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Oxidative Stress, Antioxidant Status and DNA Damage in Type 2 Diabetes Mellitus

Shankar Shetty[1], Suchetha Kumari N[2]*, and Madhu LN[3]

[1] PRIST University, Thanjavoor.
[2] Department of Biochemistry, K. S. Hegde Medical Academy, Nitte University, Mangalore
[3] Department of Allied Health Science, Nitte University, Mangalore

ABSTRACT

Diabetes mellitus is known to be associated with increased oxidative stress. Oxygen free radicals are implicated in pancreatic beta cell damage and genesis of diabetes as well as in the pathogenesis of diabetic complications like cardiovascular disease, neuropathy, retinopathy and nephropathy. The present study is to evaluate lipid peroxidation, Myeloperoxidase level, antioxidant status and DNA damage in type 2 diabetic persons and to compare the results with non diabetic healthy control subjects. Serum Malondialdehyde (MDA) Myeloperoxidase (MPO), Total antioxidants, (TAC) Superoxide dismutase (SOD), Catalase (CAT) and Total cholesterol, (TC), HDL cholesterol (HDL-C), Triglycerides (TG) were estimated spectrophotometrically. A comet assay was employed to evaluate DNA damage. Type 2 diabetic subjects had higher levels of plasma MDA and MPO concentration, but lower TAC, GSH, CAT and SOD activity. Correlation analysis shows that there is a positive association between MPO and MDA concentration but negative correlations with TAC status and SOD activity to the fasting blood glucose level and duration of diabetes. The comet assay revealed higher percentage of DNA damage in diabetics when compared to control subjects. The results of our present study suggests that hyperglycemia in diabetic subjects is the predominant cause for oxidative stress over antioxidant defense systems leading to lipid peroxidation and oxidative DNA damage which possibly contribute to the development of diabetes and its complications.

Keywords: Type 2 diabetes mellitus, Oxidative stress, Cardiovascular disease, Antioxidants, Comet assay, DNA damage.

Corresponding author

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INTRODUCTION

Type 2 diabetes mellitus and its complications are frequently seen today, and with an increasing incidence. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications. Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death [1]. Factors that strongly affect the risk of diabetic complications are disease duration and degree of glycemic control. These observations have given the 'glucose hypothesis' which suggests that glucose mediates many of the deleterious effects of diabetes. Both the studies Diabetes Control and Complications Trial and the United Kingdom Diabetes Study found that strict glycemic control dramatically lowered the incidence of retinopathy, nephropathy and neuropathy. This important finding suggests that hyperglycemia promotes or even initiates these complications [2]. Oxidative stress mediated by reactive oxygen species alter the fundamental properties of cholesterol, cholesteryl esters phospholipids and lipoproteins as well as other proteins to make them dysfunctional, immunogenic and proatherogenic [3,4]. Oxidative stress can be enhanced by non enzymatic pathway such as copper and iron cat ions as wells as by lipoxygenases, myeloperoxidase and NADPH oxidase [5]. These pro oxidant pathways are balanced by antioxidant mechanism such as antioxidant vitamins, (vitamin E, Carotenoids and Vitamin C) antioxidant enzymes such as SOD, Catalase and Glutathione peroxidase etc [6]. Under normal conditions free radicals are formed in minute quantities and are rapidly scavenged by natural cellular defense mechanism. Lipidperoxidation products (MDA), which increases in clinical and experimental diabetes, are important results of oxygen derived free radicals stress. These products may be important in the pathogenesis of vascular complications in diabetes mellitus [7].

Oxygen- free radicals induce a variety of lesions in DNA, including oxidized bases, abasic sites, DNA strand breaks and formation of cross-links DNA and Proteins. Rehaman et.al (1991) showed that the products generated by oxidative DNA damage are significantly elevated in type 2 diabetes mellitus and the pattern of modification was the same as the attack of the hydroxyl radical(OH.) upon DNA . Choi et. al (2005) reported that lymphocyte DNA damage was significantly higher type 2 diabetes mellitus patients with poor glycemic controls. DNA damage can be assessed by a number of different ways, including techniques to measure strand breakage and baseless sites such as single cell gel electrophoresis or Aldehyde Reactive Probe assays. Singh et.al (1998) first demonstrated the potential of single cell gel electrophoresis assay (comet assay) as a measurement of DNA damage [8]. Thus the intent of the present study is to assess the extent of lipid peroxidation, antioxidant status and DNA damage among the type 2 diabetic individuals.

