

Original Article

Oxidant Stress in Children With Resistant Nephrotic Syndrome: Effect of Vitamin E Supplementation

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ABSTRACT

Background: Experimental data indicate that excessive production of reactive oxygen molecules contributes to progressive renal injury and that treatment with antioxidants may attenuate this damage. Several studies indicate the pathophysiological importance of reactive oxygen species in patients with nephrotic syndrome.

Objectives: To test the hypothesis that oxygen free radicals are mediators of excessive protein permeability in steroid resistant nephrotic syndrome (SRNS) and to assess the therapeutic effect of vitamin E (VE) supplementation as antioxidant on the level of proteinuria in those children.

Methods: The present study included 26 patients with SRNS, subdivided into 2 equal groups and 15 matched healthy children as controls. Patients in group I received standard therapy of methylprednisone and cyclophosphamide plus VE (200 IU twice a day by mouth) for 4 months, group II patients received the standard medical treatment. Protein : creatinine ratio (mg/mg) in an early morning specimen of urine was determined in all studied children at the beginning and at follow-up. Erythrocyte and plasma glutathione peroxidase (GSH-Px) and erythrocyte superoxide dismutase (SOD) activities, and erythrocyte and plasma levels of malondialdehyde (MDA) were measured as oxidative stress indices.

Results: Initially patients in both groups showed significantly lower activities of erythrocyte GSH-Px (14.2 ± 2.2 & 16.5 ± 4.5 U/gHb) and plasma GSH-Px (143.56 ± 20.3 & 169.25 ± 25.4 U/L) in comparison to controls (24.34 ± 2.5 U/gHb & 278.43 ± 37.34 U/L respectively) [$p < 0.01$ for each]. They also had significantly lower erythrocyte SOD activities (1484.24 ± 228.13 & 1373.54 ± 265.53 U/gHb) than that of the controls (2004.53 ± 229.72 U/gHb) [$p < 0.01$]. There were significant increases in the levels of MDA of both erythrocytes (346.5 ± 32.5 & 312.6 ± 29.7 nmol/gHb) and plasma (5.6 ± 1.5 & 4.9 ± 1.3 nmol/ml) of patients in the two groups compared with the controls (189.5 ± 24.2 nmol/gHb & 1.52 ± 0.2 nmol/ml respectively) [$p < 0.001$ for each]. After 4 months patients who received VE supplementation showed significant reduction in protein : creatinine ratio from 9.25 ± 3.16 to 3.75 ± 1.3 ($p < 0.01$) and significant correction of oxidative stress indices as evident by significant decrease in MDA levels with concomitant increase in the activities of the antioxidants GSH-Px & SOD. However patients in group II showed no evidence of such significant improvement.

Conclusions: The results obtained indicate an abnormality in the antioxidative system of children with SRNS and oral administration of VE to children with SRNS leads to significant reduction in proteinuria. Thus, VE may be a useful adjunct in treating children with SRNS.

INTRODUCTION

Reactive oxygen species (ROS) have been considered to have role in pathogenesis of glomerular diseases^(1,2). The importance of ROS in kidney diseases has been suggested by detection of oxidative injury products in kidney tissue or in urine and by protection with inhibitors of ROS⁽³⁾. Adler et al.⁽⁴⁾ and Bulucu et al.⁽⁵⁾ reported that renal cells, neutrophils or other cells in

circulation may be the sources of reactive oxygen species. An oxidant stress has been shown to prevail in experimental and clinical nephrotic syndrome⁽⁶⁻⁸⁾. Decreased glutathione levels as an indicator of oxidative stress were reported by Ginevri et al.⁽⁹⁾ and Asami et al.⁽¹⁰⁾ in children with nephrotic syndrome. It has also been reported that there is a correlation between the presence of active glomerular disease

and evidence of oxidative changes in pediatric and adult nephrotic patients^(6,11,12). The experimental studies performed in nephrotic rats indicate roles for intrinsic antioxidant enzyme activities and antioxidant deficiencies in the nephrotic syndrome⁽¹³⁻¹⁶⁾.

