SIALIC ACID AS A PREDICTOR OF TYPE 2 DIABETES MELLITUS

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ABSTRACT... drmusman@hotmail.com Inflammatory markers predict type 2 diabetes. Gestational Diabetes Mellitus (GDM) predicts type 2 diabetes. **Objective:** To examine the association of inflammatory markers with GDM, we investigated total sialic acid (TSA) in women with and without previous GDM. **Design & methods:** All women with GDM and a random sample of women from diabetic center of Sir Ganga Ram Hospital, Lahore were taken after an interview, an oral glucose tolerance test and anthropometry were performed. A total of 46 women with and 50 women without previous GDM completed the protocol. **Results:** Mean TSA was significantly higher in women with (71.8±11.1 mg/dl) than without (67.5±9.8 mg/dl) previous GDM (P< 0.05). In a linear regression model, TSA was 4 mg/dl (P< 0.05) higher in women with previous GDM, after adjustment for BMI and fasting insulin sensitivity. In a similar model, current 2-h plasma glucose levels were associated with higher TSA levels after adjustment for waist-to-hip ratio and the log of triglycerides. TSA was strongly correlated with individual components and aggregates (r = 0.55, P< 0.001) of the metabolic syndrome. **Conclusions:** Increased TSA levels are associated with previous GDM and are strongly linked to the metabolic syndrome. These findings in young women suggest that a chronic mild systemic inflammatory response is an early feature of the metabolic syndrome and that GDM may be a window for its investigation.

Abbreviations: GDM, gestational diabetes mellitus, OGTT, oral glucose tolerance test, TSA, total sialic acid, WHO, World Health Organization.

INTRODUCTION
Gestational diabetes mellitus (GDM) and type 2 diabetes have been said to be part of the same disease process, although they generally occur at different points during the life span. Indeed, GDM has been shown in several prospective cohort studies to increase the risk of developing type 2 diabetes. Additionally, it has been suggested that GDM should be included as part of the insulin resistance syndrome, also known as the metabolic syndrome.

Sialic acid, an inflammatory marker, has previously been shown to be a strong predictor of coronary heart disease as well as stroke and cardiovascular mortality. Total sialic acid (TSA) levels are also increased in type 2 diabetes and have been found to be significantly associated with the development of diabetes, suggesting that these elevations may reflect the pathogenic processes involved in diabetes. Inflammatory markers, although to our knowledge not sialic acid, have been linked to composite measures of the metabolic syndrome.
To examine the association of inflammatory markers with GDM, this study investigates the association of TSA with a previous diagnosis of GDM in women of reproductive age. Further, it investigates the relationship of TSA levels with hyperglycemia and other components of the metabolic syndrome.

METHODS

Study population
All women with a previous diagnosis of GDM (n=75) and a randomly selected sample of control subjects (n=151) from diabetic center of Sir Ganga Ram Hospital, Lahore were invited to participate in this study. Women who were pregnant when contacted were rescheduled for examination after completion of the pregnancy. After a mean of 6.8±0.6 years after their index pregnancy, a total of 46 women (61.3%) with previous GDM and 50 women (33.1%) without previous GDM were localized, agreed to participate, completed the protocol and had biochemical determinations for the analyses. A written consent was obtained from all participants.

Pregnancy assessment
During the index pregnancy, all women completed a standard interview, including educational level and pre-pregnancy recall weight. Authenticity was determined, according to the women’s skin color, by a trained interviewer. Weight and height measurements were made following standardized protocols. A 2-h 75-g oral glucose tolerance test (OGTT) was performed according to the 1985 World Health Organization (WHO) recommendations between 24 and 28 weeks of gestation. Plasma glucose levels were measured using an enzymatic method. In these current analyses, GDM was defined according to the 1999 WHO recommendations.

Follow-up assessment
During the follow-up assessment, each participant’s weight, waist-to-hip circumference, and systolic and diastolic blood pressures were measured and a 2-h 75-g OGTT was performed following standardized protocols. Blood pressures used in analyses were the second of two determinations, obtained in sitting position.

TSA was measured using an enzymatic method (Boehringer Mannheim, Lewes, Sussex), adapted for use on a Roche Cobas Fara analyzer (Roche, Pak). This technique involves a coupled enzyme assay reaction, incorporating neuraminidase, N-acetylneuraminic acid aldonolase, and pyruvate oxidase linked to a peroxidase dye system. The between batch coefficient of variation was 3.8% or better. Glucose measurements were recorded using an enzymatic technique and insulin was measured by radio-immunoassay (ICN Pharmaceuticals, Pak). Triglycerides were measured with an enzymatic method (Merck, Pak), and HDL cholesterol was measured after dextran-magnesium precipitation of non-HDL lipoproteins. Microalbuminuria was measured by nephelometry.

