</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.69 ± 0.44</td>
<td>5.11 ± 0.44**</td>
<td>5.84 ± 0.66***; ###</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Insulin ((\mu)U/mL)</td>
<td>6.69 ± 0.99</td>
<td>7.77 ± 1.91</td>
<td>10.06 ± 2.47***; ###</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>214 ± 45</td>
<td>234 ± 51</td>
<td>226 ± 56</td>
<td>0.657</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>152 ± 45</td>
<td>172 ± 67</td>
<td>214 ± 60*</td>
<td>0.014</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>67 ± 12</td>
<td>72 ± 20</td>
<td>67 ± 16</td>
<td>0.823</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>124 ± 34</td>
<td>138 ± 56</td>
<td>122 ± 48</td>
<td>0.749</td>
</tr>
<tr>
<td>1hGCT (mg/dL)</td>
<td>113.2 ± 9.1</td>
<td>146.9 ± 8.1***</td>
<td>162.4 ± 10.8***; ###</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

GDM, gestational diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; hs-CRP, high sensitivity C-reactive protein; TNF-\(\alpha\), tumor necrosis factor-\(\alpha\); IL-1\(\beta\), interleukin-1\(\beta\); IL-6, interleukin-6; IL-18, interleukin-18; HbA1c, glycosylated hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein; 1hGCT, the glucose levels of 1 h after 50 g glucose challenge test. \(^{*}P < 0.05\), \(^{**}P < 0.01\), \(^{***}P < 0.001\) vs healthy control group; \(^{*}P < 0.01\), \(^{**}P < 0.001\) vs Glucose intolerance group.
Inflammatory markers in pregnant women with GDM

Measurements

Venous blood samples were obtained from all participants after overnight fasting. After centrifugation, serum samples were stored at -80°C until they were analyzed. Levels of serum glucose, total cholesterol, triglyceride, high-density lipoprotein (HDL-cholesterol), and insulin were determined using commercially available kits (Roche Diagnostics GmbH, Mannheim, Germany), with a Roche/Hitachi Modular Analytics System. LDL-cholesterol, hs-CRP, TNF-α, IL-1β, IL-6 and IL-18 were measured using commercial kits (Elabscience Biotechnology Co., Ltd., Wuhan, China). HbA1c was measured in serum samples using Glycosylated hemoglobin assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis

Data are presented as mean ± standard deviation (SD) for each group. All statistical analyses were performed using PRISM version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Inter-group differences were analyzed using one-way analysis of variance followed by Tukey’s multiple comparison test as a post-hoc test to compare the group means. Non-parametric Kruskall-Wallis test was used for comparisons of continuous variables among three groups. Spearman’s linear regression analysis was used to identify the correlation between inflammatory cytokines and BMI, HbA1c, insulin or 1hGCT. \( P < 0.05 \) was considered to indicate a statistically significant difference.

Results

Demographic characteristics and physiological and biochemical parameters of subjects

Using glucose challenge test (GCT) and oral glucose tolerance test (OGTT) criteria, 32 (31.4%) had normal glucose tolerance, 41 (40.2%) pregnant women were diagnosed with glucose intolerance (abnormal GCT and normal OGTT), 29 (28.4%) had overt GDM (abnormal GCT and abnormal OGTT). Demographic characteristics and physiological and biochemical parameters are presented in Table 1. The results demonstrate that SBP, DBP, TNF-α, IL-1β, total cholesterol, HDL-cholesterol, and LDL-cholesterol had no obvious difference among the three groups \( (P > 0.05) \). However, maternal age, pre-pregnancy BMI, hs-CRP, IL-6, IL-18, HbA1c, insulin, triglycerides, and 1hGCT were highly statistically significant among the three groups \( (P < 0.05) \). Compared with healthy controls, the age of pregnant women with glucose tolerance or GDM was older. Pre-pregnancy BMI, hs-CRP, IL-6, IL-18, HbA1c, insulin, triglycerides, and 1hGCT were highly statistically significant among the three groups \( (P < 0.05) \). 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 or glucose intolerance group. Pregnant women with glucose intolerance or GDM had higher hs-CRP, IL-6 and IL-18 levels than the healthy control group. In addition, HbA1c, insulin and 1hGCT levels increased significantly in glucose intolerance and GDM group when compared with the healthy control group. HbA1c, insulin, and 1hGCT levels in the GDM group were significantly increased as compared to glucose intolerance group.