There is increasing evidence that vitamin E has a beneficial effect on free radical-mediated diseases, including progressive renal injury⁽¹⁷⁻¹⁹⁾. In animal models of IgA nephropathy and focal segmental glomerulosclerosis in which excessive production of oxygen free radicals is implicated in the pathogenesis of disease, dietary supplementation with vitamin E protects against renal dysfunction and structural damage to the kidney⁽²⁰⁻²³⁾.

Proteinuria is a non-specific symptom of kidney damage associated with glomerular disease regardless of the etiology. The extent of proteinuria correlates with the morphologic severity of glomerular damage, and persistent proteinuria can lead to irreversible renal scarring and loss of function ultimately requiring renal replacement therapy⁽²⁴⁻²⁶⁾.

AIM OF THE WORK

The aim of this work was to test the hypothesis that oxygen free radicals are mediators of excessive protein permeability in SRNS and to study the effect of VE supplementation as antioxidant on the level of proteinuria.

SUBJECTS AND METHODS

The study included 26 children (14 males and 12 females) admitted to the Nephrology Unit, Department of Pediatrics, Assiut University Hospital, with the clinical

diagnosis of nephrotic syndrome satisfying the ISKDC (International Study of Kidney Disease in Children)⁽²⁷⁾ criteria, who did not respond to a standard regimen of corticosteroids. None of them was diabetic nor had any kind of active inflammatory disease. Their mean age was (10.5 ± 2) years). Patients were divided into two groups randomly, each group consisting of 13 patients. Group I received standard therapy of methylprednisone and cyclophosphamide plus VE (200 IU twice a day by mouth) for 4 months, whereas group II patients received the standard medical treatment only. Fifteen healthy children of matched age and sex were enrolled as control. None of them had proteinuria and all had normal renal function. All children were subjected to full history and thorough clinical examination. Venous blood samples (5 ml) were collected on EDTA as an anticoagulant. Plasma was separated after centrifugation and stored in aliquots at -70°C till analysis. Erythrocyte SOD was determined according to the method of Misra and Fridovich⁽²⁸⁾. Erythrocyte and plasma GSH-Px was measured by Hopkins and Tuddhope method⁽²⁹⁾. Vitamin E was estimated by extraction to an organic solvent⁽³⁰⁾; and MDA was estimated by its thiobarbituric reactivity⁽³¹⁾. Proteinuria was determined by measuring the protein : creatinine ratio (mg/mg) in an early morning specimen of urine at the beginning of the study and at follow-up. Glomerular filtration rate was calculated using the formula validated for children by Morris et al.⁽³²⁾, $[\text{glomerular filtration rate (ml/min/1.73m}^2) = 40 \times \text{height (cm)/plasma creatinine (\mu\text{mol/l})}]$.

Data obtained were calculated and statistically analyzed using student t-test; p value ≤ 0.05 was considered significant.

RESULTS

Table 1 shows demographic and laboratory data in studied children with SRNS. It was observed that there were significantly higher levels for mean systolic blood pressure [SBP], diastolic blood pressure [DBP], protein : creatinine ratio and serum cholesterol in patients than controls. Glomerular filtration rate [GFR] and serum albumin showed significantly lower mean values in patients than in controls.

Table 2 shows the means \pm SD of various studied oxidative stress indices in the red blood cells of patients and controls before treatment.

Table 3 shows the means \pm SD of various studied oxidative stress indices in plasma of patients and controls before treatment.

Table 4 shows the means \pm SD of

various studied oxidative stress indices in red blood cells in both VE supplemented and non-supplemented groups of patients at follow-up after 4 months.

Table 5 shows the means \pm SD of various studied oxidative stress indices in plasma in both VE supplemented and non-supplemented groups of patients at follow-up after 4 months.

Figures 1a & 1b show urinary protein excretion [protein : creatinine ratio (mg/mg) in an early morning specimen] in both VE supplemented and non-supplemented groups of patients initially and after 4 months.

Figures 2a, 2b and 2c show various studied oxidative stress indices in the red blood cells of both VE supplemented and non-supplemented groups of patients initially and after 4 months.

Figures 3a and 3b show various studied oxidative stress indices in the plasma of both VE supplemented and non-supplemented groups of patients initially and after 4 months.

