Antioxidant Status in Children with Nephrotic Syndrome

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ABSTRACT

Background: It has long been recognized that primary childhood nephrotic syndrome (NS) is an immunological disorder. In recent years it has been proposed that NS is a consequence of an imbalance between oxidant and antioxidant activity. Reactive oxygen metabolites play an important role in the pathophysiologic process of a surprisingly wide variety of clinical and experimental renal diseases.

Objectives: The aim of the present study was to examine the activity of the main antioxidant enzymes and the total antioxidant status in children with NS.

Methods: The study included three groups of children. Group I included 20 children during the proteinuric phase of nephrotic syndrome. Group II included 20 children, 6 months after remission which was induced by steroid therapy. The age range was 4-10 years, with a mean of 6.2 ± 1.4 years. Group III included 20 healthy children, matched for age and sex as a control group. Total antioxidant status (TAS), glutathione reductase (GR) and glutathione peroxidase (GPX) were measured for all.

Results: TAS was significantly reduced in both Group I and Group II compared to the control (p = 0.01). In children from Group I the activity of GR and GXP were significantly lower than in controls. In children from Group II GR activity was also significantly lower than in the control group (p = 0.006), but there was no significant difference in GXP level. The activity of TAS & GXP were lower in those with first attack of NS (Group I) and those in remission (Group II). There was no significant difference in GR level between children in Group I and those in Group II. The total cholesterol (TChol) and low density lipoprotein cholesterol (LDL) concentrations in Group I were significantly higher than those in Group II and in the controls. There were no significant differences in high density lipoprotein cholesterol (HDL) & triglyceride (TG) levels in the two Groups I & II. In children with first attack NS a negative correlation appeared between TAS and TChol and LDL. There was no correlation between TAS and other laboratory results. In children with remission there was a negative correlation between TChol and TAS. But no correlation between TAS and other laboratory results.

Conclusions: We concluded that total antioxidant status is diminished in children with NS. Abnormal antioxidant status may be partly related to abnormalities of some antioxidant enzyme activity and high lipid concentration.

INTRODUCTION

It has long been recognized that primary childhood nephrotic syndrome is an immunological disorder. In recent years it has been proposed that NS is a consequence of an imbalance between oxidant and antioxidant activity. Reactive oxygen metabolites play an important role in the pathophysiologic process of a surprisingly wide variety of clinical and experimental renal diseases.

Evidence that reactive oxygen species (ROS) play a major role in many glomerular diseases has been obtained indirectly by the detection of products of lipid peroxidation in renal tissue and by the demonstration...
of protective effects of administered anti-
oxidant\(^7\).

Nephrotic syndrome is a stressful
condition for children. Free radicals have a
negative influence on renal tissues, locally
increased concentration of ROS may lead to
tissue damage by several mechanisms. As a
result of direct oxidative modification of
cellular components, cell structure and func-
tion can be altered substantially. Oxidation
of lipids generates lipid radicals which can,
in turn, initiate and self sustain lipid
oxidation\(^8\). Thus cell and basement
membranes that depend on the integration
of non-oxidation lipids to maintain their
orderly architecture may be deranged, a
process that could be important for
glomerular proteinuria. Oxidative modifi-
cation of protein residues can promote the
loss of the scaffolding property of structural
proteins, can inactivate enzymes, and
finally can alter the degradation and
clearance of these molecules\(^9\). Oxidation
of purines, pyrimidines and of the attached
ribose moiety can give rise to crosslinking
or fragmentation of nucleic acids, leading to
altered gene expression\(^10\). The effects of
enhanced oxidative stress on renal function
are stimulation of renin release from
juxtaglomerular cells, glomerular injury,
stimulation of mesangial cell growth, and
hypertrophy of tubular epithelial cells.
These result in derangement of the
glomerular filtration barrier, leading to
proteinuria\(^11\). As NS is usually accom-
panied by marked hyperlipidaemia that has
been involved in the deterioration of renal
function\(^12\), lipids are the target molecules
for free radicals\(^13\). Reduced antioxidant
protection is one of the factors leading to
renal injury and may be a consequence of
lipid abnormality\(^4\).

