Original article

Evaluation of the electrophoresis of serum proteins in cases of nephrotic syndrome in Senegalese children

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ABSTRACT

Introduction: Child nephrotic syndrome (NS) is defined by the association of proteinuria > 50 mg/kg/24h, proteinemia < 60 g/l and albuminemia < 30 g/l. α2-globulinemia is frequently increased i.e. > 11 g/l. There are reportedly very few data on the evaluation of biological parameters of NS in Senegal. We therefore sought to evaluate the electrophoretic profile of serum proteins in child NS. Materials and methods: This is a retrospective observational analytical study of 48 nephrotic children. Proteinuria and proteinemia were determined; serum protein electrophoresis was performed. The nephrotic index (NI) defined by the α2-globulins/albumin ratio and the disturbance frequencies of the protein fractions were calculated. The averages were compared using the Student T test. The children had a median age of 85 months [15 - 180] and the sex-ratio was 2.27. The study included 31 pure nephrotic (PNS) and 17 impure (INS). Proteinuria, proteinemia and albuminemia were 114.87±8.62 mg/kg/24 hours, 47.2 ± 0.99 g/l and 16.05±0.94 g/l. The electrophoresis showed a decrease in serum protein and albumin in all nephrotics and a frequent decrease in γ-globulins (50%) and β-globulins (41.67%). α1-globulins were significantly increased in INS compared to PNS (3.46±3.18 versus 1.78±0.82; p= 0.046). The rise in α1-globulins was quite frequent (33.33%), especially among INS (58.82%) compared to PNS (19.35%). A more frequent increase in NI (95.03%) than α2-globulins (87.50%) was observed. Compared to non hyper-γ-globulinnemics, hyper-α2-globulinnemics showed a significant decrease in proteinemia, albuminemia and γ-globulinemia. Conclusion: The association between hypoalbuminemia and increased NI is reported to be a more sensitive marker of NS in Senegalese children than the association between hypoalbuminemia and hyper-α2-globulinemia. The increased synthesis of α2-globulins would be by a phenomenon of amino acid remobilization from albumin and γ-globulins. α1-globulins appear to be the only protein fraction that can be used as a differential marker between pure and impure nephrotic.

INTRODUCTION

Nephrotic syndrome (NS) of the children is defined by the association of 24-hour proteinuria (Pu 24h) > 50 mg/kg, proteinemia < 60 g/l and albuminemia < 30 g/l [1, 2]. Nephrotic syndrome is very often associated with an increase in plasma concentration of α2-high molecular weight (HMW) glycoproteins, particularly α2-macroglobulin (MW = 725 kDa). The increase in HMW globulins at α2-globulins would compensate for the decrease in vascular oncotic pressure caused by the decrease in plasma concentration of the protein most representative of total serum proteins (55 - 65%), namely albumin (MW = 70 kDa) [3, 4]. These changes in the composition of total proteins naturally result in changes in the electrophoretic profile of nephrotic serum proteins. Thus, Longworth L.G. and MacInnes DA in 1940, characterized the electrophoretic silhouette of nephrotic plasma proteins as a collapse of the wings and a strengthening of the center [5]. In 2000, Sall ND et al. described, in particular, the serum protein electrophoresis of Senegalese nephrotic children as marked by a decrease in all protein fractions except α2-globulins, which were significantly elevated, up to 40% of total protein [6]. However, data on children's NS would generally be scarce in sub-Saharan Africa, and in particular in Senegal, where there are very few studies on biological parameters, among others [7, 8]. The evaluation of the biological parameters of interest to the NS is therefore a challenge to be met for a better description, understanding and management of nephrotic syndrome, with an
estimated hospital prevalence in paediatrics of 0.27% in Senegal [8]. This is why we have undertaken this study, the objective of which is to evaluate the electrophoretic profile of serum proteins in Senegalese children with nephrotic syndrome.