Table 1: Demographic and laboratory data in studied children.

| Items | Control n = 15 | SRNS patients before treatment | |
|------------------------------------|--------------------|--------------------------------|----------------------|
| | | Group I n = 13 | Group II n = 13 |
| Male/female | 8/7 | 7/6 | 7/6 |
| Mean age (years) | 10.2 \pm 1.8 | 10.8 \pm 2.3 | 9.7 \pm 1.8 |
| Mean SBP (mmHg) | 107.6 \pm 4.8 | 143.5 \pm 7.5** | 139.6 \pm 7.2** |
| Mean DBP (mmHg) | 68.2 \pm 2.8 | 92.5 \pm 5.54** | 90.3 \pm 4.5** |
| Protein : creatinine ratio (mg/mg) | 0.20 \pm 0.04 | 9.78 \pm 3.40*** | 9.13 \pm 1.91*** |
| GFR ml/min/1.73m ² | 119.6 \pm 21.67 | 85.54 \pm 17.06* | 82.17 \pm 19.3* |
| Serum albumin (gm/dl) | 4.2 \pm 0.34 | 2.1 \pm 0.55** | 1.98 \pm 0.35** |
| Serum cholesterol | 178.53 \pm 24.34 | 325.39 \pm 41.5** | 355.67 \pm 39.75** |

* p < 0.05, ** p < 0.01, *** p < 0.001.

Table 2: Mean \pm SD of the concentrations of various studied oxidative stress indices in the red blood cells of SRNS patients and controls before treatment.

| Groups | SOD U/gHb | GSH-Px U/gHb | MDA nmol/gHb | Vitamin E μ g/100ml |
|--|----------------------|-----------------|------------------|----------------------------|
| I. Patients (group I) n = 13 | 1484.24 \pm 228.13 | 14.2 \pm 2.2 | 346.5 \pm 32.5 | 79.19 \pm 15.52 |
| II. Patients (group II) n = 13 | 1373.54 \pm 265.53 | 16.5 \pm 4.5 | 312.6 \pm 29.7 | 82.42 \pm 13.55 |
| III. Controls n = 15 | 2004.53 \pm 229.72 | 24.34 \pm 2.5 | 189.5 \pm 24.2 | 140.58 \pm 10.67 |
| Significance | | | | |
| I vs II | N.S. | N.S. | N.S. | N.S. |
| I vs III | p < 0.01 | p < 0.01 | p < 0.001 | p < 0.001 |
| II vs III | p < 0.01 | p < 0.01 | p < 0.001 | p < 0.001 |

Table 3: Mean \pm SD of the concentrations of various studied oxidative stress indices in plasma of SRNS patients and controls before treatment.

| Groups | GSH-Px U/L | MDA nmol/ml |
|--------------------------------|-------------------|----------------|
| I. Patients (group I) | 143.56 \pm 20.3 | 5.6 \pm 1.5 |
| II. Patients (group II) | 169.25 \pm 25.4 | 4.9 \pm 1.3 |
| III. Controls | 278.43 \pm 37.3 | 1.52 \pm 0.2 |
| Significance | | |
| I vs II | N.S. | N.S. |
| I vs III | p < 0.001 | p < 0.001 |
| II vs III | p < 0.001 | p < 0.001 |

Table 4: Means \pm SD of the concentrations of various studied oxidative stress indices in red cells in SRNS patients in relation to VE supplementation.

| Groups | SOD U/gHb | GSH-Px U/gHb | MDA nmol/gHb | Vitamin E μ g/100ml |
|--|---------------------|-----------------|-------------------|----------------------------|
| Patients with VE supplementation | 1835.34 \pm 205.2 | 19.3 \pm 2.12 | 204.54 \pm 30.4 | 126.57 \pm 12.86 |
| Patients without VE supplementation | 1297.26 \pm 385.5 | 13.86 \pm 2.5 | 356.21 \pm 32.7 | 81.25 \pm 17.32 |
| Significance | p < 0.05 | p < 0.05 | p < 0.01 | p < 0.01 |

Table 5: Means \pm SD of the concentrations of various studied oxidative stress indices in plasma in SRNS patients in relation to VE supplementation.

