Study of biochemical and oxidative stress markers in the first-degree relatives of persons with type 2 diabetes stratified by glucose tolerance test

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Summary. Background and Aim: The present study has been attempted to compare the relative tolerance to glucose in first-degree relatives of type 2 diabetic patients and estimate the anthropometric, biochemical parameters and markers of oxidative stress in subjects with the different degree of glucose tolerance. Methodology: The study consisted of 34 subjects aged between 20–45 years, with established family history of type 2 diabetes mellitus, who were subjected to oral glucose tolerance test (OGTT). Those without any family history of diabetes served as controls. Results: Out of the 34 subjects, 5 subjects exhibited high tolerance, 18 showed a moderate degree of glucose tolerance and 9 subjects were with low glucose tolerance. Only 2 subjects were categorized as highly intolerant after OGTT. In almost all subjects with glucose tolerance test, the peak plasma glucose level was recorded at 60 minutes after oral glucose administration. In the present study subjects, with a high degree of glucose intolerance showed significantly higher levels of triglyceride (171±9.8**) mg/dl and VLDL levels (34.2±1.9**) mg/dl. A significant increase in the TBARS levels (2.9±0.053**) µg/ml was recorded in subjects with a high degree of glucose intolerance. A corresponding decrease in the reduced glutathione (1.6±2.2) mg/ml and superoxide dismutase activity (0.7±0.08) units/min/mg protein was also recorded. Conclusion: The study revealed disturbance in the lipid parameters and antioxidant defenses in the first degree relative of diabetic patients even before the establishment of disease.

Key words: impaired fasting glucose, impaired glucose tolerance, family history of diabetes, oxidative stress, antioxidant enzymes, human subjects

Introduction

Prevention is better than cure; however, in scenarios where this mark has been crossed, early intervention is the best way to tackle diabetes and its associated complications. Screening of diabetes in the general population is neither an easy task nor cost effective but screening for prediabetes and diabetes among high-risk individuals may be more appropriate (1). High-risk population includes the first degree relatives or offspring from the diabetic person who are at a higher risk of developing diabetes in future and therefore this group is more important and vulnerable to the development of diabetes and needs a special assistance so that the diabetic burden in the society can be reduced.

Prediabetes is a condition where our blood glucose levels are higher than the normal range whereas lower than diabetic range. The two terms that have been used to diagnose prediabetes are impaired fasting glucose and impaired glucose tolerance. The range of
impaired fasting glucose in an individual is 100-125 mg/dl. However, the range of diagnosing impaired glucose tolerance (2h post-load glucose) in an individual is 140-199 mg/dl (2).

Type 2 diabetes poses a major risk of the cardiovascular mortality in the world (3). Several risk factors of diabetes are known which may accelerate the diabetic complication including a family history of type 2 diabetes, increased age, obesity, and a sedentary lifestyle are important. The first-degree relatives of diabetic patients have a lifetime rate of development of diabetes near up to 40% if one parent is affected with diabetes (4). The first-degree relatives of individuals with type 2 diabetes are about 3 folds more likely to develop type 2 diabetes when compared with participants with no prior family history (5). Therefore, the family history of diabetes is one of the risk factors that may need focus and can be considered as an important approach to prevent prediabetes and type 2 diabetes. However, enough data are not available to correlate metabolic abnormalities in the people who have a parental history of diabetes.

In the present study, an attempt has been made to assess the metabolic parameters mainly focused on the biochemical and oxidative stress markers in the first degree relatives of diabetes. It has been proven that these people are at a higher risk of developing prediabetes and type 2 diabetes in the future. Having said that, it is imperative to check their blood glucose based on the criteria of impaired glucose tolerance and impaired fasting glucose and to understand the biochemical and oxidative stress parameters in them.

Materials and Methods

Selection of subjects for the study

For our study, participants were recruited from the diabetic clinic of School of Studies in Biochemistry, Jiwaji University Gwalior, India. A total number of thirty-four subjects in the age group of 20-45 years who have a parental (mother or father) history of type 2 diabetes were included in the study. A group of 20 normal subjects was selected as a control group for the study. All the participants had given their written consent for this study and appreciated to take part in this study. The subjects were also advised not to change their routine lifestyle during the study period. The study was approved by Institutional Ethics committee at Jiwaji University Gwalior Madhya Pradesh (India). Subjects with fasting blood glucose levels between 100-125 mg/dl were considered Pre-diabetic as per the American Diabetes Association (ADA) criteria (2).

