Original Article

Estimation of total antioxidant capacity in type 2 diabetic and normal healthy subjects

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Abstract

Background: Diabetes mellitus (DM) is a highly prevalent non-communicable disease in the world. Current investigations evolved that oxidative stress is also a major risk factor to cause type 2 diabetes mellitus due to impairment of antioxidant defense system in various biological fluids.

Methodology: In this cross-sectional study, 70 type 2 diabetes mellitus subjects and 30 normal healthy subjects of both genders were selected from various health care centers of Karachi, Pakistan for a study period of six months June 2017 – December 2017. The total antioxidant capacity (TAC) concentration was measured in serum by enzyme-linked immunosorbent assay (ELISA) technique using Caymans Antioxidant Assay. The biochemical parameters and anthropometric measurements were estimated by standardized methods. Data was analyzed using the statistical program Statistical Package for the Social Sciences (SPSS) version 10.0.

Results: According to the study results TAC was significantly reduced (**0.05 ± 0.00 mmol /L) in type 2 diabetes mellitus subjects compared to normal healthy subjects (0.13 ± 0.02 mmol /L). It was noted that diastolic blood pressure (DBP), body mass index (BMI), and triglycerides (TG’s) were significantly increased while high density lipoprotein-cholesterol (HDL-C) was significantly reduced in diabetic subjects than the comparative healthy individuals.

Conclusion: This study showed that decreased levels of TAC and HDL-C in type 2 DM patients with increased levels of BMI, systolic blood pressure (SBP), fasting blood sugar (FBS), DBP, and total cholesterol (TC) which may cause oxidative stress and increase the progression of cardiovascular disease (CVD) and other metabolic diseases. Modifications in dietary habits and intake of antioxidant foods or supplements may diminish the process of oxidative stress which may consequently decrease CVD and other severe clinical outcomes.

Keywords

Total Antioxidant Capacity (TAC), Type 2 Diabetes Mellitus (DM), BMI, ELISA, Cardiovascular Disease (CVD).
Introduction

DM is a major health hazard all over the world. Currently, more than 415 million world's population is affected by diabetes and it is expected that it will reach up to 642 million by the year 2030. World Health Organization (WHO) reported that DM will be a 7th most important factor causing mortality by the year 2030. The incidence of diabetes mellitus is constantly increasing in Pakistan. WHO affirm that more than 10% (12.9 million) of Pakistani population suffered from diabetes mellitus and stands at 7th position amongst the highest ten countries. It is predicted that it will reach 5th position in 2030 due to increased consumption of dietary fat/low fiber diet and physical inactivity. Oxidative stress is considered as another factor resulting in increased risk of type 2 DM by means of cytotoxicity in pancreatic beta cells, insufficient insulin production or action, and endothelial dysfunction.

Antioxidants shows a defensive role in the progression of type 2 DM by reduction of oxidative stress via glucose oxidation reaction, non-enzymatic glycation of proteins, and lipid peroxidation. TAC is a parameter to estimate the status of all antioxidants present in plasma/serum & other body fluids. TAC also provides overall information regarding the capacity of reactive oxygen species (ROS). ROS cause oxidation damage in tissue. It causes hindrance in the metabolic mechanism of macromolecules (lipids, carbohydrates, and proteins etc.) and cause non-communicable diseases such as type 2 DM, CVD, obesity, hypertension, neurodegenerative diseases and cancer. In human beings, a highly complex antioxidant system developed in various biological fluids, which depends upon the enzymatic and non-enzymatic antioxidants including glutathione peroxidase, superoxide dismutase, glutathione and uric acid that perform different functions interchangeably and sometimes symbiotically to neutralize the effect of free radicals and protect body from free radical toxicity. Under normal circumstances, a critical balance is maintained between oxygen free radicals and antioxidant defence systems. Impairment in the equilibrium of oxidant and antioxidant gives rise to oxidative stress, resulting in type 2 DM and CVD. Intake of antioxidant supplements such as ascorbic acid, tocopherols, and carotenoids reduce the effect of ROS and prevent type 2 diabetes mellitus and its complications like nephropathy, retinopathy, and CVD. Various studies suggested that dietary supplements of antioxidants along with usual food consumption decrease the morbidity and mortality rate.

