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Study of Serum MDA, Serum Vitamin C, Serum Vitamin E Levels in Patients with Diabetes and Diabetes with Hypertension

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Abstract

Introduction: Diabetes mellitus is one of the most common systemic diseases in the world. Diabetic patients are exposed to increased oxidative stress. However very few studies have measured and compared oxidative stress, antioxidant status in patient of diabetes with hypertension. Hence present study was conducted, which was aimed to measure antioxidant levels (Vitamin C, vitamin E) and oxidative stress (Malondialdehyde-MDA level) in type 2 diabetic patients, in type 2 diabetic patients with hypertension and to compare them with age and gender matched healthy controls.

Male volunteers of 40-60 years were divided into

Group I - 30 normal healthy controls,

Group II - 30 patients having type II diabetes mellitus since 3 to 6 years,

Group III - 30 patients having type 2 diabetes mellitus along with hypertension since 3 to 6 years.

Serum levels of MDA were estimated by method described by Buege and Aust. serum levels of Vitamin C and vitamin E were estimated by method described by Ayekyaw and Baker respectively. Serum MDA levels, serum Vitamin C and vitamin E levels compared by applying appropriate statistical test. We found statistically highly significant increase (<0.001) in serum MDA levels and significant decrease (<0.05) in both serum Vitamin C levels and serum vitamin E levels in patients of diabetes with hypertension as compared to patients having only diabetes. *Conclusion:* Our study showed that patients having both diabetes and hypertension have increased oxidative stress than patients having only diabetes. So, these patients will be more susceptible for developing complication related to oxidative stress. So proper precaution in the form for antioxidant supplementation to these patients can help them to live better quality of life.

Keywords: Hypertension; Malondialdehyde; Type 2 Diabetes Mellitus; Vitamin C; Vitamin E.

Introduction

Diabetes mellitus is one of the most common chronic diseases globally and in many countries it is now considered as one of the leading cause of death [1]. The prevalence of diabetes for all age groups worldwide was 2.8% in 2000 and is expected to raise up to 4.4 % (360 million) by 2030 [2]. Prevalence of Type 2 diabetes mellitus (DM) has increased from 1.2% to 11% over last 3 decades [3]. Diabetes mellitus is metabolic diseases characterized by high levels of blood sugar and it results from defects of insulin production, defects in insulin action or both. Uncontrolled hyperglycemia in diabetes prone for various long-term diabetic complications. This long-term complication in diabetic patients can be due to increased oxidative stress which results from increased reactive oxygen species and reactive nitrogen species. It has been found that diabetic patients are exposed to increased oxidative stress [4].

Various mechanism that explain oxidative stress in diabetic patients are- autooxidation of glucose, shifts in redox balance, decreased tissue concentration of antioxidant (Vitamin E, reduced glutathione-GSH) and impaired activities of antioxidant defence enzymes (superoxide dismutase-SOD, catalase-CAT) [5,6].

It has been also found that Type 2 DM patients have increased prevalence of hypertension than those without DM and most common cause of death in

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diabetic patients are heart disease [7,8]. Occurrence of Diabetes and Hypertension (HTN) are increasing in developing countries because of western, unhealthy and stressful lifestyle. Type 2 DM and HTN among the most common chronic non-communicable Diseases in developing countries and in most of the patients both diseases occur together because they share common risk factors [9].

It has been thought that patients having both diseases will have greater oxidative stress than patients having only diabetes. Recent study showed that complication of diabetes and hypertension has common aetiology involving oxidative stress and endothelial damage. Thus it has also been observed that patients of diabetic with hypertension have more oxidative stress than patient having only diabetes

So in the present study, we have compared antioxidant levels (Vitamin C vitamin E) and oxidative stress (Malondialdehyde-MDA level) in patients having type 2 diabetes and patients having both type 2 diabetes and hypertension

Materials and Method

The present cross sectional study was conducted in type 2 diabetic and diabetic with hypertension patients selected from diabetic OPD of the B. J. Medical College Pune. Duration of study was August 2010 to July 2012. After approval from Institutional Ethics Committee, male Patients were selected from diabetic outpatient department (OPD) of the hospital.

