

ORIGINAL RESEARCH

CORRELATION OF VITAMIN C WITH HbA1C AND OXIDATIVE STRESS IN DIABETES MELLITUS WITH OR WITHOUT NEPHROPATHY

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ABSTRACT

Introduction: Hyperglycemia has generally been considered as the key initiator of kidney damage associated with diabetic nephropathy. Impaired antioxidant defense mechanism with increase oxidative stress has been proposed as the root cause underlying development of insulin resistance, beta cell dysfunction and type II DM.

Methods: Present study compared the oxidant and antioxidant levels in type II diabetes mellitus with nephropathy to without nephropathy. 157 patients with type 2 diabetes mellitus with nephropathy (DN) and 162 patients of type 2 diabetes mellitus without nephropathy (DM) along with 165 unrelated age and sex matched healthy controls were included in study.

Results: An inverse relationship was observed of HbA1c with vitamin C and superoxide dismutase (SOD) levels in DM and DN groups. A significant inverse relationship was also observed between Vitamin C and malondialdehyde (MDA) levels in all the three groups. When comparing the levels of vitamin C and SOD in all the groups we observed the lowest concentration in DN group followed by DM and control groups. Oxidative stress was found increased in DN group as compared to DM and control groups as highest concentration of MDA levels were observed in this group. Multinomial logistic regression showed none of the variable independently associated with diabetic nephropathy.

Conclusion: There is an imbalance between the oxidative stress and antioxidants in diabetes mellitus with and without nephropathy as compared to healthy groups.

Keywords: Diabetes, Diabetic Nephropathy, Vitamin C, Oxidative stress

INTRODUCTION

Nephropathy complicates approximately 30% of type 2 diabetic patients.¹ Hyperglycemia has generally been considered as the key initiator of kidney damage associated with diabetic nephropathy by activation and dysregulation of several metabolic pathways. Impaired antioxidant defense mechanism with increase oxidative stress has been proposed as the root cause underlying development of insulin resistance, beta cell dysfunction and type II DM.^{2,3} It is also implicated in long term micro and macro vascular complications of diabetes.

Vitamin C is an important antioxidant in human, capable of scavenging oxygen-derived free radicals'. Several studies showed decreased basal vitamin C level in diabetic patients^{4,5} and also it is suggested that oxidative stress is increased in diabetes.^{6,7} Sergeant et al also observed a inverse relationship between vitamin C and glycated hemoglobin.⁸ However no study has been performed that compared the oxidant and antioxidant levels in type II diabetes mellitus with nephropathy to without nephropathy. Therefore aim of this study was

to evaluate the status of oxidants & antioxidant system & their association with diabetic nephropathy.

MATERIAL AND METHODS:

During 2011 to 2013 we recruited 157 patients with type 2 diabetes mellitus with nephropathy (DN) and 162 patients of type 2 diabetes mellitus without nephropathy (DM) from Outpatient department of Medicine, of a tertiary care center. The inclusion criteria for patients were onset of diabetes after the age of 35 years and no episodes of ketoacidosis.

Patients on any kind of multivitamin, lipid lowering agents, anti-inflammatory drugs, analgesics, anticoagulants like aspirin, Pregnant or lactating women, Alcoholics, smokers and individuals with tobacco or drug addiction, Past or present history of chronic illness like tuberculosis, rheumatoid arthritis other autoimmune disorders and patients of juvenile and type I DM were excluded from study group.

Diabetic nephropathy is clinically defined by the presence of persistent microalbuminuria (>30mg/day) in a

diabetic patient in the absence of clinical or laboratory evidence of other kidney or urinary tract disease.⁹ For comparison, we recruited 165 unrelated age and sex matched healthy controls.

This study was conducted with the approval of the Institutional ethical committee. Written informed consent was taken from the subjects prior to the study.

