ROLE OF RENOPROTECTIVE THERAPY ON ANTIOXIDANT STATUS IN CHILDREN WITH NEPHROTIC SYNDROME

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ABSTRACT

To prove that reactive oxygen species play a major role in nephrotic syndrome (NS) where in it could have an influence on the effectiveness of renoprotective therapy (RPT) in nephrotic children. Plasma protein thiols and ferric reducing antioxidant power of plasma (FRAP) were estimated in 30 children with NS, before the onset of RPT and also in 19 children in relapse as well as in 30 healthy controls. These parameters were also estimated in 6 children twice that is, before starting RPT and after a period of four years of RPT. There was a significant decrease in plasma protein thiol values in pre – RPT and children in relapse when compared to controls. No significant difference was observed in plasma FRAP levels in these groups. However, in 6 children after four years of RPT treatment, a noticeable decrease in plasma protein thiol and a significant decrease in FRAP levels was observed when compared to their initial values. Hence, this study indicates that the antioxidant status of children with NS probably has a role to play in their prognosis. A comparison of antioxidant status in pre - and post -RPT children further justifies this role as after therapy the turnover of the antioxidants decreases due to less demand with improvement in the clinical condition.

KEYWORDS: Nephrotic Syndrome; Plasma; Protein Thiols; Renoprotective Therapy; Total Antioxidant Power

INTRODUCTION

Nephrotic syndrome of childhood is characterized by a considerable degree of proteinuria and hypoalbuminuria. There is a wide spectrum of disorders where one of the underlying causes would be oxidative stress which could exacerbate the clinical symptoms including those in cases of pediatric NS [1-3]. Reactive oxygen species can bring about damage to tissues, cells and macromolecules within them altering the dynamics of normal function of that organ, in this case the nephron [4]. Therefore in the present study, plasma...
protein thiols and ferric reducing antioxidant power were assessed to gauge the antioxidant status of children with NS in relapse, before onset of renoprotective therapy (RPT) and after four years of treatment.

Material and Methods
This study was carried out in pediatric patients aged 1-17 years, suffering from NS and attending the Pediatrics Department of Kasturba Hospital, Manipal, India, (tertiary care hospital) between August 2008 – March 2010. Thirty children who visited the hospital for a routine health checkups between the ages of 1-17 years (mean age 6.4 years, 21 males and 9 females), served as controls. The study was approved by the institutional ethics committee. Informed consent was obtained from all parents/subjects. The study design was a nonrandomized clinical trial. The pediatric cases were divided as follows: 49 cases with NS were enrolled for this study, with biopsy proven mesangial proliferative glomerulonephritis as the pathologic basis.

Of these cases, 19 children with relapse in NS formed group 2 (mean age 6.4 years, 15 males and 4 females) and 30 children undergoing RPT formed group 3 (mean age 9.4 years, 24 males and 6 females). The diagnosis was based on clinical signs associated with significant proteinuria (24 h urine protein >40 mg/m²/h), hypoalbuminemia and symptoms of edema with oliguria (<1 ml / kg body weight/h) in cases of relapse. Children on RPT included those with a significant non-nephrotic range of proteinuria (4-40 mg/m²/h) who received oral prednisolone (40-60 mg/m²/day) for four weeks but did not go into remission phase (urine protein <4 mg/m²/h). RPT was comprised of oral administration of enalapril, 0.2-0.5 mg/kg/day, aspirin, 5-10 mg/kg/day and dipyridamole, 5mg/kg/day. None of the patient and control groups received any form of antioxidant medications. Further, regarding immunosuppresants, steroid administration itself acted as one of them.

Sample collection
Under aseptic conditions venous blood (2 ml) was collected in EDTA vacutainers both, from patients and normal children. The collected blood was centrifuged at 3000 rpm for 10 minutes for separation of plasma. The timing for sampling blood from NS cases in relapse was at the initiation of steroid therapy when there was significant proteinuria, edema and children on RPT, it was at the onset of therapy. Moreover, in the present study, the
parameters were also measured twice (prospective design), that is, before the onset of RPT and after four years of treatment in 6 nephrotic children (mean age 9.4 years, 5 males and 1 female).

Plasma protein thiols were assessed spectrophotometrically at 412 nm using dithionitrobenzoic acid \[^5\]. The absorbance was directly proportional to the amount of thiol groups present on the proteins. FRAP assay was done using 2,4,6-tripyridyl-s-thiazine and the complex formed was measured at 593 nm in a spectrophotometer \[^6\]. Statistical analysis was performed according to the non-parametric Mann-Whitney test, Kruskall Wallis test and paired samples t test using SPSS package, version 11.5.