The status of TAC in type 2 DM subjects is unknown in Pakistani population. Therefore, the aim of the study was to estimate TAC in type 2 DM patients and normal healthy subjects. It may open the doors towards new trends and most effective dietary modifications to attain a healthy lifestyle.

Methodology

A total of 100 subjects of both genders between the age group of 35 – 75 years were recruited for the study. Of which 70 subjects were type 2 DM patients while 30 subjects were normal healthy individuals. Type 2 DM and normal healthy subjects were selected by setting the screening camps at various public places and health care centers. The patients with chronic diseases, pregnant women, lactating mothers, and subjects taking vitamin and mineral supplements were excluded from the study sample. The patients were selected after obtaining written consent forms. The questionnaire was designed to gather the information regarding age, sex, occupation, place of residence, marital status, education, socioeconomic status and family history related to diabetes, hypertension, cardiovascular disease, renal disease and the time when they were diagnosed as diabetics.
The anthropometric measurements such as weight and height were measured without shoes and socks with the help of measuring scale. BMI was calculated through the standard method. Furthermore, three reading of SBP and DBP were taken in a sitting position with the 5-minute interval from the right arm extended at shoulder level by using mercury sphygmomanometer. The venous blood samples were collected aseptically after 12-14 hours overnight fast. Blood samples were collected in two separate labeled collecting tubes i.e. sodium fluoride-potassium oxalate tube for glucose estimation and gel tube for lipid and TAC. The blood samples were stored in an icebox and immediately to the laboratory for further processing. The serum and plasma were collected by centrifugation of blood for 10-15 minutes at 2500 rpm. The serum was separated in the sterile plastic containers and stored at a temperature of (-80°C).

Serum was thawed and allowed to attain room temperature before analysis. FBS was estimated by glucose oxidase - /4 aminophenazone (GOD-PAP) method(GL 364, Randox reagents, UK) method, TC, HDL-C, LDL-C were determined by cholesterol oxidase/peroxidase aminophenazone CHOD PAP methods while TG's was measured by glycerophosphate - Oxidase/4 aminophenazone GPO-PAP method using Microlab 300. TC was determined by the enzymatic hydrolysis and oxidation by endpoint method (Cat. No. CH 259, Randox reagents, UK). HDL-C (Cat. No. CH 201) and LDL-C (Cat. No. CH 1351) were estimated after precipitation by centrifugation. TG was determined after enzymatic hydrolysis with lipases (Cat. No. TR 210, Randox reagent, UK). TAC was measured in serum by Caymans Antioxidant Assay ELISA technique. Combined aqueous and lipid-soluble antioxidants were assessed which include superoxide dismutase, catalase, and glutathione peroxidase; macromolecule such as albumin, ceruloplasmin, and ferritin. Presence of antioxidant in the sample inhibit the oxidation of ABTS (2, 2'- Azino-di- [3- ethylbenzthiazoline sulphonate]) to ABTS + by metmyoglobin. The amount of ABTS, produced was measured at 750 nm or 405nm which proportionally gave their concentration. Trolox, a water-soluble tocopherol analog was used as standard and was quantified as millimolar Trolox equivalents.

Data analyzed by the statistical program SPSS (version 10.0). The values were presented as the mean ± standard deviation. Continuous variables were measured by Student’s t-test and categorical variables were analyzed by the Chi- square test. P-value ≤ 0.05 was considered statistically significant.

