

OXIDATIVE STRESS IN PATIENT WITH DIFFERENT HISTOPATHOLOGICAL TYPES OF IDIOPATHIC GLOMERULONEPHRITIS

Nitya Nand*, D. Kumar*, M. Sharma**, R.K. Yadav***

*Department of Nephrology, ***Medicine & **Biochemistry,

Post Graduate Institute of Medical Sciences, Rohtak-124001 (Haryana), India

Abstract: The present study was carried out to evaluate oxidative stress/status in idiopathic glomerulonephritis and to find out its correlation with degree of histopathological lesions in patients with different types of glomerulonephritis (GN). Thirty three (33) adult patients of idiopathic glomerulonephritis with nephritic range proteinuria and twenty one adult healthy controls (Group-I) were enrolled in the study. The patients in study (group II) were divided into two subgroups on basis of histopathological diagnosis. Group IIA included patients with histopathological diagnosis consistent with minimal change disease. Group IIB, included twenty three patients with significant glomerular changes on histopathology. This subgroup included 6 patients each of membranous GN, membranoproliferative GN, mesangioproliferative GN and 5 patients of focal segmental glomerulosclerosis, respectively. Informed written consent was obtained from all the subjects. Serum levels of malondialdehyde (MDA) glutathione peroxidase (GPX) superoxide dismutase (SOD) and Vitamin C were measured in all subjects. For comparison between Study group, group II Group IIA, Group IIB and Control Group I, unpaired t-test was applied and p value was calculated. Patients with idiopathic glomerulonephritis showed a significant increase in MDA and decrease in SOD, GPX and vitamin C levels, as compared to controls ($p < 0.05$). Significantly increase levels of MDA, and decrease in SOD, GPX and vitamin C levels were observed in patients with significant glomerular changes disease as compared to minimal change disease and controls ($p < 0.05$). A positive correlation between serum MDA ($r = +0.354$), vitamin C level ($r = +0.047$) and the number of patients with histopathological lesions was observed in patients with significant glomerulonephritis ($p > 0.05$). Patients with significant change glomerulonephritis showed a negative correlation between serum glutathione peroxidase ($r = 0.19$), superoxide dismutase ($r = 0.28$) and the numbers of histopathological lesions ($p < 0.05$). **Conclusion:** Oxidative stress levels are significantly higher in idiopathic glomerulonephritis, the levels were much higher in significant, suggesting that more is the histopathological damage, the higher were the levels and vice versa.

INTRODUCTION

Glomerulonephritis is the leading cause of end stage renal disease world wide and immune mechanism is the most accepted pathogenetic mechanism. Reactive oxygen species have been proposed to be primary mediator in glomerulonephritis and has been blamed for modification of glomerular permeability to proteins, alteration of glomerular hemodynamic and development of morphological lesions. This process results in imbalance between oxidants and antioxidants and raised levels of oxidants results in increased oxidative stress¹. Oxidative stress is involved in a variety of clinical and experimental renal disease in which a spectrum of seemingly unrelated diseases from minimum change lesion to obstructive nephropathies are known. An impaired antioxidative system has been observed in patients with nephritic syndrome, lupus nephritis and IgA nephropathy^{2,3}. Recently Markan et al⁴ evaluated oxidative stress status in patient with different primary glomerular diseases (PGD) which were grouped in to non proliferative glomerulonephritis and proliferative glomerulonephritis and found significantly higher oxidative stress in proliferative glomerulonephritis.

Various studies show that oxidative stress is increased in various glomerulonephritis. However, there is only single study available where oxidative stress has been evaluated in different histopathological types of glomerulonephritis⁴. Therefore in this study we have assessed the level of oxidative stress in different histopathological types of glomerulonephritis using malondialdehyde, super oxide dismutase, glutathione peroxidase and vitamin C as markers of oxidative stress.

MATERIAL AND METHODS

A total of thirty three histopathologically proved adult patients of idiopathic glomerulonephritis with nephritic range proteinuria with normal renal functions were enrolled in study group (II) Twenty one adult healthy volunteers were also included as control in study procedure and with associated disease which are likely to influence oxidative stress or cases on steroids / immunosuppressant drugs, were excluded from study.