Inclusion Criteria

1. Male patients (age 40 – 60 years)
2. Diagnosed type 2 DM patients and type 2 DM patients with hypertension having diabetes since 3 to 6 years and on regular oral hypoglycemic and antihypertensive medication respectively.
3. Normal Healthy controls with no history of diabetes and hypertension.

Exclusion Criteria

1. Patients with acute complications of diabetes and hypertension.
2. Patients with life threatening diseases like cancer.
3. Patients on antioxidant drug therapy.
4. Fasting blood Sugar Level > 130 mg% and Blood pressure >130/80

5) Smokers, alcoholic, tobacco chewers.

Normal healthy controls were selected from relatives of the patient. Blood glucose levels were estimated in controls, diabetic patients and patients of diabetes with hypertension. Blood glucose levels of all the three groups were within normal range.

Based on inclusion and exclusion criteria, a total of 90 subjects were finally selected for the present study and were subdivided as follows –

Group I	Normal controls.
Group II	Type 2 diabetes since 3 to 6 years and taking regular oral antidiabetics medication.
Group III	Type 2 diabetes mellitus with hypertension since 3-6 year taking regular oral antidiabetics and antihy pertensive medication.

The study protocol was explained in detail to all the subjects and informed written consent regarding participation in the study was obtained from them. Serum MDA, serum vitamin C and serum vitamin E levels were estimated in all subjects.

Estimation of Blood Glucose Levels

Fasting and postprandial blood samples were collected to measure blood sugar levels. Blood glucose levels were estimated by glucose oxidase- peroxidase method [10].

Normal range for fasting blood glucose is 70-110 mg/dl.

Measurement of serum MDA, serum vitamin C, and vitamin E levels

To assess the oxidative stress, serum MDA levels of all subjects and controls were analysed. Serum levels of vitamin C and vitamin E were measured to know the antioxidant status.

Serum separated from the venous blood specimens was used for estimation of the serum MDA, vitamin C and vitamin E levels. All samples were stored in refrigerator and the estimations were done within 24-48 hours of specimen collection.

Estimation of Serum Malondialdehyde (MDA)

(Buege and Aust) Thiobarbituturic acid method [11]

Malonyldialdehyde is major aldehyde product of lipid peroxidation; is highly reactive three carbon dialdehyde produced from lipid hydroperoxides. It can, however, also be derived by the hydrolysis of pentose, deoxyriboses, hexoses, from some amino

acids and from DNA. MDA has most frequently been measured by the thiobarbituric acid reaction.

Normal range of MDA is 2-5 nanomol/ml.

Estimation of Serum Vitamin C (Ascorbate)

Method: described by Kyaw A [12]

Normal range of vitamin C is 0.5-1.5 mg%.

Estimation of Serum Vitamin E (Á-Tocopherol):

Method: described by Baker and Frank [13]

Normal range of vitamin E is 0.5-0.8 mg%

Serum MDA levels and Vitamin C, Vitamin E levels of 3 groups were compared by applying ANOVA test.

*p<0.05 statistically significant **p<0.001 statistically highly significant

Results

Table 1 shows that difference in means of age, height, weight and body mass index were not statistically significant between three groups. Though the difference in mean values of fasting blood glucose level, post prandial blood glucose level as well as systolic and diastolic blood pressure were statistically significant in all three groups (p< 0.05), their values were within normal limits (Table 1)

Table 2 and Graph 1 show that serum level of MDA in diabetes patients (4.83±0.15) and patients having both diabetes and hypertension (5.07±0.25) were significantly higher than control group (3.72±0.34). Also we found that increase in serum MDA level in patients of DM with HTN was statistically significant as compared to diabetes patients (p<0.001).

Table 2 and Graph 2 show that the serum levels of vitamin C in diabetes patients (0.50±0.10) and patients having both diabetes and hypertension (0.39±0.16) were significantly lower than control group (0.87±0.22).

