Role of oxidative stress in associated complications in type 2 diabetes mellitus patients, District Faisalabad, Pakistan

Muzammal Sattar¹, Haseeb Anwar¹*, Muhammad Naeem Faisal², Imran Mukhtar¹, Ghulam Hussain, Humaira Muzaffar, Muhammad Umar Sohail³

Health Biology Lab, Department of Physiology, Government College University Faisalabad, Pakistan

Institute of Pharmacy, Physiology and Pharmacology, University of Agricultural Faisalabad, Pakistan

Biomedical Research Center Qatar, University Qattar.

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Abstract

The present study was designed to evaluate the serum total antioxidant capacity and total oxidative stress in type 2 diabetic (T2D) patients without associated complications and T2D patients with associated complications. Fifty T2D patients (with or without secondary complications) and 25 healthy volunteers were selected from district head quarter hospital, Faisalabad, Pakistan. Participants were divided into three groups; healthy control (HC), diabetic (Diab), and diabetic with secondary complications (DSC). Blood samples were collected to harvest serum. Total antioxidant capacity (TAC) and serum total oxidative stress (TOS) in the serum samples were evaluated by colorimetric method. Results were analyzed through one way ANOVA by using SPSS software. Diab and DSC groups had significantly (p=0.05) higher blood glucose, and total oxidative stress as compared to HC group. But the total anti-oxidant capacity was significantly (p=0.05) lower in Diab and DSC groups as compared to HC group .Oxidative stress was increased in T2D patients with secondary complications and diabetic patients without associated complications.

*Corresponding Author: Haseeb Anwar drhaseebanwar@gcuf.edu.pk
**Introduction**

Diabetes mellitus is a pandemic metabolic disorder characterized by an abnormal glucose metabolism / regulation and is associated with a number of secondary complications such as cardiovascular diseases, retinopathy, nephropathy, and osteoporosis and impaired bone healing (Janghorbani et al., 2007; Dominguez et al., 2004). Diabetes mellitus may be type-1 due to absolute deficiency of insulin or type-2 due to insulin resistance. Out of all diabetic patients, about 90%-95% are suffering from type-2 diabetes (Duarte et al., 2005). Type-2 diabetes is a multifactorial metabolic disorder and its exact cause is not fully understood so far. However, there are many factors which causes more likely to develop type-2 diabetes i.e. genetic factors, ethnic origin, obesity, age, life style, and others (Seeleang, 2011). Major features contributing pathogenesis of type-2 diabetes are insulin resistance and reduced secretion of insulin (Reavan, 2000). A number of evidences proved that chronic hyperglycemia in T2DM patients contribute to impairment of β-cells functions (Grodsky et al., 2000). Normally, glucagon which is released from pancreatic α-cells plays a key role in maintaining glucose homeostasis through its stimulatory effects in production of hepatic glucose. Moreover, these disturbances are accompanied with changes in a variety of biochemical processes, especially lipid peroxidation (Maharjan et al., 2008). Peroxidation of the lipid membrane is related to the pathogenesis of many degenerative diseases, such as atherosclerosis, aging, rheumatoid arthritis, carcinogenesis, heart disease and DM. Reactive oxygen species (ROS) and lipid peroxides are responsible for the pathogenesis of mentioned disorders (Smriti et al., 2016; Tangvarasittichai et al., 2015). Elevated ROS production and reduced antioxidant defense of body is referred as oxidative stress, having contributory role to develop chronic diabetes complications (Gentile et al., 2017). On other hand, antioxidants i.e. ascorbic acid, thiols, α-tocopherol, bilirubin, ceruloplasmin, albumin, uric acid (UA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) in sufficient concentration have a role in prevention of macromolecular oxidation. Antioxidants play a role to convert free radicals to inactive substances by electron-donating processes and prevent toxicity of oxidized products (Smriti et al., 2016; Verma et al., 2018; Tangvarasittichai et al., 2017). Diabetic patients are sustained with elevated oxidative stress, due to increased lipid peroxidation, production of free radicals, and decreased antioxidant defenses (Bosch et al., 2012).

The present study was designed to compare the serum total antioxidant capacity and total oxidative stress in type 2 diabetic (T2D) patients and T2D patients with associated complications.

**Material and methods**

A total of 50 diabetic (T2D) patients (age group 40 to 60 years) with or without associated complications were selected from district headquarter hospital, Faisalabad, Pakistan and 25 healthy volunteer from same region district Faisalabad. All patients and healthy subjects were male. The human subjects were carefully selected in terms of their medical history. It was made sure that diabetic group without secondary complications have authentic medical history and clinical profile to prove their condition. The healthy group was examined clinically to be healthy. The diabetic group with secondary complications was selected based on their provided medical history and current clinical profile. Before the start of study, a written informational consent was taken from each volunteer with permission from Ethical review board, Government College University, Faisalabad (Reference no. GCUF/ECR/13). Participants were divided into three groups on the basis of their previous medical history. Each group was comprised of 25 subjects. 1st group was (DSC) was composed of diabetic patients with associated complications with no any other disease, 2nd group was (diab) comprising recently diagnosed patients without any other associated complications and 3rd group as healthy control (HC) without any apparent disorder.