| Groups | GSH-Px U/L | MDA nmol/ml |
|-------------------------------------|-------------------|----------------|
| Patients with VE supplementation | 235.58 \pm 32.6 | 1.5 \pm 0.21 |
| Patients without VE supplementation | 156.36 \pm 28.5 | 3.4 \pm 0.27 |
| Significance | p < 0.001 | p < 0.01 |

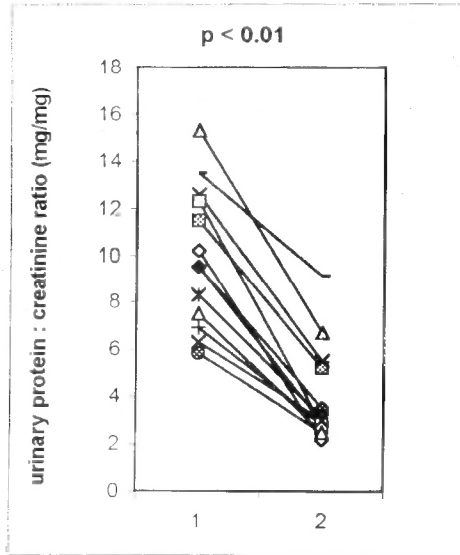


Fig. 1a: Urinary protein excretion in patients with SRNS (group I) supplemented with VE (the lines connect the values at start & follow-up).

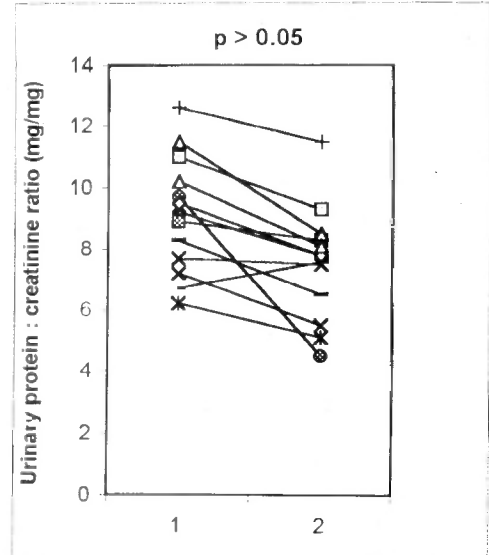


Fig. 1b: Urinary protein excretion in patients with SRNS (group II) not supplemented with VE (the lines connect the values at start & follow-up).

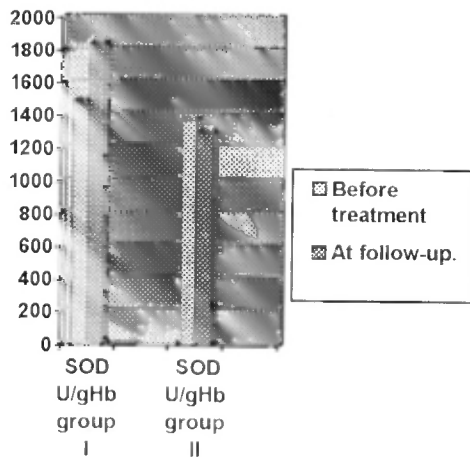


Fig. 2a: Mean values of SOD (U/gHb) in RBCs of both groups of patients before treatment and at follow-up.

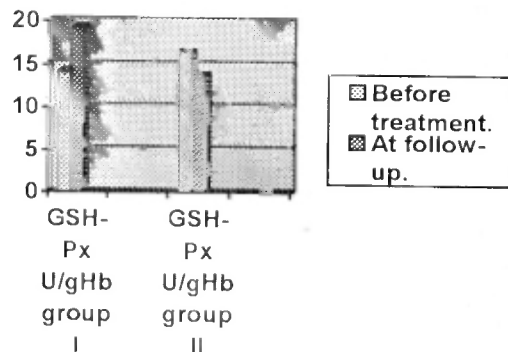


Fig. 2b: Mean values of GSH-Px (U/gHb) in RBCs of both groups of patients before treatment and at follow-up.