Correlation between inflammatory cytokines and BMI, HbA1c, insulin, or 1hGCT

By using the Spearman linear regression analysis, BMI, HbA1c, insulin, or 1hGCT was corre-
Inflammatory markers in pregnant women with GDM

As shown in Figure 1, there was a positive correlation between hs-CRP and BMI (R = 0.429; P < 0.001), HbA1c (R = 0.409; P < 0.001), insulin (R = 0.213; P = 0.032) and 1hGCT (R = 0.648; P < 0.001). Moreover, we also found that IL-6 was significantly and positively correlated with HbA1c (R = 0.329; P < 0.001; Figure 2B), insulin (R = 0.427; P = 0.032; Figure 2C) and 1hGCT (R = 0.565; P < 0.001; Figure 2D). However, the correlation between IL-6 and BMI had no statistical difference (Figure 2A). Furthermore, there were positive correlations between IL-18 and BMI (R = 0.366; P < 0.001; Figure 3A), HbA1c (R = 0.489; P < 0.001; Figure 3B), insulin (R = 0.410; P = 0.032; Figure 3C) and 1hGCT (R = 0.733; P < 0.001; Figure 3D).

Interestingly, IL-18 and 1hGCT had the highest correlation (R = 0.733; Figure 3D).

Relationship between inflammatory cytokines and odds ratio in pregnant women with glucose intolerance or GDM

Tables 2 and 3 presented the Odds Ratios (OR) for the association of hs-CRP, IL-6 and IL-18 with glucose intolerance or GDM, respectively. Before covariate adjustment, hs-CRP, IL-6, and IL-18 were significantly and positively associated with increased risk of glucose intolerance or GDM. After adjusting for maternal age, hs-CRP, IL-6, and IL-18 remained significantly and positively associated with increased risk of glucose intolerance. After adjusting for maternal age and BMI, hs-CRP, and IL-18 also remained significantly and positively associated with increased risk of GDM. However, IL-6 was no longer statistically significantly associated with risk of GDM (P = 0.095).

Discussion

In the present study, we found that IL-18 is the most important inflammatory biomarker associated with increased risk of glucose intolerance and GDM in the late second and early third trimester. Our results are similar to previous studies showing elevated levels of IL-18 in pregnant women with GDM [19, 20]. In addition, we report up-regulation of hs-CRP and IL-6 in pregnant women with glucose intolerance and GDM. After adjusting for maternal age and...
Inflammatory markers in pregnant women with GDM

BMI, only IL-6 was no longer statistically significantly associated with risk of GDM. In contrast to that, Kuzmicki et al. has been announced that the significant difference expression of IL-18 becomes insignificant, whereas the difference in IL-6 levels remains highly significant in Polish women with GDM after adjusting for glucose, insulin and BMI values [19]. These findings suggest that deregulated circulating pro-inflammatory 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 puerperae [1]. Cumulative evidence has proved that GDM is a high risk factor for abnormal glucose tolerance, obesity and T2DM in postpartum women [2, 21, 22]. In recent years, there has been increased interest to uncover the role of inflammation in 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 [23]. In our study, elevated circulating levels of hs-CRP and IL-6 had been observed in pregnant women with glucose intolerance and GDM. Increased hs-CRP and IL-6 levels are often found together in pregnancy [24]. Although IL-6 might not be a risk factor for GDM after adjusting for maternal age and BMI, hs-CRP, and IL-6 had a stronger association with increased glucose intolerance even though adjusting for maternal age. These findings suggest that hs-CRP and IL-6 may have a role in the pathophysiology of glucose intolerance and serve as the potential serum markers for the early screening of glucose intolerance. Other authors also indicate an increase in IL-6 mRNA in adipose tissue of pregnant women with GDM [25]. In contrast, the majority of studies show no significant differences in the expression of IL-6 in placentas of women with GDM compared with healthy controls [25, 26]. One of the major functions of IL-6 is to accelerate the release of hs-CRP from the liver [27]. Therefore, IL-6 may induce pro-inflammatory role by the up-regulation of hs-CRP.

