International Journal of General Medicine
and Pharmacy (IJGMP)
ISSN(P): 2319-3999; ISSN(E): 2319-4006
Vol. 5, Issue 3, Apr - May 2016; 77- 82

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

A COMPREHENSIVE STUDY OF OXIDANTS AND ANTI-OXIDANTS IN TYPE 2 DIABETICS

SAHOO HARAMOHAN[1], SHRI RAM[2], TANDON RAVI[3], MISHRA SP[4] & KHANNA HD[5]

[1,2]Junior Resident, Department of General Medicine, IMS, BHU, Varanasi, (U.P.), Indian

[3]Former Head, Department of General Medicine, IMS, BHU, Varanasi, (U.P.), Indian

[4]Head of Department, Department of Biochemistry, IMS, BHU, Varanasi, (U.P.), Indian

[5]Professor Emeritus, Department of Biophysics, IMS, BHU, Varanasi, (U.P.), Indian

ABSTRACT

Diabetes mellitus is a non-communicable disease characterized by increased blood sugar. It contributes to significant morbidity and mortality in the human race affecting every organ of the human body. Epidemiological data suggest that the number of people affected by the disease is increasing each year globally. Hence the disease is being researched intensively to find out the grass root level pathophysiology. As a result, it has been proved again and again that in this disease there is an apparent increase in oxidants and decrease in anti-oxidants resulting in oxidative stress that ultimately leads to injury to various organs. We conducted the present study to look into the level of oxidative stress in the treatment naïve diabetic North Indians.

KEYWORDS: Diabetes Mellitus, Oxidants, Anti-Oxidant

INTRODUCTION

Diabetes mellitus is a non-communicable disease of concern worldwide. According to data published by International Diabetes Federation (IDF) Diabetes Atlas 7th edition, 2015 there are around 415 million people with diabetes , 318 million people with impaired glucose tolerance and about 21 million women with gestational diabetes mellitus living across the globe. 75% of these people live in low and middle income countries. China and India have the highest prevalence of people with diabetes mellitus – 110 million and 69 million respectively. Diabetes is a leading cause of cardiovascular diseases, stroke, blindness, amputation, end stage renal disease in the world. Diabetes and its complications have also been demonstrated to be associated with increased production of free radicals and increased oxidative stress in various studies (Baynes J et al,1999; Opara EC, 2002; Maritim AC et al, 2003; Rahimi et al, 2005; Erejuwa et al,2010; Johansen et al,2005).

Aim of the Study

Aim of the study was to analyze the oxidative stress and the antioxidant status in the newly diagnosed type 2 diabetics.

Objective of the Study

The objective of the study was to look into the level of oxidative stress markers and level of anti-oxidants in patients of type2 diabetes and its comparison with the healthy non-diabetic controls.

MATERIAL AND METHOD

The present study was conducted in the Department of General Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi in collaboration with Department of Biochemistry during the period of month of June 2014 to June 2015.

Selection of Cases

40 patients of newly diagnosed type 2 diabetes of age more than 18 years were selected from the Department of General Medicine, IMS, BHU, Varanasi.

Selection of Controls

20 age and sex matched healthy non-diabetic individuals were selected as the controls.

INCLUSION CRITERIA

Criteria for Diagnosis of Diabetes (Adapted from American Diabetes Association 2011)

- Fasting Plasma Glucose (FPG) ≥ 126 mg/dl (7.0 mmol/l) (Fasting is defined as no caloric intake for at least 8 hours.)

Or

- 2-hours post prandial plasma glucose ≥ 200 mg/dl (11.1mmol/l) during an Oral Glucose Tolerance Test (OGTT). The test should be performed by using a glucose load containing the equivalent of 75 gram anhydrous glucose dissolved in water.

Exclusion Criteria

- Those who were on multi vitamin and mineral therapy.
- If patient was a smoker
- Patients with active infection
- Patients who were working in chemical / asbestos / metal factories
- Cancer patients receiving chemotherapy/ radiotherapy
- patients with blood pressure ≥ 140/90

Collection of Blood Samples

After informed consent, blood sample was collected from the antecubital vein of each subject. Venous blood sample of about 3ml was collected in a clean and dry plain vial without any anticoagulant. 2ml of blood was collected in EDTA vial. The blood in the plain vial was allowed to clot at room temperature and then subjected to centrifugation at a rate of 2000 rpm for 10-15 minutes. The serum, thus removed, was stored at -20 ^{0}C in a sterile plain vial until analyzed. Serum was pipetted out at the time of analysis after thawing.