Monitoring Anthropometric and Biochemical Variables

Height, weight and waist circumferences of the subjects, barefooted and lightly dressed were measured. The abdominal circumference (waist) was measured at the end of expiration, by wrapping the tape at the level of the umbilicus. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. The blood pressure of the subjects was monitored by using non-physician's electronic blood pressure machine.

Determination of blood glucose and lipid concentrations

Blood samples (3 ml) were collected individually from each subject after a 10-12 hour overnight fast. Plasma was separated by centrifugation at 8000rpm for 10 min and the sample was analyzed for fasting blood glucose. The blood glucose was determined by glucose oxidase-peroxidase method using a kit Monozyme India limited, Ahmadabad (6). Lipid parameters were estimated by spectrophotometric assay with the commercially available kits cholesterol (7) triglycerides (8). Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) were calculated with the help of Friedewald’s formula. Glycosylated hemoglobin (HbA1c) was estimated by the ion exchange resin method (9).

![Figure 1. Glycosylated Haemoglobin (%) of studies subjects](image-url)
**Oral glucose tolerance test (OGTT).**

Fasting finger prick blood samples were collected and glucose concentration was determined by Accu-check glucometer (Accu-Chek Roche Diagnostics India Pvt Ltd, Mumbai). After measuring their fasting blood sugar level, all subjects including control group drank 75g of glucose dissolved in 300 ml of water. The glucose levels were observed at 30-minute intervals for 2 hrs. All the experimental subjects were divided into four categories (High tolerance category I < 150 mg/dl, Moderate tolerance category II 150-200 mg/dl, Low tolerance Category III 200-250 mg/dl, Intolerance Category IV > 250 mg/dl) on the basis of concentration of blood sugar after the intake of glucose at 30-60 minutes.

**Estimation of biomarkers of oxidative stress**

Oxidative stress markers like reduced glutathione (GSH) (10) was estimated in the whole blood whereas, TBARS (Thiobarbituric acid reactive substances), SOD (Superoxide dismutase) and level of catalase were analyzed from the hemolysate.

**Hemolysate preparation for antioxidants estimation**

The plasma and the buffy coat were removed from whole blood by centrifugation at 2000 rpm for 10 minutes at 4°C. The red cells were washed thrice with normal saline and a hemolysate(s) was prepared as follows: For the estimation of catalase (11) and lipid per-oxidation (TBARS) (12): Haemolysate was prepared by mixing 1.9 ml of cold distilled water with 0.1 ml of packed cell volume (PCV) suspension. For estimation of SOD activity (13): The remaining red cells were haemolysed by approximately adding 1.5 volumes of water. The lipids were removed by chloroform-ethanol extraction as follows: The hemolysate was diluted four times with ice-cold distilled water. To 4 ml of the hemolysate, 1ml of ethanol and 0.6ml of chloroform were added sequentially by continuous shaking and vortexed for 1 minute. The preparation was subjected to centrifugation for 10 minutes at about 3000 rpm at 4°C. The aqueous layer was used for the estimation of SOD. Protein estimation was calculated by Lowry method (14).

**Statistical analysis**

The results were expressed as the mean ± standard deviation. The data obtained from the experiments were analyzed using one-way ANOVA (Bonferroni t-test) employing sigma stat, statistical software, version 1.0 (Jandal Corporation, USA). The values were tested for significance at $P < 0.001$, $P < 0.05$.

**Results**

**Anthropometric parameters**

The anthropometric parameters (BMI, blood pressure, waist circumference) of the subjects who participated in the study have been detailed in Table 1. A significant difference in BMI was recorded between the control group and the participants with impaired glucose tolerance. Table 1 shows a significant difference in BMI among normal participants and high tolerance subjects (27.3±0.42), low tolerance subjects (24.1±0.41), intolerance subjects (29.6±1.09). Subject with impaired glucose tolerance exhibited relatively high systolic and diastolic pressure as compared to the

<table>
<thead>
<tr>
<th>Anthropometric parameters</th>
<th>Normal subjects</th>
<th>High tolerance subjects</th>
<th>Moderate tolerance subjects</th>
<th>Low tolerance subjects</th>
<th>Intolerance subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Kg/m²)</td>
<td>21.3 ± 0.12</td>
<td>27.34 ± 0.42</td>
<td>21.7 ± 0.18</td>
<td>24.1 ± 0.41</td>
<td>29.6 ± 1.09</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>117 ± 0.54</td>
<td>122 ± 1.5</td>
<td>117.8 ± 0.54</td>
<td>126.8 ± 1.2</td>
<td>108.5 ± 0.35</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.3 ± 0.34</td>
<td>76 ± 1.08</td>
<td>77 ± 0.32</td>
<td>80 ± 0.38</td>
<td>77.5 ± 1.76</td>
</tr>
<tr>
<td>Waist Circumference (Inches)</td>
<td>35 ± 0.13</td>
<td>36.2 ± 0.77</td>
<td>35.2 ± 0.15</td>
<td>38.5 ± 0.45</td>
<td>40.5 ± 0.35</td>
</tr>
</tbody>
</table>

