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RESEARCH ARTICLE

Oxidative stress and Lipid profile in Diabetic end stage renal disease

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Abstract

Long-term uncontrolled diabetes mellitus (DM) causes dyslipidemia and enhances the production of free radicals. Conditions leading to enhanced oxidative stress (OS) can produce a wide range of lipid disturbances. This study was intended to investigate the degree of association between DM and OS, to analyze their effect on lipid profile in diabetic End-Stage Renal Disease (ESRD) and to investigate the possible factors influencing the lipid status in diabetic ESRD. The study was conducted at RLJH, Kolar attached to SDUMC, Kolar, India. Three groups, G1 (control), G2 (diabetics without complication) and G3 (diabetics with ESRD) of 50 patients each were included in the study. Erythrocyte glutathione (GSH), plasma thiols and malondialdehyde (MDA) were measured to assess OS. Lipid profile, renal profile and blood glucose levels were measured using standard methods. Multiple logistic regression and student t-test were used to analyze the result. Statistically significant ($p<0.001$) increase of OS in diabetics with ESRD than those diabetics without any complications were observed. The results suggest a continuous accumulation and damage caused by OS in the target group. Dyslipidemia observed was due to the effect of OS on TC, LDL and MDA. On the other hand, hemoglobin, HDL and GSH were found to have a more protective role on the lipid status. Hence, it is suggested that GSH and MDA in erythrocytes and plasma thiols, can be an effective prognostic tool in assessing diabetic nephropathy and can also be used as a diagnostic tool in considering patients for early dialysis.

Keywords: End-stage renal disease, GSH, MDA, oxidative stress, multiple logistic regression.

Introduction

Diabetes mellitus is a multi-factorial metabolic disorder which has been of immense interest among the researcher fraternity, health-care professionals and general population. The availability of sophisticated diagnostic tools and the emergence of newer treatment have surprisingly failed to curtail the prevalence and incidence of this disorder in most of the developing countries. Along with the increase in the incidence of diabetics, there has been a significant rise in the rate of morbidity and mortality especially among diabetics with complications. One of the complications in diabetes that is prevalent worldwide is coronary artery disease, which has assumed epidemic proportions in India especially in the rural population. Among the rural population of South India, the prevalence has increased from 2.3% in 1992 to about 12.4% in 2007 (Ramachandran *et al.,* 1992; Patandin *et al.,* 1994). The higher prevalence of insulin resistance among Indians seems to be the most plausible mechanisms responsible for the metabolic and lipoprotein abnormalities resulting in higher prevalence and mortality from coronary artery disease (Prasanna Kumar, 2006). Glycosylation occurs both on the apo-proteins and phospholipid components of lipoproteins, resulting in profound functional alterations and increasing their susceptibility to oxidative modification which is considered to be a critical step in atherogenecity.

Studies have suggested that, diabetes mellitus and its complications along with free radicals have a common pathway. Being closely related they can mutually accelerate each other (Prasanna Kumar, 2006). The combined impact of glycation and oxidation on lipoproteins, especially LDL, generates products that are more atherogenic than either glycosylated or oxidized LDL alone (Prasanna Kumar, 2006). It is known that long term uncontrolled diabetes mellitus causes dyslipidemia and also enhances the production of free radicals. We also know that conditions leading to enhanced oxidative stress can produce a range of lipid disturbances. However, there are not many studies establishing, lipid status in conjuncture with oxidative stress in diabetic complications. In this study, we intended to investigate the level of association between diabetes mellitus and oxidative stress and to analyze the effect on lipid profile parameters in the study group. The study also investigates the changes in the lipid profile in response to a rising levels of OS in the study group and finally we intended to identify the possible factors influencing the alteration in lipid status, if there was any.

Materials and methods

Sample collection: This prospective study was conducted at Sri RL Jalappa Hospital and Research Center, Kolar, India, a rural tertiary care center.

The study was performed in accordance with the regulation and guidelines of the ethical committee of Sri Devaraj Urs Medical College, Kolar, India. All chemicals used in the experiments were of analytical reagent grade (AR).

Experimental design: The control group (Group 1) consisted of 50 normal, healthy, age and sex matched individuals, the second group (Group 2) consists of 50 patients presenting with diabetes mellitus (diagnosed as per the criteria proposed by ADA, 2005) without any complications and no other concurrent disease or illness and aged between 30 to 60 years. The third group (Group 3) consisted of 50 patients, clinically and biochemically diagnosed diabetic ESRD patients as per the literature criteria (Teitz, 2005) posted for hemodialysis, with no other concurrent disease or illness and on no other medication apart for the disease under study at RL Jalappa hospital and research center.