**Results**

According to the study results, 31 (44.3%) were type 2 DM males with the mean age of 47.7 ± 9.0 years and 39 (55.7%) were DM females with the mean age of 51.8 ± 10.5 years. Whereas, in normal healthy subjects, 14 were males with the mean age of 50.8±16.2 years and 16 (53.3%) were females with the mean age of 47.18 ± 14.4 years as shown in table 1. Significant differences in BMI and SBP and DBP, FBS, TG, HDL-C, LDL-C, and TAC was observed among type 2 DM subjects and normal healthy subjects of both genders. BMI of type 2 DM subjects was significantly higher in both men and women (31.3 ± 7.5 Kg/m² & 30.6 ± 4.5 Kg/m²) than normal healthy subjects (22.2 ± 1.3 Kg/m² & 21.4 ± 1.1 Kg/m²). The blood pressure in type 2 DM male patients, increased mean SBP/DBP i.e. 134.5. ± 17.2/*100.3±18.7 mmHg as compared to normal healthy subjects 117.4 ± 19.9/77.3±9.5 mmHg. Similarly in female high levels of mean SBP/DBP i.e. 130.5 ± 18.7/*110.6 ± 19.5 mmHg are revealed in type 2 diabetic subjects than normal healthy subjects 118.1 ± 14.4 /78.9 ± 2.7mmHg.
Table 1: Comparison of anthropometric measurements in normal healthy and type 2 diabetic subjects based on gender

| Parameters | Normal Healthy Subjects (N=30) | Type 2 Diabetic Subjects (N=70) | P Value |
|------------|--------------------------------|-------------------------------|---------|
|            | Male Mean ± SD | Female Mean ± SD | Male Mean ± SD | Female Mean ± SD |         |
| Age (Years) | 50.8 ± 16.2 | 47.1 ± 14.4 | 47.7 ± 9.0 | 51.8 ± 10.5 | ≤ 0.05 |
| BMI (Kg/m²) | 22.2 ± 1.3 | 21.4 ± 1.1 | 31.3 ± 7.5 | 30.6 ± 4.5 | ≥ 0.05 |
| SBP (mmHg)  | 117.4 ± 19.9 | 118.1 ± 14.4 | 134.5 ± 17.2 | 130.5 ± 18.7 | ≥ 0.05 |
| DBP (mmHg)  | 77.3 ± 9.5 | 78.9 ± 2.7 | 100.3 ± 18.7 | 110.6 ± 19.5 | ≥ 0.05 |

Biochemical Parameters and TAC results are shown in table 2. FBS is significantly increased in type 2 DM subjects both male & female (190.7 ± 86.9 & 191.2 ± 78.6) in comparison with normal healthy subjects both males and females (90.0 ± 0.2 & 86.9 ± 10.8).

TC is considerably high in type 2 diabetic subjects both male and female (176.9 ± 53.7 & 183.6 ± 42.0) than normal healthy subjects both male and female (146.3 ± 17.5 & 157.6 ± 25.4). Similarly, TG’s levels are significantly increased in both male and female type 2 diabetic subjects (218.7 ± 63.8 & 180.5±57.4) as compared to normal healthy subjects male and female (120.2 ± 16.6 & 113.1 ± 29.7). Whereas, HDL-C showed significant decreased values 34.7 ± 8.1 & 40.7±16.1 in male and female type 2 diabetic subjects and normal healthy subjects showed normal values in both male and female i.e., 79.7 ± 41.2 & 76.3 ± 48.5. However, LDL-C in type 2 diabetic subject male and female rise to a greater extent (78.9 ± 12.8 & 85.9 ± 14.5). TAC reduced in both male and female type 2 diabetic subjects (0.05 ± 0.00 & 0.05 ± 0.00) as compared to normal healthy subjects (0.13 ± 0.02 & 0.13 ± 0.02).