Although our results showed no significant association of GDM with TNF-α, recent studies have shown that circulating maternal TNF-α levels are an independent predictor for the development of GDM regardless of BMI [10, 14]. Moreover, both previous studies [13, 15] and our conclusions have confirmed that the levels of IL-1β are not associated with GDM.

Age and BMI are associated with the homeostasis of endocrine metabolic system, such as glucolipid metabolism and blood pressure [28]. Moreover, elevated inflammatory response is observed with increases in both age and BMI [29, 30]. 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

Table 2. Relationship between inflammatory cytokines and odds ratio in patients with glucose intolerance

<table>
<thead>
<tr>
<th>Inflammatory cytokines</th>
<th>Crude OR (95% CI)</th>
<th>p-Value</th>
<th>Adjusted OR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP</td>
<td>5.9 (1.8, 16.4)</td>
<td>0.004</td>
<td>2.8 (1.1, 8.5)</td>
<td>0.012</td>
</tr>
<tr>
<td>IL-6</td>
<td>4.7 (1.3, 13.8)</td>
<td>0.007</td>
<td>2.1 (0.8, 6.4)</td>
<td>0.035</td>
</tr>
<tr>
<td>IL-18</td>
<td>6.9 (2.2, 23.5)</td>
<td>0.001</td>
<td>3.2 (1.7, 11.2)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

OR, odds ratio; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IL-18, interleukin-18; *Adjusted for maternal age of patients with glucose intolerance.

Table 3. Relationship between inflammatory cytokines and odds ratio in patients with GDM

<table>
<thead>
<tr>
<th>Inflammatory cytokines</th>
<th>Crude OR (95% CI)</th>
<th>p-Value</th>
<th>Adjusted OR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP</td>
<td>6.3 (2.0, 18.9)</td>
<td>0.002</td>
<td>2.2 (1.0, 7.3)</td>
<td>0.027</td>
</tr>
<tr>
<td>IL-6</td>
<td>4.4 (1.1, 12.5)</td>
<td>0.016</td>
<td>1.8 (0.7, 5.6)</td>
<td>0.095</td>
</tr>
<tr>
<td>IL-18</td>
<td>7.8 (3.1, 26.2)</td>
<td>0.001</td>
<td>2.9 (1.5, 10.7)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

GDM, gestational diabetes mellitus; BMI, body mass index; OR, odds ratio; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IL-18, interleukin-18; *Adjusted for maternal age and BMI of patients with glucose intolerance.
found that BMI was significantly and positively correlated with hs-CRP, IL-6 and IL-18, these cytokines also positively correlated with the up-regulation of Hba1c, insulin and 1hGCT. Therefore, the current findings provide strong evidence that BMI may play a role in determining the levels of inflammatory cytokines and contribute to the development of GDM.

Taken together, our findings demonstrated that the circular levels of hs-CRP, IL-6 and IL-18 were significantly up-regulated in pregnant women with GDM or glucose intolerance. Higher inflammatory cytokines was 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 cytokines may be under dynamic state across the pregnancy cycle. Therefore, multi-time measurement of inflammatory cytokines will be elaborated the correlation of inflammation and GDM or glucose intolerance.

Disclosure of conflict of interest

None.

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