Journal of Molecular Pathophysiology
www.scopemed.org
DOI: 10.5455/jmp.20160307103940

Original Research

Total antioxidant status in newly-diagnosed type II diabetes patients in Bangladeshi population

Muhammad Abdul Hai Siddique[1,2], Zinat Tamannaa[3], Syed Muhammad Kamaluddin[3], Muhammad Saiedullah[3], Muhammad Aminul Haque Khan[4], Matiar Rahman[2], Liaquat Ali[1]

[1]Biochemistry & Cell Biology, Bangladesh University of Health Sciences, Bangladesh.
[2]Biochemistry & Molecular Biology, University of Rajshahi, Bangladesh
[3]Applied Laboratory Sciences, Bangladesh University of Health Sciences, Bangladesh.
[4]Biochemistry, Enam Medical College, Bangladesh.

Address for correspondence:
Mohammad Abdul Hai Siddique, Biochemistry & Cell Biology, Bangladesh University of Health Sciences,
Biochemistry & Molecular Biology, University of Rajshahi, Bangladesh.
ahaisiddique06@gmail.com

Received: August 4, 2015
Accepted: March 7, 2016
Published: March 22, 2016

ABSTRACT

Background: Prevalence and complications of type 2 diabetes mellitus (DM) is increasing in Bangladeshi population. Oxidative stress plays an important role in the pathogenesis of DM and its complications. However, antioxidant status and its contribution to type 2 DM are less explored in Bangladeshi population. **Aim:** The aim of this study was to evaluate antioxidant status (TAS) in newly diagnosed never treated type 2 diabetic subjects against apparently healthy nondiabetic subjects of Bangladeshi origin. **Methods:** In this cross-sectional study, 179 adult subjects were included. Fasting and postprandial blood specimens were collected and plasma glucose concentrations were measured by standard methods. Fasting plasma total antioxidant capacity (TAC) was measured by ferric reducing ability of plasma (FRAP) assay and compared among diabetic, prediabetic and nondiabetic. **Results:** TAC was 1077±217 μmol/L, 1225 ±285 μmol/L and 1425±319 μmol/L in diabetic (n=79), prediabetic (n=42) and nondiabetic (n=58) subjects respectively. TAS in diabetic subjects was 148 μmol/L lower than that of prediabetic subjects ($p<0.05$) and 348 μmol/L lower than that of nondiabetic subjects ($p<0.001$) whereas it was 200 μmol/L lower in prediabetic subjects compared to nondiabetic subjects ($P<0.01$). Conclusion: It may be concluded that total antioxidant status is lower in type 2 diabetic subjects compared to nondiabetic subjects and it may be related to the pathogenesis of T2DM in the studied population.

KEY WORDS: Type 2 diabetes; Antioxidant status; FRAP; Oxidative stress; Total antioxidant Capacity.

INTRODUCTION

The incidence of type 2 diabetes mellitus (DM) is becoming as a serious public health concern worldwide. DM, particularly type 2 diabetes is now recognized as a major chronic public health problem in Bangladesh. Globally, the prevalence of diabetes is ~ 8%, and nearly 80% of patients with diabetes live in low- and middle-income countries [1]. A recent meta-analysis in Bangladeshi population showed that the prevalence of diabetes among adults had increased substantially, from 4% in 1995 to 2000 and 5% in 2001 to 2005 to 9% in 2006 to 2010 [2]. According to the International Diabetes Federation, the prevalence will be 13% by 2030 [3].

The generation of reactive metabolites plays a central role in cell's life. These metabolites are continuously controlled by endogenous antioxidant enzyme systems and the balance is created between pro-oxidants and antioxidants. The impairment of antioxidant status, either by exogenous or endogenous sources, may disturb the cellular redox balance and the pathological conditions would be the main characteristics and forms oxidative stress in cells or tissues [4]. Oxidative stress is implicated in the pathophysiology of DM and its chronic complications [5].

Oxidative stress may contribute to the pathogenesis of diabetes mellitus through impairment of insulin action, injury to pancreatic β-cells, increased lipid peroxidation, and vascular endothelial damage [6]. Increasing experimental and clinical evidences suggest that oxidative stress plays a major role in the pathogenesis and development of complications of both types of DM [7,8,9]. In our population, the role of oxidative stress in the pathogenesis of type 2 DM is less explored.

The main aim of the work was to explore the antioxidant status in newly diagnosed never treated type 2 diabetic subjects, prediabetic subjects and nondiabetic subjects. In the present study, we analyzed the fasting plasma total antioxidant capacity.