**MATERIALS AND METHODS**

This is a retrospective analytical observational study of the results of serum protein electrophoresis in Senegalese children with nephrotic syndrome. The study population was composed of patients under 180 months of age hospitalized at the Albert Royer National Children’s Hospital or the Aristide le Dantec University Hospital in Dakar. Anthropometric (age, sex, weight), clinical (pure or impure NS) and biological (24-hour proteinuria (24-hour PU), proteinemia, albuminemia, serum protein electrophoresis on agarose gel at pH = 8.6) data were collected from hospital records. Patients with inoperable records and those who did not benefit from serum protein electrophoresis on alkaline pH agarose gel (pH 8.6) were not included in the study. Children with 24-hour PU > 50 mg/kg, proteinemia < 60 g/L and albuminemia < 30 g/L were included, for a total of 48 children finally included in the study.

Electrophoregrams were interpreted by considering reference values those proposed by the working group of the National College of Hospital Biochemistry (2006) [9]. We empirically introduced into the analysis of electrophoresis, a data we called the nephrotic index (NI) which expressed the α2-globulins / albumin ratio. We considered that this ratio should be strictly higher than 0.37 in nephrotic syndrome since, in our study, α2-globulins were considered increased if they were > 11 g/L and albumin decreased if it was < 30 g/L and the 11/30 ratio is 0.37.

The frequency of decrease, then the frequency of increase, and finally the frequency of disturbance of each protein fraction of the electrophotogram were calculated in the study population and then particularly in patients with pure NS (PNS) and those with impure NS (INS).

The average of the biological parameters was determined for all patients. In addition, a comparison of the mean parameters was made between subjects with PNS and those with INS and between hyperα2-globulinemic and non-hyperα2-globulinemic patients. The means were compared with the Student T test after comparing the variances with the F test. The statistical analysis was carried out using 2013 Excel software.

**RESULTS**

The children had a median age of 85 months [15 - 180 months], an average weight of 24.89±1.71 kg and a sex-ratio of 2.27. Of the 48 patients included in the study, 65.58% (n=31) had pure nephrotic syndrome and 34.42% (n=17) had impure nephrotic syndrome.

The analysis of mean biological parameters of Senegalese nephrotic children in our series showed an increase in urinary proteins and serum α2-globulins and a decrease in proteinemia and albuminemia [Table I]. The serum α1-globulins, β-globulins and γ-globulins did not appear to vary [Table I].

Comparison of mean biological parameters showed a significant increase in α1-globulinemia in INS patients compared to PNS patients [Table II]. However, the other parameters studied did not show any significant difference between patients in the two subgroups [Table II].

Compared to patients without hyperα2-globulinemia, patients with hyperα2-globulinemia had a significant decrease in total protein, albumin and gamma-globulins [Table III]. However, no significant differences were found between these two groups of patients with respect to proteinuria, α1-globulins and β-globulins [Table III].

Analysis of the frequency of decrease in serum protein electrophoresis parameters showed a decrease in protein and albumin levels in all patients. Of the 48 patients studied, half (24) had a decrease in γ-globulins, 20 showed a decrease in β-globulins, 2 showed a decrease in NI (α2-globulins / Albumin). α1-globulins were decreased in 1 patient with PNS. α2-globulins were also lowered in only 1 patient with INS. In both PNS and INS, all patients had a decrease in serum protein and albumin. In the PNS, the frequency of albumin decrease was followed by those of β-globulins and γ-globulins that were superimposed. In contrast, in the INS, the decrease frequency of albumin was followed by that of γ-globulins, which was higher than that of β-globulins. [Figure 1]

Analysis of the frequency of increases in serum protein electrophoresis parameters during nephrotic syndrome revealed that total protein or albumin was not increased in any of the subjects studied. However, among the 48 patients studied, 46 had an increase in NI (α2-globulins/albumin) above 0.37. α2-globulins were elevated in 42 patients. The increase in α1-globulins was noted in 16 patients. γ-globulins were elevated in 3 patients and β-globulins in 2 children. The increase in NI was the most frequent increase observed in both the PNS and INS, followed by the increase in α2-globulins and α1-globulins (especially in INS). [Figure 2]
Analysis of the frequency of disturbance of serum protein electrophoresis parameters during nephrotic syndrome showed a decrease in total serum protein and albumin in all nephrotics and a frequent decrease in γ-globulins (50%) and β-globulins (41.67%). However, a very frequent increase in NI (95.83%) and α2-globulins (87.50%) was observed in the 48 patients. An increase in α1-globulins was quite frequent in the study population (33.33%) especially in INS patients (58.82%) compared to PNS patients (19.35%).[Figure 3]