Exclusion criteria: Pregnant and lactating women, smokers, alcoholics and obese individuals were excluded from the study groups. Individuals with any history of recent surgery or any other illness not related to diabetes were also excluded.

Method of sample collection: A detailed personal history was taken from subjects in group 1 and for group 2 and group 3 a complete case history was taken.

- Venous sample (5 mL) was taken after overnight fast form groups 1 and 2. From group 3, 5 mL of overnight fasting sample, immediately before hemodialysis.
- Of the 5 mL of blood sample, 2.5 mL was collected in vacutainers containing EDTA as anticoagulant for analysis of oxidative stress parameters and the remaining 2.5 mL in plain vacutainers for analyzing lipid profile, renal profile and glucose levels.

Preparation of sample for analysis: Blood sample from the anticoagulant containing vacutainer was centrifuged at 3000 rpm for 10 min, supernatant plasma was aspirated and stored in alliqot tubes to assay thiol groups. The buffy coat was discarded. The packed cells were suspended in equal volume of cold phosphate buffer saline and re-centrifuged. The supernatant was discarded. The washing of packed cells was repeated twice, the packed cells were used for analysis of GSH and MDA (Jain *et al.,* 1989) and the thiol levels were estimated in plasma. The blood sample from plain vacutainers was centrifuged at 3000 rpm for 10 min; the serum was aspirated and was further used for analysis of blood glucose, Lipid profile and renal profile. All of these parameters were analyzed by Auto analyzer using standard methods adopted in the clinical laboratory.

Estimation of GSH in erythrocytes membrane: The role of GSH, especially in RBC's has been of immense interest in the study of oxidative stress status. The reason being that most of the enzymes for which GSH is an important factor are present in erythrocytes. The levels of GSH present in blood are proportional to those present in various other tissues, like hepatic, brain, renal etc., and a high proportion of these are present in erythrocytes. Hence an estimate of GSH levels in Erythrocytes was considered as a good measure of oxidative stress (Beutler *et al.,* 1963). The major non-protein sulfhydryl groups of RBC's are in the form of reduced GSH. This RBC membrane glutathione is estimated using 5,5' dithiobis 2-nitrobenzoic acid (DTNB) as a disulfide chromogen that is readily reduced by sulfhydryl compounds to an intensely yellow compound (Ellman, 1959). The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to GSH concentration.

Estimation of MDA in erythrocytes membrane: Free radical activity is determined indirectly by measuring lipid peroxidation products on the erythrocyte membrane. MDA is formed from the breakdown of polyunsaturated fatty acids serves as an index for determining the extent of peroxidation. MDA reacts with Thiobarbituric acid (TBA) to give a pink chromogen, which can be read at 532 nm and is estimated as TBAR's (Jain *et al.,* 1989).

Estimation of serum protein thiols: Total serum protein thiol, included all the free SH groups containing protein in the serum, it was measured colorimetrically using dithionitrobenzoic acid (DTNB) (Ellman, 1959).

Estimation of hemoglobin, lipid and renal profile parameters: Serum total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, creatinine, urea and hemoglobin were estimated. All of these parameters were estimated by standard methods used in the clinical laboratory.

Statistical analysis: From the data obtained, the mean of different parameters were compared among the three groups using student t-test. Multiple logistic regression was used to analyze the factors affecting the lipid status. The values of all the parameters were statistically analyzed using SPSS 15 software.

Results

A comparison between the mean values of parameters between group 1 with 2, group 1 with 3 and group 2 with group 3 was done using independent 'T' test for renal profile and oxidative stress parameters as shown in Table 1, 2 and 3. The results of multiple logistic regression were shown in Table 5. The analysis showed that the comparative study for renal profile among the groups, were statistically significant with a p value of <0.001. A similar analysis was done for GSH, MDA and thiol values between the groups (Table 1-4), which suggest a statistically significant comparison among them with p value of <0.001.

Table 1. Mean and standard deviation of oxidative stress parameters among study groups.

Groups	Glutathione (mg/g of Hb) Mean ± SD	MDA (n moles/g of Hb) Mean ± SD	Thiol (μ moles/L) Mean ± SD
Group 1: Control	12.06 ± 1.96	9.02 ± 1.6	307.69 ± 44.5
Group 2: Diabetic	7.66 ± 1.6	11.73 ± 2.6	260.8 ± 36.9
Group 3: Diabetic ESRD	3.33 ± 0.82	16.40 ± 4.3	217.3 ± 57.14

Table 2. Comparative values of mean, standard deviation, and level of significance for oxidative stress parameters between study groups.