SUBJECTS AND METHODS

Study designs and population

This cross-sectional study was conducted in the Applied Laboratory Sciences, Bangladesh University of Health Sciences (BUHS), Dhaka, Bangladesh during the period of March 2014 to September 2014. Of the 205 subjects approached from the out-patient department (OPD) of

Bangladesh Institute of Health Sciences (BIHS) general hospital, 179 subjects were included according to inclusion-exclusion criteria. After written consent, past and present history about medication, diabetes, hypertension, smoking and other points of selection criteria were recorded. Subjects receiving any antihyperglycemic agents, with serious comorbid diseases (infection, stroke, myocardial infarction, major surgery, malabsorption etc.), pregnant or lactating mother, with liver or kidney dysfunction, using drugs that significantly affect glucose metabolism (anti-hyperglycemic agents, glucocorticoids, oral contraceptives, thiazide diuretics etc.) or taking vitamin supplements were excluded.

Anthropometric measurements

Anthropometric indices included height and weight. All the individuals were measured wearing light clothing without shoes and hats. Height was measured to the nearest 0.1 cm using a portable stadiometer and weight was measured to the nearest 0.1 kg using calibrated platform scales. Body mass index (BMI) of the subjects was calculated using standard formula, BMI = Weight (Kg)/[Height (m)]^2 and BMI value of 27.5 Kg/m^2 was considered as cutoff value [10].

Waist and hip circumferences were measured to the nearest 0.5 cm with a soft non-elastic measuring tape. The tape was snug, but not so tight as to cause skin indentation or pinching. The waist circumference was taken to the nearest standing horizontal circumference between the lower border of the 12th rib and the highest point of the iliac crest on the mid-axillary line at the end of normal expiration. Waist-hip ratio was calculated. Mid-upper arm circumference (MUAC) was measured according to standard procedure [11].

Blood pressure measurement

Blood pressure was measured to the nearest 1 mm Hg with mercury sphygmomanometers using standard recommended procedures [12]. Two readings each of systolic and diastolic blood pressures were recorded, and taken at 5 minutes intervals. The average of two readings was used in the data analysis. If two of the measurements differed by more than 5 mm Hg, an additional reading was taken.

Diagnosis of diabetes mellitus

Prediabetes and diabetes were defined according to WHO criteria [13].

Clinical measurements

Fasting blood was collected between 8.00-9.00 am. Venous blood (5 ml) was taken by venipuncture from the subject sitting comfortably in a chair in a quiet room. A portion of blood specimen was poured into a test tube containing sodium fluoride to measure plasma glucose concentrations. Another portion of blood specimen was poured into another test tube containing ethylenediaminetetraacetic acid (EDTA) to measure plasma antioxidant capacity. After 15 minutes blood samples were centrifuged for 10 minutes at 3000 rpm to obtain plasma. The EDTA treated plasma was preserved in capped microtubes at -20ºC until (~5 months) analysis and reported to be stable for 1 year at -20°C [14]. Two hours after breakfast, 2 mL blood was collected for the measurement of post-prandial plasma glucose concentration. Plasma glucose concentrations were measured by hexokinase method using Dimension RxL Max (Siemens, USA). Plasma antioxidant capacity was determined by a modified ferric reducing ability of plasma (FRAP) assay [15].

Statistical Methodology

Statistical analysis was performed using STATISTICA version 8/MedCalc® version 11.4/GraphPad Prism 6.01 for Windows. All data were expressed as Mean±SD (standard deviation) and/or percentage (%) as appropriate. The statistical significance of differences between the values was assessed by one-way ANOVA with Tukey's multiple comparison or Mann-Whitney U test (as appropriate). Correlation was also seen among the parameters. Univariate or multivariate linear regression analysis was applied to assess the association of TAC as dependent variable with age, sex, BMI, WHR, MUAC, SBP, DBP, FPG, PPG as independent variables. A two-tailed p value of <0.05 was considered statistically significant. Graph Pad Software, San Diego California USA)/MedCalc® version 11.4 for Windows OS was used.

RESULTS

Clinical Characteristics

From 205 subjects approached, 179 subjects were included and 26 subjects were excluded according to inclusion-exclusion criteria. Of them 58 subjects were nondiabetic, 42 subjects were never treated newly diagnosed prediabetic and 79 subjects were newly diagnosed never treated diabetic according to WHO criteria. Of the total subjects 120 (67%) were male and 59 (33%) were female. The mean age of the study subjects was 44 ± 9.6 years. In the total subjects, the mean BMI was 26.4 ± 3.8 Kg/m^2, Waist-Hip ratio was 0.98 ± 0.06 and mid upper arm circumference (MUAC) was 28.2 ± 2.8 cm. One hundred twelve (63%) of the study subjects had BMI up to 27.5 Kg/m^2 and 75 (37%) subjects had BMI>27.5 Kg/m^2. Characteristics of the nondiabetic, prediabetic and newly diagnostic never treated type 2 diabetic subjects are presented in Table 1.

