RESEARCH ARTICLE

Oxidative stress in patients with diabetic nephropathy

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ABSTRACT

Background: Diabetic nephropathy (DN) is currently the leading cause of end-stage renal disease globally. Oxidative stress which is classically defined as an event resulting from the magnitude of imbalance between oxidant and antioxidant substances, generated in a setting of oxidation-reduction reactions, and is hypothesized to play a role in the development of diabetic nephropathy. Aim and Objectives: The aim of the study was to assess lipid peroxidation by estimating serum malondialdehyde (MDA) and antioxidant status by assaying paraoxonase-1 (PON-1) in diabetes patients with nephropathy and healthy controls. Furthermore, the study aimed the correlation between MDA and PON-1 levels in patients with diabetic nephropathy.

Materials and Methods: A cross-sectional comparative study was conducted in 152 participants, which were divided into two groups as control (n = 76) non-diabetic, healthy, age-, and sex-matched individuals and diabetic patients with nephropathy(n = 76). The study was conducted in Government Medical College, Kozhikode. All the subjects who satisfied the inclusion and exclusion criteria and who gave informed consent were included in a consecutive manner till sample size is achieved. Serum MDA and PON-1 were estimated using spectrophotometry. The data were analyzed using statistical package for the social sciences (SPSS) version 18.

Results: Oxidative stress was increased in diabetic nephropathy patients as evidenced by significantly elevated MDA and reduced PON-1 than the normal controls. There was a significant negative correlation of serum MDA with serum PON-1 in patients with diabetic nephropathy.

Conclusion: Oxidative stress is an important pathophysiological process for the development of diabetic nephropathy. This study reveals the importance of screening all diabetes patients for oxidative stress. Dietary management and antioxidant supplementation would help them to prevent development of diabetic nephropathy and related complications, which, in turn, improve their quality of life.

KEY WORDS: Oxidative Stress; Malondialdehyde; Paraoxonase 1; Diabetic Nephropathy

INTRODUCTION

Diabetic nephropathy is one of the major complications associated with diabetes mellitus. About 40% of diabetes mellitus (DM) patients are affected with diabetic nephropathy which is one of the leading causes of chronic kidney disease (CKD) and end-stage renal disease (ESRD) especially in high- and middle-income countries. Among majority of patients, diabetic nephropathy remains undiagnosed for many years. The treatment of DN is mainly aimed to stop or delay the disease progression. In present scenario, the mainstay of management for DN includes glucose control and blood pressure control. The percentage of DN patients who progress to ESRD has not been substantially declined. Even though much progress has been made in decreasing diabetes-related mortality and delaying the development of kidney disease from DM. Disappointingly, there has been no progression in the development of new drugs with success
in Phase 3 clinical trials for DN. The main reason being the lack of proper understanding of the pathophysiology of human DN development and progression. Hypothesis driven researches targeting single molecules and pathways or both which play an important factor in development and progression of DN which can lead to newer interventions in the management were very less in the past few decades. The present cell culture and animal models mainly show the early stage features of human DN and fail to reproduce the whole process involved in development and progression of DN.[1]

At present, oxidative stress has emerged as a significant novel objective for DN. It is traditionally defined as an imbalance between antioxidant and oxidant substances, produced as a result of oxidation-reduction framework. The authors presently utilize the expression “imbalance of redox system” to allude to the oxidative stress. In general, called as free radicals, oxidants comprise reactive oxygen species (ROS) and reactive nitrogen species (RNS), which carry out the oxidation of lipids (lipoxidation) and glucose (glycation).[2] Human body is enabled with an exceptionally complex antioxidant framework, which works synergistically with one another, to secure the cells, tissues, and organ systems of our body against harmful free radical damage. The term antioxidant alludes to any particle which is able to do either balancing out or neutralizing free radicals before they cause cell damage. The antioxidants can be exogenous as dietary supplements or endogenous. These antioxidant agents assume a significant role in keeping up ideal cellular functions and, hence, fundamental well-being and prosperity of a person.[3]

As oxidative stress occurs as a result of imbalance between the amount of oxidant and antioxidant substances, serum malondialdehyde (MDA) can be used to assess the levels of oxidative stress and serum paraoxonase (PON-1) for antioxidant status in this study.

