ORIGINAL ARTICLE
DECIPHERING ADIPONECTIN-INSULIN RESISTANCE NEXUS IN NORMAL PREGNANT VERSUS NON-PREGNANT WOMEN

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Background: Insulin resistance in pregnancy and gestational diabetes is an important cause of mortality and morbidity. We aimed to compare serum insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and serum adiponectin between normal non-pregnant and pregnant women to see the correlation between adiponectin and insulin resistance in normal pregnant women. Methods: This case control study was conducted at Army Medical College and Centre for Research in Experimental and Analytical Medicine (CREAM) and Military Hospital, Rawalpindi. Sixty subjects were recruited. Group I had 30 normal healthy women. Group II consisted of 30 age and BMI matched 2nd trimester pregnant women with normal glucose tolerance. Women with diabetes, history of miscarriage or inflammatory disease were excluded. ELISA was used to measure serum insulin and adiponectin levels. Glucose levels were measured with glucometer. HOMA-IR was used to assess the insulin resistance (IR). Mean values were calculated. Independent samples t-test was used to compare the means of the parameters. Pearson correlation test was applied to evaluate the correlation between variables in the groups, and p<0.05 was considered as significant. Results: Serum Insulin and HOMA-IR levels were significantly different between group I and group II (p=0.000), however, serum adiponectin levels in both groups were not significantly different. Small, non-significant negative correlation was observed between serum adiponectin and HOMA-IR in group II (p>0.05). Conclusion: Non-significant negative correlation between serum adiponectin and HOMA-IR reveals that there might be factors other than serum adiponectin leading to IR in pregnant women.

Keywords: HOMA-IR (homeostatic model assessment of insulin resistance), adiponectin, gestational diabetes mellitus, Type 2 diabetes mellitus

INTRODUCTION
Impaired glucose tolerance is one of the most commonly observed complications of pregnancy that leads to adverse consequences within the mother and neonate. During normal pregnancy, insulin resistance (IR) gradually starts developing from the first trimester and as the pregnancy progresses, the insulin sensitivity declines to almost 50% of the normal. Studies indicate that this progressive decline in the insulin sensitivity is mainly due to increasing placental hormones including progesterone, human placental lactogen and estrogen. The other significant factor in causing IR in normal pregnancy is change in the levels of adipocytokines. Literature shows that adiponectin and tumour necrosis factor-α are inversely related to insulin resistance, however, the exact metabolic pathway leading to this resistance is still under study. The increased insulin resistance caused by these factors is counteracted by a significant increase in the release of insulin from maternal β-cell islets in order to maintain a normoglycaemic state.

The adipose tissue is considered as a metabolically active compartment which is responsible for the regulation and maintenance of a number of biological processes within the human body. Adipokines form the main link between the adipose tissues and other biological processes. Alterations in the levels of adipocytokines especially adiponectin can lead to development of a pro-inflammatory state in the body of the mother.

Adiponectin performs multiple roles in the human body such as insulin sensitization of target tissue, and promotes glucose uptake in skeletal muscle, decreases production of hepatic glucose, and also has an anti-inflammatory role. The adiponectin levels are negatively linked with body adiposity that implies a negative feedback control of adipose tissue on the levels of adiponectin. There is an association of the levels of maternal adiponectin with pregnancy as it causes to decline its amount as the pregnancy continues. It is established that adiponectin levels are inversely related to insulin resistance. Moreover hypoadiponectinemia has been confirmed in obesity and type-2 diabetes mellitus. Besides, this is also noticed that the condition of hyperinsulinemia that is recognized during the time of pregnancy may cause to interact with the insulin resistance. This condition may lead to a significant reduction in the adiponectin plasma concentration as insulin can reduce the levels of plasma adiponectin. A study indicated that the risk of gestational diabetes mellitus (GDM) is considered
to be 5–6 times more in women with low levels of adiponectin in comparison to those with elevated levels. In the first trimester of pregnancy, the reduced levels of adiponectin are considered as the single most important indicator of developing GDM, by augmenting the inflammatory response and reducing insulin sensitivity. There is an inverse relationship between the levels of serum adiponectin and the levels of serum insulin along with the development of insulin resistance. This measurement is considered as a reflection of insulin sensitivity of the whole body.