Five percent (5%) of the study subjects had hypertension and used anti-hypertensive medication for the management of hypertension. Among the participants, 48 men (66.66%) of mae and 27% of the total subjects) had a habit of smoking and were not excluded from this study since no significant differences were observed between smoker and nonsmoker in DM, prediabetic and nondiabetic groups (data not shown). Tukey's multiple comparison test of FPG and PPG among different groups is presented in Fig 1.

Table 1. Characteristics of the study subjects (*n* = 179)

	Diabetic (n=79)	Prediabetic (n=42)	Nondiabetic (n=58)
Age (yrs)	44±9.2	44±9.1	45±10.6
Sex			
Male	49(62%)	26(62%)	45(78%)
Female	30(38%)	16(38%)	13(22%)
Body mass index (Kg/m^2)	26.1±3.4	26.8±3.3	26.5±4.3
Waist-Hip ratio	1.0±0.05	0.97±0.05	0.96±0.05
Mid upper arm circumference (cm)	27.9±2.6	28.3±2.3	28.7±3.3
Systolic Blood Pressure (mmHg)	124±15	121±9	121±14
Diastolic Blood Pressure (mmHg)	83±13	81±8	80±12
Fasting plasma glucose (mmol/L)*	11.1±3.8	5.9±0.9	5.2±0.7
Postprandial plasma glucose (mmol/L)*	19.0±4.5	8.9±1.3	5.5±0.9

*One way ANOVA

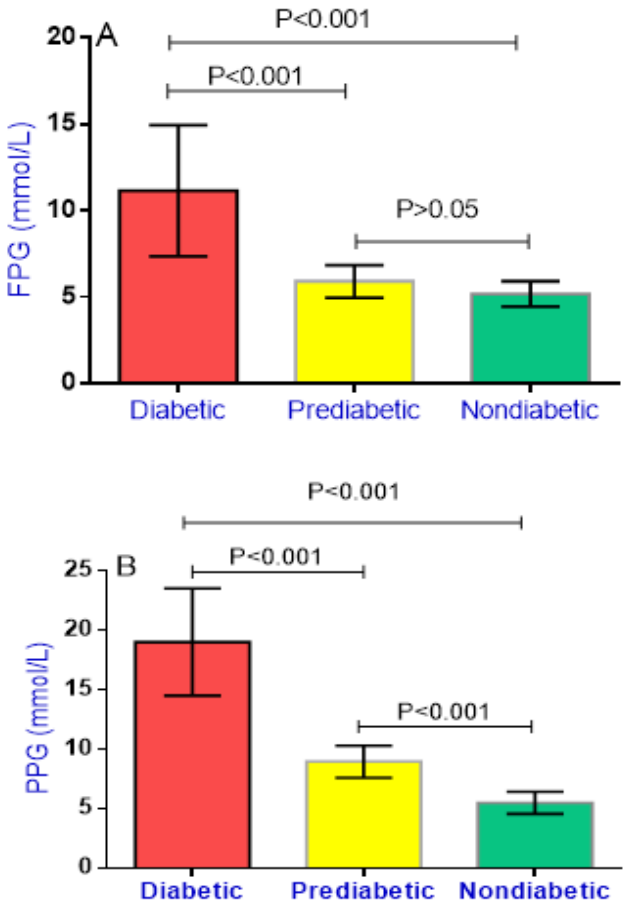

Fig 1. Comparison of fasting plasma glucose (A) and postprandial plasma glucose (B) among groups (Tukey's test)

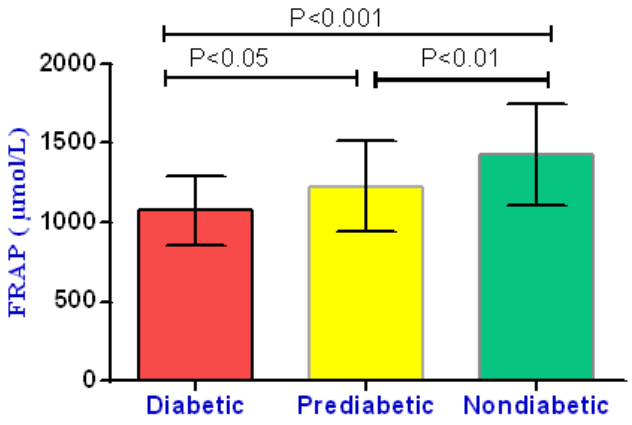

Fig 2. Comparison of TAC among diabetic, prediabetic and nondiabetic subjects.

