ORIGINAL ARTICLE

ROLE OF REACTIVE OXYGEN SPECIES IN PATHOGENESIS OF NEPHROTIC SYNDROME

S R Ghodake, A N Suryakar*, R D Ankush* K Shaikh and A V Katta

Department of Biochemistry, KBN Institute of Medical Sciences, Gulbarga (Karnataka), India.

*Dr. V M Govt. Medical College, Solapur, Maharashtra.

ABSTRACT

Nephrotic syndrome is the common chronic disorder characterized by alteration of permeability of the glomerular capillary wall, resulting in its inability to restrict the urinary loss of proteins. Nephrotic syndrome is characterized by heavy proteinuria, hypoalbuminemia, hyperlipidemia associated with peripheral edema. The molecular basis of glomerular permselectivity remains largely unknown. In recent years it has been proposed that Nephrotic syndrome is a consequence of an imbalance between oxidant and antioxidant activity. The present study was aimed to test that the reactive oxygen species are the mediators of excessive protein permeability and other complications of Nephrotic syndrome. For this 30 adults with Nephrotic syndrome were studied. The control group comprised 30 healthy adults matched for age. Serum levels of lipid peroxides, nitric oxide (NO•), α -tocopherol, ascorbic acid, erythrocyte superoxide dismutase activity, serum albumin, uric acid, cholesterol and plasma total antioxidant capacity were measured. Student's 't' test was applied for statistical analysis. There was a significant increase in lipid peroxide (1.58 ± 0.42 in controls, 3.64 ± 1.3 in patients) ($P < 0.001$) levels in study group as compared with controls. α -tocopherol (12.95 ± 1.04 in controls, 9.93 ± 1.43 in patients) ($P < 0.001$), erythrocyte SOD activity (1.88 ± 0.9 in controls 1.07 ± 0.5 in patients) ($P = 0.01$), serum albumin (4.06 ± 0.50 in controls, 3.04 ± 0.11 in patients) ($P < 0.001$), and plasma total antioxidant capacity (847.33 ± 126.83 in controls, 684.00 ± 102.94 in patients) ($P < 0.001$) were significantly decreased. There was non-significant increase in uric acid ($P > 0.05$), a non-significant decrease in NO• (38.48 ± 15.47 in controls 37.47 ± 14.27 in patients) ($P > 0.05$) and ascorbic acid levels ascorbic acid, (0.95 ± 0.31 in controls 0.79 ± 0.30 in patients) ($P > 0.05$) in study group as compared with controls. Imbalance between oxidants and antioxidants may contribute to pathogenesis of proteinuria and related complications in nephrotic syndrome.

KEY WORDS

Nephrotic syndrome, Lipid peroxide, Antioxidants, Erythrocyte Superoxide dismutase.

INTRODUCTION

Nephrotic syndrome (NS) is characterized by heavy proteinuria and hypoalbuminuria, is an important chronic disorder. Edema is the chief clinical manifestation, which may vary from mild periorbital puffiness to anasarca (1).

The basic functional defect in NS is an increased permselectivity of the glomerular filtration barrier (GFB) to molecules that ordinarily would not be filtered. Numerous factors affect the filtration of molecules, such as properties of molecules themselves, the properties of the filtration barrier, and hemodynamic factors. Properties of molecules themselves include the size and the charge of the molecule. The GFB has a negative charge imparted to it by a heparin sulfate proteoglycan that retards the transport of negatively charged molecules across the GFB while facilitating the passage of positively charged molecules (2). The increase of permeability of the GFB can be caused by damage of many macromolecules, including degradation of heparin sulfate. This damage is suggested to be caused at least in part, by free

Address for Correspondence :

Ms. Santoshi R. Ghodake

Department of Biochemistry,
K. B. N. Institute of Medical Sciences,
Gulbarga 585104, Karnataka
E-mail: santoshi163@yahoo.com

radicals (2). The extremely short half life of free radicals seems that they cannot be measured directly and that oxidative activity must be measured indirectly by the levels of antioxidant substances in the body. The scavengers of superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\bullet OH$) were protective with different efficiencies. Whereas Total antioxidant capacity (TAC) analysis reflects the overall performance of the antioxidant system. Controversial reports are available regarding involvement of free radical mediated injury in NS in adults.