BMI was calculated as the ratio of weight (kg) to the square of height (m²). A fasting insulin sensitivity index was estimated from the OGTT using the formula: Insulin sensitivity index=10,000/[fasting insulin (μU/ml) x fasting glucose (mg/dl)]. Current glucose regulation status was defined according to the 1999 WHO recommendations.

Statistical analyses
Means and proportions were compared with Student’s t test and χ² test, respectively. Pearson’s correlation coefficients were calculated.

Multiple linear regression was used to model adjusted associations between variables. Variables contributing minimally to the model or whose P values were >0.05 were excluded from final models.

We examined the strength of the association of TSA with elements of the metabolic syndrome and their clustering using categorical and continuous expressions of syndrome components.

For the categorical variable approach, we defined as abnormal a value higher than the 75th percentile for BMI (29.3 kg/m²), waist-to-hip ratio (0.822), systolic blood pressure (127 mmHg), triglycerides (1.46 mmol/l),
microalbuminuria (9.80 g/l), and 2-h glucose (7.63 mmol/l) and a value lower than the 25th percentile for HDL cholesterol (0.99 mmol/l) and fasting insulin sensitivity (4.68). We then summed the number of abnormal syndrome components, so defined, for each woman.

For the continuous variable approach, we created a metabolic syndrome z score as the average of the SD, or z, scores of its components for each woman. To do this, each of the six components of the metabolic syndrome suggested by the WHO Consultation Group’s 1999 report was expressed in SDs of difference from its population mean, if necessary after logarithmic transformation. The metabolic syndrome score was then calculated using the following formula: Mean z score = \( \frac{2-h\ glucose\ score + mean\ blood\ pressure\ score + mean\ dyslipidemia\ score + mean\ obesity\ score + log\ (microalbuminuria)\ score - insulin\ sensitivity\ score}{6} \), where mean blood pressure score = (systolic blood pressure score + diastolic blood pressure score)/2; mean dyslipidemia score = [log(triglycerides) score – HDL cholesterol score]/2; and mean obesity score = (BMI score + waist-to-hip ratio score)/2.

P values <0.05 were considered statistically significant unless otherwise specified. All statistical analyses were performed in Public Health Institute, Lahore, Pakistan.

RESULTS

The characteristics of the sample of women studied as a group and according to their previous GDM diagnosis are shown in Table I. The groups of women with and without a previous diagnosis of GDM are not significantly different in most aspects. However, compared with women without previous GDM, women with a previous diagnosis of GDM have significantly higher TSA levels (P = 0.048), a significantly greater proportion of women with a parental history of type 2 diabetes (P = 0.01), and a greater proportion of women in whom impaired glucose tolerance developed (P = 0.03) at follow-up. Whereas, Table II displays the correlation matrix for TSA levels with elements of the metabolic syndrome, separating women with and without previous GDM. Most of the associations found were statistically significant. TSA, log of triglycerides, and glucose levels were inversely correlated with estimates of fasting insulin sensitivity. Larger correlation coefficients were seen between glucose levels and TSA in women with a previous diagnosis of GDM. Additionally, larger coefficients for TSA were seen with 2-h than with fasting glucose values.

We next examined the adjusted association of current TSA (dependent variable) with a previous diagnosis of GDM and related variables, using a linear regression model. Age, race and nonglucose metabolic syndrome elements from Table II showing statistical significance were included in the model, in addition to quadratic terms where appropriate. Factors that were not statistically significant were excluded. The resulting model included previous GDM (\( \beta = 4.11; P = 0.03 \)), BMI (\( \beta = 0.53; P = 0.01 \)), insulin sensitivity index (\( \beta = -0.34; P = 0.004 \)), and years in school (\( \beta = -0.55; P = 0.04 \)). The model presented an \( r^2 \) of 0.31; in other words, the set of variables explains 30% of the variation of TSA levels. Assuming a linear relationship and taking into account the effects of BMI, fasting insulin sensitivity, and number of years spent in school, a previous diagnosis of GDM is associated with an increase in current TSA levels of 4 mg/dl. An additional model, adding 2-h plasma glucose levels, presented an \( r^2 \) of 0.36, with the 2-h glucose term being statistically significant (P = 0.01). In this model, previous diagnosis of GDM was of borderline statistical significance (P = 0.11). To investigate the association of 2-h plasma glucose levels (dependent variable) with TSA, a strategy similar to the one shown above was used Table III. Two-hour plasma glucose values were significantly associated with TSA levels (P < 0.001); even after the effects of waist-to-hip ratio and triglycerides were taken into account. The \( r^2 \) for the final model was 0.48. Similar associations were seen in separate models for women with and without previous GDM with \( r^2 \) values of 0.49 and 0.47, respectively, although the contribution of TSA to the model was not statistically significant in women without a previous diagnosis of GDM.
Table I. Sample characteristics at follow-up assessment of all women and according to previous GDM