The patient in study group (II) were divided in to two subgroups on basis of histopathological diagnosis. Group IIA included 10 patients with histopathological diagnosis consistent with minimal change disease, where a Group IIB, included twenty three patients with significant glomerular changes on histopathological examination, including 6 patients in each of membranous glomerulonephritis, membranoproliferative GN, mesangioproliferative GN and 5 patients of focal segmental glomerulosclerosis, respectively. Informed written consent was obtained from all the subjects.

The mean age of patients was 31.45 ± 14.4 years and that of control was 35.14 ± 13.1 years. There was 24 males and 9 females in study group. Besides routine biochemical parameters, oxidative stress was assessed in all subjects by estimation of lipid peroxidation – Malondialdehyde, ascorbic acid and glutathione peroxidase, superoxide dismutase.

Statistical Analysis: For comparison between study group (Group II), IIA, Group IIB and Control Group I, unpaired t-test was applied and p value was calculated. Correlation coefficient – r was also calculated to find any correlation between oxidative stress markers

and number of histoathological lesions.

RESULTS

Biochemical parameters in the various groups of patients, including creatine clearances, were comparable and were within normal limits (Table -1). Patients with idiopathic glomerulonephritis had significantly increased level of serum MDA, and decreased levels SOD, GPX and vitamin C as compared to healthy volunteers (p<0.05 Table 2).

Table 1: Showing baseline renal profile and other investigations

| Parameter | Group A | Group B | Control | Total cases |
|------------------------------|-------------------------|-------------------------|-----------------------|----------------|
| Hemoglobin (gm%) | 10.78± 1.54 | 10.33 ±0.89 | 13.04 ±0.80 | 10.64 ±1.38 |
| Serum bilirubin (mg%) | 0.99± 0.15 | 0.92 ±0.13 | 0.95 ±0.15 | 0.96 ±0.15 |
| Blood Sugar (mg%) | 88.6 ±10.38 | 87.2 ±8.8 | 91.85 ±8.79 | 88.18 ±9.81 |
| Blood urea (mg%) | 24.52± 8.62 | 27.2 ±4.91 | 33 ±4.2 | 28.82 ±7.68 |
| Serum Creatinine (mg%) | 0.95± 0.16 | 0.87± 0.12 | 1.27± 0.15 | 0.93± 0.15 |
| Serum Calcium (mg%) | 8.4 ±0.8 | 9.35 ±0.68 | 9.16 ±0.17 | 8.38 ±0.76 |
| Serum phosphate (mg%) | 4.67± 0.99 | 4.79 ±0.75 | 4.36 ±0.24 | 4.70 ±0.95 |
| Serum protein with A/G ratio | 6.32 ±0.95 / 1.07± 0.32 | 6.29 ±0.83 / 1.07 ±0.32 | 7.1 ±0.1 / 1.17 ±0.1/ | |
| SGOUT (IU) | 32.47± 6.57 | 37.8 ±5.37 | 31.04±4.12 | 34.09 ±6.63 |
| SGPT (IU) | 30.52 ±7.63 | 33.2 ±4.54 | 24.71 ±3.05 | 37.33 ±6.88 |
| Serum cholesterol (mg/d) | 302.43± 126.54 | 232.88 ±56.9 | 147.09±4.38 | 281.33 ±113.91 |
| Proteinuria (gm/day) | 5.9 ± 2.07 | 5.11 ±1.51 | 4.36 ±0.24 | 5.57 ±1.93 |

Table II: Oxidative stress markers in patients with idiopathic glomerulonephritis, minimal change disease, significant change glomerulonephritis and controls

| | Contyrol Goup I (n=33) | Study Group II (n=33) | Group IIA (n=10) | Group IIB(n=23) |
|-----------------|------------------------|-----------------------|------------------|-----------------|
| MDA(nmol/l) | 2.431± 0.213 | 3.206± 0.334 | 2.99± 0.242 | 3.3± 0.329 |
| SOD(uit/ml) | 49.010 ± 2.527 | 35.112 ± 7.391 | 42.618 ± 4.649 | 31.848 ± 5.819 |
| GPX (unit / ml) | 0.0710 ± 0.0367 | 0.0453 ± 0.014 | 0.0598± 0.014 | 0.0391 ± 0.0092 |
| VIT.C (mg %) | 1.242 ± 0.092 | 0.893 ± 0.149 | 1.057 ± 0.109 | 0.821± 0.098 |

MDA Malondialdehyde, GPX Glutathione peroxidase, SOD: Superoxide distumase.