In the light of above, the present study was undertaken to evaluate the role of Reactive Oxygen Species (ROS) and assorted antioxidants in the etiopathogenesis of NS in adult patient.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, Dr. V.M. Govt. Medical College, SCSM General Hospital, Solapur, with approval of institutional Ethical Committee. Present study included a total of 60 subjects between 19 to 55 years of age. The subjects were further divided into two groups- Group 1: 30 healthy controls, Group 2: 30 patients with NS.

The NS patients diagnosed as per the criteria such as heavy proteinuria, hypoalbuminemia and high blood pressure. Healthy control subjects were matched for age and not suffering from the conditions which may alter oxidative status. NS patients and control subjects were not on supplementation such as vitamins or minerals.

About 10-12 ml of random venous blood was collected of which 5-6 ml was collected in a sterile plain bulb for the estimation of malondialdehyde (MDA) as an index of lipid peroxidation (3), $NO^{\bullet}$ (4), albumin (5), cholesterol (kit provided by Star diagnostics Pvt.Ltd.,Mumbai) and uric acid (kit provided by Star diagnostics Pvt. Ltd., Mumbai). Remaining 4-5 ml blood was poured into sterile bulb containing heparin for the estimation of erythrocyte Superoxide dismutase (SOD) activity (6), levels of α -tocopherol (7), ascorbic acid (8) and TAC (9). The values are expressed as mean $\pm$ SD and Student's 't' test was applied for the statistical analysis of the data.

RESULTS AND DISCUSSION

All the data of MDA, $NO^{\bullet}$, Erythrocyte SOD, α -tocopherol, Ascorbic acid, Albumin, Uric acid, Total cholesterol and TAC are summarized in Table. There was a significant elevation

($P<0.001$) in the levels of serum MDA, in patients (3.64 ± 1.3) as compared to control (1.58 ± 0.42). In NS, infiltrating phagocytes were the first cells identified to produce ROS upon activation. NS is associated with hypoalbuminemia and hyperlipidemia which are independently associated with increased risk of cardiovascular disease, possibly by increasing oxidant stress (10). Increased generation of ROS results in increased lipid peroxidation of glomerular basement membrane. Since accumulation of even small amount of oxidized lipid is enough to induce protein oxidation and thereby cell/tissue damage, it can be stated that lipid peroxidation is one of the possible cause of NS progression (11). Also, oxidative damage to other biomolecules such as heparin sulfates may lead to increased glomerular permeability thereby proteinuria (12, 13). Furthermore, many abnormalities within antioxidant system either enzymatic or nutritional also add up to the progression of lipid peroxidation that may lead to an increase in oxidant levels within cells (1).

A non-significant decrease ($P>0.05$) in $NO^{\bullet}$ levels in patients (37.47 ± 14.27) was observed when compared with control group (38.48 ± 15.47). In NS decrease $NO^{\bullet}$ may result from decrease in NO synthase (NOS) like inducible NOS (iNOS) and neuronal NOS (nNOS) activities. The possible mechanism behind decreased NOS activities could be severe proteinuria and concomitant hypoalbuminemia in NS. These are reported to be primary mediators of kidney iNOS and nNOS deficiency (14). Yet another mechanism may be urinary loss of $NO^{\bullet}$ that circulates bound to albumin (15). Therefore, inactivation of $NO^{\bullet}$ by ROS does not seem to be the most obvious explanation for a reduced $NO^{\bullet}$ bioavailability in patients with NS (16). As NS is associated with hypertension with usually normal or increased blood volume, which may be associated with impaired $NO^{\bullet}$ system. Therefore, it seems possible that hypertension in NS may be associated with impaired $NO^{\bullet}$ system (17).

It has been reported that over production of superoxide ($O_2^{\bullet-}$), in glomeruli while the activity of the principal scavenger SOD is reduced (18). In this study we found that, erythrocyte SOD activity was significantly decreased as compared to controls (1.88 ± 0.9 in controls, 1.07 ± 0.5 in patients). SOD is an intracellular scavenging enzyme that destroys the highly reactive radical by converting it into the less reactive H_2O_2 , which can be subsequently destroyed by catalase and Glutathione peroxidase reaction. Therefore, the primary defense against the oxidative damage is by SOD (19, 20). Reduction in erythrocyte SOD activity could be due to excessive consumption in antioxidation procedure and increased excretion from the kidney in NS (21).