Similarly, Table 2 and Graph 2 shows that the serum levels of vitamin E in diabetes patients (0.48±0.14) and patients having both diabetes and hypertension (0.40±0.15) were significantly lower than control group (0.64±0.08).

Table 3 and Graph 3 show negative correlation between serum MDA (oxidative stress) and vitamin C, E (antioxidant status) in control group, which is statistically highly significant.

Table 1: Descriptive characteristics of baseline parameters in the three groups:

Parameters	Control (n=30) Mean ± SD	DM (n=30) Mean ± SD	DM with HTN (n=30) Mean ± SD	p value
Age (years)	53±5.55	54.0±4.32	54.7±4.43	p>0.05
Weight (Kg)	64.9±8.06	66.1±7.82	67.4±10.65	p>0.05
Height (Meters)	1.65±0.07	1.64±0.07	1.63±0.08	p>0.05
Body mass index (Kg/m^2)	23.9±1.49	24.5±1.13	25.4±4.69	p>0.05
Blood glucose level fasting (mg/dl)	95.2±3.61	106.0±9.40	108.4±8.76	p<0.05*
Blood glucose level post-prandial (mg/dl)	124.5±7.03	149.2±12.35	152.4±8.21	p<0.05*
Systolic blood pressure (mm Hg)	118.6±4.64	122.1±6.19	127.1±2.80	p<0.05*
Diastolic blood pressure (mm Hg)	78.3±2.39	79.2±1.63	80.1±1.36	p<0.05*

* p<0.05 statistically significant ** p<0.001 statistically highly significant DM – diabetes mellitus, HTN – Hypertension

Table 2: Comparison of serum MDA (oxidative stress) and serum vitamin C, vitamin E levels (Antioxidant Status) among three groups (By ANOVA test):

Parameters	Control n=30 Mean ± SD	DM n=30 Mean ± SD	DM with HTN n=30 Mean ± SD	p value
MDA	3.72±0.34	4.83±0.15	5.07±0.25	<0.001 **
Vitamin C	0.87±0.22	0.50 ± 0.10	0.39 ± 0.16	<0.001 **
Vitamin E	0.64±0.08	0.48 ± 0.14	0.40 ± 0.15	<0.001 **

* p<0.05 statistically significant ** p<0.001 statistically highly significant DM-Diabetes mellitus, HTN-Hypertension, MDA- Malondialdehyde

Table 3: Correlation of MDA and vitamin C, vitamin E levels in control group

Oxidative stress levels	Antioxidant status	Pearson's correlation coefficient r	p value
MDA	Vitamin C	-0.908	<0.001**
	Vitamin E	-0.883	<0.001**

* p<0.05 statistically significant ** p<0.001 statistically highly significant MDA- Malondialdehyde

Table 4: Correlation between MDA and vitamin C, vitamin E levels in diabetes patients

Oxidative Stress levels	Antioxidant Status	Pearson's Correlation Coefficient r	p Value
MDA	Vitamin C	-0.942	<0.001**
	Vitamin E	-0.982	<0.001**

* p<0.05 statistically significant ** p<0.001 statistically highly significant MDA- Malondialdehyde

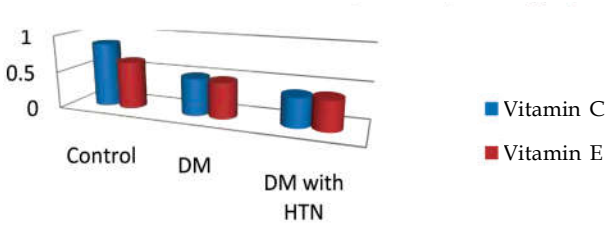
Table 5: Correlation between MDA and vitamin C, vitamin E levels in diabetes with hypertension patients

Oxidative stress levels	Antioxidant Status	Pearson's Correlation Coefficient r	p VALUE
MDA	Vitamin C	-0.9740	<0.001**
	Vitamin E	-0.9850	<0.001**