Sample collection: Fasting venous blood sample was drawn from all the subjects in EDTA tube (2ml) and plain tube (5ml), the serum was carefully separated and transferred to micro tubes and stored at -20°C until analysis. Post prandial venous blood sample was collected 2 hours after meal. For collection of 24 hour urine samples, wide mouth 5 litre containers were provided to the patients and control subjects and instructions regarding urine collection were given. The urine volume was measured and 20 ml sample was preserved for analysis.

Biochemical Analysis: Fasting and Post prandial blood glucose was measured by fully automated biochemistry analyzer. HbA1C was measured by Immuno-turbidimetric Method.¹⁰

Estimation of Malondialdehyde: Malondialdehyde (MDA) was estimated using the thiobarbituric acid-reactive substances (TBARS) test.¹¹ The MDA in the sample was reacted with Thiobarbituric Acid (TBA) to generate the MDA-TBA adduct. The MDA-TBA adduct was quantified at $\lambda = 532 \text{ nm}$ on spectrophotometer. A plot was plotted against OD and Standard concentration. Concentration in patient sample was calculated from the graph.

Estimation of super oxide dismutase (SOD): Superoxide radical generation by photo reduction of riboflavin is combined with nitrite formation from hydroxylamine hydrochloride to detect superoxide radicals.¹² Superoxide radicals are allowed to react with hydroxylamine hydrochloride to produce nitrite. The nitrite in turn reacts with sulphanilic acid to produce a diazonium compound which subsequently reacts with naphthylamine to produce a red azo compound whose absorbance is measured at 543 nm . SOD scavenges superoxide radicals produced by photoreduction of riboflavin. Therefore nitrite formation is inversely proportional to the amount of SOD.

Estimation of Serum Vitamin C: Ascorbic acid is oxidized by copper to form dehydro ascorbic acid and diketogulonic acid. These products are treated with 2,4-DNPH to form the derivative bis-2,4 DNPH. This compound in strong sulphuric acid undergoes a rearrangement to form a product with an absorption band that is measured at 520 nm .¹³

Vitamin C is highly sensitive against oxidation. Therefore samples were stabilized immediately after arrival in the laboratory. For stabilization, the precipitating reagent Trichloro acetic acid (TCA) was added. Serum, containing the precipitating reagent is stable for 24 h at $2-8^{\circ}\text{C}$. After centrifugation the supernatant is stable for 3 month at -20°C .

RESULTS

As per inclusion criterion there was no significant difference in age and sex between all groups (table 1). We observed a significant higher fasting and post prandial blood sugar as well as HbA1C in DN group as compared to DM and control groups. The mean duration of diabetes was 5.00 ± 2.2 and 6.55 ± 2.7 years in DM and DN groups respectively

On analysis by Karl Pearson correlation coefficient test, an inverse relationship was observed in HbA1c and vitamin c levels in DM ($r = -0.373$, $p \text{ value} < 0.0001$) and DN groups ($r = -0.362$, $P \text{ value} < 0.0001$). However no association was observed in vitamin C and HbA1c levels in control groups. A significant inverse relationship was also observed between Vitamin C and MDA levels in all the three groups. Superoxide dismutase was also found inversely correlated with HbA1c levels in all the three groups (Figure 1).

When comparing the levels of vitamin C in all the groups we observed the lowest concentration in DN group followed by DM and control groups. The difference was also found statistically significant by one way ANOVA test (Table 1). Bonferroni test was also applied to see the difference in intergroup and we observed the vitamin C levels were significantly different in all groups. Similar to vitamin C, superoxide dismutase levels were also statistically different in all three groups with highest concentration in control group and lowest in DN group. Oxidative stress is found increased in DN group as compared to DM and control groups as highest concentration of malondialdehyde levels were observed in this group (table 1).

To find out the effect of variables such as vitamin C, SOD, MDA, and other factors independently for the development of diabetic nephropathy we performed the multinomial logistic regression analysis with diabetes mellitus as a control variable. None of the variable was found independently associated with diabetic nephropathy (Table 2).

DISCUSSION

Hyperglycemia has generally been considered as the key initiator of kidney damage associated with diabetic nephropathy by activation and dysregulation of several metabolic pathways.

