Prediction of steroid response in nephrotic syndrome by humoral immunity assessment

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ABSTRACT

The purpose of this study was to estimate the serum levels of IgG, IgM, and IgA in nephrotic syndrome (NS) cases, in activity or in remission, and to detect their levels in relation to steroid response by evelautingthe relationship between IgG/IgM ratio and response to steroids. We investigated 27 cases with NS in activity and in remission and 20 healthy children as controls. Group A included 16 NS patients (12.3±1.4 years) who were steroid-resistant, frequent relapsers, or steroid dependent. Group B included 11 steroid-sensitive NS patients with a mean age of 11.6±2.1 years. Group C included 20 healthy children with a mean age of 12.1±2.3 years who were the control group. We found lower serum IgG level in NS cases compared with the control group; and it was lower in activity than in remission. The levels were lower in Group A compared with those of Group B. Serum IgG levels in Group A were as follows: in activity, 2.29±1.13 g/L and in remission, 4.3±2 g/L. In Group B, they were 6.2±1.2 g/L and 6.5±1.15 g/L in activity and in remission, respectively, and 11.8±2.5 g/L in the healthy control group (P<0.05). There was a direct correlation between serum albumin and serum IgG. We found no significant difference in serum IgM and IgA levels among studied groups whether in activity or in remission. Serum IgG/IgM ratio was lower in activity and in remission in the patient groups than in the control group as it was 9.3±4.7 in healthy subjects. It was 1.8±1.5 in Group A in activity and 3.2±2 in remission, and in Group B 4.8±2.39 in activity and 4.8±2.4 in remission. We conclude that IgM and IgA show no significant difference in NS patients. Serum IgG is lower in NS than in the control group and is much lower in activity than in remission. It is lower in patients with poor steroid response. We propose a predictive value of IgG/IgM ratio in activity, that is, the higher the IgG/IgM ratio in activity, the better the prognosis.

Key words: Humoral immunity, nephrotic syndrome, steroids

Introduction

Nephrotic syndrome (NS) results from excessive urinary loss of albumin and other plasma proteins of similar mass and presents clinically as a syndrome complex of low serum albumin levels, edema, high blood lipid levels, and lipids in the urine.[1]

It is the most common renal disorder in Africa and accounts for 40% of renal disorders in Egypt. Minimal change NS (MCNS) constitutes 88% of the cases of NS.[2]

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Access this article online	
Quick Response Code:	Website: www.indianjnephrol.org
	DOI: 10.4103/0971-4065.82638

In recent years, different studies have been performed in relation to NS and immunity. These studies can be classified under 2 main categories, namely cellular immunity and humoral immunity. Shalhoub was the first to postulate that MCNS could be produced by a systemic abnormality of T-cell function.[3]

The mechanisms by which T cells increase glomerular permeability have remained elusive. There is evidence that idiopathic MCNS may be due to a circulating factor released from activated T cells. Efforts have been made to identify this specific cytokine as well as to understand the mechanism(s) for the increased release of this factor.[4]

In MCNS, serum IgG and IgA levels are reduced, whereas serum IgM level is elevated. It was postulated that the primary defect in idiopathic NS is the deficiency in the T-cell function that mediates the switch from IgM synthesis to IgG synthesis.[5] found abnormal levels of immunoglobulins both in active and in remission stages of the disease, whereas elevated levels of circulating immune complexes were seen only in patients with relapse.

Similar to albumin, IgG is lost in the urine, its serum concentration is decreased, and the fractional rate of its catabolism is increased, suggesting that the kidney contributes to IgG catabolism in conditions of proteinuria.[5]

IgG synthesis responds in a variable fashion in the NS, and may be decreased, thus contributing to its reduced serum concentration. In contrast, the serum concentration of the high–molecular weight immunoglobulin IgM is increased, similar to the serum concentration of a variety of high–molecular weight liver-derived proteins.[6]

A study performed by,[7] suggested that hypogammaglobulinemia of steroid-sensitive NS (SSNS) is characterized by a different constitution of IgG subclasses. In relapse, a reduction of serum levels of IgG-1, IgG-3 occurs, while low concentrations of IgG-2 might be the explanation for hypogammaglobulinemia in remission of SSNS.

Our objectives were to estimate the serum levels of IgG, IgM, and IgA in NS cases during activity and remission and to compare these levels with those of normal children. We tried to detect their levels in relation to steroid response, and to find out the relationship between IgG/IgM ratio and response to steroid therapy.

Patients and Methods

This case–control study was conducted in the Pediatric Nephrology Unit, Zagazig University Hospital, Egypt, from June 2009 to November 2009. We studied 27 cases (15 males and 12 females) of NS.