This study was conducted to examine the levels of serum adiponectin among pregnant women without recognized insulin resistance and non-pregnant healthy women, and the correlation among the levels of adiponectin and insulin resistance among pregnant women in their second trimester.

**METHODOLOGY**

This case-control study was conducted at the Department of Physiology, Army Medical College, and Centre for Research in Experimental and Analytical Medicine (CREAM) in association with Military Hospital, Rawalpindi, Pakistan. The research was formally approved by the ethical review committee of the Army Medical College and Military Hospital. Consent form was written in Urdu language and was signed by all subjects who took part in the study. There were a total of 60 study subjects selected according to the inclusion and exclusion criteria by convenient non-probability purposive sampling. Group I included 30 healthy non-pregnant women and served as control group. Group II included 30 age and BMI matched pregnant women in their second trimester. Women having diabetes, on insulin therapy, with history of recent miscarriage, or suffering from inflammatory diseases like rheumatoid arthritis, pre-eclampsia, and eclampsia were excluded.

Blood samples of all subjects were collected by peripheral venipuncture after a fasting period of at least 8 hrs and transferred to the gel separator tube and centrifuged at 2,200–2,500 rpm for 5 min at room temperature. Pipetted serum was transferred to the polypropylene tubes and stored at -20 °C.

Insulin levels were assessed by Sandwich ELISA technique using Human Insulin (INS) ELISA kit, Catalogue No. 10811, Elabscience, Inc. Fasting blood glucose levels were measured with a glucometer (Accu-Chek®) after at least an 8 hr fasting, and the upper limit for normoglycemia was taken as 5.1 mmol/L. Fasting levels of serum insulin and blood glucose were used in order to calculate HOMA-IR.

Serum adiponectin levels were measured by Human ADP/Acrp30 (Adiponectin) ELISA Kit, Elabscience Co., Ltd, USA. Catalogue No. E-EL-H0004. This ELISA kit uses sandwich technique for measurement of quantitative levels of adiponectin in the samples.

Data was entered on and analysed using SPSS-22. Mean and standard deviation was measured for all the quantitative variables including fasting glucose levels, HOMA-IR values, and serum adiponectin levels. Independent Samples t-test was used to compare the means of the parameters. Pearson correlation test was applied to evaluate the correlation between variables in the groups, and p<0.05 was considered as significant.

**RESULTS**

The data was normally distributed (Shapiro-Wilk Test, p>0.05), no outliers were found as assessed by box-plots and homogeneity of variances was found as assessed by Levene’s Test for Equality of Variances (p>0.05). There was a statistically significant difference in mean serum insulin (μU/ml) between non-pregnant and pregnant women, with mean insulin levels higher in pregnant women (t = -13.771, p=0.000). A statistically significant difference was also found in mean serum HOMA-IR between non-pregnant and pregnant women, with mean HOMA-IR levels higher in pregnant women (t = -11.953, p=0.000). (Table-1).

There was a statistically non-significant, small negative correlation between HOMA-IR and Serum Adiponectin level, (r = -0.182, p=0.336). Pearson’s correlation was run to assess the relationship HOMA-IR and Serum Adiponectin levels in pregnant women (n=30). A linear relationship was found between the two variables (Figure-1).

**Table-1: Comparison of mean values of fasting serum glucose, serum insulin HOMA-IR and serum adiponectin (n=60)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (n=30)</th>
<th>Group II (n=30)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose (mmol/L)</td>
<td>4.36±0.60</td>
<td>4.31±0.51</td>
<td>0.372</td>
<td>0.711</td>
</tr>
<tr>
<td>Serum insulin (μU/ml)</td>
<td>4.301±0.39</td>
<td>12.79±3.04</td>
<td>-13.771</td>
<td>0.000*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.841±0.34</td>
<td>2.446±0.65</td>
<td>-11.953</td>
<td>0.000*</td>
</tr>
<tr>
<td>Serum adiponectin (ng/ml)</td>
<td>13.99±2.8</td>
<td>13.91±2.37</td>
<td>0.133</td>
<td>0.894</td>
</tr>
</tbody>
</table>

*Significant

**Figure-1: Linear correlation between HOMA-IR and serum adiponectin levels in pregnant women (n=30)**
DISCUSSION
The present study was aimed to compare serum insulin, HOMA-IR and serum adiponectin between non-pregnant and normal pregnant women in their second trimester and to find out the correlation between adiponectin and IR in normal pregnant women.