* p<0.05 statistically significant ** p<0.001 statistically highly significant MDA - Malondialdehyde

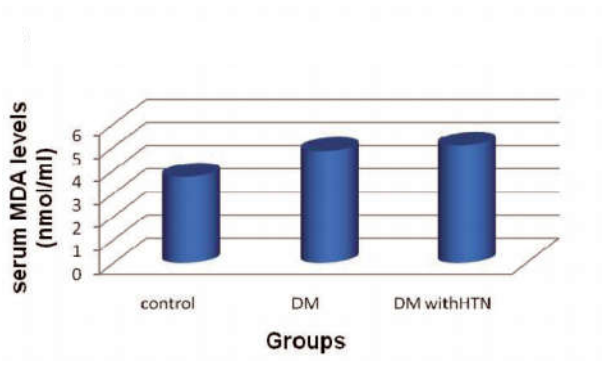
Table 4 and Graph 4 show negative correlation between serum MDA (oxidative stress) and vitamin C, E (antioxidant status) in diabetes group, which is statistically highly significant.

Table 5 and Graph 5 shows negative correlation between serum MDA (oxidative stress) and vitamin C, E (antioxidant status) in diabetes with hypertension group, which is statistically highly significant. Thus tables 3, 4, 5 and Graphs 3, 4, 5 show negative correlation between serum MDA (oxidative stress) and vitamin C, E (antioxidant status). The correlation analysis shows that higher the oxidative stress lowers are the levels of antioxidants



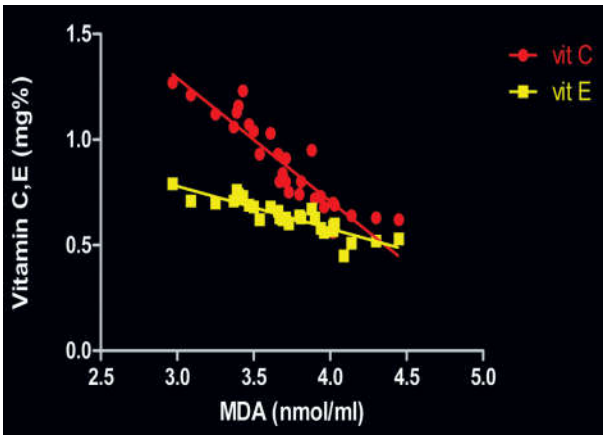
Graph 2: Serum vitamin C and Vitamin E levels (antioxidant) in three group

DM- Diabetes mellitus HTN- Hypertension



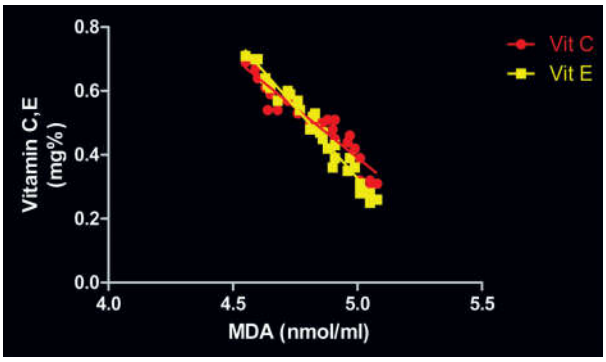
Graph. 1: Serum MDA levels (oxidative stress) in three groups

MDA- Malondialdehyde DM- Diabetes mellitus HTN- Hypertension



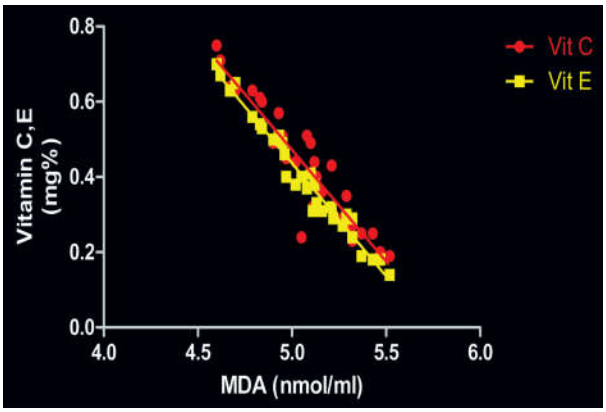
Graph 3: Correlation of MDA levels with vitamin C and vitamin E levels in Control group