Lipid peroxidation is the process by which free radicals damage lipids. The decay of lipid peroxides in presence of iron or copper gives rise to cytotoxic compounds such as malondialdehyde (MDA) and hydroxynonenal (NNE). These compounds lead to chemical change in cellular phospholipids, proteins, and DNA. Therefore, MDA is a significant marker utilized for evaluating lipid peroxidation.[4,5]

An antioxidant is defined as any substance that forestalls the process of oxidation or the substance that restores the damage caused due to the activity of oxidants in a living organism. A polymorphic protein enzyme named paraoxonase, located on chromosome 7 has three gene families, PON-1, PON-2, and PON-3. Human serum paraoxonase-1 (PON-1) is synthesized by liver and get released into blood. The enzyme combines with HDL in blood. It acts as an antioxidant that hydrolyzes the peroxides in the oxidized lipids in both LDL and HDL.[5]

MATERIALS AND METHODS
This is a comparative cross-sectional study that was done at Government Medical College, Kozhikode. The study was conducted after getting the Institutional Ethics Committee clearance. It was conducted for a period of 1 year. A total of 76 clinically diagnosed patients with diabetic nephropathy and 76 age- and sex-matched normal healthy subjects were included in the study. The participants were selected from the Nephrology Outpatient Department, hospital staff, and the patient bystanders at Government Medical College, Kozhikode. The mean age of participants was 51.26 years ranging from 35–65 years. For selecting participants in this study, DM was diagnosed on the basis of classical symptoms, fasting blood sugar (FBS), and postprandial blood sugar (PPBS) values according to the American diabetic associations diagnostic criteria 2015. Nephropathy was diagnosed using investigations including urinary albumin to creatinine ratio >30 mg/g and estimated glomerular filtration rate < 30 mL/min per 1.73 m² found out using CKD-EPI 2009 formula.[4] History of cardiovascular diseases, liver diseases, malignancy, infective diseases, habit of alcoholism, and smoking was considered as exclusion criteria. An informed written consent was taken from each participant before the study so that voluntary participation can be ensured. Detailed history of each participant was taken with the help of a proforma. The participants were subjected to clinical examination after history taking. Following clinical examination, blood samples were collected for investigations.

Estimation of Serum Malondialdehyde (MDA)
MDA usually exists as a highly reactive compound enol. It is the most commonly used indicator for lipid peroxidation. Estimation of serum MDA was done using the thiobarbituric acid test based on Pasha and Sadasivadu’s procedure.[6] The thiobarbituric acid reacts with MDA and produces a colored product. This product can absorb light at 530 nm. The results of the test were expressed in nmol/dL. Normal value ranges from 70 to 110 nmol/dL.

Estimation of Paraoxonase-1 Activity
Estimation of serum PON1 activity was done using automated microtiter plate method developed by Rel Assay Diagnostics. Paraoxonase enzyme reacts with tris buffer which contain calcium ion. It acts as a cofactor for PON1 enzyme to produce a colored product p-nitrophenol. The p-nitrophenol can absorb light at 412 nm at 37°C.

The unit of expressing PON-1 activity was international units (IU). One IU was defined as 1 micromol of p-nitrophenol produced/min/L at 37°C. Monitoring of hydrolysis of the paraoxonase (diethyl-p-nitro phenylphosphate) was done by follow-up of the increase of absorbance at 37°C and 412 nm. The molar absorption coefficient 18,920 M⁻¹cm⁻¹ (at pH 8)
is used for calculating the amount of p-nitrophenol. For estimating the PON-1 activity, the mean absorbance has to be determined. For this, kinetic measurement was taken immediately at every minute for 5 min at 412 nm in 37°C in microplate spectrophotometer.[6] Then, a graph is plotted using 1 micromol P-nitrophenol as standard. The results are expressed as International Units/Liter. One IU/L is considered to be equal to hydrolysis of 1 micromol substrate in 1 min and in 1 L.[6]

Statistical Analysis

This study compares the oxidant-antioxidant status (MDA, PON-1) between Type 2 diabetic nephropathy patients and normal healthy subjects. Data were entered into Excel sheet and statistical analysis was done using SPSS (statistical package for the social sciences) version 18. Continuous variables in the study were expressed as Mean ± SD. The mean value was calculated by summing up all the individual values in the group and dividing it by number of subjects in that group. To test for statistical significance of mean differences between the groups, independent non-parametric tests (Kruskal–Wallis Test) were used. For all statistical tests, $P \leq 0.05$ was taken as level of significance.