Although the underlying patho-mechanisms remain incompletely understood, it can be postulated that oxidative stress due to chronic hyperglycemia may play a significant role in the pathogenesis of diabetic nephropathy, retinopathy and neuropathy. Several biochemical pathways have emerged as being predominant potential pathophysiological mechanisms of oxidative stress that can be associated with hyperglycemia in diabetes mellitus.¹⁴ Furthermore, diabetes associated oxidative stress is probably a result of both an increased production of plasma free radical concentrations and a significant reduction in antioxidant defense mechanisms.¹⁵

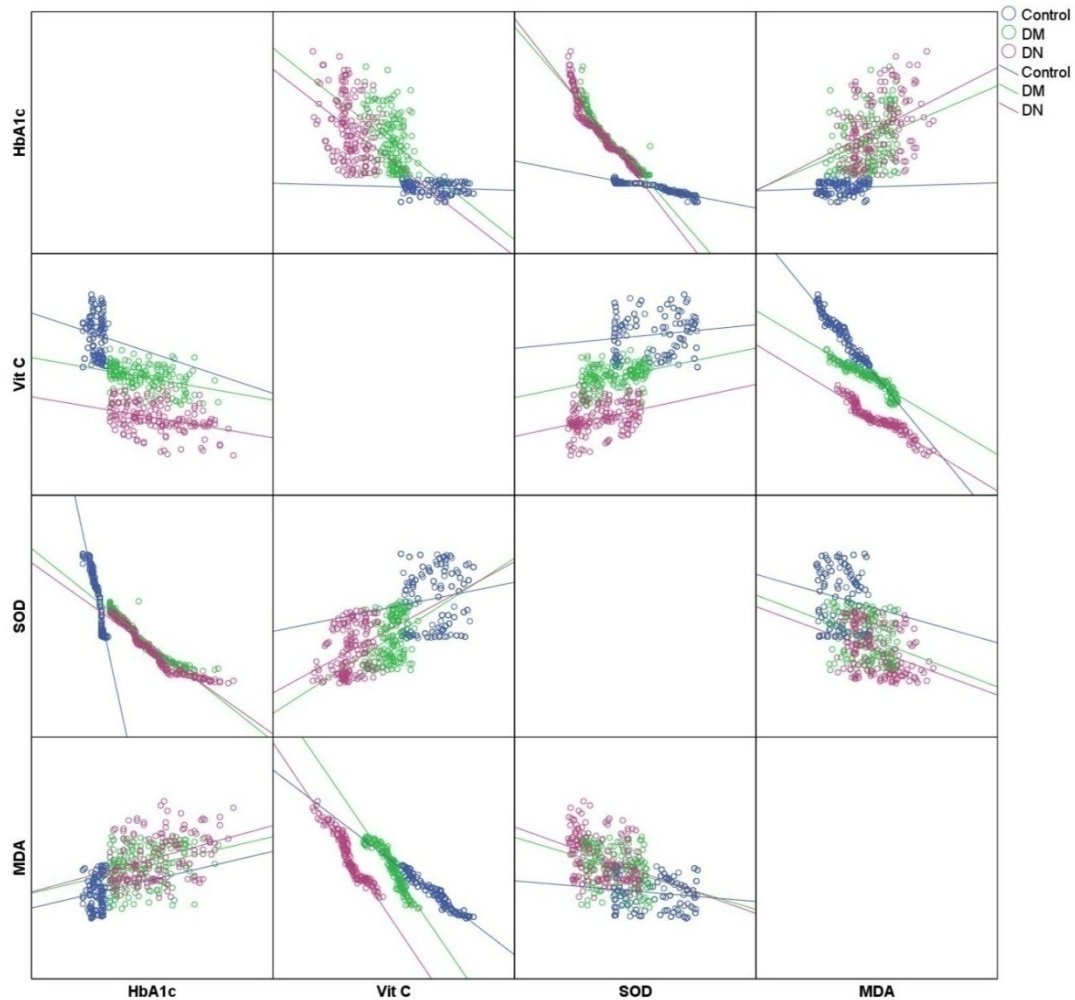


Figure 1: Scatter dot plot showing correlation of different variables

Table 1: Demographic and Biochemical profile in three groups

Parameter	Control	DM	DN	P value
Age(years)	54.35±11.0	53.18±10.4	54.59±7.85	0.392
Sex(Male: Female)	83:82	78:84	60:97	0.069
FBS(mg/dl)	85.97±9.57	152.05±57.9	7.50±64.7	<0.0001
PPBS(mg/dl)	114.56±13.598	239.25±97.02	287.49±116.91	<0.0001
HbA1c %	5.732±0.358†	8.46±1.5	9.21±1.84	<0.0001
Creatinine(mg/dl)	0.883±0.107	0.929±0.243	1.46±0.924†	<0.0001
Urine Microalbumin (mg/day)	9.85±3.4	11.69±4.09	175.91±234.84	<0.0001
Vitamin C	1.427±0.169	1.076±0.103	0.739±0.121	<0.0001
SOD	6.01±0.87	4.86±0.65	4.47±0.68	<0.0001
MDA	1.77±0.57	2.67±0.69	3.00±0.81	<0.0001

Table 2: Multinomial Logistic Regression Analysis

Parameter	B Value	P value	EXP(B)	95% CI
Age	-0.366	0.999	0.693	-0.616 - 0.242
Duration of diabetes	0.810	0.999	2.248	-0.536 - 2.341
FBS	-0.046	0.999	0.955	-0.109 - 0.053
PPBS	0.047	0.999	1.048	0.007- 0.079
HbA1C	-1.905	1.000	0.149	-6.118 - 2.105
SOD	-0.006	1.000	0.994	-11.658 - 9.069
MDA	-38.877	0.993	1.305X10 ⁻¹⁷	-40.528 - -31.916
Vitamin C	-266.465	0.990	1.88X10 ⁻¹¹⁶	-273.827- -213.041