MATERIALS AND METHODS

The present study was carried out at the Central Research Laboratory A.B Shetty Memorial Institute of Dental Sciences, after getting the approval from the concerned ethical committee of the institution. 246 cases of Type 2 diabetic subjects (Both male and female) aged between 35 to70 years and 130 age sex matched healthy subjects as control were recruited for

the study after taking written consent. Patients with acute and chronic inflammatory conditions were excluded from the study. 8 ml of venous blood sample was collected in fasting condition processed and preserved for the analysis of various parameters: Blood glucose, Total cholesterol, HDL cholesterol, Triglycerides (Spinreact-Spain) and HbA1c(Reckon diagnostics) were estimated by standard diagnostic laboratory methods. LDL cholesterol was calculated by Friedwald formula. Plasma Total antioxidant capacity is measured on the basis of the antioxidants in the samples to reduce Fe^{3+} TPTZ to Fe^{2+}-TPTZ a, stable blue product proportional to the total antioxidants concentration which was measured at 593 nm [9]. GSH was estimated by 5, 5 dithiobis-2-nitrobenzoic acid (DTNB) method [10]. SOD was estimated by nitro blue tetrazolium (NBT) method [11]. MDA was estimated by Thiobarbutyric acid (TBA) method [12] Catalase (CAT) was estimated by Spectrophotometric method of L.Goth [13]. Serum Myeloperoxidase (MPO) was estimated by method of Matheson etal [14]. DNA damage in PBMC was assessed by single cell gel (SCG) comet assay (Singh N.P. Tice R.R; McCoy, MT) [15], a well developed method for assessment of DNA strand breaks in individual cell. Samples from the subset of subjects of study population (34 subjects with type 2 diabetes and 20 subject of normal control group) were taken for comet assay for assessment of DNA damage

Statistical analysis:

All the values are expressed as their mean± SD. Statistical comparisons were performed by analysis of variance (ANOVA) test. The comparisons between two parameters were performed by Student t –test. Relationship between continuous variables were assessed using Pearson's correlation coefficient analyzed by bivariate correlation method

RESULTS

Table 1 illustrates the baseline values of participants both type 2 diabetic and non diabetic normal subjects. Age, sex, BMI, fasting blood glucose level, and HbA1c, duration of diabetes and blood pressure are shown.

Age ,BMI, blood pressure , blood glucose and HbA1 c were higher in the diabetes group than the normal non diabetic group(table-1).By Independent samples t-test, blood glucose was significantly different between the nondiabetic group($p-<0.001$) and systolic blood pressure and HbA1c also showed significant differences between the nondiabetic and diabetes group ($p<0.05$)(table-1). The average levels of serum total cholesterol, LDL cholesterol and triglycerides were higher, while the average concentration of HDL cholesterol was significantly lower in type 2 diabetes when compared to normal subjects($p<0.05$)

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Table 1: Characteristics of study groups (Normal and Type 2 diabetes subjects)

	Healthy Subject (n=130)	Type 2 Diabetic Subject (n=240)
Age (years)	44.3±11.4	48.5±12.15
Sex (male/Female)	80/50	150/90
BMI (Kg/m^2)	22.4±3.3	26.6±4.2*
Systolic blood pressure (mmHg)	124.7±12.8	143.6±18.6*
Diastolic blood pressure (mmHg)	74.3±5.4	82.6±6.72
Fasting blood glucose (mg/dl)	93.3±8.4	178.6±78.3**
Duration of diabetes (years)	-	12.43±10.5
HbA1c (%)	4.9±0.8	7.8±2.1**
Serum cholesterol (mg/dl)	160.61±18.2	218.4±30.1*
HDL cholesterol (mg/dl)	49.41±7.8	40.43±8.8*
Triglycerides (mg/dl)	136.4±35.5	202.2±76.32*
LDL cholesterol l(mg/dl)	108.81±26.5	132.3±31.84*

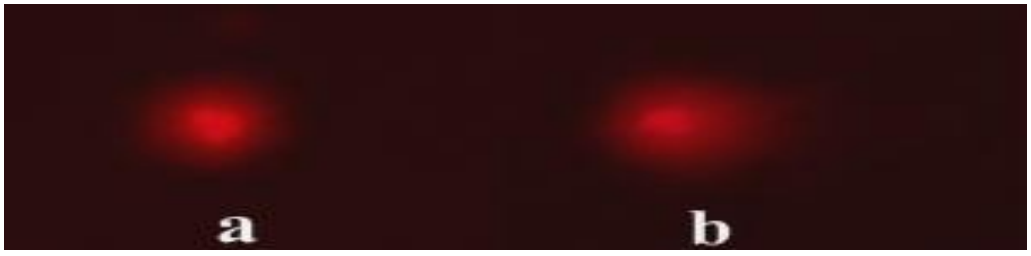
**Significant difference between normal and type 2 diabetic subjects (p value <0.001)
*significant difference between normal and control subjects (p-value<0.05)

Table 2: Oxidative stress, antioxidant status and DNA damage in type 2 diabetic subjects compared with non diabetic.