Value in Mean ± SE, $P > 0.05$ (‘), $P > 0.001$ (‘’), BMI, Body mass index. Significant compared with normal control group value with different superscript latter in row and column are significantly different from each other by Pair wise multiple comparision procedure (ANOVA Banferroni t test)
normal one (Table 1). Subjects with a high degree of glucose intolerance showed significantly high waist circumference when compared with the normal participants. In almost all subjects with glucose tolerance test, the peak plasma glucose level was recorded at 60 min. after oral glucose administration.

**Oral glucose tolerance test**

34 subjects with established parental history of type 2 diabetes exhibited no hyperglycemia and were compared for tolerance of glucose in different groups of participants. Participants with a high glucose tolerance showed a mean fasting blood glucose concentration of 102 ± 1.4 mg/dl. Following the glucose administration (75 gm orally) the peak value (139 ± 4.75) was reached at 30 min. and thereafter a gradual decline in the blood glucose concentration was recorded (Table 2). All the healthy control subjects exhibited peak plasma glucose (153 mg/dl) at 60 minutes. Table 2 revealed that all the subjects of moderate glucose tolerance exhibited less tolerance to glucose in comparison to the participants with high tolerance of glucose.

Table 2 showed that low tolerance participants exhibited lower tolerance to glucose in comparison to the first and the second categories. The peak glucose levels following oral glucose administration reached at 60 min in participants with a lower tolerance of glucose failed to reach the baseline level at 120 min. The lowest tolerance to glucose was categorized as the fourth group called intolerant participants which showed the highest peak of glucose when compared to other groups. This category exhibited very less tolerance to glucose (P>.001) at all monitoring level (Table 2). In almost all subjects with OGTT, the peak plasma glucose level was recorded at 60 min. after oral glucose administration.

**Glycosylated (or glycated) hemoglobin**

HbA1c is a form of hemoglobin which is used primarily to identify the plasma glucose concentration over a prolonged period of time. It is formed in a non-enzymatic pathway by hemoglobin’s normal exposure to high plasma levels of glucose. (Fig.1) represents the glycosylated hemoglobin levels of normal and impaired glucose tolerant participants. The HbA1c concentration was found to be highest in participants with the intolerant group and the percentage of difference was (6.1%) as compared to normal participants.

**Lipid profile parameters**

Table 3 represents the comparison of lipid profiles in normal and impaired glucose tolerance subjects. The slight elevation was recorded in total cholesterol in intolerance to glucose participants and moderate tolerance to glucose participants when compared to normal subjects or control group. The triglyceride and VLDL level were elevated in moderate and low tolerance to glucose participants (P>.001), while no change was recorded in the LDL level when compared with the normal subjects.

**Antioxidant enzymes and lipid peroxidation**

Table 4, shows the antioxidant enzymes status in normal and impaired glucose tolerance subjects. The GSH level, Catalase & SOD activities were the lowest in participants with the highest intolerance to glucose.

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Normal subjects</th>
<th>High tolerance subject</th>
<th>Moderate tolerance subject</th>
<th>Low tolerance subject</th>
<th>Intolerance subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>97.75 ± 0.34</td>
<td>101.8 ± 1.39</td>
<td>102.8 ± 0.56</td>
<td>110.8 ± 1.38</td>
<td>114 ± 5.65</td>
</tr>
<tr>
<td>30 min</td>
<td>146 ± 0.88</td>
<td>139.4 ± 4.75</td>
<td>167.4 ± 0.37</td>
<td>190.9 ± 3.23</td>
<td>197.5 ± 1.76</td>
</tr>
<tr>
<td>60 min</td>
<td>153 ± 0.14</td>
<td>132 ± 5.38</td>
<td>163.2 ± 1.50</td>
<td>204.0 ± 3.89</td>
<td>251 ± 0.70</td>
</tr>
<tr>
<td>90 min</td>
<td>133.15 ± 0.80</td>
<td>129.6 ± 3.64</td>
<td>139.7 ± 1.32</td>
<td>166.3 ± 3.75</td>
<td>225 ± 6.36</td>
</tr>
<tr>
<td>120 min</td>
<td>110.6 ± 0.67</td>
<td>120.2 ± 4.06</td>
<td>128 ± 0.69</td>
<td>135.6 ± 4.08</td>
<td>161 ± 4.24</td>
</tr>
</tbody>
</table>