Table 2: Comparison of biochemical parameters and total antioxidant capacity (TAC) based on gender

| Parameters | Normal Healthy Subjects (N=30) | Type 2 Diabetic Subjects (N=70) | P Value |
|------------|--------------------------------|-------------------------------|---------|
|            | Male Mean ± SD | Female Mean ± SD | Male Mean ± SD | Female Mean ± SD |         |
| FBS (mg/dL) | 90.0 ± 0.2 | 86.9 ± 10.8 | 190.7 ± 86.9* | 191.2 ± 78.6* | ≤ 0.05 |
| TC (mg/dL)  | 146.3 ± 17.5 | 157.6 ± 25.4 | 176.9 ± 53.7 | 183.6 ± 42.0 | ≥ 0.05 |
| TG (mg/dL)  | 120.2 ± 16.6 | 113.1 ± 29.7 | 218.7 ± 63.8* | 180.5±57.4* | ≤ 0.05 |
| HDL-C (mg/dL) | 79.7 ± 41.2 | 76.3 ± 48.5 | 34.7 ± 8.1* | 40.7±16.1* | ≤ 0.05 |
| LDL-C (mg/dL) | 78.9 ± 12.8 | 85.9 ± 14.5 | 93.1±28.3 | 111.7 ± 29.1 | ≥ 0.05 |
| TAC (mmol/L) | 0.13 ± 0.02 | 0.13 ± 0.02 | 0.05 ± 0.00** | 0.05 ± 0.00** | ≥ 0.05 |

Comparisons of biochemical parameters and TAC with duration of type 2 diabetes mellitus are shown in table 3. Results showed that FBS showed variation between <05 years to >15 years of type 2 diabetes mellitus. Whereas, TC, TG, and LDL-C were constantly increased and HDL-C was gradually decreased in <5 years to >15 years of type 2 diabetes mellitus. TAC levels decreased and there was no difference among <05 years to >15 years duration of type 2 DM. The levels of all biochemical parameters and TAC were increased and decreased irrespective of the duration of the type 2 DM. It is presumed that when the duration of diabetes increases the possibilities of cardiovascular diseases increased.
Table 3: Comparison of biochemical parameters and total antioxidant capacity in type 2-DM subjects based on the duration of diabetes

| Parameters   | < 5 years Mean ± SD | 6 - 10 years Mean ± SD | II - I5 years Mean ± SD | >15 years Mean ± SD | P Value |
|--------------|---------------------|------------------------|-------------------------|---------------------|---------|
| FBS (mg/dL) | 194.7 ± 81.8        | 185.5 ± 74.8           | 192.0 ± 97.2            | 193.0 ± 55.0        | ≥ 0.05  |
| TC (mg/dL)  | 173.0 ± 39.6        | 185.8 ± 44.1           | 179.2 ± 70.4            | 185.4 ± 44.7        | ≥ 0.05  |
| TG (mg/dL)  | 189.6 ± 56.4        | 186.8 ± 42.5           | 200.0 ± 71.5            | 213.4 ± 75.2        | ≥ 0.05  |
| HDL-C (mg/dL) | 44.0 ± 24.5        | 38.2 ± 10.1            | 38.4 ± 4.9              | 32.0 ± 7.0          | ≥ 0.05  |
| LDL-C (mg/dL) | 97.9 ± 27.5        | 99.4 ± 36.6            | 104.3 ± 25.4            | 112.2 ± 33.3        | ≥ 0.05  |
| TAC (mmol/L) | 0.05 ± 0.00        | 0.05 ± 0.00            | 0.05 ± 0.00             | 0.05 ± 0.00         | ≥ 0.05  |

Discussion

DM is a chronic non-communicable disease which is caused by persistent hyperglycemia. The major cause of type 2 DM is insulin resistance and deficient production or complete absence of insulin. TAC is a biological marker to provide information related to all antioxidants present in various biological fluids\(^{17,18}\). The current study results showed that BMI was significantly increased in type 2 DM patients among both males and females as compared to the normal healthy subjects (Table 1). A similar study was conducted in U.S. elder population and they found BMI is high in type 2 diabetes mellitus subjects along with its complications than in non-diabetic group\(^{19}\). Correspondingly, in a Korean study, type 2 diabetic patients showed raised values of BMI in association with non-diabetic subjects\(^{20}\). When we compare our results with the above-stated findings it is confirmed that BMI plays a vital role to recognize the obesity both in diabetic / healthy subjects and also a major risk factor to cause type 2 DM and its severe clinical outcomes.

