Serum immunoglobulin G, M and IgG:IgM ratio as predictors for outcome of childhood nephrotic syndrome

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Background: **Nephrotic syndrome is an immune mediated disorder of the kidney associated with T cell dysfunction and secondary disturbance of B cell with changes in levels of immunoglobulin and IgG:IgM ratio. These changes in immunoglobulin levels can be used as a proxy marker to understand the clinical variety and prognosis of nephrotic syndrome.**

Methods: **We studied 43 children with nephrotic syndrome during January 2003 to January 2005 in the Pediatric Nephrology Unit, Department of Pediatrics, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Blood samples were collected from the 43 patients, and serum levels of IgG, IgM and IgG:IgM were measured by liquid phase immunoprecipitation assay. Another 20 healthy children attending the laboratory for blood grouping and hepatitis B screening test were enrolled as controls.**

Results: **In the 43 children with nephrotic syndrome, 24 had steroid sensitive nephrotic syndrome (SSNS) and 19 steroid resistant nephrotic syndrome (SRNS). Compared with healthy children, the IgG level was low, IgM level was high, and IgG:IgM ratio was low (P<0.05). The serum IgG level and IgG:IgM ratio were significantly lower in children with SRNS and in children with frequent relapse (FRNS) combined with steroid dependent nephrotic syndrome (SDNS) than in those with infrequent relapse nephrotic syndrome (IFRNS) (P<0.05, respectively).**

Conclusions: **Management of different nephrotic syndromes is based on the levels of immunoglobulins along with clinical and biochemical parameters. The decrease of IgG level as a predictive marker for unfavorable prognosis of nephrotic syndrome in children needs further evaluation in larger scale studies.**

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Introduction

Childhood nephrotic syndrome is an immune mediated kidney disease associated with T cell dysfunction and secondary disturbance of B cell that leads to changes in levels of immunoglobulins. Researchers have hypothesized that this systemic disorder of T cell function results in the production of humoral factors or lymphokines responsible for the increase of glomerular basement membrane permeability.[1,2] Estimate on the annual incidence of nephrotic syndrome is 2-7 per 100 000 children, and the prevalence is 12-16 per 100 000. There is epidemiological evidence of a higher incidence of nephrotic syndrome in children from south Asia and Africa.[3-6]

The disease is characterized by massive proteinuria (urinary total protein >1 g/m^2 per day or urinary spot protein creatinine ratio of >200 mg/mmol), hypoalbuminemia (serum albumin ≤2.5 g/dL), edema, and hypercholesterolemia (serum cholesterol ≥200 mg/dL).[7] The clinical outcome of the disease depends upon the age at presentation, histological changes, absence of hematuria, hypertension, impaired renal function, and responsiveness to steroid therapy.[6] Although recurrence is common in nephrotic syndrome, 90%-95% of children with minimal change nephrotic syndrome (MCNS) are responsive to steroid therapy with complete clinical and biochemical remission and

have excellent long-term prognosis.[6,8-10] Primary or idiopathic nephrotic syndrome is commonly seen in 95% of patients,[8] 80% of whom show histological features of MCNS and have good prognosis.[9,11]

Nephrotic syndrome after the first diagnosis is treated with a standard steroid regimen.[12] Eighty to ninety percent of patients respond to this therapy and 10%-20% are steroid resistant.[12] Giangiocomo et al[13] correlated serum immunoglobulin with steroid treatment outcome in various types of nephrotic syndrome. They reported T cell defect in the conversion from IgM-secreting T cell to IgG-secreting T cell (switching defect), leading to a high level of IgM and a low level of IgG.[13] Therefore the estimation of IgG and IgM levels and their ratio as well as correlation of these values with clinical responsiveness to steroid therapy, relapse, and dependency is important to monitor management strategies and to know the outcome.

The objectives of this study were to compare immunoglobulin levels in children with nephrotic syndrome and healthy children, to understand changes in diseased state, and to assess differences in immunoglobulin levels among the steroid resistant (SRNS), frequent relapse and steroid dependent (FRNS+SDNS), and infrequent relapse (IFRNS) groups. Changes in IgG:IgM ratio were compared in SSNS, SRNS, FRNS+SDNS, IFRNS and healthy children.

