Original Research Article

Antioxidant status of type 2 diabetic patients attending a tertiary care hospital

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Abstract

Background: Insulin insufficiency causes diabetes mellitus, the most prevalent endocrine condition. It has been shown that chronic hyperglycemia and increased oxidative stress are linked to the pathology of diabetic vascular disease. These patients are more prone to adverse cardiovascular events which occur due to the accelerated rate of atherosclerosis in diabetic patients. This study was conducted to determine and compare *FBG, HbA1C, TAS, Vitamin C, Vitamin E and Lipoprotein levels* in T2DM patients and healthy control subjects. **Materials And Methods:** Total *50 patients with type 2 diabetes and other 50 healthy controls were studied. SOD, Vitamin C and Vitamin D levels were tested in the blood of all subjects to determine their antioxidative status. FBG, HbA1C were all measured, and the lipid profile was estimated by determining total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels in all the participants.* **Results:** Diabetic patients had significant higher levels of Fasting blood glucose (P<0.001), HbA1c (P<0.001), TC (P<0.001), TG (P<0.001), LDL-C (P<0.05), MDA (P<0.001) and significant lower levels of HDL-C (P<0.001) than control subjects. The Mean values of SOD, Vitamin C, E were significantly decreased in patients as compared to control. **Conclusion:** The findings of this study are in accordance with earlier studies, that there is increased oxidative stress in diabetics compared to controls; and the oxidative stress further increases as diabetes to cardiovascular diseases. This study emphasizes the importance of assessing the antioxidant status in diabetes in addition to the markers of oxidative stress and lipid profile to formulate the specific therapies for early therapeutic intervention and better treatment of diabetes mellitus.

Keywords: oxidative stress, Lipid Profile, Type 2 Diabetes, superoxide dismutase, Vitamin C, Vitamin E.

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INTRODUCTION

Diabetes mellitus is a metabolic condition characterised by high blood sugar levels and elevated free radical activity. Glucose autoxidation, protein glycation, development of advanced glycated end products, and activation of the polyol pathway are all processes that result in oxidative stress in a number of tissues.[1] A redox imbalance occurs when natural antioxidant systems fail to provide enough compensatory mechanisms, resulting in the activation of stress-sensitive intracellular signalling pathways.[2] As a result, oxidative stress may have a role in the pathogenesis of diabetes. The increased production of reactive oxygen species canlead to damage of proteins, lipids, and DNA. In addition, the activation of stress-sensitive signaling pathways thatregulate gene expression can also result in cellular damage.[3] Proteins, lipids, and DNA may be damaged as a result of increased reactive oxygen species generation. Furthermore, cellular damage may be caused by the activation of stress-sensitive signaling pathways that control gene expression.[3] Superoxide dismutase (SOD) is a dimeric antioxidant enzyme responsible for the quenching of superoxide

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radicals which are released during the chemical reactions of the various metabolic pathways. Vitamin c (Ascorbic acid) is a six-carbon lactone that is synthesized from glucose in the liver of mammalian species but not by humans, human primates, and guinea pigs. It acts as a chain-breaking antioxidant.[4] Vitamin E is a lipid-soluble antioxidant present in all cellular membranes protecting against lipid peroxidation. It acts as a chain-breaking antioxidant.[5] With this background, the present study was done to determine and compare *FBG, HbA1C, TAS, Vitamin C, Vitamin E* and *Lipoprotein levels* in T2DM patients and healthy control subjects

MATERIALS AND METHODS

This study is a cross-sectional case-control study, conducted in the Department of Biochemistry in association with the Central lab at Koppal institute of medical sciences and Hospital, koppal, Karnataka. The study was conducted on successive patients after informed consent was obtained from them and approvedby the Ethical Clearance Committee of the institution.100 consented individuals were enrolled in this study. They were grouped into two groups, and the study group consisted of 50 patients (22 males and 18 females) aged 30-70 years with type 2 diabetes mellitus without complications. They were recruited during their routine medical care visits from an outpatient diabetic clinic at Koppal institute of medical sciences and Hospital, Koppal, Karnataka. All patients with diabetes were being treated with stable doses of oral hypoglycaemic agents. The control group consisted of 50 (25 males and 25 females) systemically healthy subjects aged 30–70 years.

Inclusion Criteria: The patients who were having Type-2 Diabetes mellitus

Exclusion Criteria: The patients who were having Type-I. DM History Suggestive of Complications of T2DM-Angiopathy, Cardiopathy, Retinopathy, Nephropathy. smokers, and alcoholics**.** Renal failure

Determination of Biochemical Parameters: After an overnight fast 10 ml of venous blood was drawn from participants and 3 ml was put into ethylenediaminetetraacetic acid (EDTA) bottles for fasting lipid analysis and 2 ml into fluoride oxalate bottles for fasting plasma glucose analysis. The remaining 5 ml was placed into a lithium heparin bottle for analysis of vitamins C, E, and SOD. Within 30 minutes of collection, plasma was separated from blood cells by centrifugation at 2500 g for 15 minutes and put into simple bottles with Pasteur pipettes. All assay samples were batch analyzed, and fasting plasma glucose was assessed within 24 hours following blood collection. The lipid profile, vitamin C, vitamin E, and TAS analysis samples were kept frozen and evaluated within two weeks of collection. The glucose oxidase technique was used to determine plasma glucose levels (Randox kit).[6] The enzymatic method of analysis was used to determine plasma triglyceride (Randox kit).[7] The enzymatic method of analysis was used to quantify plasma total cholesterol and HDL (Randox kit).[8,9] The LDL concentration was determined using Friedewald *et al.* equation's using total cholesterol, HDL, and triglyceride concentrations.[10] The concentrations of vitamins C and E in plasma were measured using high-performance liquid chromatography (HPLC) (Agilent HPLC 1100 series).[11,12] The ABTS(2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) spectrophotometric technique was used to determine the total antioxidant status of plasma (Randox kit).[13]

RESULTS

The clinical parameters of the controls and the type 2 diabetics were shown in Table 1 Among 50 controls, 25 were females and 25 were males and among 50 T2DM patients, 23 were females and 27 were males. The age duration of participants was from 30 to 70 years.