There are many different enzymes and
proteins protecting human cells from the
effects of free radicals. The antioxidant
defences interact to form an integrated
system\(^14\). Decreased activities of glome-
ricular antioxidative enzymes may be at
least partly responsible for increased ROS
levels\(^15\). It may therefore be inappropriate
to rely on the measurement of a single
component as an indicator of the function-
ing of the entire antioxidant system\(^13\).

AIM OF THE WORK

The aim of the present study was to
examine the activity of the main antioxidant
enzymes and the total antioxidant status in
children with NS.

SUBJECTS AND METHODS

The study was a hospital-based, pro-
spective cohort study. Total antioxidant
status (TAS), glutathione reductase (GR)
and glutathione peroxidase (GPX) were
measured in 40 children with NS. Of those
20 children were in the proteinuric phase of
the disease (Group I) whereas Group II
consisted of 20 children 6 months after
remission which was induced by steroid
therapy. The age range was 4-10 years, with
a mean of 6.2 ± 1.4 years. The same
biochemical parameters were measured in
20 healthy children matched for age and
sex, who constituted the control group. All
patients had normal blood pressure, normal
serum creatinine level and had no evidence
of bacterial or viral infection at the time of
the study. Laboratory investigations for
children in Group I were performed before
steroid treatment. This is important because glucocorticoids can raise glomerular antioxidant enzyme activity(16). Nephrotic syndrome was defined as daily urinary protein excretion ≥ 40 mg/hr/m². Total antioxidant status, GPX & GR were estimated using antioxidant kits (Randox Laboratories). Total antioxidant status was estimated using the two reagent Randox Total Antioxidant status in plasma. ABTS was incubated with a peroxidase and (H₂O₂) to produce the radical cation ABTS⁺. This has a relatively stable blue-green color, which was measured at 600 nm. Antioxidants in the added plasma caused suppression of this color production to a degree that was proportional to their concentration. GPX activity in red blood cells was measured using the method described by Poglia & Valentine(17). Glutathione peroxidase catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of GR and NADPH, the oxidized glutathione is converted to reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbency was measured at 340 nm. Glutathion reductase activity was measured using the method described by Goldberg & Spooner(18).

The basic laboratory tests including serum TG, LDL, HDL and cholesterol levels were measured using Cobas Integra 400, Roche Diagnostics, UK (a fully automated chemistry analyser).

Statistical Analysis

The mean and standard deviation were determined for each variable in all groups. Comparisons between groups were based on the Mann-Whitney rank test, and the significance level was (< 0.05). Bivariate associations between variables of interest were analyzed by Pearson's linear regression.

RESULTS

All results in children with first attack NS were compared with values found in healthy children (Group III) and those in remission (Group II). The results of children in remission were compared with those in the two other groups (Groups I and III).

The basic laboratory results are summarized in Table (1), while Table (2) summarizes the results of TAS, GR, and GPX activity.

The TChol and LDL concentrations in Group I were significantly higher than in Group II (p = 0.0001 and p = 0.001 respectively) and controls (p < 0.000 and p < 0.0001 respectively). There were no significant differences in HDL & TG levels in Groups I and Group II (p = 0.17) and (p = 0.19).

In children from Group I, the activity of TAS, GR, GXP was significantly lower than in controls (p = 0.0001, p < 0.001 and p = 0.01 respectively). In children from Group II, TAS & GR activity were significantly lower than in controls (p < 0.003 and p < 0.006 respectively). There was no significant difference in GXP (p = 0.5). The activity of TAS & GXP were also significantly lower in those with first attack of NS (Group I) compared to those in remission (Group II) (p = 0.001 and p = 0.035 respectively), but not GR level.