Table: Serum MDA, NO• end products, RBC-SOD activity, α -tocopherol, Ascorbic acid, Albumin, Cholesterol, Uric Acid and TAC in controls and patients with nephrotic syndrome

Parameters	Controls	Patients
MDA (nmols/ml)	1.58 $\pm$ 0.42	3.64 $\pm$ 1.37*
NO• (μ mol/L)	38.48 $\pm$ 15.47	37.47 $\pm$ 14.27
RBC-SOD (U/mg of Hg)	1.88 $\pm$ 0.9	1.07 $\pm$ 0.5*
Vitamin E (mg/L)	12.95 $\pm$ 1.04	9.93 $\pm$ 1.43*
Vitamin C (mg/dl)	0.95 $\pm$ 0.31	0.79 $\pm$ 0.30
Albumin (gm/dl)	4.06 $\pm$ 0.50	3.04 $\pm$ 0.11*
Cholesterol (mg/dl)	149.88 $\pm$ 28.06	311.90 $\pm$ 81.38*
Uric Acid (mg/dl)	4.65 $\pm$ 1.09	4.06 $\pm$ 1.17
TAC (μ mol/L)	847.33 $\pm$ 126.83	684.00 $\pm$ 102.94*

Values are expressed as Mean $\pm$ SD; *Significant

In this study, highly significant decrease ($P < 0.001$) in levels of α -tocopherol in adults with NS (12.95 $\pm$ 1.04 in controls, 9.93 $\pm$ 1.43 in patients) has been observed. Significant lower levels of α -tocopherol may indicate restricted antioxidant function and enhanced lipid peroxidative action resulting in damage to glomerular cells. Reduction in α -tocopherol levels due to excessive consumption in the antioxidant procedure and increased excretion from inflammatory kidney in NS. During utilization of α -tocopherol, tocopherol radicals are formed.

However, a non-significant decrease ($P > 0.05$) in plasma ascorbate level was observed which is in accordance with other authors (0.95 $\pm$ 0.31 in controls, 0.79 $\pm$ 0.30 in patients) (24). The regeneration of α -tocopherol from involves synergistic reaction between α -tocopherol and ascorbic acid. This recycling reaction leads to formation of dehydroascorbate which is further reduced to ascorbate by a non- enzymatic with reduced glutathione. (19, 22, 23). Depletion in ascorbic acid levels were expected in the process of regeneration of α -tocopherol.

Our results shows non-significant increase ($P > 0.05$) in uric acid levels in study group (4.06 $\pm$ 1.17) when compared with controls (4.65 $\pm$ 1.09). This might be due to marked inhibition of renal clearance of uric acid in response to oxidative stress

or a consequence of tubular dysfunction or hypertension (25). Significantly lower serum albumin ($P < 0.001$) was observed in study group (3.04 $\pm$ 0.11) as compared in controls (4.06 $\pm$ 0.50). A low level of albumin in NS is obviously due to increased/disturbed glomerular permeability leading to proteinuria. In fact, this molecule may represent the major low levels and predominant circulating antioxidant in plasma known to be exposed to continuous oxidative stress (26). Also, serum albumin was negatively correlated with serum MDA was found to the conclusion that consequence of hypoalbuminemia leads to the oxidative damage.

Plasma TAC assessed by FRAP assay was significantly lower ($P < 0.001$) in the NS group (684.00 $\pm$ 102.94) as compared to control group (847.33 $\pm$ 126.83). The antioxidant system consists of many different components interact to form an integrated system. Decreased TAC values represent a decreased ability to trap free radicals, low antioxidant enzyme activity, increased consumption of certain antioxidants or may be partly related to abnormalities in lipids (27).

NS is associated with profound hyperlipidemia. Our finding showed that, cholesterol levels were significantly increased ($P < 0.001$) in study group (311.90 $\pm$ 81.38) as compared with controls (149.88 $\pm$ 28.06). Lipid disorder in NS patients was a mixed hyperlipidemia of relatively recent onset and increase in peroxidative damage may be a longer term consequence of sustained hypercholesterolemia (10, 28).

In conclusion, this study provides an evidence for increased ROS generation and total antioxidant status is diminished in NS. Abnormal antioxidant status may be partly related to abnormalities of some antioxidants activity and to high lipid concentrations.

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