Groups	Glutathione (mg/g of Hb) Mean ± SD	P value	MDA (n moles/g of Hb) Mean ± SD	P value	Thiol (μ moles/L) Mean ± SD	P value
Group 1: Control	12.06 ± 1.96	<0.001	9.02 ± 1.6	<0.001	307.69 ± 44.5	<0.001
Group 2: Diabetic	7.66 ± 1.6		11.73 ± 2.6		260.8 ± 36.9	

Table 3. Comparative values of mean, standard deviation and level of significance for renal profile and oxidative stress parameters between study groups.

Groups	Urea (mg/dL) Mean ± SD	P value	Creatinine (mg/dL) Mean ± SD	P value	Glutathione (mg/g of Hb) Mean ± SD	P value	MDA (n moles/ g of Hb) Mean ± SD	P value	Thiol (μ moles/L) Mean ± SD	P value
Group 1	25.4 ± 6.68	<0.001	0.79 ± 0.14	<0.001	12.06 ± 1.96	<0.001	9.02 ± 1.6	<0.001	307.6 ± 44.5	<0.001
Group 3	114.4 ± 13.4		7.284 ± 1.4		3.33 ± 0.82		16.40 ± 4.3		217.3 ± 57.14	

Table 4. Comparative values of mean, standard deviation and level of significance for renal profile and oxidative stress parameters between study groups.

Groups	Urea (mg/dL) Mean ± SD	P value	Creatinine (mg/dL) Mean ± SD	P value	Glutathione (mg/g of Hb) Mean ± SD	P value	MDA (n moles/ g of Hb) Mean ± SD	P value	Thiol (μ moles/L) Mean ± SD	P value
Group 2	32.94 +/- 7.0	<0.001	0.928 +/- 0.26	<0.001	7.66 +/- 1.6	<0.001	11.73 +/- 2.6	<0.001	260.8 ± 36.9	<0.001
Group 3	114.46 ± 13.4		7.284 ± 1.4		3.33 ± 0.82		16.40 ± 4.3		217.3 ± 57.14	

Table 5. Multiple logistic regressions (final model).

Parameters affecting the lipid profile status	P value	Odds ratio	95% CI
TC	0.0001	1.06	1.035, 1.085
TG	0.006	1.056	1.016, 1.097
LDL	0.017	1.256	1.041, 1.514
Hb	0.001	0.632	0.482, 0.831
GSH	0.002	0.713	0.576, 0.883
MDA	0.004	1.238	1.072, 1.430
HDL	0.015	0.799	0.667, 0.958

Discussion

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to either insufficiency of secretion or action of endogenous insulin. Although the etiology of diabetes is not well defined, genetic components, viral infection, autoimmune disease and environmental factors have been implicated in this disease. Diabetes has become a leading cause of blindness, ESRD, atherosclerosis and a variety of debilitating neuropathies (Ramachandran *et al.,* 1992; Patandin *et al.,* 1994).

Almost half of those diagnosed as diabetic before the age 31, die before they reach 50, largely as a result of cardiovascular or renal complications (Deckert *et al.,* 1978). ESRD is one of the eventual late renal complications in diabetes, having high morbidity and mortality rates. Joshi (2005) have shown that among ESRD patients, 42-45% was found to be as a consequence of diabetes. Michael Browniee (2001) have shown that in pathophysiology of diabetes and its complications, free radicals plays a major role either in the formation of advanced glycated end products (AGE) or through increased polyol pathway or increased activation of protein kinase C or through flux of glucose into hexosamine pathway or either any of the above in combination. All of the above pathways generate various free radicals, which eventually leads to oxidative stress (Michael Browniee, 2001). There are a good number of studies documenting free radical induced oxidative stress injury in diabetic patients. However, studies on cases with raised oxidative stress in late diabetic complication, particularly diabetic ESRD has been scarcely done.

Hyperlipidemia has been well documented in diabetes mellitus, but its pattern in diabetic complications like diabetic ESRD are studied less. Hence, it was found worthwhile to evaluate the behavior of dyslipidemia in diabetic ESRD, thereby assessing its predictive value for a cardiovascular event. In this study, two control groups of 50 each were considered, one with normal healthy individuals (group 1) and the other comprising of diabetics with no systemic or metabolic complications. The above mentioned two groups were compared with the third group of 50 individuals clinically diagnosed of diabetic ESRD. A thorough assessment of all the groups were made to exclude the confounding factors mentioned in the exclusion criteria. The present study shows that in group 1, the mean glucose, renal and lipid profile parameters were all in the normal limits for the method used. Oxidative stress parameters which included Glutathione (GSH), Malondialdehyde (MDA) and thiols, were found to be in the normal range, as proposed in the studies done by Joshi (2005) and Prakash *et al.* (2004). In group 2, which included diabetic patients with normal renal profile, they showed an increase in the mean serum glucose levels in comparison with group 1.