Unit- mg/dl, Value in Mean ± SE; P>0.05 (.), P > 0.001 (*) Significant compared with normal control group value with different superscript latter in row and column are significantly different from each other by pair wise multiple comparison procedure (ANOVA Banferroni t test)
Study of biochemical and oxidative stress markers in the first-degree relatives of persons with type 2 diabetes stratified by glucose tolerance test

In the present study, we have reported abnormal biochemical and antioxidant levels in the first degree relatives of type 2 diabetes mainly in the group of low tolerance and intolerance to glucose participants. In addition to this, lipid peroxidation was also higher low tolerance and intolerant to glucose participants. Studies have predominantly linked prior familiar diabetes history to the positive incidence of obesity and glucose intolerance, ultimately increase the risk of type 2 diabetes (15). However, the study on the antioxidant levels and lipid peroxidation in participants with a family history of diabetes are limited to understand their pathophysiology.

Impaired glucose tolerance is a pre-diabetic state of hyperglycemia that is associated with insulin resistance and may precede type 2 diabetes in future (16). The abnormal metabolic state between normal glucose tolerance and diabetes consists of two distinct disorders: impaired fasting glucose and impaired glucose tolerance. Compared with subjects who have normal glucose tolerance, patients with impaired fasting glucose or impaired glucose tolerance, unless treated, have considerably a higher risk of developing diabetes and cardiovascular disease (17-19) and thus, can be used as a significant target group for the primary prevention of type 2 diabetes.

Our study corroborates the finding of the previous study by Rizvi et al (2009) on antioxidant and lipid peroxidation levels in participants with a family history of type 2 diabetes (20). Our result indicates

Table 3. Lipid parameters among different groups of participants.

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th>High tolerance subject</th>
<th>Moderate tolerance subject</th>
<th>Low tolerance subject</th>
<th>Intolerance subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>152.82 ± 3.15</td>
<td>129.02 ± 7.7*</td>
<td>174.8 ± 3.09</td>
<td>167.0 ± 3.1</td>
<td>183.9 ± 6</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>102.2 ± 2.64</td>
<td>90 ± 3.23</td>
<td>102.6 ± 2.97</td>
<td>122.3 ± 5.3*</td>
<td>171 ± 9.8**</td>
</tr>
<tr>
<td>VLDL-Cholesterol (mg/dl)</td>
<td>20.43 ± 0.53</td>
<td>18 ± 0.64</td>
<td>21.9 ± 0.55</td>
<td>24.4 ± 1.07**</td>
<td>34.2 ± 1.9**</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>92.62 ± 2.9</td>
<td>63.94 ± 8.1**</td>
<td>100.8 ± 3.5</td>
<td>89.3 ± 3.5</td>
<td>88.15 ± 3.3</td>
</tr>
</tbody>
</table>

The values expressed as mean ± SE, *P<0.05, **P<0.001 compared to normal subjects. VLDL- Very low density lipoprotein; LDL- Low density lipoprotein. Significant compared with normal control group value with different superscript letter in row and column are significantly different from each other by pair wise multiple comparison procedure (ANOVA Banferroni t test)

Table 4. Antioxidant status in normal, High, Moderate, low and Intolerance subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
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<th>Moderate tolerance subject</th>
<th>Low tolerance subject</th>
<th>Intolerance subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (mg/ml)</td>
<td>1.95 ± 0.045</td>
<td>2.1 ± 0.013</td>
<td>1.9 ± 0.038</td>
<td>1.8 ± 0.098</td>
<td>1.6 ± 0.22</td>
</tr>
<tr>
<td>Catalase (µ mol/min/mg protein)</td>
<td>13.9 ± 0.4</td>
<td>14.3 ± 0.396</td>
<td>13.5 ± 0.445</td>
<td>13 ± 0.68</td>
<td>12.2 ± 1.3</td>
</tr>
<tr>
<td>SOD (units/min/mg protein)</td>
<td>1.15 ± 0.076</td>
<td>1.2 ± 0.084</td>
<td>1.1 ± 0.026</td>
<td>0.9 ± 0.022</td>
<td>0.7 ± 0.08</td>
</tr>
<tr>
<td>TBARS (µg/ml)</td>
<td>1.8 ± 0.065</td>
<td>1 ± 0.123</td>
<td>2.1 ± 0.041*</td>
<td>2.3 ± 0.122*</td>
<td>2.9 ± 0.053**</td>
</tr>
</tbody>
</table>

