Maternal serum leptin concentration in gestational diabetes mellitus

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ABSTRACT

OBJECTIVE: Pregnancy simulates diabetes like condition in which insulin resistance starts to appear from mid pregnancy along with the changes in body weight and energy homeostasis. Hormonal changes seen during pregnancy can lead on to hyperglycemia. Leptin, apart from playing a major role in energy metabolism, also regulate fetal growth and development. Thus objective of the study was to evaluate maternal serum leptin levels and its correlation with BMI and lipid profile in gestational diabetes mellitus (GDM) patients.

MATERIAL AND METHODS: 30 healthy pregnant females and 30 age and gestation (28-32 weeks) matched females with singleton pregnancy; newly diagnosed with GDM satisfying the inclusion and exclusion criteria were included in the study. Serum leptin, BMI and lipid profile were done in both the groups.

RESULTS: Mean serum leptin was significantly higher (p<0.001) in GDM patients (30.0±5.98 ng/mL) than in control group (20.30±4.48 ng/mL). Mean values of BMI was also found to be significantly higher (p<0.05) in GDM patients (26.20±3.74 kg/m²) as compared to controls (24.17±3.72 kg/m²). Serum Triglycerides, Serum Cholesterol, Serum VLDL-C were found to be significantly elevated in patients as compared to controls (p<0.05) while HDL-C and LDL-C showed statistically no difference between patients and controls (p>0.05).

CONCLUSION: Our study showed that serum leptin level was raised in GDM patients and a significant positive correlation was seen between serum leptin and BMI (r=0.414, p= 0.023). A significant positive correlation was also found between serum leptin and TG (r= 0.509, p=0.004) and serum leptin and VLDL-C (r=0.442, p= 0.014). This suggests that increased body fat may be one of the contributing factors for elevating serum leptin in GDM patients as leptin is mainly produced by white adipose tissue. Also raised leptin has an effect on lipoprotein metabolism along with the effect on glucose homeostasis.

Keywords: Gestational diabetes mellitus; insulin resistance, insulin secretion, Leptin, body weight, lipid profile.

Introduction

Diabetes mellitus is one of the most common chronic diseases affecting around 300 million people worldwide. By 2030, diabetes is expected to affect 552 million people globally with India having largest number of diabetic patients correctly, being termed as “The Diabetes Capital of the World”.¹ The incidence of diabetes is continuously increasing and affecting individuals of all ages including
young adults and children, especially women of reproductive age group (i.e. 18-44 years) are at increased risk of developing diabetes during pregnancy. Therefore, implementation of effective and affordable strategies are required to reduce the incidence of diabetes and its associated health burden more so in women with Gestational Diabetes Mellitus (GDM) to further reduce perinatal morbidity and mortality.2

The World Health Organization (WHO) and the American Diabetes Association (ADA) define GDM as "Any degree of glucose intolerance with its onset or first recognition during pregnancy."3,4 It is a pathological condition characterized by insulin levels that are insufficient to meet increased insulin demands during late pregnancy. Pathogenesis of GDM is not clearly known till date. Increased insulin resistance irrespective of hyperinsulinemia and chronic sub-clinical inflammation are the two main proposed mechanisms for development of GDM. Also the hormones produced by placenta like estrogen, progesterone, human placental lactogen (HPL), prolactin, glucocorticoids etc and increased maternal adiposity play a major role in pathogenesis of GDM.5 It is due to insulin desensitizing effect of these placental hormones.6 Researchers have found the role of other novel markers in the pathogenesis of GDM. These include leptin, adiponectin, resistin, visfatin, retinol binding protein-4 (RBP-4) and other inflammatory mediators [CRP, IL-6, plasminogen activator inhibitor (PAI-1), TNF-α] which seems to play an important role in glucose tolerance and insulin sensitivity dysregulation in women with GDM.5

Leptin is a 16 kilodalton (KDa) protein predominantly synthesized in white adipose tissue in proportion to adipose tissue mass.7 In addition, it can also be produced by variety of other tissues, including placenta, ovaries, mammary epithelium, skeletal muscle, bone marrow, and lymphoid tissues, stomach, pituitary and liver.8 It acts via leptin (LRb) receptors in mediobasal hypothalamus resulting in decreased food intake and increased energy expenditure.5 It also regulates endocrine function, sex maturation, inflammation, immune response, and angiogenesis, reproduction, wound healing, cardiovascular functions. In pregnant women leptin concentration increases due to increased adipocyte leptin synthesis along with the placental synthesis. Maternal leptin concentration increases 2-3 times above the non-pregnant concentration reaching its peak at around 28 weeks of gestation.9 Leptin affects glucose homeostasis as it alters insulin sensitivity.10 By causing insulin resistance, it inhibits the insulin mediated glucose uptake in tissues resulting in hyperglycemia. This decreased insulin sensitivity is also implicated in changes in lipid metabolism as well.11