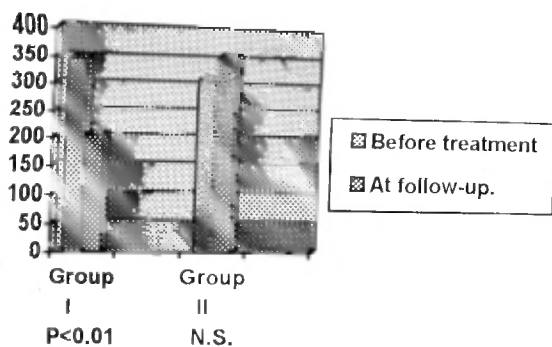


Fig. 2c: Mean values of MDA nmol/gHb in RBCs of both groups of patients before treatment and at follow-up.

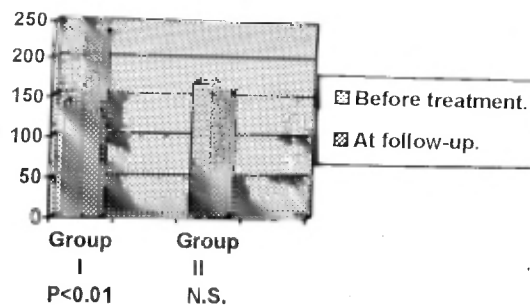


Fig. 3a: Mean values of GSH-Px (U/L) in plasma of both groups of patients before treatment and at follow-up.

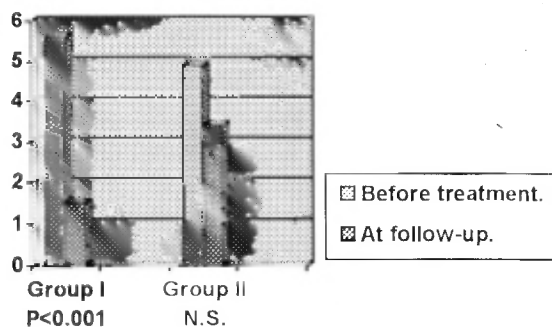


Fig. 3b: Mean values of MDA (nmol/ml) in plasma of both groups of patients before treatment and at follow-up.

DISCUSSION

Free oxygen radicals are the molecules which contain unpaired electrons⁽³³⁾. Reactive oxygen metabolites are constant products of normal aerobic cell metabolism⁽¹⁾. Healthy organisms are protected by various defense mechanisms against free oxygen radicals which are ceaselessly generated⁽³⁴⁾. These oxidants are degraded by enzyme systems such as SOD, GSH-Px and antioxidants such as ceruloplasmin, transferrin, reduced glutathione (GSH), methionine, vitamin E and vitamin C⁽³⁵⁻³⁹⁾. MDA is the product of lipid peroxidation which occurs as a result of free radical injury and its

formation is accelerated by oxidative stress^(38,40). In the present study, a defect in the antioxidative system was suggested by significantly decreased levels of GSH-Px, SOD and increased levels of MDA in both red blood cells and plasma in SRNS patients than controls. Our results are in agreement with the previous studies^(5,41). Asami et al.⁽¹⁰⁾ reported decreased glutathione levels as an indicator of oxidative stress in children with the nephrotic syndrome. Others have also reported that there is a correlation between the presence of active glomerular disease and oxidative changes in pediatric and adult nephrotic patients^(6,11,12,42). Experimental studies performed in nephrotic rats indicate roles for intrinsic antioxidant enzyme activities and antioxidant deficiencies in nephrotic syndrome⁽¹³⁻¹⁶⁾. ROS produce the proteinuria by inducing injury to glomerular epithelial cells and subsequent alterations in glomerular filtration barrier components synthesized by these cells, by reduction of the electronegative charge of glomerular filtration barrier or by other unknown mechanisms⁽¹⁾. Glomerular toxicity of hydrogen peroxide, activation of proteolytic

capacity of metalloproteinases, inactivation of circulatory inhibitors of some proteolytic enzymes and/or sustained leucocytic recruitment into the glomerulus resulting in continuous generation of ROS and reduction in single nephron GFR due to reactive oxygen species have been incriminated in glomerular damage related to oxidative stress⁽³⁾. Solin et al.⁽²⁾ could show distinct deposition of MDA and 4-HNE (4-hydroxynonal) adducts in glomeruli in human proteinuria, where they appear to directly perturb the function of glomerular basement membrane. This is in good agreement with the earlier results showing splitting of glomerular basement membrane in the kidney of patients with congenital nephrotic syndrome⁽⁴⁵⁾.