According to our study results, SBP and DBP were considerably elevated in type 2 diabetic subjects of both genders (Table 1). Parallel results were found in another study, which showed that the prevalence of hypertension was significantly increased in type 2 diabetes patients in Southern Ethiopia\(^{21}\). A similar result was recorded in India i.e., the prevalence rate of hypertension is increased in type 2 diabetic subjects along with micro and macrovascular complications\(^{22}\). It is clear from the study results that hypertension is an independent risk factor to cause atherosclerosis and cardiovascular diseases in type 2 diabetes patients.

FBS, TC, TG’s, and LDL-C were significantly higher in both male and female type 2 diabetic subjects than normal healthy male and female subjects (Table 2). However, HDL-C showed lower values in male and female type 2 diabetic subjects as compared to normal healthy subjects. It is perceived that by increasing values of FBS, TC, TG, & LDL-C and decreasing levels of HDL-C the risk of CVD will be raised in our population (Table 2). Analogous findings were noted in India, the mean total cholesterol, triacylglycerol’s, LDL-C and the fasting blood sugar levels were greatly increased along with low levels of HDL-C in diabetic subjects as compared to those in the controls subjects\(^{23}\). Similar results were reported in a study from Nepal\(^{24}\). In summary, the study assumed that type 2 diabetes mellitus is related with a group of interconnected abnormal values of BMI,
SBP, DBP, and lipid profiles that are a well-documented risk factor of cardiovascular diseases and other metabolic disorders.

The present study showed that TAC significantly decreases among type 2 diabetic subjects as compared to normal healthy subjects (Table 2 & 3). Our result also indicated that lower levels of TAC do not affect the duration of diabetes. Subsequent result was found in India, in this study total antioxidant status significantly low in type 2 diabetic subjects than healthy controls. The corresponding result was noted in Iran i.e., serum TAC in type 2 diabetic patients has substantially declined as compared to the comparative group. Similarly, TAC level was reduced in type 2 DM subjects of Bangladeshi population than non-diabetic mellitus subjects which was also supported by a study on Nigerian population. Whereas, In Palestinian population total antioxidant status significantly high in diabetic patients than control subjects due to modifications in dietary habits. Another study was conducted to polish adult population to determine the association between the metabolic risk factors and dietary antioxidants. They found that a high intake of dietary antioxidants reduced the prevalence of metabolic risk factors such as obesity, CVD, hypertension and oxidative stress.

**Conclusion**

The overall summary of this study is suggested that decrease levels of TAC increase lipid peroxidation which may cause oxidative stress in type 2 DM. Furthermore, high levels of BMI, SBP, DBP, FBS, TC, TG, LDL- C and low levels of HDL- C may also enhance the progression of oxidative stress in type 2 DM subjects which promotes the development of obesity, CVD, hypertension, and other metabolic diseases. In type 2 diabetic subjects, Oxidative stress is prevented by dietary modifications and healthy lifestyle by means of scavengers of ROS. Dietary antioxidants along with early interventions improve the durability and quality of life of type 2 diabetic subjects. Further advance studies along with specific interventions and dietary modifications are required in the progression and prevention of type 2 DM.

**Conflicts of Interest**

None.

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Humera Jabeen was involved in experimental work and writing manuscript. Sumreen Begum was contributing her efforts in data analysis, graphical presentation, and writing manuscript. Mehwish Zeeshan provides support in data collection; writing manuscript and review manuscript systematically according to the publication requirements. Muhammad Imran performed the data collection and experiments. Nazia Ahmed was helping with data analysis. Ms. Tajallee Saleem was assisting in writing the manuscript. Rashida Qasim conceived and designed the experimental work.

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