Serum Immunoglobulin G and M as Predictors for Outcome of Childhood Nephrotic Syndrome

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ABSTRACT
Background: Nephrotic syndrome (NS) is characterised by a loss of albumin, proteins, and other plasma components with comparable bulk. Symptoms include decreasing serum albumin levels, increased blood lipid levels, lipids in urine, and edema. **Objective:** To estimate serum levels of IgG, IgM, in nephrotic syndrome cases in addition to detect the relationship between IgG/IgM ratio and response to treatment with steroids.
Patients and Methods: The present study was carried out in Benha University Hospital, it comprised 60 children admitted to Nephrology Unit of Pediatric Department of Benha University Hospitals. The cases were divided into four groups: Group A included 15 cases of frequent relapse N.S. (FRNS), Group B comprised 15 cases of steroid-resistant nephrotic syndrome (SRNS), and Group C consisted of 15 cases with infrequent relapses. Additionally, a control group of 15 cases.
Results: In our study, serum IgM showed a significant difference between the studied groups (P < 0.001). Pairwise analysis revealed that it was significantly lower in the control group (median = 0.71 mg/ml) than in groups A (median = 1.48 mg/ml), B (median = 1.27 mg/ml), and C (median = 1.62 mg/ml). ROC analyses were conducted to assess the discriminatory potential of serum IgG and IgM levels among the different study groups (Group A, Group B, and Group C) compared to controls. For serum IgG, the analysis for all groups demonstrated significant AUC values, ranging from 0.938 to 0.958, with confidence intervals indicating excellent discrimination.
Conclusion: Our findings highlight the potential of serum IgG and IgM levels as diagnostic markers for differentiating nephrotic syndrome cases and provide valuable insights into the pathophysiology and treatment response in these patients.
Keywords: Serum Immunoglobulin G - Serum Immunoglobulin M – Nephrotic Syndrome.

INTRODUCTION

The term "NS" describes a collection of clinical symptoms that are brought on by several glomerular illnesses. These symptoms include severe edema, hyperlipidemia, hypoproteinemia (<30 g/L), excessive proteinuria (>3.5 g/day), and edema. The loss of immunoglobulins and complement proteins causes a reduction in immunological function. The best medications for treating NS are corticosteroids and immunosuppressive medicines, although these medications exacerbate immune system suppression. As a result, among the most frequent side effects among NS patients is infection. According to a prior study, at least 20% of patients with NS had serious infections at some point during the illness, including invasive fungal infections and even meningitis caused by cryptococcal bacteria [1].

The most common glomerular condition in children is idiopathic nephrotic syndrome (INS). Its occurrence is uncommon, though 1–17 instances per 100,000 children annually. The hallmark of INS is glomerular permeability barrier impairment, which results in a significant leakage of proteins into the urine and is linked to edema and hypoalbuminemia. During the active period of the illness and occasionally even during remission, there is often a significant decrease in blood IgG levels accompanied by elevated serum IgM levels. It's debatable if the severe proteinuria alone or a compromised immune system is to blame. A number of T cell dysregulations have been reported in both relapse and remission, and changes in memory B cell counts

have been noted from the outset of the disease, prior to the administration of any immunosuppressive treatments. The protracted and severe immunosuppression given in severe forms of the disease may potentially contribute to the decrease of protective antibodies seen in INS patients, raising the possibility that these patients may have serious infections [2].

Patients are treated with a standardised regimen of oral prednisone medication from the outset of the condition; most react to this treatment within 4-6 weeks (referred to as "steroid-sensitive NS" patients, or SSNS). The majority of pediatric SSNS patients have highly variable clinical evolutions, ranging from non-relapsing to severely steroid-dependent forms that necessitate repeated cycles of steroid therapy and additional immunosuppression with one or more steroid-sparing medications, such as B-cell depleting medications, calcineurin inhibitors, and anti-proliferative medications [2].