Methods

Patients

The prospective study was carried out in the Pediatric Nephrology Unit of Bangabandhu Sheikh Mujib Medical University (BSMMU) from January 2003 to January 2005. The hospital is a tertiary referral hospital with diagnostic and treatment facilities.

A total of 480 children diagnosed with primary nephrotic syndrome were admitted in BSMMU during the study period. Forty-three children were randomly selected who were aged 1-15 years. Among them, 19 had steroid resistant nephrotic syndrome (SRNS, group B) and 24 steroid sensitive nephrotic syndrome (SSNS, group C). In group C, 11 children had FRNS+SDNS (group C_1) and 13 infrequent relapsing nephrotic syndrome (IFRNS, group C_2). Meanwhile, 20 healthy children attending the outpatient department for blood grouping and hepatitis B virus screening were enrolled as controls (group A). Among the 19 children with SRNS, the histological reports on biopsy were mesangial proliferative glomerulonephritis (9 children), MCNS (3), focal segmental glomerulosclerosis (3), membranoproliferative glomerulonephritis (2), and membranous glomerulonephritis (2).

Excluded were children below one year or above 15 years, and those with congenital nephrotic syndrome, nephrotic syndrome secondary to systemic disease like systemic lupus erythematosus, hepatitis B, Henoch-Schönlein purpura, falciparum malaria, lymphoma, or amyloidosis. Those with severe protein energy malnutrition or Down's syndrome were also excluded from the study.

Parents and guardians of the enrolled children were informed about the purpose of data collection and the procedures of the study, and consent was obtained at the enrollment. They were given the choice to withdraw from the study at any time during the course of the study. The study was approved by the Ethical Review Committee of BSMMU.

Laboratory and clinical investigations

Urine samples were collected on admission, and routine microscopic and physical examinations included culture sensitivity (C/S) with colony count (C/C). Spot urinary protein creatinine ratio or urinary total protein was measured by an autoanalyzer (RA 50 chemistry analyzer). 5 ml of venous blood sample was collected for estimation of serum albumin, total protein, cholesterol, creatinine, and blood urea. Serum immunoglobulin (IgG, IgM) levels were measured by liquid phase immunoprecipitation assay in all groups. Reference values of IgG and IgM were 7-14 g/L and 0.4-2.4 g/L, respectively. Cut-off value for low IgG was <7 g/L, high IgM >2.4 g/L, and IgG:IgM >3.

Mantoux test (MT) and Bacillus Calmette-Guerin (BCG) acceleration tests were performed when indicated. Antinuclear antibody (ANA) and anti-DNA double stranded antibody (anti dsDNA) were used for enzyme-linked immunosorbent assay (ELISA) in the children when indicated to rule out systemic disease. HBsAg was tested by screening and ELISA.

Chest X-ray and ultrasonography of the kidneys, ureter, and bladder were performed for all the patients. Renal biopsy was done for SRNS patients.

Study definition

SSNS was defined as responding to steroid therapy within 4 weeks after initiation of the therapy.[3,6] IFRNS was defined as less than 4 relapses within one year or less than 2 relapses within six months after initial responsive episode, and FRNS was defined as more than 4 relapses in one year and more than 2 relapses within six months after initial responsive episode. Remission was defined as protein-free urine for 3 consecutive days, and relapse was defined a proteinuria (urine albumin 3+ or more) for 3 consecutive days after initial responsive episode.[3,6] The occurrence of 2

consecutive relapses during alternate day prednisolone therapy or within 2 weeks of its discontinuation was defined as SDNS.[3,6] No remission after 4 weeks of standard prednisolone therapy at 60 mg/m^2 per day was defined as SRNS.[3,6]

Treatment regimens

All children with first attack and infrequent relapse were given prednisolone 60 mg/m^2 per day at divided doses for 6 weeks, followed by 40 mg/m^2 on alternate days at a single dose for another 6 weeks provided they responded by 4 weeks.[3,12]