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Whole group</th>
<th>GDM</th>
<th>Non-GDM</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>96</td>
<td>46</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Parental history of type 2 diabetes</td>
<td>20 (20.8)</td>
<td>15 (32.6)</td>
<td>5 (10)</td>
<td>0.01</td>
</tr>
<tr>
<td>Glucose tolerance status diabetes</td>
<td>4 (4.2)</td>
<td>3 (6.5)</td>
<td>1 (2)</td>
<td>0.27</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>20 (20.8)</td>
<td>14 (30.4)</td>
<td>6 (12)</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 5.0</td>
<td>27.0 ± 4.6</td>
<td>26.2 ± 5.3</td>
<td>0.43</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.8 ± 6.5</td>
<td>156.7 ± 6.7</td>
<td>157 ± 6.5</td>
<td>0.82</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.79 ± 0.06</td>
<td>0.80 ± 0.05</td>
<td>0.79 ± 0.06</td>
<td>0.35</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.0 ± 5.5</td>
<td>36.7 ± 5.1</td>
<td>35.4 ± 5.8</td>
<td>0.27</td>
</tr>
<tr>
<td>Parity</td>
<td>2.0 ± 2.0</td>
<td>1.8 ± 2.2</td>
<td>2.2 ± 1.8</td>
<td>0.36</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>119.8 ± 15.0</td>
<td>120.9 ± 15.2</td>
<td>118.8 ± 14.9</td>
<td>0.50</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79.3 ± 12.2</td>
<td>80.4 ± 11.4</td>
<td>78.2 ± 12.8</td>
<td>0.37</td>
</tr>
<tr>
<td>TSA (mg/dl)</td>
<td>69.6 ± 10.6</td>
<td>71.8 ± 11.1</td>
<td>67.5 ± 9.8</td>
<td>0.048</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>0.76</td>
</tr>
<tr>
<td>Triglycerides (log mmol/l)</td>
<td>0.03 ± 0.22</td>
<td>0.07 ± 0.21</td>
<td>-0.01 ± 0.22</td>
<td>0.09</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.6 ± 1.0</td>
<td>5.7 ± 0.9</td>
<td>5.5 ± 1.1</td>
<td>0.24</td>
</tr>
<tr>
<td>2-h plasma glucose (mmol/l)</td>
<td>6.9 ± 2.3</td>
<td>7.4 ± 2.0</td>
<td>6.4 ± 2.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Fasting insulin sensitivity index</td>
<td>6.5 ± 3.0</td>
<td>6.5 ± 3.3</td>
<td>6.6 ± 2.7</td>
<td>0.85</td>
</tr>
<tr>
<td>Microalbuminuria (log g/l)</td>
<td>0.77 ± 0.47</td>
<td>0.77 ± 0.48</td>
<td>0.78 ± 0.47</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Data are means ± SD or n (%).

*P values are for continuous and categorical variables comparing the GDM group with the non-GDM group derived from t tests and x² tests, respectively;

†urine albumin values were missing for five women with and four women without previous GDM.

Given the multiple correlations of TSA with elements of the metabolic syndrome Table II, we next examined the strength of the association of TSA with combinations of these variables using categorical (number of abnormalities) and continuous (z score) approaches. The categorical variable approach showed that TSA levels were higher in women with greater numbers of abnormal elements: women with one or no abnormalities had a mean TSA level of 64.8 ± 9.2 mg/dl, compared with 73.8 ± 10.5 mg/dl (P < 0.001) and 78.3 ± 8.4 mg/dl (P < 0.001) for women with two to four and five to eight abnormalities, respectively.
In women with previous GDM, this correlation coefficient was 0.48 (P < 0.005), whereas it was 0.59 (P<0.001) in women without such a previous diagnosis. Similarly, correlation coefficients were 0.46 (P=0.055) in women with a family history of diabetes and 0.57 (P<0.001) in women without a family history of diabetes.