The pathogenesis of idiopathic NS is caused by a number of variables, including structural abnormalities, systemic characteristics, and immunology. Idiopathic NS contained MCNS, MesPGN, and FSGS in its histology. Serum IgM levels rise in NS, whereas IgG and IgA levels fall. IgM and IgG syntheses can be reversed by T-cell malfunction [3].

The aim of this study was to estimate serum levels of IgG, IgM, in nephrotic syndrome cases in addition to

detect the relationship between IgG/IgM ratio and response to treatment with steroids.

PATIENTS AND METHODS

The present study was carried out in Benha University Hospital, it comprised 60 children admitted to Nephrology Unit of Pediatric Department of Benha University Hospitals of both sexes.

The cases were divided into four groups: Group A included 15 cases of frequent relapse NS (FRNS), **Group B** included 15 cases of steroid-resistant nephrotic syndrome (SRNS), **Group C** included 15 cases with infrequent relapses, and **Control group** included 15 cases.

Inclusion criteria:

- During the research period, patients visiting Benha University Hospital and were followed up in the outpatient clinic and inpatient pediatric division.
- Patients with NS have various origins.
- Ages vary from two to eighteen
- Both sexes (Males and females).
- Every patient with edema, hyperlipidemia, hypoalbuminemia (less than 2.5 grams/dl), and significant proteinuria (more than 40 mg/m^2/h) at the active stage of the illness.

Exclusion criteria:

- Refusing and uncooperative patients or parents.
- Younger than one year old or older than eighteen.
- Childrens who had NS as a result of systemic conditions such cancer, amyloidosis, hepatitis B, systemic lupus erythematosus, or Henoch-Schönlein purpura.
- Childrens who were severely malnourished in protein and energy.

All subjects underwent to:

Complete history taking: Complete assessment of history, with a focus on symptoms of NS such as puffiness in the eye lid and lower limb swelling.

Complete clinical examination: Clinical examination focusing on signs of NS such as lower limb edema, ascites, and scrotal edema, and blood pressure and any signs of infection.

Laboratory investigations include: Serum IgG and IgM, serum albumin, blood urea and creatinine, CBC with differential WBC count, urine analysis, and serum cholesterol.

Blood Sampling:

Five millilitres of venous blood were drawn in order to measure blood urea, creatinine, and serum albumin. A liquid-phase immunoprecipitation technique was used to assess the amounts of serum Ig (IgG, IgM) in each group. IgG and IgM reference ranges are 7–16 and 0.4–1.5 g/l, respectively. The threshold values were as follows: IgG:IgM greater than 5, low IgG < 7 g/l, and high IgM > 1.5 g/l.

Ethical approval:

The Ethics Committee of the Benha Faculty of Medicine approved this investigation. All patients' parents gave their informed permission after being fully told about the study's hazards and benefits. The Helsinki Declaration was adhered to at every stage of the investigation.

Statistical methods

SPSS V. 28.0 was used for data administration and statistical analysis. Using direct data visualisation techniques and the Shapiro-Wilk test, quantitative data were evaluated for normalcy. Normality dictated that mean±standard deviation (SD), or medians and interquartile ranges (IQRs), be used to summarise quantitative data. Numbers and percentages were used to summarise categorical data. The one-way ANOVA or the Kruskal Wallis test were used to compare quantitative data between the groups under study. When there was a significant overall impact, pairwise analysis was performed, and all post hoc analyses were Bonferroni corrected. X^2-test was used to compare categorical data. ROC analyses were performed on serum immunoglobulins G and M to distinguish between study groups and controls. The areas under the curve, 95% confidence intervals, optimal cutoff points, and diagnostic indices were computed. Each statistical test was two-sided. P-values < 0.05 were deemed significant.