GSH- Reduced Glutathione; SOD- Super oxide dismutase; TBARS- thiobarbituric acid reactive substances.
The values expressed as mean ± SE, *P<0.05, **P<0.001 compared to normal subjects. Significant Compared with normal control group value with different superscript letter in row and column are significantly different from each other by pair wise multiple comparison procedure (ANOVA Banferroni t test)
that participants with a family history observe higher oxidative stress and lower antioxidant levels compared to the normal control group of subjects. The risk in these individuals is probably due to the imbalance in the pro-oxidant and antioxidant levels.

OGTT is the best procedure to predict the future risk of diabetes in a population at large. The data of OGTT indicate the glucose tolerance in the subjects. Studies conducted on the offspring’s of type 2 diabetics have shown that genetic factors are an important aspect of the disease pathogenesis. Besides, insulin resistance is the harbinger of type 2 diabetes, and its presence in unconventional lean and young subjects of diabetic parents can be used as a positive indicator of type 2 diabetes incidences (15).

Given the magnitude of the problem and the seriousness in the complications of diabetes, prevention appears to be a logical approach to curbing the rising menace to the disease. People who develop type 2 diabetes pass through a phase of impaired glucose tolerance. Any intervention in the impaired glucose tolerance phase that reduces resistance to insulin to protect the beta cells or both, should prevent or delay progression to diabetes. If diagnosed, impaired glucose tolerance presents an opportunity for intervention that potentially could delay or prevent the development of diabetes.

Our study characterized the first degree relatives of type 2 diabetic subjects into four categories based on the tolerance for glucose viz., high tolerance (n=5), moderate tolerance (n=18), low tolerance (n=9) and intolerance (n=2). Previous studies reported an association between impaired glucose tolerance, BMI, waist circumference and blood pressure etc. (15, 21, 22). We reported a high systolic blood pressure in the subjects with a moderate degree of intolerance and other groups. Additionally, the BMI and waist circumference were significantly higher in subjects with a relatively high degree of glucose intolerance. However, subjects with high tolerance to glucose also exhibited a high BMI which was statistically significant (Table 1) this is an unexpected result and further need to clarify the association with more number of participants.

Determination of lipid profile may provide clues for cardiovascular risk factors in subjects with prediabetes. Individuals with impaired glucose tolerance are at a slightly elevated risk of developing high blood pressure, elevated lipid profiles, and further risk of developing type 2 diabetes (23). Excess adipose tissue in the truncal region is an important cardiovascular disease risk factor and it adversely affects serum lipid profile (24, 25). In the present study subjects with a high degree of glucose intolerance shows significantly a higher level of total cholesterol and triglyceride and VLDL level. However, there is no corresponding elevation in LDL levels (Table 3). Impaired insulin action and relative insulin deficiency are associated with complex alterations in plasma lipid viz., plasma VLDL levels are raised (26). A controlled blood pressure and lipid profiles substantially reduce the risk of cardiovascular disease in patients with prediabetes.

Increasing evidence in both the experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of prediabetes and diabetes mellitus (20). Changes in oxidative stress biomarkers including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins, lipids peroxidation, nitrite concentration, non-enzymatic glycosylated proteins have been reported in type 2 diabetes (27). The same has been substantiated by several studies that have indicated that there occurs a significant reduction in the enzymatic activity of the radical scavenging enzymes as SOD, catalase with a reduction in the level of antioxidants (28-31). Enhanced oxidative stress and changes in antioxidant capacity observed in both the clinical and the experimental prediabetes and diabetes mellitus are thought to be the etiology of diabetic complications. A significant increase in TBARS levels was recorded in subjects with a high degree of glucose intolerance (Table 4). Corresponding decrease in the GSH and SOD activity was also recorded. Participants with impaired glucose tolerance show disturbances in lipid metabolism and antioxidant defenses in subjects with a high degree of glucose intolerance.

Conclusion

The study reported a disturbance in the lipid parameters and antioxidant defenses in the first-degree relatives of diabetic patients even before the establish-
ment of disease. Our finding of this study may be useful for recommending the people who had a family history of diabetes to change their lifestyle and dietary factors that could help in the preventing type 2 diabetes and prediabetes. The study supports the basis of screening of prediabetes (impaired fasting glucose/impaired glucose tolerance subjects) in participants with a family history and advocates the importance of reducing the lipid peroxidation and enhancing the levels of antioxidant enzymes.

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