On investigation, it was suggestive that poor dietary habits and/or poor compliance to treatment by the patients could be the reason. The mean values of urea and creatinine analyzed were within the normal range. The measure of oxidative stress parameters in group 2, suggests a significant decrease in GSH ($p<0.001$), a considerable increase in MDA levels ($p<0.001$) and plasma thiol levels was also significantly decreased ($p<0.005$) when compared to with normal's, which concurred with other studies (Margus *et al*., 2001). A careful selection of cases was done to eliminate major factors that influence the oxidative stress parameters like, smoking, alcoholism and antioxidant therapy in all the groups. Reports have suggested that increased oxidative stress as result of increased ROS generation and fall in the levels of antioxidants can structural and functional damage of β-cells of pancreas (Wolff, 1993).

The measure of lipid profile in group 2 patients suggests, a significant increase in total cholesterol ($p<0.005$), triglycerides ($p<0.005$) and LDL ($p<0.005$) levels along with a significant decline in HDL ($p<0.005$) which confirms the literature evidence. The altered lipid status among the individuals in group 2 is suggested due to glycation of phospholipids and apoprotein resulting from chronic hyperglycemia. The oxidative stress parameters measured in group 3 have shown a highly significant reduction in Glutathione ($p<0.005$) levels in comparison with group 1, along with a significant increase in lipid peroxidation as suggested by MDA levels ($p<0.005$) and a significant increase in plasma thiols. All of these values observed were in accordance with previous studies (Veronique *et al.,* 1996; Toshio *et al.,* 1997; Paik-Seong *et al*., 1999).

This suggests of enhanced oxidative stress in this group probably influenced by chronic hyperglycemic status (Toshio *et al.,* 1998) and as a result of uremia (Toshio *et al.,* 1997). Chronic uremia is a state of increased oxidative stress, as suggested by increased lipid peroxidation which was directly assessed by measuring erythrocytic malonedialdehyde (Tatsuya *et al.,* 2003) along with increased ratio of oxidized glutathione to reduced glutathione (Canestrari *et al.,* 1994). Miyata *et al.* (2006) have postulated in their study that in uremia, there exist factors catalyzing the formation of AGE's (Toshio *et al.*, 1997) and these AGE products generate H_2O_2, reactive oxygen intermediates and ketoaldehydes which contribute to alteration of proteins (Veronique *et al.*, 1996). It is suggested that in patients on maintenance hemodialysis, in whom blood interaction with bio-incompatibile membranes, triggers circulating neutrophils to produce reactive oxygen species, including superoxide anions, hydrogen peroxide, hydroxyl radical and hypochlorous acid (Nguyen *et al.,* 1985). There are also evidence that suggest cells of renal structures are capable to produce and secrete ROS in presence of various stimulating factors like, acute hypertension, radiation exposure, hyperoxia and effect of various drugs. In addition, circulating infiltrating cells, which are present in many inflammatory renal processes, are capable to produce large amount on ROS (Basilla, 2003). The study could not associate any specific pattern of lipid dysfunction in group 3. However, the results convincingly suggest that there are factors that could affect the lipid status which concurred with a study done by Kimoto *et al*. (2002). Raised oxidative stress, uremia, altered dietary patterns, frequent hospitalization and hemodialysis could explain the inconsistency in lipid profile in group 3. Hemoglobin, GSH, Thiols and HDL have a protective influence, whereas, LDL, MDA, TC, and TG have a destructive effect on the plasma lipoproteins. Thus, observations made from this study suggest a defined inter-relation between oxidative stress parameters and uremic status in diabetic ESRD patients. And the influence on the lipoprotein is not predictable. Hence, in chronic diabetic patients, who are manifesting renal insufficiency, it is worthwhile to investigate oxidative stress parameters as it might warrant as an early indicator for the need for hemodialysis. Further a measure of lipid profile might not prove as a reliable indicator for predicting CAD in patients with diabetic ESRD.

Conclusion

The study emphasize that GSH, MDA and Thiol levels were significantly altered in diabetics when compared to normal suggesting the role of uncontrolled hyperglycemia as a cause and consequence of oxidative stress. And prolonged uncontrolled diabetes, associated late complications like diabetic ESRD had a significantly increased measure of oxidative stress, when compared to the cases of uncomplicated diabetes.

This suggests that the ongoing production and damage caused by reactive oxygen species, had a strong relationship with diabetic status of an individual. The study also shows an inconsistent pattern of lipid profile among diabetics with ESRD, suggesting an ambiguity in its use to predict CAD. Erythrocyte GSH, MDA and plasma thiols along with renal profile and GFR measure can be considered as a good index of the progression in pathophysiology of diabetic nephropathy, thus providing a better diagnostic spectrum, in considering the patients for early hemodialysis.

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