RESULTS

No significant differences were observed between the studied groups regarding age, sex, weight, and height **(Table 1).**

Table (1): Demographic characteristics of the studied groups

	Group A (n = 15)	Group B (n = 15)	Group C (n = 15)	Controls (n = 15)	P-value
Age (Year)	9 (1.8 - 14)	9 (2.5 - 14)	6.5 (4 - 12)	6 (2 - 13)	0.431
Sex					
Males	10 (66.7)	9 (60)	11 (73.3)	9 (60)	0.848
Females	5 (33.3)	6 (40)	4 (26.7)	6 (40)	
Weight (Kg)	30 ±11.7	27.6 ±10.7	26 ±7.7	24.4 ±10.3	0.488
Height (cm)	126 ±22	124 ±23	119 ±14	118 ±22	0.666

Quantitative data are presented as median and interquartile range (IQR) or as mean ± standard deviation, while qualitative data are presented as frequency (percentage).

Serum IgG was significantly higher in the control group than the other 3 groups. Serum IgM was significantly lower in the control group than in groups A, B, and C **(Table 2).**

Table (2): Serum immunoglobulins G and M in the studied groups

	Group A (n = 15)	Group B (n = 15)	Group C (n = 15)	Controls (n = 15)	P-value
Serum IgG (mg/ml)	1.82 ±0.25 [4]	1.71 ±0.29 [4]	1.57 ±0.14 [4]	3.07 ±0.67 [1,2,3]	**<0.001***
Serum IgM (mg/ml)	1.48 ±0.35 [4]	1.27 ±0.29 [4]	1.62 ±0.38 [4]	0.72 ±0.16 [1,2,3]	**<0.001***

*Significant P- value; 1: Significant difference from group A; Significant difference from group B; 3: Significant difference from group C; 4: Significant difference from controls.
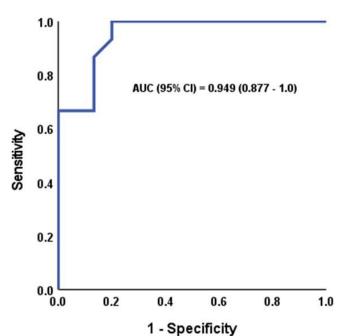
Regarding serum IgG, ROC analysis was done to differentiate between group A and controls. It revealed a significant AUC of 0.949, with a 95% confidence interval ranging from 0.877 to 1, indicating excellent discrimination ability. The best cutoff point was ≤ 2.33. ROC analysis was done to distinguish between group B and controls. It revealed a significant AUC of 0.958, with a 95% confidence interval ranging from 0.895 to 1, indicating an excellent discrimination ability. The best cutoff point was ≤ 2.12. ROC analysis was done to distinguish between group C and controls. It revealed a significant AUC of 0.938, with a 95% confidence interval ranging from 0.854 to 1, indicating an excellent discrimination ability. The best cutoff point was ≤ 2.52 **(Table 3, Figures 1-3).**

Table (3): ROC analysis of IgG to differentiate between (group A and controls), (group B and controls), and (group C and controls).
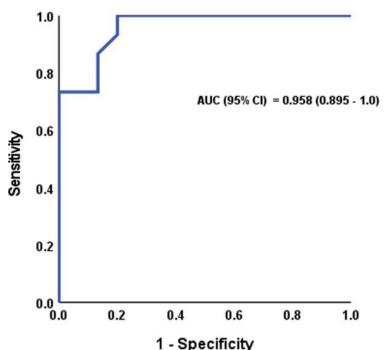
ROC characteristics	Group A and controls
AUC	0.949
95% CI	0.877 - 1
Best cutoff point	≤ 2.33
Sensitivity	100%
Specificity	80%
P-value	**<0.001***
ROC characteristics	**Group B and controls**
AUC	**0.958**
95% CI	**0.895 - 1**
Best cutoff point	**≤ 2.12**
Sensitivity	**100%**
Specificity	**80%**
P-value	**<0.001***
ROC characteristics	**Group C and controls**
AUC	**0.938**
95% CI	**0.854 - 1**
Best cutoff point	**≤ 2.52**
Sensitivity	**100%**
Specificity	**80%**
P-value	**<0.001***

*Significant P-value; AUC: Area under the curve; 95% CI: 95% confidence interval

Figure (1) ROC analysis of IgG to differentiate between group A and controls.