RESULTS

Serum MDA level was significantly increased in diabetic nephropathy patients compared to healthy controls ($P < 0.001$ [Table 1]. Serum PON-1 was significantly reduced in diabetic patients with nephropathy compared to healthy controls ($P < 0.001$) [Table 2]. There was a significant negative correlation of serum MDA with serum PON-1 in patients with diabetic nephropathy [Figure 1]. The above observations indicate that there is enhanced oxidative stress and decreased antioxidant defense in diabetic nephropathy patients with the mean age of 51.26 years ranging from 35 to 65 years. This can cause potential oxidative injury in the cells leading to easy progression of DN patients to ESRD.

DISCUSSION

This study was aimed at comparing the oxidant-antioxidant status (serum MDA, PON-1), among diabetic nephropathic patients, healthy controls, and also at finding the correlation of oxidant – antioxidant levels in diabetic nephropathy patients. In this study, serum MDA in patients with diabetic nephropathy was significantly elevated compared to healthy controls. The study also observed that the level of serum PON-1 was significantly reduced among patients with diabetic nephropathy. The mean value of malondialdehyde (nmol/dL) among the DN group was found to be 247.95 ± 85.97 and it was 89.16 ± 10.95 among the healthy controls. The mean value of paraoxonase (IU/L) among DN group was 146.23 ± 80.61 and that of healthy controls was found to be 360.04 ± 24.66. The mean difference of serum MDA levels and serum PON-1 levels between the groups was statistically significant ($P < 0.001$).

This study revealed that there is an increased level of oxidative stress with increased production of reactive oxygen species among patients with DN. This leads to decreased level of antioxidant enzyme among them. Another study conducted by Gawde et al. in 2015 among 40 diabetic nephropathy patients, 69 diabetic non-nephropathic patients, and 40 healthy controls, observed a significant fall in serum PON-1 activity in diabetic group as well as in diabetic nephropathy group as compared with normal healthy control group, with greater reduction in diabetic nephropathy patients.[7] Suvarna et al. in her study on “paraoxonase activity in Type 2 diabetes mellitus patients with and without complications” concluded that patients with...
Type 2 DM complications have reduced level of HDL-C and PON1 activity, which was statistically significant. This result indicates their decreased biochemical roles in these patients. Another study by Seres et al. reported that there was a progressive decrease in PON-1 activity among elderly patients of their study. They also observed that this decrease can be due to the oxidative stress which develops with aging. A study done by Mohamed et al. observed a significantly reduced level of PON1 activity among diabetic patients with and without nephropathy compared to the control group. They also observed that patients with diabetic nephropathy had more significant decreased level of PON-1 activity. Another study by Varma et al. reported that reactive oxygen species (ROS) produced in hyperglycemia can increase the peroxidation of lipids of cell membrane. The ROS can also cause increase in oxidation of proteins that yield protein carbonyl derivatives. This will lead to production of the high level of MDA in the diabetic nephropathy subjects which indicate oxidative stress in long standing Type 2 diabetes. Bigagli et al. reported that in their study, there was a significant negative correlation between serum paraoxonase and serum malondialdehyde levels in diabetic and diabetic nephropathy patients. The study, hence, concluded that increased level of oxidative damage represents an underlying mechanism of glucose toxicity in T2DM and its related micro- and macrovascular complications.