In children with first attack NS a negative correlation appeared between TAS and TChol (p < 0.001; r = -0.58; Fig. 1) and TAS and LDL (p = 0.01, r = 0.29; Fig. 2).
There was no correlation between TAS and other laboratory results. Serum cholesterol by multiple linear regression analysis was dependent upon serum albumin, TAS, GR, in Group I and upon TAS and GXP in Group II.

### Table 1: Basic laboratory results in nephrotic children & controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. Albumin (g/l)</strong></td>
<td>2.4 ± 0.5</td>
<td>2.9 ± 0.8</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td><em>p</em> = 0.04</td>
<td><em>p</em> = 0.08</td>
<td><em>p</em> = 0.001</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td>250.3 ± 101.8</td>
<td>135 ± 48</td>
<td>128.3 ± 16.01</td>
</tr>
<tr>
<td></td>
<td><em>p</em> = 0.0001</td>
<td><em>p</em> = 0.5</td>
<td><em>p</em> &lt; 0.0001</td>
</tr>
<tr>
<td><strong>LDL (mg/dl)</strong></td>
<td>109.3 ± 26.2</td>
<td>75.8 ± 29.5</td>
<td>68.3 ± 11.7</td>
</tr>
<tr>
<td></td>
<td><em>p</em> = 0.001</td>
<td><em>p</em> = 0.3</td>
<td><em>p</em> = 0.0001</td>
</tr>
<tr>
<td><strong>HDL (mg/dl)</strong></td>
<td>55.3 ± 11.7</td>
<td>49.1 ± 8.5</td>
<td>41.4 ± 3.6</td>
</tr>
<tr>
<td></td>
<td><em>p</em> = 0.17</td>
<td><em>p</em> = 0.08</td>
<td><em>p</em> = 0.003</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dl)</strong></td>
<td>82.1 ± 16.8</td>
<td>71.7 ± 20.4</td>
<td>73.7 ± 15.1</td>
</tr>
<tr>
<td></td>
<td><em>p</em> = 0.09</td>
<td><em>p</em> = 0.7</td>
<td><em>p</em> = 0.06</td>
</tr>
<tr>
<td><strong>Proteinuria (mg/24 hr)</strong></td>
<td>4.5 ± 2.9</td>
<td>3.4 ± 0.9</td>
<td>No proteinuria</td>
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<tr>
<td></td>
<td><em>p</em> = 0.03</td>
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</tbody>
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Table 2: TAS, GR and GXP activity in Group I, Group II and controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (nmol/l)</td>
<td>0.74 ± 0.06</td>
<td>0.78 ± 0.19</td>
<td>1.05 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>p = 0.001</td>
<td></td>
<td>p = 0.003</td>
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<tr>
<td></td>
<td></td>
<td>p = 0.0001</td>
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<tr>
<td>GR (U/gHb)</td>
<td>7.8 ± 1.28</td>
<td>8.05 ± 1.6</td>
<td>10.6 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>p = 0.08</td>
<td></td>
<td>p = 0.006</td>
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<tr>
<td></td>
<td></td>
<td>p &lt; 0.001</td>
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<tr>
<td>GXP (U/gHb)</td>
<td>27.1 ± 4.8</td>
<td>31.06 ± 4.41</td>
<td>33.3 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>p = 0.035</td>
<td></td>
<td>p = 0.5</td>
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<tr>
<td></td>
<td></td>
<td>p = 0.01</td>
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Fig. 1: Correlation between total cholesterol and total antioxidant status.

Fig. 2: Correlation between LDL cholesterol and total antioxidant status.
DISCUSSION

Small amounts of reactive oxygen species (ROS) are constantly produced in aerobic metabolism and have important roles in normal cell physiology. However in pathophysiological conditions with increased levels of ROS, these molecules become relevant factors in the initiation and amplification of deleterious processes\(^{(19)}\).