Following precautions were taken to ensure that there was no hemolysis:

- Use of tourniquet was avoided

- The blood was drawn slowly and steadily into the syringe and later expelled into the vial after removing the needle and the tip of the syringe touching the side of the container.

These collected samples were subjected to estimation of the following parameters in the Department of Biochemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Following parameters were analyzed using standard protocols by colorimetric method:

A .Oxidants

- Protein carbonyl
- Malondialdehyde

B .Anti-oxidants

- Reduced glutathione
- Serum nitrite
- Superoxide dismutase
- Uric acid
- Total anti-oxidant capacity

Observation and Results

The present study was conducted in the Department of General Medicine and Department of Biochemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi. The study consisted of 40 patients of newly diagnosed type 2 Diabetes and 20 healthy non diabetic age and sex matched controls. The baseline clinical characteristic of the study population is shown in Table 1.

Table 1: Clinical Characteristics of Study Population

Parameter	Cases	Control
Age (in years)	58.72±10.936	57.60±6.91
Male / Female	25 / 15	12 / 8
HbA1C	10.0±2.36	4.34±0.8
Fasting blood sugar(mg/dl)	180±20	80.86±8.52
Post Prandial blood sugar (mg/dl)	300±60	130.43±6.33

Table 2: Sex Distribution

Sex	Cases		Control	
	No.	%	No.	%
Male	25	62.5	12	60
Female	15	37.5	8	40
Total	**40**	**100**	**20**	**100**

In our study, males were slightly higher in number in both groups. The number of male patients were25 (62.5%) and controls had 12 male (60%). Females were 15 (37.5%) in cases and 8 (40%) in control group. Male to female (M: F ratio) ratio in cases was 1:0.60, in controls it was 1:0.67.

Table 3: Age Group of Study Population

Age (In Years)	Cases		Control	
	No.	%	No.	%
40-50	10	25	3	15
50-60	10	25	6	30
60-70	15	37.5	9	45
≥ 70	5	12.5	2	10

In our study, among the cases 10 (25%) were in age group of 40-50 whereas in the control the count was 3 (15%). 10 (25%) of cases were in 50-60 year age compared to 6 (30%) of control. 15 (37.5%) of cases were 60-70 years age group and 9 (45%) in control group. 5 (12.5%) of cases were≥ 70 years age and 2 (10%) in control group. The maximum number of patients were 15 (37.5%) in the age range of 60-70 years, while minimum number of cases were 5 (12.5%) in the age range of more than 70 years.

Table 4: Compressive Lab Characteristic of Study Population

Parameters	Case	Control	P-Value
MDA (μmol/L)	0.122±0.188	0.0042±0.0067	<0.0001
Protein carbonyl (ηmole/ml)	21.43±1.44	7.60±0.92	<0.007
SOD (U/L)	1.59±0.57	3.430±0.58	<0.0001
Uric Acid (mg/dl)	6.045±1.84	6.96±0.84	<0.039
Glutathione (mg/L)	0.185±0.130	0.40±0.14	<0.0001
Serum Nitrite (μg/dl)	0.04±0.156	0.30±0.05	<0.0001
Total Antioxidant Capacity	**2.98±110.6**	**4.101±102**	**<0.0001**

According to table 4, it is clear that there is a significantly increased level of oxidants like malondialdehyde and protein carbonyl in type 2 diabetics as compared to non-diabetic controls. The values of anti-oxidants were also decreased in type 2 diabetic as compared to non-diabetic controls which is statistically significant.

DISCUSSIONS

In the present study, oxidative stress was measured indirectly by malondialdehyde and protein carbonyl estimation in the serum. Anti-oxidant status was measured by the estimation of serum nitrite, Glutathione, Superoxide Dismutase, Uric Acid, and Total Antioxidant Capacity.

Malondialdehyde (MDA) is a highly toxic byproduct of lipid peroxidation of unsaturated fatty acids by free radicals. Since it is a stable product, it is used as the marker of oxidative damage of unsaturated fatty acids. In the present study serum malondialdehyde (MDA) was found to be significantly elevated in the diabetic patients 0.122±0.188μmol/L) when the compared to healthy controls (0.0042±0.0067 μmol/L) ($p < 0.0001$).

The modification of native amino acid side chains in protein to carbonyl (aldehyde and Ketone) derivatives is known as protein carbonylation. Oxidative stress leads to enhanced protein carbonylation. In the present study protein carbonyl was found to be significantly elevated in the diabetic patients (21.43±1.44 ηmole/ml) when the compared to healthy controls (7.60±0.92 ηmole/ml) ($p < 0.007$).