Patients with significant change glomerulonephritis (group IIB) had significantly increased levels of serum MDA and decreased levels SOD GPX and vitamin C as compared to minimal change disease (group II A) (p <0.05) and healthy volunteers (Table II). Within various histopathological types, the mean MDA levels were found highest in MPGN and lowest in MCD group, and mean SOD, GPX and vitamin C levels were lowest in MPGN and highest in MCD group.

A positive but statistically non significant (p>0.05) correlation was found between serum MDA (r=+0.35) vitamin C level (r=0.047) and the numbers of histopathological lesions in patients with significant glomerulonephritis (Table 3). Patients with significant change glomerulonephritis showed a negative correlation (p>0.05) between serum glutathione peroxidase (r=0.19), superoxide dismutase level (r=0.28) and the numbers of histopathological lesions.

Table III: Oxidative stress markers in patients with significant change Glomerulonephritis with numbers of histopathological lesions

| Number histopathological of lesions | One lesion (n=10) | Two lesions (n=9) | Three lesions (n=3) | Four lesions (n=1) | Pearson's correlation coefficient | P-value |
|-------------------------------------|-------------------|-------------------|---------------------|--------------------|-----------------------------------|---------|
| MDA(nmol/l) | 3.17± 0.35 | 3.35± 0.24 | 3.37± 0.27 | 3.68 | R=+0.35 | P>0.05 |
| SOD(uit/ml) | 33.08 ± 4.52 | 31.72 ± 6.7 | 31.5 ± 7.79 | 22.96 | r=-0.28 | P>0.05 |
| GPX (unit / ml) | 0.04 ± 0.007 | 0.04 ± 0.011 | 0.035± 0.009 | 0.03 | r=-0.19 | P>0.05 |
| VIT.C (mg %) | 0.81 ± 0.08 | 0.83 ± 0.07 | 0.87 ± 0.21 | 0.73 | r=+0.047 | P>0.05 |

DISCUSSION

Oxidative stress plays a key role in pathophysiological processes in various renal disease, including inflammatory lesions such as glomerulonephritis and interstitial nephritis, ischemic reperfusion injury, hemolytic uremic syndrome and toxic nephropathies, and possibly in progression of chronic renal failure². Malondialdehyde is product of lipid peroxidation, which normally occurs at low level in all cells and tissues. But this process is accelerated by increase oxidative stress, causing increase production of Malondialdehyde^{9,10}. Significantly higher MDA levels in significant change glomerulonephritis as compared to minimal change disease and controls indicate an increased production of Reactive Oxygen Species (ROS) and severe lipoperoxidative damage in these patients. ROS react with polyunsaturated fatty acid in lipids, which are highly susceptible to oxidation, producing lipid hydroperoxides. Most of the lipid hydroperoxides are unstable and undergo decomposition through peroxy radical dependent chain reaction to smaller and more stable lipid hydroperoxides products, such as malondialdehyde¹¹. This process is accelerated by increased production of reactive oxygen species, causing increase production of malondialdehyde. Increased MDA levels might be due to lipoperoxidative damage caused by excessive amount of reactive oxygen species generation by infiltrating inflammatory (neutrophils, monocytes) or proliferating glomerular cells (mesangial and endothelial cells and podocytes) as a result of different sites of glomerular damage and cellular proliferation, and their propensities to generate reactive oxygen species in these patients.

We also observed significantly decreased level of SOD, GPX (anti oxidant enzymes) and vitamin C (non enzymatic anti oxidants) in Patients with significant change glomerulonephritis, thus indicating an increased oxidative stress in patients with significant glomerulonephritis. Glutathione peroxidase is a selenoenzyme which catalyzes the reduction organic hydro peroxides and hydrogen peroxide by using glutathione as reducing agent and play an important role of extra cellular fluid component and cell surface against peroxide mediated damage¹². The exact mechanism of decreased glutathione peroxidase levels in patients with significant glomerulonephritis as compared to minimal change disease is not known, but these different levels of glutathione peroxidase in different histopathological types or lesions might be result of increased utilization of enzymes to cope up with reactive oxygen species, which might be generated in excessive amount by proliferating glomerular cells (mesangial cells, endothelial cells and podocytes) which are a source of ROS or could be due to infiltration of the macrophage and the neutrophils or impaired synthesis of extra cellular GPX in the damaged kidneys^{13,14}.