IFRNS was treated with prednisolone 60 mg/m^2 per day at divided doses until urine was albumin free for 3 consecutive days and then 40 mg/m^2 on alternate day for 4 weeks.[3,6]

FRNS was treated with prednisolone at a single morning dose of 0.5 mg/kg on alternate days for 12 months.[3,6] In case of steroid toxicity, a 12-week course of cyclophosphamide (2.5 mg/kg per day) or levamisole (2.5 mg/kg) on alternate days for one year was given after remission with prednisolone.[3,6] Initial prednisolone was given on alternate days at 1 mg/kg for a few weeks, then it was discontinued.[3,6]

SDNS was treated with a minimum threshold dose of prednisolone, and it was 0.25 mg/kg on alternate days, which was continued for 12 months provided there were no major side effects. The patients who developed steroid toxicity were treated with levamisole or cyclophosphamide.[3,6]

SRNS was treated with intravenous methyl prednisolone at 30 mg/kg on alternate days for 6 doses, followed by tapering doses of oral prednisolone on alternate days starting at 1.5 mg/kg for 12 months.[3] Supportive measures were also taken, including control of edema, infection, salt restriction, treatment of hypertension, supplementation of protein rich diet, calcium and micronutrient, and albumin infusion.[3,12]

Statistical analysis

Statistical analyses were performed using computer-based software, Statistical Package for Social Science (SPSS). ANOVA was used to compare differences in mean immunoglobulin levels between groups. The Chi-square test and Odd's ratio were compared between the groups. A P value <0.05 was considered statistically significant.

Results

Age distribution

Of the 63 children enrolled in the study, 41 were male and 22 female. There was no significant difference in sex distribution among groups A, B, C_1, and C_2 (P>0.50). The mean (±SD) ages of children in groups A, B, and C were 7.70±4.00, 8.70±4.81, and 8.43±3.92 years, respectively (P>0.50).

Comparisons of IgG, IgM and IgG:IgM ratio

The IgG level was lower, IgM level was higher, and IgG:IgM ratio was lower in the patient groups than in healthy controls (P<0.05) (Table 1).

The mean (±SD) IgG levels in the patient groups are shown in Table 2. The mean (±SD) IgG level was normal (11.60±5.24 g/L) in the healthy controls (group A). The IgG level decreased in FRNS+SDNS group (group C_1) and IFRNS group (group C_2), and was the lowest in SRNS group (group B). The difference in the mean IgG levels between groups B and C_2 was significantly significant (P<0.001), but no statistical difference was found between groups B and C_1. There was similar difference in the mean IgG (±SD) level between groups C_1 and C_2 (P<0.05).

The mean (±SD) IgM levels in the patient groups are shown in Table 2. The mean (±SD) IgM level was normal (1.62±0.76 g/L) in the healthy controls.

Table 1. IgG, IgM levels and IgG:IgM ratio in the patients and controls

Parameters	Controls *n* (%)	Patients *n* (%)	Odd's ratio	95% CI	*P*
IgG (g/L)					
<7 (low)	4 (20.0)	38 (88.4)	0.03	(0.01, 0.16)	<0.05
7-14 (normal)	16 (80.0)	5 (11.6)			
IgM (g/L)					
0.4-2.4 (normal)	7 (85.0)	18 (41.9)	7.8	(1.7, 39.9)	<0.05
>2.4 (high)	3 (15.0)	25 (58.1)			
IgG:IgM					
<3 (low)	3 (15.0)	35 (81.4)	0.05	(0.01, 0.22)	<0.05
>3 (high)	17 (85.0)	9 (20.9)			

Table 2. Comparison of IgG, IgM and IgG:IgM ratio between different types of nephrotic syndrome