Table 1: Clinical characteristics of Control and T2DM subjects

	Control	T2DM patients
Number	50	50
Sex (Female/Male)	25/25	23/27
Age (duration in years)	30-70	30-70

Biochemical parameters like Fasting Blood sugar, HbA1c, MDA and Lipid profile were shown in Table 2 There was a significant increase (P<0.001) in the Fasting Blood Glucose, HbA1c, Total Cholesterol, Serum Triglycerides in diabetic Group compared to control group. There was a significant increase (P<0.05) in LDL-Cholesterol in diabetic Group compared to control group and there was a significant decrease (P<0.001) in the HDL-Cholesterol in Diabetic group compared to control group.

Table 2: Fasting Blood sugar, HbA1c, and lipid profile

Biochemical parameters	controls	T2DM patients	P value
FBS (mg/dl)	90.6±12.8	172.6±22.4	<0.001

HbA1c (%)	5.64±0.54	7.85±1.3	<0.001
Total Cholesterol (mg/dl)	146.7±13.8	210.7±18.6	<0.001
Triglycerides (mg/dl)	103.2±15.8	208.8±34.8	<0.001
LDl-C (mg/dl)	86.4±12.6	131.6±17.8	<0.05
HDL-C (mg/dl)	44.2±1.9	37.6±2.4	<0.001

Antioxidant levels were shown in Table 3.

There was a statistically significant decrease in the Vitamin C, Vitamin E and Total Antioxidant status in T2DM patients compared to control group.

Table 3: Antioxidant levels

Antioxidant level	controls	T2DM patients	P value
Vitamin C (µmol/l)	44.65 ± 5.88	27.68 ± 8.42	<0.001
Vitamin E (µmol/l)	30.23 ± 5.20	16.31 ± 5.03	<0.001
Total antioxidant status (mmol/l)	1.54 ± 0.24	1.15 ± 0.26	<0.001

DISCUSSION

Multiple metabolic abnormalities are linked to type 2 diabetes mellitus, resulting in an excess of reactive oxygen species and oxidative stress. Chronic illness and cell death are characterised by oxidative stress and the resulting tissue damage. Increased generation and/or inefficient scavenging of such reactive oxygen species may play a critical role in determining tissue damage in certain pathologic situations, according to growing data. Endothelial dysfunction is thought to be a key factor in the development of diabetic angiopathies. The consequences of hyperglycemia, advanced glycation end products (AGE), and dyslipidaemia have all been proposed as possible reasons for the onset of endothelial dysfunction in type 2 diabetes.[14,15] Hyperglycemia has also been demonstrated to cause free radical release and impair antioxidant defences, both of which are linked to endothelial dysfunction.[16] Patients with NIDDM often have abnormal lipid metabolism.[17] Hypertriglyceridemia is often associated with a reduction in HDL-C, which is also a common hallmark of diabetic lipid abnormalities.[18,19,20] A significant aspect of NIDDM is the low level of HDL-C, which has anti-atherogenic and antioxidative properties when present in appropriate concentrations (also known as type 2 diabetes mellitus). Reduced HDL-C levels are often accompanied by increases in plasma TG levels, which is mediated by cholesterol ester transfer protein (CETP).[21] Insulin resistance is thought to be at the root of alterations in lipid parameters in NIDDM, and it's frequently related with greater TC and TG levels and lower HDL-C levels.[22] Higher hepatic secretion of VLDL and delayed clearance of TG-rich lipoproteins may be the cause of hypertriglyceridemia, which might be owing to increased levels of substrates for TG synthesis, free fatty acids, and glucose. Reduced activity of lipoprotein lipase (LPL), a crucial enzyme for lipoprotein-TG, might be the cause of the latter.[23] Vitamin C (ascorbic acid), vitamin E (alpha tocopherol), and beta carotene are antioxidants, as are enzymes such as catalase, superoxide dismutase, and glutathione peroxidase, and transition metal binding proteins such as caeruloplasmin. Vitamins C and E, in addition to albumin and urate, have been shown to play a significant role in serum total antioxidant activity.[24,25,26] Vitamin E is the most abundant lipid-soluble antioxidant found in cell membranes and lipoproteins. Vitamin C is the most significant antioxidant for breaking aqueous phase chains.[25] Because of the synergistic interactions between antioxidants, they operate better and more effectively against ROS when used together than when used alone. Plasma total antioxidant status is a quantitative evaluation of the state of balance of these various components under specific reaction conditions. It includes all antioxidants in plasma, even those that have yet to be found or that are difficult to measure. It also provides a more accurate assessment of plasma antioxidant capacity.[27] Previous study has revealed that low antioxidant levels may be linked to their increased consumption during the process of countering excessive free radicals produced in diabetes.[24,28] As a result, antioxidant reserves, such as vitamins C and E, are depleted.

CONCLUSION

In this study, as compared to healthy control subjects, T2DM patients exhibited significantly reduced plasma levels of TAS, vitamins C, and E, which could be due to oxidative stress. Increased consumption of antioxidant-rich foods or supplements may help people with T2DM improve their cardiovascular and metabolic health.

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