Some studies reported that antioxidant deficiencies affect the course of nephrotic syndrome negatively. Supplementation by antioxidants such as VE may have ameliorating effects on the disease. VE is an important lipid-soluble antioxidant that acts synergistically with vitamin C, which regenerates VE by reducing the tocopherol radical produced when VE scavenges peroxy radicals. VE is transported in serum with serum lipids, and VE levels correlate with serum lipid concentrations. As the serum lipid level increases, VE appears to partition out of the cellular membranes into circulating lipoproteins. In accordance with this observation, Fydryk et al.⁽⁴⁴⁾ found a decreased VE concentration in erythrocyte membranes during relapse of nephrotic syndrome when serum lipids were typically elevated. The high ratio of serum VE to lipid fractions observed in nephrotic syndrome demonstrated sufficient VE in

nephrotic patients. However, a high serum VE level does not imply an equally high content of this vitamin in lipid membranes, where it acts as a principal antioxidant⁽⁴²⁾.

In the present study the mean value of VE levels in red blood cells was significantly lower in groups I & II of patients (79.19 ± 15.52 & 82.42 ± 13.55) in comparison to controls (140.58 ± 10.67) [$p < 0.01$ for each]. Our finding of reduction of VE in the red blood cells is in accordance with Kinra et al.⁽⁷⁾. After VE supplementation, its level in the red blood cells of the supplemented patients significantly increased (126.57 ± 12.86 $\mu\text{g}/100\text{ml}$) compared to non-supplemented patients (81.25 ± 17.32 $\mu\text{g}/100\text{ml}$) [$p < 0.01$]. This increment was associated with significant reduction of proteinuria from 9.25 ± 3.16 to 3.75 ± 1.3 mg/mg ($p < 0.01$), and significant correction of oxidative stress indices as evidenced by significant decrease in MDA levels with concomitant increase in the activities of the antioxidants GSH-Px & SOD [Fig 2 & 3].

Proteinuria is a hallmark of glomerular disease and reduction in urinary protein excretion is often used as a surrogate marker for diminished glomerular barrier dysfunction⁽⁴⁵⁾. The observed benefit of VE treatment may be due to a direct effect on immunoeffector cells that may mediate the increased glomerular permeability to protein⁽⁴⁶⁾. Alternatively, the antioxidant may act in a non-specific manner e.g., reduction in transforming growth factor- β bioactivity and extracellular matrix protein gene expression, to diminish interstitial inflammation and fibrosis⁽⁴⁷⁾. Tahzib et al.⁽⁴⁸⁾ reported that the reduction in urinary

protein excretion that can be achieved with VE therapy may not be sufficient in magnitude to favorably impact on edema and other complications of nephrotic syndrome. However others state that if proteinuria per se is injurious to the renal parenchyma, then any decrease in proteinuria may be associated with a parallel reduction in the rate of progression of glomerular disease^(49,50). Also Kueemmerle et al.⁽⁵¹⁾ found that administration of a diet that is enriched in α -tocopherol clearly ameliorates renal injury at 4-8 weeks of experimental IgA nephropathy. This protective effect was associated with a reduction in renal cortical malondialdehyde content and suppression of the elevation of TGF β 1 mRNA in the kidney. This confirms that oxidative injury contributes to the pathogenesis of IgA nephropathy^(21,52), and that antioxidant therapy can decrease

glomerular dysfunction. The amelioration of glomerular injury by α -tocopherol supplementation has also been observed in chronic puromycin aminonucleoside nephropathy, a severe glomerulonephropathy⁽⁵³⁾. Schena & Pertosa⁽⁵⁴⁾ demonstrated that diabetes-induced accelerated apoptosis is related to free radical injury of the rat kidney. This supports the use of α -tocopherol in this most-common cause of end stage renal disease⁽⁵⁴⁾.

In conclusion, the results obtained indicate an abnormality in the antioxidative system of children with SRNS. We have demonstrated that short-term oral administration of VE to children with SRNS leads to significant reduction in proteinurea. The availability of a safe and highly effective treatment such as VE supplementation would represent an advance in the care of children with SRNS.

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