Parameters	Mean ± SD	Median	Range	95% CI
IgG (g/L)				
SRNS (B)	2.67±1.65	2.30	0.60-7.50	(1.88, 3.47)
FRNS + SDNS (C_1)	3.34±1.44	3.50	0.50-5.50	(2.37, 4.31)
IFRNS (C_2)	5.84±3.37*	4.60	1.00-12.50	(3.80, 4.87)
IgM (g/L)				
SRNS (B)	3.17±1.54	3.60	0.75-5.10	(2.42, 3.91)
FRNS + SDNS (C_1)	2.27±0.87	2.11	1.25-3.69	(1.69, 2.85)
IFRNS (C_2)	2.92±1.63	2.90	0.37-5.70	(1.93, 3.90)
IgG:IgM				
SRNS (B)	1.27±1.25	0.70	0.14-4.55	(0.67, 1.88)
FRNS + SDNS (C_1)	1.74±1.10	1.50	0.28-3.52	(1.01, 2.48)
IFRNS (C_2)	3.51±3.78†	1.88	0.20-12.16	(1.22, 5.79)

*: P<0.05, compared with groups B and C_2, respectively; †: P<0.05, compared with groups B and C_1, respectively.

It was higher in group B than in group C, though the difference was not statistically significant. The median values of IgM level in groups A, B, C_1 and C_2 were 1.50, 3.60, 2.11 and 2.90 g/L, respectively.

IgG:IgM ratio was significantly lower ($P<0.05$) in groups B and C_1 than in group C_2, but the ratio did not differ between groups B and C_1. The median values of IgG:IgM ratio in groups A, B, C_1 and C_2 were 8.5, 0.70, 1.50 and 1.88, respectively.

Discussion

The children with nephrotic syndrome had low levels of IgG and high levels of IgM compared with the controls in this study, which is consistent with a previous study by Giangiocomo et al[13] where low IgG and IgA levels and high IgM level in idiopathic and secondary nephrotic syndrome have been reported. They concluded that T cell dysfunction is responsible for failure to convert surface IgM-bearing B lymphocyte (plasma cell) into subsequent IgG and IgA secreting plasma cell apart from urinary loss of immunoglobulin as was suggested previously by Peterson et al.[14] This hypothesis was further supported by Shakib et al[15] who have shown asymmetric depression of IgG subclasses (I to IV) in serum of patients with nephrotic syndrome. Subsequent works supporting similar view of T cell defect were made by Warshaw and Check,[16] Sobel et al,[17] Ingelfinger et al,[18] and Rashid et al.[19] Chen et al[20] reported that enhanced suppressor T cell activity resulted in increased IgM and decreased IgG production in children with nephrotic syndrome. Dall'Aglio et al[21] observed significantly increased IgG-bearing (sIgG-C) B cells. Vascular permeability factor produced by T lymphocytes had been observed.[22-24]

The IgG levels were lower in the SRNS, FRNS+SDNS groups compared to the IFRNS group in our study. Similar low levels of IgG were found in a study by Andal et al[24] who observed frequent relapses had lower IgG than infrequent relapsers, but there was no difference in IgM level between the two groups. In another study, Das et al[25] noticed very low IgG level in SRNS patients. Mishra et al,[26] however, did not find any significant difference between IFRNS and FRNS patients, but observed decreased IgG and IgA levels and increased IgM level and circulating immune complex in the serum of patients with active nephrotic syndrome. Mishra et al[26] showed significantly higher circulating IgG complexes in relapse than in remission or controlled children. Kemper et al[27] found no difference in relapse and remission children; in both situations they observed low IgG and high IgM levels.

The IgM level was elevated significantly in the patient group compared with the healthy controls. Similar findings of low IgG and high IgM levels in the patient group of idiopathic nephrotic syndrome are consistent with previous reports.[13,17,19,26,28] Low IgG and high IgM levels are seen in hyper IgM syndrome, a condition in which hypogamma globulinemia occurs with increased IgM and decreased IgG levels in the absence of features of nephrotic syndrome.[29] Therefore, decreased IgG and increased IgM levels associated with other features of nephrotic syndrome may be of diagnostic value.

In this study, IgG:IgM ratio was found to be significantly lower in the patient group than in the healthy controls. Similarly, a study in Taiwan 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.[30] The finding indicates that the lower the ratio the worse the outcome.

In conclusion, IgG level is decreased and IgM level is increased in all patients with nephrotic syndrome compared with healthy children. Immunoglobulin levels can be used as an important serological marker to predict responsiveness to treatment in different nephrotic syndromes. Serological makers for quick diagnosis are extremely important in management of the disease.

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