Superoxide dismutase is one of the key antioxidant enzymes that participates in the cellular defense system against oxidative damage by catalyzing the dismutation of superoxide (O₂⁻) to oxygen and H₂O₂¹⁵, and ascorbic acid is one of the most effective water soluble antioxidants (non enzymatic anti oxidants) in biological fluids and can scavenge physiologically important reactive oxygen species and

reactive nitrogen species. However, the significant decrease in SOD and vitamin C levels in patients with significant glomerulonephritis might be the result of inactivation of enzyme by reactive oxygen species, could have been generated in excessive amount due to the rapid proliferation of the glomerular cells (mesangial cells, endothelial cells and podocytes) which are a source of ROS or could be due to infiltration of the macrophage and the neutrophils in the patients with significant change glomerulonephritis. Superoxide dismutase inactivation by hydrogen peroxide (H_2O_2), a dismutation product of O_2 through destruction of histidine residue has been reported by Bray and Cockle. Like in present study, significant increase in oxidative stress also has been supported by various other studies: Markan S et al⁴ reported that mean serum MDA levels were significantly higher ($p < 0.05$) and lower SOD levels ($P < 0.05$) in patients with proliferative glomerulonephritis (MPGN and RPGN) as compared to non proliferative glomerulonephritis (MCD, MGN and FSGS).

Kuo HT et al¹⁸ also reported increased plasma malondialdehyde (MDA) levels in the patients with FSGS as compared to patients with MCD which were associated with the degree of glomerulosclerosis, suggesting that oxidative stress occurs early and may play an important role in the pathogenesis of glomerulosclerosis. Hung Chun C et al¹⁹ reported that plasma glutathione peroxidase levels were significantly lower (both $p < 0.01$) in FSGS patients than in either MCD patients or normal control subjects.

From the observations made in this study, it can be concluded that oxidative stress levels were significantly higher in idiopathic glomerulonephritis; the levels were much higher in significant change glomerulonephritis (membranous glomerulonephritis, membranoproliferative glomerulonephritis, mesangiol proliferative glomerulonephritis and focal segmental glomerulosclerosis) as compared to minimal change disease. Suggesting that more is the histopathological damage, higher were the levels and vice versa. Also the oxidative stress difference in different histopathological types can be used for clinicopathological correlation and perhaps is a prognostic indicator in different histopathological types. Thus future research should focus on decreasing oxidative stress by using various antioxidants, to halt the disease process and improve survival.

REFERECES

1. McCord JM The evolution of free radicals and oxidative stress. *Am J Med* 2000; 108: 652-59.
2. Turi S, Nemeth I, Torkos A, Saghy L, Vargar I, Matkovic SB, Nagy, J. Oxidative Stress and antioxidant defense mechanism in glomerular disease. *Free Rad Biol Med* 1997; 22:161-8.
3. Chen HC, Tomino Y, Yaguchi Y et al. Detection of polymorphonuclear cells, superoxide dismutase and poly C 9 in glomeruli of patients with IgA nephropathy. *Nephron* 1991; 59: 338.
4. Markan S, Kohli HS, Sud K, Ahuja M, Ahluwalia TS, Sakhuja V, Khukkar M. Oxidative stress in primary glomerular disease: a comparative study. *Mol Cell Biochem* 2008; 31: 105-10.
5. Kumar R, Seth R.K, Sekon MS, Bhargava JS. Serum lipid peroxide and other enzyme levels of patients suffering from thermal Injury. *Burn* 1995; 21: 96-97.
6. Mc Cormick DB, Greene HC, Vitamins In Burtis CA, Ashwood ER, eds. *Teitz, text book of clinical chemistry* 3rd ed. US wb Saunders. 1999. 1023-25.
7. Hopkins J, Tudhope GR. Glutathione peroxidase in human red cells in health and disease. *J. Haematol* 1973; 25: 563-65.
8. Misra H, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for SOD. *J. Biol Chem* 1972; 247: 3170-5.
9. Brent JA, Rumach HH. Role of free radicals in toxic hepatic injury. *Free radical biochemistry. Clin. Toxicol* 1993; 31: 139-71.
10. Horic K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sabai H, Strihou CY, Monnier VM, Witztun JL, Kurokawa K. Immuno histochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions: implications for glyoxidative stress in the pathogenesis of diabetic nephropathy. *J. Clin Invest* 1997; 100: 2995-3004.
11. Chic D, Lubin B, Shohet SB. Peroxidation reactions in red cell biology. *Free radicals in biology San Diego Academic* 1982; 5:115-60.
12. Flohe L. Glutathione Peroxidase brought into focus. *Free radicals in biology Academic Press* 1980; 14:223-54.
13. Yoshimura S, Suemiza H, Nomoto Y, Sakai H, Katsuoka Y, Kawamuran et al. Plasma glutathione peroxidase deficiency caused by renal dysfunction. *Nephron* 1996; 73: 207-11.
14. Avissar N, Ornt DB, Yagil Y, Horowitz S, Watkins R.H, Kerl EA, et al. Human kidney proximal tubule are main source of plasma glutathione peroxidase. *Am J Physiol* 1994; 226: 367-75.
15. Getzoff ED, Tainer JA, Wemer PK, Koollman PA, Richardson JS, Richardson DC. Electrostatic recognition between superoxide and copper, zinc superoxide dismutase. *Nature* 306: 1983: 287-90.
16. Frei B, Stocker R, Amas BN, Ascorbate: The most effective antioxidants in human blood plasma. *Adv. Exp. Med Biol* 1990; 264: 153-6.
17. Bray RC, Cockle SA. Reduction and inactivation of superoxide dismutase by hydrogen peroxide. *Biochem J* 1974; 134: 43-48.
18. Kuo HT, Kuo M.C, Chiu YW, Chang JM, Guh JY, Chen HC. Increased glomerular and extra cellular malondialdehyde levels in patients and rats with focal segmental glomerulosclerosis. *Eur J. Clin Invest* 2005; 35: 245-50.
19. Hung-Chun C, Jinn-Yun G and Yung Hsiung L. Alterations of glomerular and extra cellular glutathione peroxidase levels in patient and rats with focal segmental glomerulosclerosis. *J Lab Clin Med* 2001: 279-83.