**Table 1** : Mean biological parameters of Senegalese nephrotic children

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Average</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuresis (ml/kg/h)</td>
<td>1.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Proteinuria (mg/kg/24h)</td>
<td>114.87</td>
<td>8.62</td>
</tr>
<tr>
<td>Proteinemia (g/l)</td>
<td>47.19</td>
<td>0.99</td>
</tr>
<tr>
<td>Albuminemia (g/l)</td>
<td>16.05</td>
<td>0.94</td>
</tr>
<tr>
<td>Alpha 1-globulinemia (g/l)</td>
<td>2.37</td>
<td>0.3</td>
</tr>
<tr>
<td>Alpha 2-globulinemia (g/l)</td>
<td>16.82</td>
<td>1.04</td>
</tr>
<tr>
<td>Beta-globulinemia (g/l)</td>
<td>7.12</td>
<td>0.39</td>
</tr>
<tr>
<td>Gamma-globulin (g/l)</td>
<td>5.69</td>
<td>0.49</td>
</tr>
</tbody>
</table>

**Table 2** : Comparison of mean biological parameters between subjects with pure and impure nephrotic syndrome

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pure nephrotic syndrome (n=31)</th>
<th>Impure nephrotic syndrome (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuresis (ml/kg/h)</td>
<td>1.68±0.56</td>
<td>1.76±0.58</td>
<td>0.628</td>
</tr>
<tr>
<td>Proteinuria (mg/kg/24h)</td>
<td>106.75±62.54</td>
<td>129.67±52.76</td>
<td>0.207</td>
</tr>
<tr>
<td>Proteinemia (g/l)</td>
<td>46.22±6.88</td>
<td>48.98±6.81</td>
<td>0.188</td>
</tr>
<tr>
<td>Albuminemia (g/l)</td>
<td>15.83±5.72</td>
<td>16.46±8.01</td>
<td>0.752</td>
</tr>
<tr>
<td>Alpha-1-globulinemia (g/l)</td>
<td>1.78±0.82</td>
<td>3.46±3.18</td>
<td>0.046</td>
</tr>
<tr>
<td>Alpha-2-globulinemia (g/l)</td>
<td>15.97±4.29</td>
<td>18.37±10.76</td>
<td>0.390</td>
</tr>
<tr>
<td>Beta-globulinemia (g/l)</td>
<td>6.79±2.71</td>
<td>7.78±2.82</td>
<td>0.261</td>
</tr>
<tr>
<td>Gamma-globulin (g/l)</td>
<td>5.78±3.27</td>
<td>5.51±3.73</td>
<td>0.789</td>
</tr>
<tr>
<td>Nephrotic Index</td>
<td>1.67±1.61</td>
<td>1.18±0.58</td>
<td>0.243</td>
</tr>
</tbody>
</table>

**Table 3** : Comparison of mean biological parameters between hyperα2-globulinemia and non-hyperα2-globulinemia patients with nephrotic syndrome

**DISCUSSION**

The analysis of the results from the protein electrophoresis profiles of Senegalese nephrotic children revealed that proteinemia and albuminemia were the most frequently disrupted parameters in our series. Indeed, 100% of the nephrotics in the study showed hypoproteinemia and hypoalbuminemia [Figures 1, 3]. This finding remained consistent across the two groups of PNS and INS patients and could be explained by the selection of our study population. Indeed, only patients who met the NS definition criteria were included in the study: 24-hour proteinuria > 50 mg/kg, proteinemia < 60 g/l and albuminemia < 30 g/l.