**Collection of Samples**

The blood sample from each subject was collected
between (11.00 am to 12.00 pm) and placed in vacuum containers without anti-coagulant for the serum collection. Serum was analyzed for serum blood sugar, Total antioxidant capacity and total oxidative stress. Commercially available Elisa kits were used to examine serum glucose level (Bioclin® Glucose Monoreagent diagnostic kit), antioxidant capacity (TAC) and serum total oxidative stress (TOS) in the serum samples were evaluated by colorimetric method (Erel, 2004; Erel, 2005).

**Statistical analysis**

All datasets were expressed as mean ± SEM and statistical significance between groups was determined by using one way ANOVA followed by post hoc test. The result was considered statistically significant at p<0.05.

**Results and discussion**

**Results**

**Body Mass Index (Kg/m²)**

The mean BMI (Kg/m² ± SEM) was comparable in diabetic (Diab) (29.98 ± 1.16) and healthy control (HC) (30.3 ± 2.1) groups. Whereas the BMI in Diabetic with secondary complications (DSC) (26.51 ± 0.96) group was slightly low as compared to Diab and HC groups.

**Table 1.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DSC</th>
<th>Diab</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Kg/m²)</td>
<td>26.51</td>
<td>29.98</td>
<td>30.3</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>170.77</td>
<td>258.79</td>
<td>120.94</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>1.08</td>
<td>1.72</td>
<td>2.42</td>
</tr>
<tr>
<td>TOS (μmol/L)</td>
<td>47.37</td>
<td>37.005</td>
<td>27.5</td>
</tr>
</tbody>
</table>

**Serum glucose concentration (mg/dl)**

The mean serum glucose concentration (mg/dl) was significantly higher (P≤0.05) in Diab group (258.79 ± 39.62)b and DSC group (170.77 ± 16.12)b groups as compared to the HC group (120.94 ± 3.86)a. The difference between diabetic and diabetic with complication groups was also significant.

**Total antioxidant capacity (TAC) (mmol/L)**

The mean TAC level (mmol/L ± SEM) was significantly lower (P≤0.05) in Diab group (1.72 ± 0.07)b and DSC (1.08 ± 0.35)a groups as compared to HC (2.42 ± 0.15)c group. The level of TAC was found to be significantly lower in Diab group as compared to DSC group.

**Fig. 1.** Mean BMI (Kg/m² ± SEM) of Diabetic with secondary complications (DSC), diabetic (Diab) and Healthy control (HC) groups.

**Fig. 2.** Mean serum glucose level (mg/dl ± SEM) in Diabetic with secondary complications (DSC), diabetic (Diab) and Healthy control (HC) groups (p≤0.05).
Total oxidative stress (TOS) (µmol/L)
The mean serum TOS (µmol/L ± SEM) was significantly (P≤0.05) higher in Diab (37.005 ± 3.1) and DSC (47.37 ± 2.15) as compared to HC (27.5 ± 2.95) groups. The level of TOS was also significantly higher in DSC groups as compared to Diab group.

Discussion
In current study the serum glucose level is significantly higher (P≤0.05) in diabetic patients (Diab) and diabetic patients with secondary complications (DSC) as compared to healthy volunteers (HC) (Fig. 2) These results were supported by a previous study by Moussa, (2008), showing significant increase in blood glucose level and significantly reduced insulin levels in diabetic patients as compared to healthy individuals in Egyptian population. This increased serum glucose level and decreased insulin level in recently diagnosed diabetic patients as compared to control healthy is hypothesized to occur due to increased oxidative stress supported by Evans et al., (2003), who described that increased generation of ROS could worsen pancreatic β cells activity resulting in decreased insulin secretion. Obesity is one of the major contributor factors in the development of T2DM. In our study, all groups (DSC, Diab and HC) exhibit over weight (on BMI basis) and were non-significantly differ from each other groups (Fig. 1) which were in accordance with Farasat et al., (2009), showing non-significant differences in BMI of healthy & diabetic patients. However, these results are discordance with the findings of Fawwad et al., (2006), who observed strong correlation between BMI & T2DM. This may be due to genetically prone diabetic patients selected for their study. In our study, the total antioxidant capacity was significantly reduced in Diab and DSC groups as compared to the HC group as shown in (Fig.3).

These finding are supported by the results of Varadhara et al., (2017) and Zargari et al., (2018) in which reduced TAC were founded in diabetic patients as compared to healthy However few studies showed contradiction to our results by declaring elevated total antioxidant capacity in diabetic patients (Verma et al., 2018). Similarly, Total oxidative stress were founded to be elevated significantly in Diab and DSC groups in comparison to HC group in our study. These are supported by the findings of Savu et al., (2012) in which elevated level of total oxidative stress were founded in diabetic patients as compared to the healthy individuals.

Conclusion
Conclusively, it was founded that total oxidative stress
was elevated and total antioxidant capacity was decreased in T2DM patient in comparison to normal healthy individuals, which ultimately leads to development of associated complications.

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