LITERATURE REVIEW

Initiation of dialysis at higher GFRs: Is the apparent rising tide of early dialysis harmful or helpful?

Steven Jay Rosansky et al. *Kidney International* 2009;76,257-261.

Over the past decade a trend of increasing estimated glomerular filtration rate (eGFR) at the initiation of dialysis for treatment of end-stage renal disease (ESRD) has been noted in the United States. In 1996, only 19% of patients began dialysis therapy with an eGFR of greater than 10 ml/min/1.73m² (denoted as 'early start'), but by 2005 the fraction of early start dialysis patients had risen to 45%. This review examines US dialysis data, national guidelines, and publications relevant to the early start phenomenon. It is not known whether early start of dialysis is beneficial, harmful or neutral with respect to the outcome of dialysis treatment for ESRD. Available data indicate that mortality while on dialysis therapy may be higher in those subjects with early start. Comorbidities present at the time of dialysis initiation do not appear to be a major driving force for early start patients. As well, residual kidney function in these patients is a major contributor to total urea or creatinine clearance. This can be a positive factor for patient outcomes and might be compromised by early start. Finally, we estimate the dollar cost of early start to the US Medicare-supported ESRD program. Properly designed, prospective and randomized studies may help to clarify the benefit or harm of early start of dialysis for ESRD.

Check-list

- (i) Copyright statement/declaration (not submitted or published elsewhere) signed by all the authors.
- (ii) Three hard copies of manuscript with illustrations attached to each; **must send an electronic copy of text with photographs loaded on CD.**
- (iii) **Title page**: Title of Manuscript, Name(s) and affiliation of author(s); Institution(s) and city(ies) address of corresponding author (**Tel; Fax; e-mail**)
- (iv) The text of the article should contain **Abstract** highlighting objectives, methods, results, conclusions.
- (v) Original Article (double-spaced on A-4 size paper): should contain introduction material & methods, results, discussion; **Indian Literature**

MANUSCRIPT SUBMISSION : FOR JIMSA

- (vi) **must be referred**, references numbered in text as they appear. Update/Review/Therapy update should have appropriate headings, with reference numbers in the text; Indian Literature cited, wherever available.
- (vii) References: maximum number of references for update-30, original-20, Case reports-6-8.
- (viii) Each table on separate sheet; maximum number-4 in original article; 6 in update.
- (ix) Photographs/figures in envelope, each marked figure number on reverse with legends on separate sheet, numbers not to exceed 4 in original, 2 in case report.
- (x) Statement signed by all authors regarding adherence to Standard ethical guidelines prescribed by ICMR 2000.