Group A included 16 patients (10 males and 6 females) with a mean age of 12.3±1.4 years. They had NS, received steroids and other immunosuppressants. They were steroid-resistant NS (SRNS) [whether frequent relapser (FRNS) or steroid dependent (SDNS)]. Of the 16 cases with SRNS, 9 cases had focal segmental glomerulosclerosis (FSGS), 1 case had IgM nephropathy, 1 case had global sclerosis, and renal biopsy was not available in the other 5 cases. Group B included 11 patients (5 males, 6 females) with a mean age of 11.6±2.1 years with SSNS. Group C included 20 healthy children (12 males, 8 females) with a mean age of 12.1±2.3 years, the control group.

Patients below 1 year of age or above 15 years, those with systemic diseases, and hepatitis B or C positive cases were excluded.

Treatment regimen was prednisolone 60 mg/m^2/day for 4 weeks in SSNS with withdrawal of steroid gradually on alternate days. In those with SRNS, another cytotoxic medication was added in the form of levamisole, cyclophosphamide, or cyclosporine A.

Activity or Relapse were defined as increased urinary protein excretion > 40 mg/m^2 body surface area/h or Albustix ++ or more on 3 consecutive days, having previously been in remission. Remission was defined as urinary protein excretion < 4 mg/h/m^2 or Albustix negative or trace for 3 consecutive days.[8] The patients were classified as SSNS when remission could be achieved with steroid therapy alone with 4 weeks treatment. SRNS is defined as failure to achieve response after 4 weeks of 60 mg/m^2/day of prednisone. FRNS was defined as 2 or more relapses within 6 months after the initial response or more than 4 relapses within any 12-month period. SDNS was defined as 2 consecutive relapses occurring during the corticosteroid treatment or within 14 days of its cessation.[8]

All the patients were subjected to complete history taking, thorough clinical examination, and routine investigations as protein in 24 hours urine, serum albumin, and complete blood count with differential white blood cell count. Serum immunoglobulin G, immunoglobulin M, and immunoglobulin A were measured in all subjects, and in patient groups they were obtained both in activity and in remission.

Serum (IgG, IgM) levels were measured by enzyme-linked immunosorbent assay (ELISA) for all subjects supplied by Genway Biotech, Inc., GenWay Biotech, Inc. 6777 Nancy Ridge Drive San Diego USA. Serum IgA was measured by ELISA kit supplied by Immunodiagnostic Systems GmbH, Mainzer landstrasse, 49,60329 Frankfort, Am, Main, Germany. Reference value of IgG was 8–18 g/L, IgM was 0.4–2.3 g/L, and IgA was 0.7–4 g/L.

Statistical analysis was performed using a SPSS version 11. The quantitative data are presented as mean± standard deviation. Unpaired independent *t* test was used to compare independent groups, and paired *t* test was used to obtain paired quantitative data. For more than 2 groups, ANOVA was used. *P* value less than 0.05 indicated a statistical significance.

Results

There is a highly significant decrease ($P = 0.0001$) in the serum levels of IgG, IgG/IgM ratio, and serum albumin in the patient groups compared with the control group as shown in Table 1.

A highly significant increase in urinary protein excretion was observed in the patients, while there were no

significant differences with regard to serum IgM or IgA among the study groups.

There is a significant decrease (P=0.0001) in the serum level of IgG and IgG/IgM ratio in the patient groups compared with the control group and there is . No difference was seen in IgM or IgA and urinary proteins among the study groups.as shown in Table 2.

There is a significant difference between NS cases (P=0.015) in activity as there is a significant decrease in the IgG/IgM ratio in Group A (FRNS and SDNS) in comparison to Group B (SSNS). But there was no significant difference between both the groups in remission. Table 3 shows also a significant decrease in the IgG/IgM ratio in Group A in activity than remission, but this difference was not significant in Group B.

Figure 1 shows a significant positive correlation (P=0.05, r=0.7) between serum IgG in activity and serum albumin in all the studied groups.

Figure 2 shows a significant negative correlation (P=0.05, r=−0.3) between serum IgG/IgM in activity and urinary protein (mg/m^2/h) in all the studied groups.