Diabetic nephropathy (DN) is a significant microvascular complication of diabetes mellitus which represents 30–47% instances of end-stage renal disorders. Hyperglycemia prompted vascular dysfunction is the major pathology in diabetic nephropathy. Initial phases of DN are characterized by microalbuminuria which is present even when there is not yet evidence of abnormal glomerular filtration. On progression, microalbuminuria advances to a broad proteinuria that is macroalbuminuria. Given the expanding frequency of diabetes, numerous specialists hold the view that in the long run, DN will also progress toward pandemic proportions. About 20% of the individuals who need kidney transplantation or dialysis in India usually have diabetic nephropathy. Progression of diabetic nephropathy is driven by a heterogeneous factors, including inflammation, fibrosis, and oxidative stress. Oxidative stress plays an important role in cell–injury due to hyperglycemia and hence plays a significant role in the pathogenesis of diabetic nephropathy. The activity of reactive oxygen and nitrogen species (ROS/NS), which are by-products of the diabetic milieu, has been found to correlate with pathological changes observed in the diabetic kidney. Oxidative stress is defined as a disturbance in the equilibrium between free radicals (FR), reactive oxygen species (ROS), and the endogenous defense mechanisms. Hence, it can be considered as the disturbance in the balance between oxidant-antioxidant states that result in increased production of oxidant species. The human body needs both oxidant and antioxidant species for normal metabolism, signal transduction, and regulation of cellular functions. Therefore, a state of homeostasis needs to be maintained between the oxidant and antioxidant species. Oxidative stress results in damage of almost all the major cellular components such as proteins, DNA, and membrane lipids ultimately lead to cell death. Mitochondria generates ROS and plays a key role in apoptotic cell death in the diabetic kidney. Oxidative stress has also been associated with necrosis through induction of mitochondrial permeability transition. Thus, it can be concluded that the development of diabetic nephropathy can be delayed by treatment methods which stabilize oxygen metabolism and regulate oxidative stress. In diabetic nephropathy, there is excessive formation of free radicals, which, in turn, decrease the activities of antioxidant enzymes. MDA is one among the reactive electrophile species which can cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products (ALE), in analogy to advanced glycation end-products (AGE). The production of MDA is considered as a biomarker for measuring the level of oxidative stress. Malondialdehyde reacts with deoxyyadenosine and deoxyguanosine in DNA, forming DNA adducts, the primary one being M1G (pyrimido[1,2-a]purin-10(3H)-one), which is mutagenic finally leading to cell destruction. Therefore, MDA is used as an index marker of oxidative stress. An ideal antioxidant should be readily absorbed and quench free radicals and chelate redox metals at physiologically relevant levels. The ideal antioxidant should also work in both aqueous and/or membrane domains and effect gene expression positively. An important role in maintenance of optimal cellular functions is played by the endogenous antioxidants and thus leading to systemic health and well-being. Whenever there is an increased oxidative stress, these endogenous antioxidants would not be sufficient and exogenous antioxidants from diet may be required for maintaining optimal cellular functions. Paraoxonases are a family of three enzymes called PON-1, PON-2, and PON-3. Among the three enzymes, PON-1 is the most studied one. It is synthesized in the liver and appears mainly in serum. It is seen associated to high-density lipoproteins (HDL) in the serum. They are multifunctional enzymes which play crucial role in many biochemical pathways such as protection against oxidative damage and lipid peroxidation, contribution to innate immunity, detoxification of reactive molecules, bioactivation of drugs, modulation of endoplasmic reticulum stress, and regulation of cell proliferation/apoptosis. As they are able to perform multiple autonomous and often unrelated functions, they are also named as “moonlighting proteins.”

One limitation of this study is that it did not assess any markers of oxidative stress other than serum MDA and PON-1 for comparing the oxidant-antioxidant status. Assessment of other markers along with MDA and PON-1 and inclusion of a larger sample size can give more conclusive and accurate results.
CONCLUSION

At present, India has the dubious distinction of being the diabetes capital of the world. In parallel with the increase in diabetes, a dramatic increase in the prevalence of diabetic nephropathy has been noted. An increased level oxidative stress and decreased antioxidant defense were observed among diabetic nephropathy patients. This will lead to remarkable oxidative injury in the cells, which will result in progression of DN patients to ESRD and the development of various complications such as carotid artery stenosis, coronary heart disease, stroke, and peripheral vascular disease, which is the major cause of mortality rather than ESRD for patients with diabetic nephropathy. Our study on oxidative stress in diabetic nephropathy patients can provide some insight about the susceptibility profile of diabetic nephropathy patients to oxidative stress. Moreover, any association of the difference in serum levels of paraoxonase-1 (PON-1) and malondialdehyde (MDA) may give future direction toward management of diabetic nephropathy in our population. Screening for oxidative stress among diabetic nephropathy patients can be conducted at health centers as a part of health program for prevention and control of non-communicable diseases. Antioxidant supplementation reduces the effects of oxidative damage. Hence, diabetic nephropathy patients should be advised to follow a diet rich in antioxidants and micronutrients. Researches should be done for understanding the exact pathophysiologic pathways of oxidative stress in diabetic nephropathy that leads to its progression. This would help to prevent the patient from progressing to the end stage renal disease, and reverse the condition; thus reducing mortality and morbidity along with improvement in the survival rate.

REFERENCES


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