The mean values observed in our series were for proteinemia and albuminemia of 47.19±0.99 g/l and 16.05±0.94 g/l respectively [Table 1]. The average value of our proteinemia was lower than that found by Sall ND et al. which was 52.10±4.32 g/l but higher than that found by Moyen G. et al. which was 42.4 g/l [28 - 66 g/l] or by...
Hypothesis that their HMW would contribute to increase oncotic pressure and thus macroglobulin. HMW protein (MW = 725 kDa). 0.390 [Table II]. The increase in not significantly different between PNS patients in our study especially in impure nephrotic syndrome. 2, 3. It goes without saying that the NI could be considered as a nephrotic group [Figures 2, 3]. In addition, we noted an increase in the NI for 94.11% of patients compared to 82.35% for α2-globulins in the group of patients with impure nephrotic syndrome [Figures 2, 3]. It goes without saying that the NI could be considered as a more sensitive marker than α2-globulins in nephrotic syndrome, especially in impure nephrotic syndrome. The average blood count was 16.82±1.04 g/l for our nephrotic patients. This mean was lower than that obtained by Sall ND et al. which was 21.36±8.27 g/l but higher than that found by Moyen G. et al. which was 9.8 g/l [2, 5 - 25 g/l] [6, 10]. α2-globulinemia was not significantly different between PNS patients in our study compared to INS patients [15.97±4.29 g/l versus 18.37±10.76; p = 0.390] [Table II]. The increase in α2-globulins is explained by the increased hepatic synthesis of α2-globulins, especially α2-macroglobulin, HMW protein (MW = 725 kDa), α2-globulins with their HMW would contribute to increase oncotic pressure and thus to counteract the abundant diuresis noted in nephrotics. Nevertheless, the hypothesis that α2-globulins increase to compensate for urine protein leakage could be questioned in light of our results. Indeed, if this hypothesis were true, then patients with a higher protein leakage rate should be those who showed an increase in α2-globulins. However, in our study, 24-hour proteinuria was not significantly different between hyperα2-globulinemic and non-hyperα2-globulinemic patients (109.28±52.39 mg/kg versus 153.97±94.46 mg/kg; p = 0.086). Moreover, the trend was that α2-globulins were much higher in patients with lower proteinuria. In other words, the increase in α2-globulins would prevent protein leakage from the urine, but massive proteinuria would not induce increased synthesis of α2-globulins. The increase in the synthesis of α2-globulins by the liver would be at the expense of albumin and γ-globulins. It would rather appear as a phenomenon of remobilization of amino acids by degradation of albumin and γ-globulins and reuse of their amino acids, stimulated by a decrease in oncotic pressure, in order to synthesize α2-globulins of HMW to increase oncotic pressure. Indeed, the comparison of protein fractions between hyperα2-globulinemia and non-hyperα2-globulinemia patients in our study revealed a significant difference between the two subgroups only with albumin and γ-globulins [Table III]. Albuminemia was significantly lower (p = 7.52×10-5) among hyperα2-globulinemia (14.73±5.78 g/l) compared to non-hyperα2-globulinemia (25.3±3.29 g/l) [Table III]. γ-globulinemia was also significantly decreased (p = 0.027) among hyperα2-globulinemia (5.28±3.32 g/l) compared to non-hyperα2-globulinemia (8.52±3.48 g/l) [Table III].

The most frequent disturbance recorded after the increase in α2-globulins was the decrease in γ-globulins (< 5 g/l) [Figures 1, 3]. A collapse of γ-globulins was observed in 50.00% of nephrotics, 45.16% of PNS patients and 58.82% of INS patients [Figures 1, 3]. In other words, half of the patients in our study had a very significant decrease in γ-globulins, so the mean γ-globulinaemia was only 5.69±0.49 g/l [Table I]. An average that was lower compared to the results of Sall ND et al. and those of Moyen G. et al. who had found γ-globulinaemia at 7.28±4.62 g/l and 9.7 g/l respectively with extremes of [0.8 - 18.3 g/l] [6, 10]. Gamma-globulinemia was not different (p = 0.789) between our PNS patients (5.78±3.27 g/l) and our INS patients (5.51±3.73 g/l) [Table II]. This observation seems paradoxical in a Senegalese context marked by bacterial infections and recurrent parasitic infestations. Nevertheless, it could be explained, in part, by the massive urinary leakage of plasma proteins which would hardly spare low MW antibodies, in particular class G immunoglobulins (IgG) of MW = 150 kDa, and, probably by the phenomenon of remobilization of amino acids from γ-globulins to α2-globulins of HMW.

