ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

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

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Manuscript Info

Manuscript History:

Received: 18 February 2016
Final Accepted: 29 March 2016
Published Online: April 2016

Key words:
Diabetes mellitus, Hypertension, Oxidants, Anti-oxidant.

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Abstract

Diabetes and hypertension are two most common non communicable diseases globally. Both the diseases contribute to significant morbidity and mortality in the human race due to affection of every organ of the human body. Epidemiological data suggest that the number of people affected by these diseases is increasing each year. Hence both these diseases are being researched intensively to find out the grass root level pathophysiology. As a result,it has been proved time and again that in both the diseases 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 when both the diseases are present simultaneously in the treatment naïve North Indians.

Introduction:-

Hypertension is one of the major global health problems. The biggest burden of hypertension is in the low and middle income countries. Approximately, 7.5million deaths (12.8% of total) and 57 million disability adjusted life years worldwide are attributable to high blood pressure (Global Health Observatory Data by WHO, 2015). Hypertension increases the risk of cardiovascular diseases (including coronary artery disease and congestive heart failure), ischemic and hemorrhagic stroke, renal failure etc. and hence attributes to high morbidity and mortality. From various experimental models and human studies it has been proved that the complications arising out of hypertension is directly related to the increasing levels of oxidants and decreasing levels of anti-oxidants (Landmesser U et al, 2003; White CR et al,1994; Touyz RM et al,2004; Redon J et al,2003).

Similarly diabetes mellitus is another 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; Rahimiet 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 with hypertension.

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 with hypertension and its comparison with the healthy normotensive, 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 with hypertension 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 & normotensive 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.

Diagnostic criteria for Hypertension According to JNC 8(2014)

Types	Systolic BP	Diastolic BP
Normal	<120 mm Hg	<80 mm Hg
Prehypertension	120-139 mm Hg	80-89 mm Hg
Stage I Hypertension	140-159 mm Hg	90-99 mm Hg
Stage II Hypertension	≥160 mm Hg	≥100 mm Hg

Exclusion criteria:

i. Those who were on multi vitamin and mineral therapy.
ii. If patient was a smoker
iii. Patients with active infection
iv. Patients who were working in chemical / asbestos / metal factories
v. Cancer patients receiving chemotherapy/ radiotherapy

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
   i. Protein carbonyl
   ii. Malondialdehyde
B. Anti-oxidants
   i. Reduced glutathione
   ii. Serum nitrite
   iii. Superoxide dismutase
   iv. Ascorbic acid
   v. Uric acid
   vi. 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 with hypertension and 20 healthy non diabetic and non-hypertensive age and sex matched controls. The baseline clinical characteristic of the study population is shown in Table 1.

Sex distribution:-

In our study, males were slightly higher in number in both the groups. The number of males in the study group were 21 (52.5%) while in controls were 12 (60%). Females were 19 (47.5%) in cases and 8 (40%) in the control group. Male to female (M: F ratio) ratio in cases was 1:0.90 while it was 1:0.67 in controls. (Table 2)

Age group of study population:-

In our study, nine patients (22.5%) were in the age group of 40-50 years whereas in the control group the count was 3 (15%). 12 (30%) of cases were in 50-60 years age group compared to 6 (30%) in the control group. 16 (40%) patients and 9 (45%) controls were in 60-70 years age group. 3 (7.5%) cases were ≥ 70 years while 2 (10%) were ≥70 years in the control group. So the maximum number of cases was in the age group of 60-70 years while the minimum number was documented in the age group of more than 70 years. Best effort was made for case and control selection to match the age and sex. (Table 3)

Blood Pressure in study population:-

In our study, there were 40 patients of diabetes with hypertension of which 18 (45%) patients had stage I hypertension while 22 (55%) patients had stage II hypertension. Among the controls, 13 (65%) were having normal BP while 7 (35%) were in prehypertension group. None of the controls had stage I or stage II hypertension. (Table 4)

Lab characteristics of study population:-

According to table 5, it is clear that there is a significantly increased level of oxidants like malondialdehyde and protein carbonyl in type 2 diabetics with hypertension as compared to non-diabetic normotensive controls. The values of anti-oxidants were also decreased in type 2 diabetic hypertensives as compared to non-diabetic normotensive controls which is statistically significant.