Discussion

In SSNS, various *in vitro* and *in vivo* immunologic abnormalities have been demonstrated, such as change of lymphocyte subsets, different cytokine profiles, and alterations of serum immunoglobulins.[9-11]

We found a lower serum IgG level in NS cases than in the control group and it was lower in activity than in remission. Comparing the level according to steroid

Table 1: Serum levels of IgG, IgM, IgA, albumin, and protein in urine in activity or relapse

Immunoglobulin measured	Group A n=16	Group B n=11	Group C n=20	*P* value
IgG (g/L)	2.29±1.13 (1–5)	6.2±1.2 (4.9–8.5)	11.8±2.5 (7.8–16)	0.000
IgM (g/L)	1.7±1.2 (0.7–5)	1.5±0.69 (0.7–3)	1.5±0.6 (0.4–2.4)	0.717
IgG/IgM ratio	1.8±1.5 (2–7)	4.8±2.39 (2.3–10.6)	9.3±4.7 (3.9–22)	0.000
IgA (g/L)	1.98±0.88 (1–4)	1.7±0.3 (1.2–2.3)	2.4±0.96 (0.9–4)	0.045
Serum albumin (g/dL)	2±0.6 (1.2–3)	1.9±0.39 (1.4–2.7)	3.9±0.21 (3.5–4.3)	0.000
Protein in urine (mg/m^2/h)	95.6±28.7 (60–150)	92.9±23.7 (65–130)	2.29±0.5 (1–3.1)	0.000

Table 2: Serum levels of IgG, IgM, IgA, and protein in urine in remission

Immunoglobulin measured	Group A n=16	Group B n=11	Group C n=20	*P* value
IgG (g/L)	4.3±2 (2–8)	6.5±1.15 (5.2–8.9)	11.8±2.5 (7.8–16)	0.000
IgM (g/L)	1.6±1 (0.4–4.2)	1.6±0.7 (0.9–2.8)	1.5±0.6 (0.4–2.4)	0.816
IgG/IgM ratio	3.2±2 (1.19–8)	4.8±2.4 (1.8–9.8)	9.3±4.7 (3.9–22)	0.000
IgA (g/L)	2.3±0.6 (0.9–3)	2±0.97 (0.9–4)	9.3±4.7 (3.9–22)	0.328
Urinary protein (mg/m^2/h)	2±0.43 (1.5–3)	1.9±0.47 (1.3–3)	(2.29±0.5) 1–3.1	0.19

Table 3: Comparison between the patient groups (A and B) with regard to serum IgG/IgM ratio both in activity and in remission

IgG/IgM ratio	Group A n=16	Group B n=11
In activity	1.8±1.5 (2–7)	4.8±2.39 (2.3–10.6)
In remission	3.2±2 (1.19–8)	4.8±2.4 (1.8–9.8)

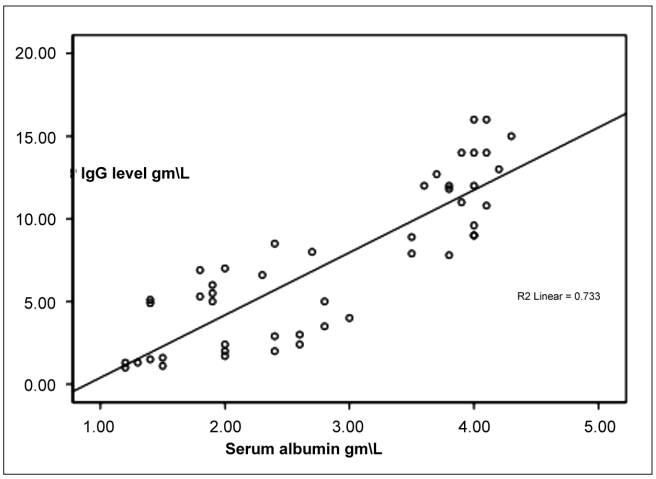

Figure 1: Correlation between serum IgG in activity and serum albumin in all cases

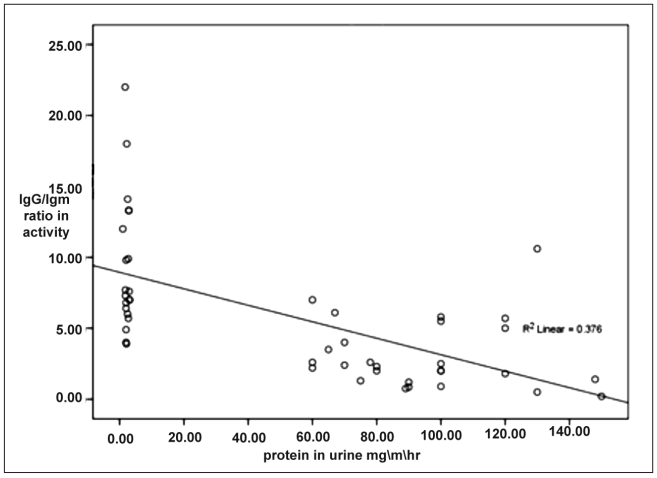

Figure 2: Correlation between serum IgG/IgM ratio in activity and urinary protein in all cases

response, it was lower in Group A (SRNS) either FRNS or SDNS compared with patients of Group B with SSNS. There was a directly proportional correlation between the serum albumin values and serum IgG levels.