MDA- Malondialdehyde



Graph 4: Correlation of MDA levels with vitamin C and vitamin E levels in diabetes patients

MDA- Malondialdehyde DM- Diabetes mellitus



Graph 5: Correlation of MDA levels with vitamin C and E levels in DM with HTN patients

MDA- Malondialdehyde DM- Diabetes mellitus HTN- Hypertension

Discussion

Various studies have shown that oxidative stress is increased in type 2 diabetes mellitus patients. However, patients having both diabetes and hypertension seem to have greater oxidative stress than patients having only diabetes. But there are very few published studies showing oxidative stress in type 2 diabetes mellitus patients with hypertension.

With this background, our present study was aimed to assess oxidative stress and antioxidant status in patients with type 2 diabetes mellitus and patients having type 2 diabetes mellitus with hypertension. We compared serum levels of malondialdehyde (MDA) - an index of oxidative stress, serum level of vitamin C and vitamin E -indices of antioxidant status, in patients with diabetes mellitus, in patients having diabetes mellitus with hypertension and normal healthy control subjects, each group having a sample size of 30.

Serum MDA Level: (Indicator of Oxidative Stress)

In present study we found that the serum levels of MDA in diabetic patients were statistically significant than control group (Table 2, Graph1)

Mehboob et al and other found higher serum MDA levels in diabetes patients as compared to control group due to oxidative damage to lipids [14,15].

Role of Oxidative Stress

In normal healthy individual there is balance between rate of free radical production and elimination and it is important for healthy life. Excess free radical can lead to oxidative stress either by increasing free radical generation or decrease in free radical elimination [16,17,18].

Oxidative stress results from increased reactive oxygen species and / or reactive nitrogen species.

Possible sources of oxidative stress in diabetes patients include

i. Increased non enzymatic glycosylation of proteins –in which chemical reaction between glucose and amino group of proteins take place and leads to formation of glycated protein. In turn, this glycated protein can give an electron to the molecular oxygen and form free radical [19].

ii. Auto-oxidation of glucose- when glucose reacts with trace amounts of transition metals like copper generating free radicals, (hydrogen peroxide, reactive ketoaldehydes) [20,21].

Other Mechanisms are- [6,16,22,23]

a. Metabolic stress resulting from changes in energy metabolism,

b. Alterations in sorbitol pathway

c. Changes in the level of inflammatory mediators,

d. Decrease tissue concentration of low molecular weight antioxidants –Vitamin E

e. Impaired activities of antioxidant defense system

In diabetes mellitus because of predominance of oxidative stress over antioxidant defense system there is damage to various biomolecules like lipid, protein and nucleic acid which prone for the diabetic complication like diabetic retinopathy, neuropathy, accelerated coronary artery disease. Oxidative stress also contributes to pancreatic â cell dysfunction and insulin resistance resulting in more prominent hyperglycemia. This vicious cycle leads to deterioration of diabetes.

Long term Oxidative stress in diabetes mellitus responsible for hypertension. Various mechanisms

postulated are [24]

1. Oxidative stress limits bioavailability of Nitric oxide (NO) in key tissues and organs involved in blood pressure regulation by several mechanism
   a. ROS (reactive oxygen species) avidly react and inactivate NO
   b. ROS reduce NO production by uncoupling endothelial NO synthesize

This reduction of NO in vascular tissue can raise systemic vascular resistance and hence blood pressure by lowering the NO-mediated vasodilator tone

2. ROS species can increase vascular smooth muscle tone by increasing cytoplasmic calcium concentration
3. ROS result in nonenzymatic oxidation of lipoproteins and cell membrane phospholipids, which leads to generation of vasoconstrictor proinflammatory products which in turn lead to endothelial injury and dysfunction which contributes to the rise in blood pressure

The hypertension in diabetes in turn can exaggerate the oxidative stress in diabetes. Thus the vicious cycle sets between oxidative stress and hypertension.