Kampmann et al.10 elucidated in their study that maternal insulin resistance increases gradually as the pregnancy progresses, even in normal pregnant women. Insulin sensitivity decreases 50–60% even in healthy pregnant women. Our study effectively endorses this fact as the serum insulin levels were significantly higher in group II subjects compared to group I. This increase in serum insulin helps to counteract the insulin resistance caused by several placental hormones along with adipokinetes.10

The severity of insulin resistance was assessed using HOMA-IR index, values being significantly different in the two groups. Alptekin et al stated that HOMA-IR levels ≥2.08 represent considerable insulin resistance and decreased insulin sensitivity.11 Pregnant women in Group II of our study showed normal fasting glucose but raised serum insulin levels which lead to insulin resistance. Significantly raised HOMA-IR in group II suggests the customary pattern of hyperinsulinemia and IR characteristically present in the mother’s body to fulfill the requirements of foetus.12 In our study, group I non-pregnant women showed normal fasting serum glucose and serum insulin levels. Therefore, HOMA-IR values in group I were less than the cut-off value suggesting absence of IR in the healthy non-pregnant subjects. In the normoglycemic pregnant women, the pancreatic beta cell reserve compensates for the insulin resistance that is present during normal pregnancy. As insulin resistance is linked with decreased sensitivity of cells to insulin, it is counteracted by the proliferation of beta cells up to 50% to increase insulin release.13 Therefore significantly raised levels of insulin are expected in insulin resistance which can lead to gestational diabetes.

We observed that serum adiponectin levels were not significantly different between non-pregnant and healthy pregnant women. Fuglsang et al.14 enrolled eleven healthy pregnant women in their study and concluded that serum adiponectin levels kept changing during pregnancy peaking during mid-pregnancy and then decreasing through the third trimester but these changes were not significantly different from normal healthy non-pregnant women. Our findings are in agreement with Fuglsang et al.14

The role of adipokines in development of GDM is gaining strength over the years. Adiponectin is considered the most significant one, which is a physiologically active polypeptide secreted by adipocytes. It shows anti-inflammatory and insulin-sensitizing actions. Serum adiponectin levels of group I came out to be in the normal range in the present study. Women with higher levels of serum adiponectin have 30% lesser risk of developing GDM or T2DM.15

In our study, group I and group II did not show a significant difference in serum adiponectin levels. A possible explanation for this alleged inconsistency of normal serum adiponectin and raised insulin resistance can be in the fact that serum adiponectin is subjected to regulation by various hormones of pregnancy like estrogen, progesterone and testosterone etc.16 The results of our study are consistent with a research carried out by Mazaki et al.17 who reported that the mean serum adiponectin levels in normal pregnant women in their first and second trimester are not significantly reduced. Moreover, serum adiponectin levels in normal pregnant women were not significantly different than non-pregnant healthy women. Another possible explanation for the normal levels of adiponectin in presence of IR in healthy pregnant women can be the changes in regulation of adiponectin during pregnancy. These alterations are mainly due to effect of pregnancy hormones on the secretion and expression of serum adiponectin.17

CONCLUSION
Non-significant negative correlation between serum adiponectin and HOMA-IR reveals that there might be some other factor other than serum adiponectin leading to insulin resistance in pregnant women.

LIMITATIONS
Small sample size is a limitation of the present study. Future studies are suggested to be conducted with a larger sample size to establish a significant correlation between parameters, if any.

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SN: Drafting, Critical review  
SA: Interpretation of data, Final review  
TN: Interpretation of data, Critical revision

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