The decrease in β-globulins was frequent although it was less frequent than that of γ-globulins [Figures 1, 3]. The decrease in β-globulinemia (< 6.4 g/l) was observed in 41.67% of patients, 48.38% of PNS patients and 29.41% of INS patients in our series [Figures 1, 3]. The average recorded for β-globulins was 7.12±0.39 g/l [Table I]. This average was superimposed on that obtained by Moyen G et al. which was 7.7 g/l with extremes of [3-15 g/l] [10]. On the other hand, Sall ND et al. reported a β-globulinemia of
5.33±3.76 g/l lower than ours [6]. In our study, β-globulinemia was not statistically different (p = 0.261) between PNS patients with 6.79±2.71 g/l and INS patients with 7.73±2.82 g/l [Table II]. The lowering of β-globulins was not in line with a context of nephrotic syndrome in which an increase in β-globulins was expected. Indeed, β-globulins, often have a high MW, e.g. fibrinogen (MW = 340 kDa) and apoprotein B100 from β-lipoproteins (MW = 540 kDa). Therefore, their increase could contribute to increasing oncotic pressure and counteract the abundant diuresis often observed in nephrotics. Thus, the increase in β-globulins was described in nephrotics as early as 1940 by Longsworth LG and MacInnes DA [5]. These authors were also able to experimentally show that the increase in β-globulins observed in their nephrotics was linked to the increase in certain β-globulins associated with lipids (phospholipids and cholesterol) [5] suggesting that the increase in β-globulins was probably related to the increase in apoprotein B100 in β-lipoproteins. However, from their observation, a statistical inference could certainly not be made to all nephrotics since the number of their patients did not exceed 2.

For α1-globulins, the average recorded in our series was 2.37±0.30 g/l [Table I]. This value was superimposed on that reported by Sall ND et al. which was 2.06±1.27 g/l and that obtained by Moyen G et al. which was 3 g/l with extremes of [1.3-9] [6, 10]. The α1-globulins represented the fraction with the rarest disruptions in our series. Indeed, we found an increase in α1-globulins in only one-third (33.33%) of our nephrotics [Figures 2, 3].

In addition, the comparison of the means of the different protein fractions between PNS and INS patients revealed only one significant difference which related to α1-globulins [Table II]. Indeed, the mean of α1-globulins was 1.78±0.82 g/l in PNS patients compared to 3.46±3.18 g/l in INS patients with a value of p = 0.046 [Table II]. In addition, the increase in α1-globulins was noted in 19.35% of NPS patients and was more pronounced in the NIS where it occurred in more than half of the patients, 58.82% [Figures 2, 3]. This implies that α1-globulins would be the only protein fraction that would allow a differential diagnosis between PNS and INS to be made during electrophoresis. These observations would be explained by the fact that INS would be accompanied by an inflammatory state of varying intensity depending on the multiple and different etiologies of the patients. This would not be the case in the PNS.

CONCLUSION

Serum protein electrophoresis and 24-hour proteinuria should be the two biological parameters to be prescribed as first-line treatment for any suspicion of nephrotic syndrome since, in the report of results, not only 24-hour PU, total serum proteins and albuminemia defining nephrotic syndrome will appear, but also α1-, α2-, β- and γ-globulins and possibly the nephrotic index. The association of hypoalbuminemia and increased nephrotic index would be a more sensitive marker of nephrotic syndrome in Senegalese children compared to the association of reason for the determination of fibrinogen and apoprotein B100 from β-lipoproteins that migrate into the beta zone of the electrophoregram. The elevation of α1-globulins very present in patients with im pure nephrotic syndrome compared to those with pure nephrotic syndrome could be considered as a differential biological diagnostic criterion between these two pathological entities.

REFERENCES


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