In our present study, we also found that serum levels of MDA were significantly higher in patients of diabetes with hypertension as compared to diabetes patients and controls. (Table 2, Graph1).

Gallou G et al and other found higher MDA levels in diabetes patients with complications such as hypertension, as compared to diabetes patients without complications [25,26,27].

Serum Levels of Vitamin C and Vitamin E

We found that the serum levels of vitamin C and vitamin E in diabetes patients were significantly lower than control group (Table 2, Graph 2).

Ford ES et al also found that there was a decrease in vitamin C and vitamin E in diabetic patients as compared to controls [28].

Our results are comparable with the results obtained by Pasupathi P et al [29] and Paramesha S et al [30].

In present study, we also observed that serum levels of vitamin C and vitamin E were significantly lower in patients having diabetes with hypertension as compared to diabetes patients and controls (Table 2, Graph 2,).

Gupta MM et al found that there was decreased level of vitamin C and vitamin E in diabetes patients with complication such as hypertension as compared to diabetes patient [31].

Oxidation reactions can produce free radicals. In turn these radicals can start chain reactions that damage cells and result in oxidative stress. Vitamin C and vitamin E terminate these chain reactions by removing free radical and inhibiting other oxidation reactions. They do this by getting oxidized themselves, so they are often reducing agents.

Vitamin C: (an essential water-soluble Vitamin), acts as a primary antioxidant in plasma and within cells that quenches ROS. Under physiological conditions, Vitamin-C predominantly exists in its reduced form Ascorbate; it also exists in trace quantities in the oxidized form, DHA (Dehydroascorbic Acid) transducers. Increased levels of Vitamin-C and DHA suppress the formation of ROS and induce the antioxidant defense mechanism [32,33].

Pasupathi P etal has shown that there is a significant decrease in the level of vitamin C in patients of diabetes and diabetes with hypertension because of utilization of vitamin C as an antioxidant defense against reactive oxygen species [29].

Vitamin E: Vitamin E is well known physiological antioxidant and membrane stabilizer. It breaks the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals, thus prevent the cell membrane from damage [29]. Vitamin E can quench a variety of oxyradicals including superoxide anion radicals, singlet oxygen and hydroxyl radical. Vitamin E protects sulfhydryl groups in membrane proteins from peroxidation by quenching of $^1O_2$ (singlet oxygen). This activity depends upon a free hydroxyl group (OH) in position 6 of the chromane ring [32].

Vitamin E also acts as a potential immunoenhancing nutrient. It acts as a free radical scavenger and prevents free radicals from reacting with proteins in cell and thus protects against oxidation. It prevents formation of lipid peroxidation products. Vitamin E supplementation can prevent the development of abnormality of glucose metabolism and diabetes [28].

Table 3, 4 ,5, and Graph 3,4,5 show strong negative correlations between serum MDA and serum vitamin C and vitamin E levels in all the three groups.

Sureda A et al found negative correlation between oxidative stress and antioxidant status in normal subjects[34], While Ahmed RG and Cerilello A found negative correlation between oxidative stress and antioxidant status in diabetes patients and in diabetes with hypertension patients respectively [35,36].

Thus the correlation analysis showed that higher the oxidative stress lowers are the levels of antioxidants. This is mainly because antioxidants neutralize the oxidative stress i.e. free radicals, resulting in their consumption and decreased levels.

Conclusion

Thus the present study showed that patients having both diabetes and hypertension have much more oxidative stress as compared to those having only diabetes.

So, serum MDA and antioxidant levels can be good markers to find out the patients who are at increased risk of developing various complications along with other parameters of diabetes such as blood glucose, lipid profile and $HbA_{1C.}$

In addition to strict measures to control the blood glucose levels, Proper precaution in the form of antioxidant supplementation and yoga practices to these patients can help them to live better quality of life.

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