Locally increased concentrations of ROS may lead to tissue damage by several mechanisms; as a result of direct oxidative modification of cellular components, cell structure and function can be altered substantially\(^{(7)}\). Oxidation of lipids generates lipid radicals which can, in turn, initiate and self-sustain lipid oxidation\(^{(9)}\). Thus, cell and basement membranes that depend on the integration of non-oxidative lipids to maintain their orderly architecture may be deranged, a process that could be important for glomerular proteinuria\(^{(20)}\). Oxidative modification of protein residues can promote the loss of the scaffolding property of structural proteins, can inactivate enzymes, and finally can alter the degradation and clearance of these molecules\(^{(9)}\). Oxidation of purines, pyrimidines and of the attached ribose moiety can give rise to crosslinking or fragmentation of nucleic acids, leading to altered gene expression\(^{(10)}\).

The antioxidant system consists of many different components that defend tissue against free radical attack. These components may be divided into three main groups: primary antioxidants (e.g. SOD, GPX, ceruloplasmin, transferrin, and ferritin), secondary antioxidants (e.g. vitamin E, vitamin C, beta-carotene, uric acid, bilirubin, albumin) and tertiary antioxidants (e.g. DNA repair enzymes, methionine sulfoxide reductase)\(^{(21)}\). Measurement of the functioning of the antioxidant system may indicate an individual’s susceptibility to oxidant induced disease. Total antioxidant status analysis is designed to evaluate the overall performance of the antioxidant system\(^{(4)}\).

The study results show that antioxidant defence mechanisms are diminished in patients with the nephrotic syndrome. Total antioxidant status is significantly lowered compared to healthy children. This is expressed especially in patients with higher TChol and LDL concentrations, suggesting the consumption of some antioxidant components during the acute phase of NS. However, it is difficult to interpret the lower level of TAS in those with first episode of NS compared to children in remission. We can only hypothesize that in children at the acute phase of the disease, low TAS may lead to abnormal lipid peroxidation, resulting in a high rate of glomerular injury\(^{(4)}\). On the other hand, prolonged lipid oxidation may lead to diminished antioxidant activity\(^{(15)}\). Antioxidant concentrations change considerably, indicating a compensatory mechanism to cope with the increased pro-oxidant status in such cases\(^{(22)}\).

The decreased activities of glomerular antioxidative enzymes may be at least partly responsible for increased ROS levels\(^{(15)}\). Patients with high TChol and LDL concentrations had the lowest TAS activity. This is probably the result of increased consumption of certain antioxidant components, such as vitamin E (serum and erythrocyte), vitamin C and carotene.
concentrations, which are significantly lowered in the acute phase of NS. All these tend to improve during remission although complete normalization does not occur. Deficiencies in antioxidant status can develop from low intake of dietary antioxidants. Diets deficient in selenium and vitamin E may lead to renal injury characterized by proteinuria and a reduced GFR. Low manganese intake has a negative influence on TAS. Thus improvement of antioxidant status may be a potential therapeutic target to protect from or ameliorate injury during nephrotic syndrome.

In the present study we found differences in GPX & GR activity in children with NS compared to the controls. This suggests that low TAS may be due to differences in antioxidant enzyme activity. When endogenous GPX was experimentally depressed in NS rats, the characteristic proteinuria was even more markedly augmented. Decreased GPX activity in NS, was already reported by others. Low GPX activity may be a factor limiting the antioxidant capacity in NS.

It is very interesting that in children with remission of NS, TAS does not correspond fully with GR and GPX. This is probably a result of the fact that the antioxidant system consists of many different components and these antioxidant components interact to form an integrated system. This finding is very important because it clearly indicates that we cannot rely on the activity of a single enzyme or even two as an indicator of the functioning of the entire antioxidant system.

We concluded that total antioxidant status is diminished in children with NS. Abnormal antioxidant status may be partly related to high lipid concentrations. This may be a compensatory mechanism to cope with increased pro-oxidant status in nephrotic syndrome. However, studies are required to investigate the influence of improvement of antioxidant status in ameliorating injury during the nephrotic syndrome.

REFERENCES