Discussion:-

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, Vitamin C 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 hypertensive patients (0.03±0.03μmol/L) when compared to healthy controls (0.0042±0.0067 μmol/L) ($p < 0.002$).

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 hypertensive patients (15.52±1.829 ηmole/ml) when compared to the healthy controls (7.60±0.92 ηmole/ml) ($p < 0.0001$).

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. In the present study serum Glutathione (GSH) was found to be significantly reduced in the diabetic patients with hypertension (0.01±0.008 mg/L) when compared to the healthy controls (0.40±0.14 mg/L) ($p < 0.0001$).

Superoxide dismutase is an anti-oxidant. In the present study, serum Superoxide Dismutase (SOD) was found to be significantly reduced in the diabetic hypertensive patients (1.17±0.56 U/L) when the compared to the healthy controls (3.430±0.58 U/L) ($p <0.0001$).

Ascorbic acid is a water soluble antioxidant. Its value decreases in the conditions with increased oxidative stress. In present study Vit. C was found to be decreased in the diabetic hypertensive patients (0.12±0.03 mg/L) when compared to the healthy controls (0.15±0.04 mg/L) ($p < 0.019$).

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. Uric acid can also block this reaction. In the present study, serum Uric Acid was found to be significantly reduced in the diabetic hypertensive patients (4.37±2.06 mg/dl) when compared to the healthy controls (6.96±0.84 mg/dl) ($p <0.0001$).

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 reduced in the diabetic hypertensive patients (0.15±0.09μg/dl) when compared to 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 hypertensive patients (3.32±119.4 CRE) when the compared to healthy controls (4.101±102.6 CRE) ($p <0.016$). (CRE is cupper reducing equivalent)

TABLES

Table 1: Baseline clinical characteristics of study population

Parameter	**Case**	**Control**
Age (years)	56.35±7.621	57.60±6.91
Male / Female	21 / 19	12 / 8
Systolic blood pressure (mmHg)	166.23±10.45	123.29±10.7
Diastolic blood pressure (mmHg)	93.15±7.82	75.71±6.27
HbA1C	8.64±1.41	4.34±0.8
Fasting blood sugar (mg/dl)	168.65±24.63	80.86±8.52
Post prandial blood sugar (mg/dl)	280.27±27.83	130.43±6.33

Table 2: Sex distribution

Sex	**Case**		**Control**	
	No.	%	No.	%
Male	21	52.5	12	60
Female	19	47.5	8	40
Total	40	100	20	100

Table 3: Age group of study population

Age (in years)	Case						Control					
	Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
40-50	4	19.04	5	26.31	9	22.5	1	8.33	2	25	3	15
50-60	6	28.57	6	31.57	12	30	4	33.33	2	25	6	30
60-70	9	42.85	7	36.84	16	40	6	50	3	37.5	9	45
≥ 70	2	9.52	1	5.26	3	7.5	1	8.33	1	12.5	2	10
Total	21	100	19	100	40	100	12	100	8	100	20	100

Table 4: Blood Pressure in study population

Degree of hypertension	Case		Control	
	No.	%	No.	%
Non-hypertensive	0	0	13	65
Pre-hypertension	0	0	7	35
Stage – I hypertension	18	45	0	0
Stage – II hypertension	22	55	0	0
Total	40	100	20	100

Table 5: Comprehensive table of lab characteristics of study population

Parameters	Case	Control	p-value
Malondialdehyde(μmole/L)	0.03±0.03	0.0042±0.0067	<0.002
Protein carbonyl (ηmole/ml)	15.52±1.829	7.60±0.92	<0.0001
Superoxide Dismutase (U/L)	1.175±0.56	3.430±0.58	<0.0001
Uric Acid (mg/dl)	4.37±2.06	6.96±0.84	<0.0001
Ascorbic acid (mg/L)	0.12±0.03	0.15±0.04	<0.019
Glutathione (mg/L)	0.018±0.008	0.40±0.14	<0.0001
Serum Nitrite (μg/dl)	0.15±0.09	0.30±0.05	<0.0001
Total Antioxidant capacity (CRE)	3.32±119.4	4.101±102.64	<0.016

Conclusion:-

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 with hypertension. 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.

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