Some studies indicate that there is a direct inhibitory effect of plasma leptin on insulin secretion, suggesting hyperleptinemia may be one of the reason in the causation of GDM.12 Alternatively, increased release of inflammatory mediators like TNF-α in GDM is known to enhance leptin production as proven by placental gene expression profiling study done by Lepercq et al.13 So, available data do not clarify as to whether alterations in leptin concentration are the cause or consequence of GDM. Thus present study was planned to evaluate maternal serum leptin concentrations in women with gestational diabetes and to compare the levels with those of healthy pregnant controls as well as to correlate serum leptin with body mass index (BMI) and lipid profile in GDM patients.

Materials and methods

This was a one-time cross-sectional study conducted in the department of Biochemistry in collaboration with the Department of
Obstetrics and Gynaecology, Pt. B.D. Sharma, PGIMS, Rohtak. 30 healthy pregnant females and 30 age and gestation (28-32 weeks) matched females with singleton pregnancy; newly diagnosed with GDM following OGTT based on WHO criteria\textsuperscript{14}, not having previous history of medical illness like diabetes, hypertension, cardiac, renal and liver diseases, and being on any medical treatment that affects lipid profile, hormones concentration, were included in the study. An informed written consent was taken from all the subjects. Ethical approval for the study was obtained from the institute.

Fasting venous blood sample was drawn from the antecubital vein in GDM subjects as well as controls at 28-32 weeks of gestation before starting any treatment, under all aseptic conditions in a plain red capped vacutainer. Samples were processed within one hour of collection. Serum was separated by centrifugation at 2000 rpm for 10 minutes after clotting.

BMI, lipid profile and serum leptin were done in both the groups. BMI was calculated by dividing the weight in kilograms by the height in meters squared in both the groups.\textsuperscript{15} Lipid profile was measured by enzymatic method.\textsuperscript{16} Serum leptin was estimated by Enzyme Linked Immunosorbent Assay (ELISA) using DRG Leptin ELISA Kit based on the sandwich principle.\textsuperscript{17}

**Statistical Analysis:** Results are expressed as mean and standard deviation. The data was compiled and analyzed by SPSS (Statistical package for social sciences) using appropriate statistical methods. For comparisons of means, student t-test was used to determine the significance between GDM and controls. For assessment of correlation between variables, Pearson’s correlation was used. P values <0.05 were considered statistically significant.

**Results**

Sixty pregnant women participated in this study, which included 30 women with GDM, 30 healthy pregnant women with normal OGTT. The baseline characteristics of the pregnant women are shown in Table 1.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>GDM (n=30) (Mean ± SD)</th>
<th>Non-GDM (n=30) (Mean ± SD)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.50 ± 4.21</td>
<td>26.13 ± 3.58</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>30.67 ± 1.47</td>
<td>30.56 ± 1.38</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI (Kg/m\textsuperscript{2})</td>
<td>26.20 ± 3.74</td>
<td>24.17 ± 3.72</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87 ± 0.04</td>
<td>0.78 ± 0.03</td>
<td>&lt;0.001</td>
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</tbody>
</table>

The age of pregnant women ranged from 20-35 years. The mean age of pregnant controls was 26.13 ± 3.58 years while that of GDM patients was 26.50 ± 4.21 years. The difference between both the groups was not statistically significant (p>0.05). Mean gestational age of controls and patients was 30.56 ± 1.38 and 30.67 ± 1.47 weeks respectively with p value of >0.05. Thus, both the groups were age and gestation period matched.

Mean values of BMI was found to be higher in patients as compared to controls. Mean values of BMI in GDM patients was 26.20 ± 3.74 kg/m\textsuperscript{2} while in control group values was 24.17 ± 3.72 kg/m\textsuperscript{2}.
kg/m². On statistical analysis, significant difference was found between both the groups (p<0.05) (Figure 1).

As per Table 1, it was observed that mean value of W/H was higher in GDM patients as compared to healthy pregnant controls with mean levels of 0.78 ± 0.03 in control group as compared to 0.87 ± 0.04 in patients. The difference between two groups was statistically significant (p<0.001).

Special investigation, serum leptin levels among GDM patients and control group is shown in Table 2, Figure 2.