Some of the previous studies of serum IgG in NS have not included patients in remission systematically but a low IgG value has been well described by many others both in activity and in remission. In one study,[12] IgG values of SSNS patients in remission (mostly characterized as frequent relapsers) amounted to only 76% of a reference pool, and the decrease in serum IgG during relapse may be responsible for some of the complications associated with NS.[13]

Although the pathophysiology of this decrease remains unknown, the low level of serum IgG in NS may be due to any of the following: the increased IgG catabolism, decreased IgG synthesis, or altered distribution of IgG to the extra plasma compartments.[14] Another mechanism may explain IgG decrease in NS rather than IgM; is the loss of IgG in urine because it has a lower molecular weight than IgM.[5]

A generalized depression of serum IgG subclasses in relapse has been found not only for the idiopathic NS but also for other forms of NS.[15]

A study of humoral immunity in idiopathic NS,[16] demonstrated that patients with idiopathic MCNS are capable of producing, *in vivo*, active antibodies in response to viral or bacterial infections and to antipoliomyelitis immunization. However, these patients presented with decreased IgG and increased IgM during exacerbation of the disease. On the other hand, the number of B lymphocytes and their distribution according to surface immunoglobulins were normal.

In previous studies, there was no attempt to study separately the cellular immunity in the different clinical categories of MCNS, which include infrequent relapsers, frequent relapsers who respond to long-term small dose prednisolone therapy, SNNS, and steroid nonresponders.[17]

We found no significant difference in serum IgM and IgA levels among the studied groups whether in activity or in remission. Mea and Jae found the same result of no differences in the serum values of IgA and IgM between NS and the control groups.[18] However, a study performed by,[13] demonstrated that in children with NS, the serum IgM level was significantly increased during relapse.

It is unknown by what mechanism serum IgM concentration is increased,[6] and the increase in IgM was claimed to be as a result of a defect in the switch from IgM to IgG synthesis due to an unknown immunologic defect,[19] but this theory has not been proved.[11] Chen *et al*[20] reported that enhanced suppressor T cell activity resulted in increased serum IgM and decreased IgG production in children with NS.

We found a highly significant difference between the studied groups regarding serum IgG level both in activity and in relapse, with the lowest values in Group A.

This agreed with the findings of, Andal *et al*[21] who observed that frequent relapsers had lower IgG than infrequent relapsers, but there was no difference in the serum IgM level between the 2 groups.In another study,[22] noticed very low IgG level in SRNS patients.

We also compared the IgG/IgM ratio both in activity and in remission and we found that it was lower in NS group than in the control group. It was lower in Group A (1.8±1.5) in activity and (3.2±2) in remission, than Group B (4.8±2.39) in activity and (4.8±2.4) in remission.

From these results, we have shown that with higher IgG/IgM ratio, a favorable response to steroids is more predictable and with a lower ratio, a poorer response to steroids was predicted. These results agree with some other studies,[23,24] who suggested a better response to treatment for patients with idiopathic NS (including SSNS and FSGS with a high serum IgG-1/IgM ratio and one concluded that IgG/IgM ratio was found to be significantly lower in the NS patients than in the healthy controls.

Recently, a study,[25] showed IgG/IgM ratio of more than 3.0 in most patients with SSNS and the ratio of IgG/IgM more than 1.0 in those of SRNS or FRNS. The finding indicates that the lower the ratio the worse the outcome.

Limitation to this study was that the patients had a mixed group of diseases (FSGS, IgM nephropathy, and so on), which had affected the steroid responsiveness and also the levels of immunoglobulins. Studies on larger number of cases with comparison of the result in relation to their histologic typing will help address this issue.

Conclusion

We conclude that serum IgM and IgA showed no significant difference in NS patients. Serum IgG is lower in NS group than in the control group and is much lower in activity than in remission. It is lower with poor steroid response.

So, we propose a predictive value of IgG/IgM ratio in activity having shown a poor response to steroids with lower ratio and good response with higher ratio, that is to say higher the IgG/IgM ratio in activity, better the prognosis. Further studies are needed to determine the exact ratio that would predict the type of steroid responsiveness.

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How to cite this article: Youssef DM, Abdel Salam SM, Karam RA. Prediction of steroid response in nephrotic syndrome by humoral immunity assessment. Indian J Nephrol 2011;21:186-90.

Source of Support: Nil, **Conflict of Interest:** None declared.