Total anti-oxidant capacity of the study subjects

The mean value of TAC in the total study subjects was 1224±308 µmol/L. The mean values of TAC in diabetic, prediabetic and nondiabetic subjects were 1077±217 µmol/L, 1225±285 µmol/L and 1425±319 µmol/L respectively. One way ANOVA result with Tukey's multiple comparison test is presented in Fig 2. One way ANOVA showed that TAC was significantly different among diabetic, prediabetic and nondiabetic subjects. Tukey's multiple comparison showed that TAC value in diabetic subjects was 148 µmol/L lower than that of prediabetic subjects ($p<0.05$) and 348 µmol/L lower than that of nondiabetic subjects ($p<0.001$) whereas it was 200 µmol/L lower in prediabetic subjects compared to nondiabetic subjects ($P<0.01$).

TAC in male and female

In the total subjects (n=179) comparison of TAC between male and female showed that TAC level were significantly higher in male (n=120) compared to female (n=59) (1274±304 µmol/L vs 1123±293 µmol/L). The unpaired t test (Welch test) static t value was -3.214 (p=0.002).

Relationship of TAC with different parameters

The relationship of TAC with anthropometric parameters, FPG and PPG is presented in Table 3.4. Age, BMI, WHR, MUAC and BP showed no significant relationship with TAC. Both FPG ($r=0.410$, $p<0.001$) and PPG ($r=0.451$, $p<0.001$) showed significant relationship with TAC (Table 2).

Table 2. Relationship of TAC with different parameters in total subjects

	Correlation coefficient (*r*)	*P* value
Age	0.027	0.719
BMI	0.062	0.409
WHR	-0.052	0.489
MUAC	0.105	0.163
SBP	-0.059	0.434
DBP	-0.046	0.540
FPG	-0.410	**<0.001**
PPG	-0.451	**<0.001**

Regression analysis

In multivariate linear regression analysis TAC showed no significant association with FPG (β=-0.009, p=0.954) but PPG showed significant inverse association (β=-0.443, p=0.006). When adjusted for sex, β remained significant for PPG (Table 3).

Table 3. Multivariate linear regression analysis with TAC as outcome variable

Parameters	β value	p value
FPG	-0.078	0.619
PPG	-0.359	0.024
Sex (Male)	0.186	0.006

DISCUSSION

Global health burden is increasing due to upward trend in non-communicable diseases (NCDs), and diabetes mellitus (DM) is considered as an important component of NCDs [16,17,18]. The prevalence of diabetes is rapidly increasing due to lifestyle, eating habit, population growth, aging, obesity and physical inactivity [16] and the rising rate is higher in developing countries. Nearly 80% of patients with diabetes live in low- and middle-income countries [1].

Role of some biochemical factors on insulin secretory defects, insulin sensitivity or insulin resistance in Bangladeshi prediabetic and diabetic subjects were investigated previously in this population [10,19,20,21] other biochemical factors such as endogenous antioxidant enzymes or environmental determinants of B-cell dysfunction and insulin resistance in prediabetes and diabetes still requires more studies as they should be taken as a major target for primary prevention of these disorders. Since, oxidative stress may vary with nutritional factors, lifestyle and race, its contribution in the pathogenesis of type 2 DM or in the complications of DM is required to be studied in our population. So, this study was aimed to evaluate the antioxidant status in newly diagnosed never treated type 2 diabetic subjects, prediabetic subjects and nondiabetic subjects.

In this cross-sectional study, total antioxidant capacity (TAC) in nondiabetic subjects was 1425±319 μmol/L and it is comparable to TAC in other population. It was 1580±280 μmol/L in Nigerian healthy control [22]. Furthermore, a gradual and significant decrease in total antioxidant status was observed from nondiabetic through prediabetic and diabetic subjects in this study and indicated that the gradual progression of hyperglycemia may be linked to loss of redox balance as supported by univariate or multiple regression analysis. This finding is consistent with other studies that compare TAS between diabetic and nondiabetic subjects[22,23,24]. However, no study is available to compare the TAS/TAC in prediabetic subjects in this as well as in other population. So, it may be concluded that TAS is lower in type 2 diabetic subjects compared to nondiabetic subjects and progression of T2DM may be associated with gradual loss of total antioxidant capacity in prediabetic and diabetic subjects in our population.