<table>
<thead>
<tr>
<th>TABLE 2. Leptin levels in GDM vs non-GDM</th>
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<tbody>
<tr>
<td>Investigations (Mean ± SD)</td>
</tr>
<tr>
<td>Leptin (ng/mL) Mean ± SD</td>
</tr>
<tr>
<td>30.0 ± 5.98</td>
</tr>
</tbody>
</table>

The serum leptin was significantly higher in women with GDM compared with the healthy pregnant women (p<0.001) (Fig. 2). Mean serum leptin in patients was 30.0 ± 5.98 ng/mL while in control group mean serum leptin was 20.30 ± 4.48 ng/mL.

<table>
<thead>
<tr>
<th>Table 3. Lipid profile among GDM Patients and Controls</th>
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<tbody>
<tr>
<td>Investigations (Mean ± SD)</td>
</tr>
<tr>
<td>S. Triglycerides (mg/dL)</td>
</tr>
<tr>
<td>248.40 ± 77.63</td>
</tr>
<tr>
<td>S. Cholesterol (mg/dL)</td>
</tr>
<tr>
<td>227.50 ± 51.50</td>
</tr>
<tr>
<td>S. HDL-C (mg/dL)</td>
</tr>
<tr>
<td>49.03 ± 8.67</td>
</tr>
<tr>
<td>S. LDL-C (mg/dL)</td>
</tr>
<tr>
<td>125.83 ± 42.24</td>
</tr>
<tr>
<td>S. VLDL-C (mg/dL)</td>
</tr>
<tr>
<td>50.13 ± 15.28</td>
</tr>
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</table>

Serum Triglycerides, Serum Cholesterol, Serum VLDL-C were found to be elevated in patients as compared to controls with mean levels of 248.40 ± 77.63 mg/dL, 227.50 ± 51.50 mg/dL, and 50.13 ± 15.28 mg/dL respectively in patients. In control group, mean levels were 165.70 ± 53.05 mg/dL, 194.43 ± 46.30 mg/dL, and 33.16 ± 10.59 mg/dL respectively. On statistical analysis, the difference between two groups was statistically significant (p<0.05). Mean HDL-C and LDL-C showed statistically no difference between patients and controls (p>0.05). Mean HDL-C levels was 53.06 ± 11.99 mg/dL in control which was higher when compared to 49.03 ± 8.67 mg/dL in patients but the difference was not statistically significant (p>0.05). Mean LDL-C levels was 107.10 ± 33.52 mg/dL in control group which was lower when compared to 125.83 ± 42.24 mg/dL in GDM patients but the difference was not statistically significant (p>0.05) (Table 3).
Table 4. Correlational Analysis

<table>
<thead>
<tr>
<th>Serum Leptin with other parameter</th>
<th>Coefficient of correlation ‘r’ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.415</td>
<td>0.023*</td>
</tr>
<tr>
<td>TG</td>
<td>0.509</td>
<td>0.004**</td>
</tr>
<tr>
<td>TC</td>
<td>0.251</td>
<td>0.181</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.059</td>
<td>0.755</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.161</td>
<td>0.396</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>0.442</td>
<td>0.014*</td>
</tr>
</tbody>
</table>

*Correlation was significant at the 0.01 level.
**Correlation was significant at the 0.05 level.

These results indicated that there was a positive correlation between leptin and BMI in diabetic pregnant women (r= 0.415, p= 0.023). A significant positive correlation was found between serum leptin and TG (r= 0.509, p=0.004) and serum leptin and VLDL-C (r= 0.442, p=0.014).

DISCUSSION

GDM is a pathological condition characterized by varying degrees of glucose intolerance that is first detected during pregnancy. Insulin resistance and chronic sub-clinical inflammation are the two main proposed mechanisms for development of GDM. In accordance with other studies; BMI is increased in our study in GDM patients when compared with healthy pregnant females of similar age and gestation. Increased BMI in GDM is attributed to increased fat stores in these patients. As pregnancy advances, maternal weight gain occurs due to increasing size of placenta, growing size of fetus and fetal membrane, amniotic fluid and maternal fluid expansion. But GDM induces more weight gain as it creates chronic hypoxia in placental environment leading to compensatory hyper-perfusion and thereby increasing the placental weight more as compared to normal pregnancy. Ramos and Caughey also showed that women with GDM have a greater predisposition to deposit intra-abdominal fat which is metabolically active and is strongly related to increased insulin resistance of GDM leading to hyperglycemia. This increased insulin resistance and subsequent development of T2DM and cardiovascular disease has been attributed to the ‘thrifty gene hypothesis.’