	Diabetic	Non diabetic
MPO (pMol/L)	178.3±54.33	130.92±61.7**
MDA(uMol/L)	2.95±1.53	1.56±0.712**
TAC(U/ml)	10.28±2.44	15.24±1.98*
SOD(U/ml	3.13±1.09	5.73±1.41**
GSH(mg/dl)	40.97±9.8	55.5±4.16**
CAT(U/ml)	44.7±11.3	56.38±10.8*
Tail DNA (%)	9.95±2.48	4.12±1.26*

All the values are mean ±SD,*Significant difference between normal and type2 diabetic subjects (p<0.05). ** Significant difference between normal and type 2 diabetic subjects (p <0.001)

Figure 1: Comparision between lymphocyte DNA fragmentation between Diabetic and healthy control group. (a-Control, b-Diabetic)



Oxidative stress parameters and DNA damage of type 2 diabetic and normal nondiabetic groups were shown in Table 2. Serum MPO level and MDA levels were significantly higher in diabetic group in comparison with the non diabetic normal subjects (p<0.001) and positively associated with degree of hyperglycemia and duration of diabetes. Serum Total antioxidants levels, antioxidant enzymes SOD, CAT and GSH levels were significantly decreased in type 2

diabetic subjects when compared to normal subject ($p<0.05$). There is a statistically negative correlation was found between FBS and TAC level ($r = -0.6832$), GSH ($r = -0.8322$), SOD($r = -0.7351$). In the comet assay for DNA damage subjects with type 2 diabetes had higher percentage of tail DNA (9.95±2.48) when compared with normal subjects (4.12±1.26) ($p<0.05$). (Table-2). Linear correlation analysis of the percentage of tail DNA showed significant positive correlations with BMI ($r= 0.31$, $p =0.032$) fasting blood sugar($r=0.432$, $P=0.002$).

DISCUSSION

Type2 diabetes mellitus is associated with endothelial dysfunction and oxidative stress. Chronic exposure to elevated glucose and hyper lipidemia can cause damage in different types of cells by variety of mechanisms. Lipids and proteins are the primary target of oxidative stress [16]. Lipid peroxidation of the cellular structures, a consequence of increased oxygen free radicals is thought to play an important role in atherosclerosis and micro vascular complications of diabetes mellitus [17]. Our study showed that there is increase in serum MDA concentrations in diabetic subjects which is in line with many previous studies. Increased non enzymatic glycosylation and auto oxidation of glucose is the possible mechanism in the excessive production of free radicals in diabetes mellitus which induce lipid peroxidation and increased MDA concentration. Noberasco G. et.al. has found a positive correlation between the MDA level and indices of glycemic control Fasting blood glucose and HbA1c level,[18] our studies also in line with their findings.

Myeloperoxidase (MPO) is a pro oxidant enzyme derived from leukocytes plays an important role in leukocyte- mediated vascular injury responses in inflammatory vascular diseases such as atherosclerosis.MPO produces hypochlorous acid (HOCL) from hydrogen peroxide (H_2O_2). HOCL then reacts with various amino acids and proteins to produce chloramines, such as chlorinated L-Arginine and chloro -tyrosine, and modified proteins such as modified lipoproteins. HOCL modified LDL are detected in animal and human atherosclerotic lesions [19-22]. Our study shows there is increase in the serum MPO level in Type 2 diabetes mellitus patients, in line with previous study by Wiersma JJ et.al. Indicate that MPO plays an important role in the initiation and progression of atherosclerosis in type 2 diabetes mellitus patients [23]. The decrease in the SOD activity in diabetes might be due to hyperglycemia which activates various biochemical pathways such as glucose auto oxidation, non enzymatic glycosylation of proteins and activation of protein kinase C, which in turn overproduce oxidants like superoxide and hydroxyl radicals as well as H_2O_2 [24], or the increase of glycosylated SOD that leads to inactivation of enzyme [25] or loss of its two cofactors Zn^{2+} and Cu^{2+}. This is in line with finding that in diabetic patients, as in healthy people, there is a close correlation between decreased SOD activity and loss of its two factors Zn^{2+} and Cu^{2+} [26-27]. The decrease of CAT as an inducible may be due to the decreased level of H_2O_2 generated by SOD [28]. Glutathione (GSH) participates in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen species. Thus the decrease in GSH level reflects a direct reaction between GSH and free radicals generated by hyperglycemia in diabetes mellitus [29].

Oxygen-free radicals induce a variety of lesions in DNA including oxidized bases, basic sites, DNA strand breaks and formation of cross links between DNA and proteins [30]. The comet assay, considered as a sensitive and reliable measure of DNA –strand breaks associated with incomplete excision repair and alkali-labile sites developed by Singh et.al. [15]. Subjects with type 2 diabetes had higher levels of percentage of tail DNA when compared with normal control subjects supported by previous study done by Fangfang Song et.al. They estimated the DNA damage in newly diagnosed type 2 diabetes found out that DNA damage is higher in type 2 diabetes subjects when compared with impaired glucose tolerance subjects and healthy control [31], suggesting that oxidative DNA damage in patients with type 2 diabetes mellitus .

CONCLUSION

Results of the present study showed that there were increased lipid peroxidation and oxidative DNA damage in type 2 diabetic individuals. Free radical formation along with antioxidant deficiency in type 2 diabetes mellitus increases over time due to prolonged hyperglycemic status and play an important role in the oxidative DNA damage and development of diabetic complications like diabetic nephropathy, retinopathy, neuropathy, peripheral arterial disease and atherosclerosis. Strict glycemic control along with antioxidant therapy can help to reduce the risk of developing diabetic complications

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