Strengths and weaknesses of the study: total antioxidant status in type 2 diabetic subjects was compared against nondiabetic and prediabetic subjects and confounding effects of obesity and gender were considered to find out the relation of TAS with DM but it does no necessarily reflects the causal relationship between TAS and development of DM since it was a cross-sectional study with small sample size and FRAP was used to measure TAS that accounts only nonenzymatic antioxidant capacity but unable to detect enzymatic antioxidant capacity as well as thiol groups. Future study should include other markers of redox status in prospective design to understand the possible contribution and importance of redox markers in the progression of DM as well as prevention of its complications.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas. 5th ed. Brussels, Belgium, International Diabetes Federation. 2012.
2. Saquib N, Saquib J, Ahmed T, Khanam MA, Cullen MR. Cardiovascular diseases and type 2 diabetes in Bangladesh: a systematic review and meta-analysis of studies between 1995 and 2010. BMC Public Health. 2012; 12: 434.
3. International Diabetes Federation. Country estimates table 2011. IDF diabetes atlas. 6th Ed. 2012.
4. Noori S. An Overview of Oxidative Stress and Antioxidant Defensive System. 2012; 1:413.
5. Van Campenhout A, Van Campenhout C, Lagrou AR. Impact of diabetes mellitus on the relationships between iron-, inflammatory and oxidative stress status. Diabetes Metab Res Rev. 2006; 22: 444 – 454.
6. Kim J, Wei Y, James RS. Role of mitochondrial dysfunction in insulin resistance. Circulation Research. 2008; 102: 401-414.
7. Saltiel AR. New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. Cell 2001;104:517–29.
8. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes 2003;52:1-8.
9. Kaneto H, Katakami N, Kawamori D, Miyatsuka T, Sakamoto K, Matsuoka TA, Matsuhisa M, Yamasaki Y. Involvement of oxidative stress in the pathogenesis of diabetes. Antioxid Redox Signal 2007;9:355-66.

10. Kamaluddin SM, Saiedullah M, Biswas S, Hayat S, Akhteruzzaman S, Ali L. Circulating resistin concentration associates strongly with postprandial plasma glucose in newly diagnosed Bangladeshi type 2 diabetic patients. J Mol pathophysiol. 2015; 4 (2): 59-64.
11. http://www.unicef.org/nutrition/training/3.1.3/1.html (2/27/2016)
12. Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, Morgenstern BZ. Human blood pressure determination by sphygmomanometer. Circulation. 1993; 88:2460-2470.
13. International Diabetes Federation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva: World Health Organization. 2006.
14. Jansen EHJM, Beekhof PK, Cremers JWJM, Viezeliene D, Muzakova V, Skalicky J. Long-term stability of parameters of antioxidant status in human serum. Free Radical Research 2013;47:535-40.
15. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 1996; 239, 70-76.
16. King H, Aubert RE, Herman WH. Global Burden of Diabetes, prevalence, numerical estimates & projections. Diabetes Care. 1998; 21: 1414-31.
17. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27: 1047-53.
18. Boyle JP, Thompson TJ, Gregg EW, Barker LE and Williamson DF. Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality and pre-diabetes prevalence. Population Health Metrics. 2010; 8:29.
19. Hayat S. Incretin effect in prediabetic subjects. MPhill thesis, Department of Biochemistry & Molecular Biology, University of Dhaka. 2013.
20. Hossain M, Faruque MO, Kabir G, Khan I, Sikdar D, Ali L. Association of serum tumor necrosis factor-alpha and interleukin-6 with insulin secretion and insulin resistance in subjects with type-2 diabetes in a Bangladeshi population. S Afr J Diabetes Vasc Dis. 2012; 9:21-4.
21. Kabir G, Hossain M, Faruque MO, Hassan N, Hassan Z, Nahar Q, Shefin SM, Alauddin M, Ali L. International Journal of Diabetes Mellitus. 2010; 2: 144-7.
22. Odum EP, Ejilemele AA, Wakwe VC. Antioxidant status of type 2 diabetic patients in Port Harcourt, Nigeria. Nigerian Journal of Clinical Practice. 2012; 15.
23. Maharjan BR, Jha JC, Adhikari D, Akila RS, Alurkar VM. Oxidative stress, antioxidant status and lipid profile in ischemic heart disease patients from western region of Nepal. Nepal Med. Coll. J. 2008; 10: 20–24.
24. Faure P, Wiernsperger N, Polge C, Favier A, Halimi S. Impairment of the antioxidant properties of serum albumin in patients with diabetes: protective effects of metformin. Clin Sci. 2008; 114: 251-6.

Source of Support: Nil, Conflict of Interest: None declared