Glutathione (GSH) is the most abundant non protein thiol that defends against oxidative stress. So, whenever there is an increased oxidative stress, GSH level falls. Glutathione (GSH) was found to be significantly reduced in the diabetic patients (0.185±0.130 mg/L) when compared to the healthy controls (0.40±0.14 mg/L) ($p < 0.0001$).

Superoxide dismutase is an anti-oxidant. The Superoxide Dismutase (SOD) was found to be significantly reduced in the diabetic patients (1.59±0.57 U/L) when compared to the healthy controls (3.430±0.58 U/L) ($p < 0.0001$).

Uric acid plays a protective role during the formation of free radicals. Uric acid has much higher antioxidant capacity. Urate (the soluble form of uric acid in the blood) can scavenge superoxide, hydroxyl radical, and singlet oxygen and can chelate transition metals. Peroxynitrite is a particularly toxic product formed by the reaction of superoxide anion with nitric oxide that can injure cells by nitrosylating the tyrosine residues (nitro tyrosine formation) of proteins. In the present study serum Uric Acid was found to be significantly reduced in the diabetic patients (6.045±1.84 mg/dl) when the compared to healthy controls (6.96±0.84 mg/dl) ($p < 0.039$).

Nitric oxide (NO) is the first well described representative of a class of gaseous biological mediators. NO, being a gaseous free radical, has a half-life of < 15 seconds. Since it is difficult to measure NO directly, because of its short half-life, serum nitrite is measured as an index of NO production. In the present study, Serum Nitrite was found to be significantly decreased in the diabetic patients (0.04±0.156μg/dl) when the compared to the healthy controls (0.30±0.05 μg/dl) ($p < 0.0001$).

In the present study, total anti-oxidant capacity (TAC) was found to be significantly reduced in the diabetic patients (2.98±110.6) when compared to healthy controls (4.101±102) ($p < 0.0001$).

CONCLUSIONS

From our study, it is clear that there is a significant increase in the levels of oxidants and decrease in the levels of antioxidants in the cases i.e. the newly diagnosed type 2 diabetics. Whereas in the controls, there is lower levels of oxidants and higher levels of antioxidants. Antioxidant supplementations may have clinical usefulness in the treatment of this complex disorder and in preventing complications, but the final verdict and consensus can only be obtained after large randomized controlled studies.

REFERENCES

1. ADA (2011). American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care, Vol. 34 Suppl 1, No. pp. S62-69.
2. Baynes J.W., Role of oxidative stress in development of complications in diabetes, Diabetes 40 (1991).405–412.
3. Baynes J.W., S.R. Thorpe, The role of oxidative stress in diabetic complications, Curr. Opin. Endocrinol. 31 1996.277–284.
4. Erejuwa, O. O., Gurtu, S., Sulaiman, S. A., AbWahab, M. S., Sirajudeen, K. N. &Salleh, M. S. (2010a). Hypoglycemic and antioxidant effects of honey supplementation in streptozotocin-induced diabetic rats. Int J VitamNutr Res, Vol. 80, No. 1, pp. 74-82.
5. Erejuwa, O. O., Sulaiman, S. A., Wahab, M. S., Sirajudeen, K. N., Salleh, M. S. &Gurtu, S. (2010b). Antioxidant protective effect of glibenclamide and metformin in combination with honey in pancreas of streptozotocin-induced diabetic rats. Int J MolSci, Vol. 11, No. 5, pp. 2056-2066.
6. IDF Diabetes Atlas Seventh edition 2015

7. Johansen, J. S., Harris, A. K., Rychly, D. J. & Ergul, A. (2005). Oxidative stress and the use ofantioxidants in diabetes: linking basic science to clinical practice. CardiovascDiabetol, Vol. 4, No. 1, pp. 5.

8. Maritim, A. C., Sanders, R. A. & Watkins, J. B., 3rd (2003). Diabetes, oxidative stress, and antioxidants: a review. J BiochemMolToxicol, Vol. 17, No. 1, pp. 24-38.

9. Opara E.C. Oxidative stress, micronutrients, diabetes mellitus and its complications, The journal of the Royal Society for the Promotion of Health; March 2002, 122(1), pp. 28-34.

10. Rahimi, R., Nikfar, S., Larijani, B. & Abdollahi, M. (2005). A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother, Vol. 59, No. 7, pp. 365-373