In uncontrolled diabetes, the level of SOD, the enzyme responsible for inactivating the superoxide radical, along with the levels of the antioxidants vitamins are decreased.¹⁶ Ascorbate is a powerful reducing agent capable of rapidly scavenging a number of ROS such as superoxide, H₂O₂ and singlet oxygen. Various studies have reported protective effects of antioxidants such as vitamin C against oxidative damage of diabetes.¹⁷⁻¹⁹ The level of vitamin C in plasma and renal tissues is significantly reduced in diabetic patients.^{18,20} Impaired antioxidant function also plays a role in the development of diabetic kidney disease. Human studies with antioxidants for DN are limited and have had variable results.²¹

In present study we observed a significant decrease in vitamin C and SOD levels and increase in MDA levels in diabetes patient as compared to controls. Gupta et al,²² Rysz et al,²³ Aaseth et al,²⁴ Varvarovska et al²⁵ and Mukherjee et al²⁶ in their experimental and patients studies have shown similar results for MDA, SOD and vitamin C. Kimura et al²⁷ and Moussa et al²⁸ in their studies got raised SOD levels in diabetics and suggested that increase in serum SOD may reflect decrease binding of the enzyme to the endothelium, resulting in vascular wall being more vulnerable to oxidative damage. Similar to our study Bhatia et al²⁹ showed that the Vitamin C and SOD levels were found significantly reduced in DN patients as compared to DM patients. None of the above mentioned studies had performed the multinomial logistic analysis to see the effect of individual parameter on the development of diabetic nephropathy. In present study we performed the multinomial logistic analysis and found that none of the variable was independently associated with diabetic nephropathy when compared to diabetic mellitus

CONCLUSION

In conclusion there is an imbalance between the oxidative stress and antioxidants in diabetes mellitus with and without nephropathy as compared to healthy groups however none of the factor independently contributes towards the progression of disease.

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