Similarly, maternal serum leptin was significantly higher in GDM females in comparison to healthy pregnant females. This is in concordance with the findings of Kautzky-Willer et al, who also reported similar results. Cause of this increased leptin concentration in GDM may be attributed to increased placental leptin synthesis and secretion apart from adipocytes as placentae of GDM females is big. Increase in body weight and accumulation of fat especially, visceral fat is also one of the main contributor of increased leptin release in GDM. Gestational hormones like estrogen and cortisol also stimulates leptin production by adipose tissues. Similar findings were reported by Vitoratos et al and Liu et al, who have also shown that women with GDM had higher serum leptin concentrations than non-GDM women. Similar studies of elevated maternal serum leptin in GDM were also quoted by many other researchers like Chen et al, Lappas and Qiu et al.
Gao et al also showed similar results of elevated values of leptin in GDM at 14-20 weeks of gestation. In 2011, Soheilykhah and colleagues conducted a study whose results were also in concordance with our study. But contradicting the above reported findings, Festa and others have reported a decrease in leptin levels in women with very mild abnormalities in glucose metabolism during pregnancy as compared to healthy pregnant controls. Similarly, McLachlan et al and Noureldeen et al, also demonstrated decrease in maternal serum leptin levels in GDM as compared to normal pregnancy at third trimester though the precise mechanism is not known. However one study done by Saucedo et al have found no difference in the plasma leptin concentration between GDM patients and non GDM women.

Gestational diabetes mellitus is characterized by an amplification of the low-grade inflammation already prevailing in normal pregnancy. This hypothesis is supported by increased circulating concentrations of pro-inflammatory molecules like TNFα and IL-6 in GDM pregnancies. Along with leptin, this TNFα is also synthesized and secreted from the placenta and it is supposed to be responsible for causing insulin resistance seen in GDM pregnancies. Comparison of the placental gene expression profile between normal and diabetic pregnancies by Lepercq et al indicated that increased circulatory concentration of TNFα causes a chronic inflammatory environment that enhances leptin production. Conversely, leptin itself increases production of TNFα and IL-6 by monocytes and stimulates the production of CC-chemokine ligands. Thus, a vicious circle develops, aggravating the inflammatory situation.

In our study, when serum leptin levels were correlated with BMI in GDM patients, a significant positive correlation was observed between the two parameters (r= 0.415, p= 0.023). It is well known fact that obesity is strongly associated with inflammation which also contributes to insulin resistance of gestational diabetics. Compensatory hyperinsulinemia in GDM patients may be one of the reasons for increasing the leptin secretion from adipose tissue as insulin may also regulate adipose tissue leptin secretion favoring increased leptin release from adipocytes.

These findings were in agreement with the findings of Soheilykhah et al, Kautzky-Willer et al, Lappas and colleagues who also said that women with higher BMI had a higher level of leptin concentration. Hussein et al also presented similar results in GDM patients suggesting leptin is an important mediator of glucose homeostasis in humans. Their results indicated that plasma leptin had a significant positive correlation with BMI in GDM patients (r= 0.201). Therefore, we can say that gestational diabetes is frequently found in those women who are obese.

It is also noteworthy that a significant positive correlation was found between serum leptin and TG (r= 0.509, p= 0.004) and serum leptin and VLDL-C (r= 0.442, p= 0.014). These findings were consistent with the findings of Hussein et al, who also reported positive correlation between leptin with TG, cholesterol, VLDL and LDL (r= 0.446, r= 0.484, r= 0.464, r= 0.396 respectively) in GDM patients. It is probably due to increased levels of circulating inflammatory mediators in GDM like TNF-α, IL-6. As we have discussed earlier that leptin is a TNF-α inducer and this TNF-α raises serum TG levels by stimulating VLDL production. Thus, we can say that these acute phase reactants may be responsible for causing increased dyslipidemia of GDM and thereby increasing the chances of atherosclerosis later in life.
CONCLUSION: Leptin has a considerable involvement in the pathogenesis of GDM as we observed higher leptin levels in GDM patients than non-GDM women. Either hyperleptinemia is responsible for hyperglycemia of pregnancy or visa-versa, exact mechanism is still not clear. Also women with higher BMI had a higher level of leptin concentration suggesting obesity as a major determinant of causing insulin resistance and raised leptin levels. Thus, determination of leptin levels can serve as a novel marker for the timely diagnosis of GDM and for the better maternal and child health care.

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