**Results**

There was a significant decrease in plasma protein thiols in nephrotic children in relapse and in pre-RPT groups when compared to controls. A marked decrease in protein thiols was also observed in children in relapse when compared to those at the onset of RPT (Table I). Plasma FRAP however, did not exhibit any significant change when compared to controls as well as in an inter-group comparison. Further, a comparison of plasma protein thiol and FRAP levels in the same patient twice, that is before starting RPT and after a four year follow-up, indicated a noticeable decrease in plasma protein thiols and a significant decrease (p<0.046) in the plasma FRAP values post-RPT when compared to their pre-RPT values (Table II) at the end of four years. The mean urine pre-RPT protein value was 30.08 mg/m\(^2\)/h which became nil post-RPT. Considering the fact that clinical remission is when urinary protein is <40mg/m\(^2\)/h and biochemical remission is when urinary protein is <4 mg/m\(^2\)/h, the post-RPT values of nil urinary protein are an excellent indicator of complete recovery in these children.

**Discussion**

Many studies in the past have laid emphasis on the association of oxidative injury and NS \[^1\]-\[^3\]). In our earlier studies \[^1\],\[^2\], we too have found a very important correlation between free radical toxicity and NS. Hence, in the present study we now wanted to assess the effect of RPT on clinical improvement and its association with change in the levels of plasma antioxidants. Plasma protein thiols were significantly decreased in nephrotic children in relapse (p < 0.001) and those selected for RPT (p<0.001) when compared to controls. This was also the case when the relapse and pre-RPT groups were compared (p<0.001). It may be
due to the adaptive response of the body to oxidative stress present in NS \cite{1,2}. Plasma FRAP values exhibited an insignificant increase over that of controls and also in an intergroup comparison. It would be appropriate to mention that albuminuria in NS has no relation with the lowering of plasma protein thiols as reported earlier \cite{7}. However, an interesting observation was made in 6 pre-RPT nephrotic children who turned up for follow up after 4 years (8 were lost for follow up and the rest of the 16 children presented with microalbuminuria, even after a period of 4 / less than 4 years). Along with a noticeable decrease in the plasma protein thiols and a significant decrease in FRAP levels these children also showed marked improvement in their clinical features (urine protein being nil). Thus, the decreased demand for antioxidants may be due to decreased oxidative stress in such children gradually approaching normalcy. There have been observations reported elsewhere that antioxidant status recovered completely only during long-term remission in pediatric NS \cite{8} and that the total antioxidant capacity (TAC) was significantly decreased in children with NS \cite{9}. Moreover in the present study, a positive response to the treatment in patients on RPT can be due to the antioxidant effects of the combined drug therapy i.e. enalapril, aspirin and dipyridamole along with a marked reduction in proteinuria \cite{10-13}. There are several reports on enalapril, aspirin and dipyridamole mentioning their role as regulators between oxidant stress and the antioxidant system besides their action as renoprotective agents. Further, Kaneko et al \cite{14} have reported increase in urinary 8-hydroxydeoxyguanosine (8-OH-DG) in idiopathic nephrotic syndrome thereby highlighting the interaction of reactive oxygen species with cellular DNA in acute glomerular disease.

Thus, it can be concluded that there could be a relationship between oxidative stress and etiopathogenesis of NS and probably the response to treatment (RPT) depends on the antioxidant status of the body at that point of time. However, one of the limitations of this study is the small sample size accessible in the post RPT after a period of 4 years.


Table 1. Plasma protein thiols and FRAP in pediatric NS patients in controls, relapse and on RPT, Median (IQR)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 30)</th>
<th>Relapse (n = 19)</th>
<th>RPT (n = 30)</th>
<th>Significance, p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein thiols, µmole/l</td>
<td>637 (590, 675.60)</td>
<td>202.50 (135, 234)</td>
<td>387 (264, 672)</td>
<td>&lt;0.001 (versus controls and intergroup comparison)</td>
</tr>
<tr>
<td>FRAP, µmole/l</td>
<td>680 (590, 860)</td>
<td>780 (640, 1100)</td>
<td>800 (520, 930)</td>
<td>NS (versus controls and intergroup comparison)</td>
</tr>
</tbody>
</table>

*Nonparametric tests: Mann–Whitney test for protein thiols and Kruskal Wallis test for FRAP. NS – not significant.

Table 2. Plasma protein thiols and FRAP in pre-RPT and post- RPT children, Median (IQR)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-RPT (n=6)</th>
<th>Post-RPT (n=6)</th>
<th>Significance, p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein thiol, µmole/l</td>
<td>792.50 (333,935)</td>
<td>402.40 (249.7,586)</td>
<td>0.249, NS</td>
</tr>
<tr>
<td>FRAP, µmole/l</td>
<td>810 (715, 1380)</td>
<td>390 (291,625)</td>
<td>0.046**</td>
</tr>
</tbody>
</table>

*Nonparametric Wilcoxon signed rank test, **Significant when compared to pre-RPT
NS = not significant

References