**CONCLUSIONS**

Our study demonstrates that current increased TSA levels are independently associated with a previous diagnosis of GDM and with current 2-h plasma glucose levels. Furthermore, current TSA levels are correlated with insulin sensitivity, as well as with other components of the metabolic syndrome and with aggregates of these components. These latter interrelationships were present in women with and without a previous diagnosis of GDM.

Inflammatory markers have been related to the development of diabetes in adults, supporting the hypothesis that an inflammatory component is involved in its pathogenesis. To our knowledge, this hypothesis...
has not been examined in relation to milder glucose abnormalities such as GDM nor to mild elevations in glucose after a diagnosis of GDM. Our demonstration that TSA levels are related to previous GDM, independently of indexes of obesity and other metabolic syndrome elements, suggests that mechanisms of hyperglycemia operating early in the course of the disease are related to the inflammatory response. In this regard, it is of note that correlation coefficients for TSA with fasting and 2-h glucose values were larger for GDM than for non-GDM women. However, the cross-sectional nature of the associations precludes us from inferring cause or consequence.

Previous studies have shown associations of inflammatory markers with the metabolic syndrome. In a sub-sample of 600 middle-aged Atherosclerosis Risk in Communities Study participants, serum TSA and other markers of inflammation were correlated with several elements of the metabolic syndrome. A study of 107 men and women with average age of 59 years demonstrated that other inflammatory markers, particularly tumor necrosis factor- and C-reactive protein, are correlated with insulin sensitivity, HDL cholesterol levels, and systolic blood pressure. Furthermore, this study also revealed a strong correlation between summary z scores of inflammatory markers and of the insulin resistance syndrome elements. A later study of 1,088 men and women revealed that C-reactive protein was related to the number of elements (BMI, waist-to-hip ratio, triglycerides, HDL cholesterol, blood pressure, fasting plasma glucose, and insulin) of the metabolic syndrome present.

TSA is a marker of the acute-phase response; 70% of its variability is explained by levels of three acute-phase proteins. Furthermore, it is a predictor of cardiovascular events and diabetes in adults. Chronic activation of the innate immune system, which produces the acute-phase response, has been postulated to lead to insulin resistance and abnormalities in glucose tolerance and lipid metabolism, as well as to endothelial activation, resulting in increased vascular tone and atherosclerosis. The combined effect of these and other associated irregularities, known as the metabolic syndrome, is an overall increased risk of type 2 diabetes and cardiovascular disease.

While confirming the association of inflammatory markers with the metabolic syndrome, our study relating TSA with previous GDM presents unique findings and implications. These findings suggest that a mild chronic inflammatory response is part of the metabolic syndrome in young adult women, decades younger than diabetes would be expected to develop in most individuals. They strengthen the previous suggestion that GDM be considered part of the metabolic syndrome. Furthermore, they suggest that GDM may be a window to investigate the interrelationships of the syndrome, inflammatory states and TSA. In further support of this possibility are the facts that the innate immune system is activated in pregnancy and that pregnancy is associated with insulin resistance, fat deposition, and various metabolic syndrome components such as GDM, dyslipidemia, and hypertension.

This study does not allow investigation of the reasons for the increased TSA levels, which may reflect a mild chronic inflammatory state, such as that resulting from periodontitis. In this regard, it is of note that TSA levels were higher in individuals with lesser educational attainment, a factor that may correlate with a greater burden of such infections. However, higher TSA levels may also reflect increased levels of pro-inflammatory cytokines from adipose or other tissues, unrelated to exogenous infectious stimuli. The fact that correlations were slightly stronger for the 2-h than for the fasting values in our study could reflect that TSA elevation results, in part, from postprandial events such as cytokine release from adipose tissues or even hyperglycemia.

A few potential limitations deserve comment. Given the mostly cross-sectional nature of our results, we were unable to examine whether higher TSA levels precede GDM and the metabolic syndrome. Additionally, selection bias due to incomplete follow-up could help explain the associations found. However, the fact that associations were similar in women with and without a previous diagnosis of GDM and with and without a parental history of type 2 diabetes suggests that a selection bias...

operating through differential follow-up according to these factors is unlikely.

In conclusion, increased TSA levels are associated with a previous diagnosis of GDM, with hyperglycemia and other elements of the metabolic syndrome, and with clustering of syndrome components 5–8 years after the index pregnancy. These findings strengthen the hypothesis that GDM is an early manifestation of the metabolic syndrome and suggest that it may be a window to investigate the syndrome’s interrelationships with inflammatory states. Further research is necessary to clarify the temporality and pathophysiologic explanation of the associations found.

Thus, our hypothesis is justified that, TSA estimation can be used as a predictor of Type 2 Diabetes Mellitus

REFERENCES