Figure (2): ROC analysis of IgG to differentiate between group B and controls
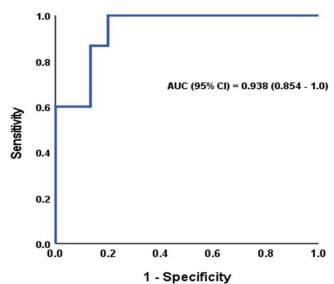
Figure (3): ROC analysis of IgG to differentiate between group C and controls.

Regarding serum IgM, ROC analysis was done to differentiate between group A and controls. It revealed a significant AUC of 0.993, with a 95% confidence interval ranging from 0.975 to 1, indicating an excellent discrimination ability. The best cutoff point was ≥ 0.95. ROC analysis was done to differentiate between group B and controls. It revealed a significant AUC of 0.869, with a 95% confidence interval ranging from 0.737 to 1, indicating a very good discrimination ability. The best cutoff point was ≥ 0.85. ROC analysis was done to differentiate between group C and controls. It revealed a significant AUC of 0.902, with a 95% confidence interval ranging from 0.792 to 1, indicating an excellent discrimination ability. The best cutoff point was ≥ 0.92 **(Table 4, Figures 4-6).**

Table (4) ROC analysis of IgM to differentiate between (group A and controls), (group B and controls), and (group C and controls).

ROC characteristics	Group A and controls
AUC	0.993
95% CI	0.975 - 1
Best cutoff point	≥ 0.952
Sensitivity	100%
Specificity	93.3%
P-value	<0.001*
ROC characteristics	**Group B and controls**
AUC	**0.869**
95% CI	**0.737 - 1**
Best cutoff point	**≥ 0.85**
Sensitivity	**73.3%**
Specificity	**93.3%**
P-value	**0.001***
ROC characteristics	**Group C and controls**
AUC	**0.902**
95% CI	**0.792 - 1**
Best cutoff point	**≥ 0.92**
Sensitivity	**80%**
Specificity	**93.3%**
P-value	**<0.001***

*Significant P-value; AUC: Area under the curve; 95% CI: 95% confidence interval
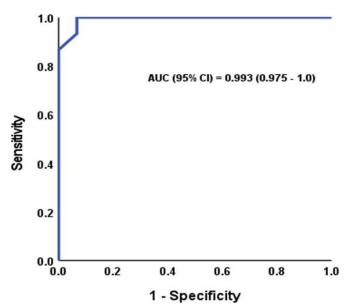
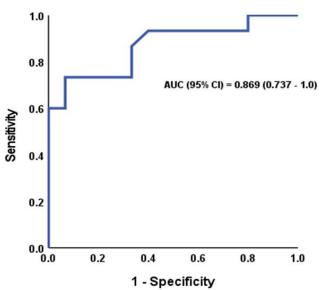**Figure (4): ROC analysis of IgM to differentiate between group A and controls.**

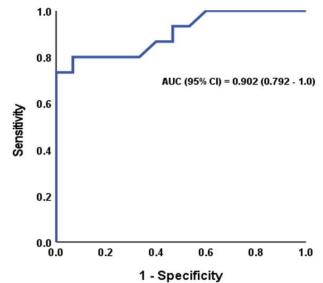**Figure (5): ROC analysis of IgM to differentiate between group B and controls.**

Figure (6) ROC analysis of IgM to differentiate between group C and controls.

DISCUSSION

In our study, the median age of patients in Group A, Group B, and the control group is around 9 years, while Group C has a slightly younger median age of 6.5 years. Regarding age, there were no discernible variations between the groups under investigation (P = 0.431).

This is consistent with the findings of **Moon et al.** [4], who examined the impact of glucocorticoids on bone geometry and BMD in children diagnosed with neural syndrome, and discovered that the average age was 10.7±3.1 years.

Abd Elaziz et al. [5], sought to determine if children with NS had fragmented QRS complex (fQRS) and to analyse the association between fQRS and cardiac functions. According to their analysis, the median age of NS patients was 4.5 years old, with a range of 2 to 15 years. Regarding age, there was no statistically significant difference (p>0.05) between the patients and the control group.

Contrary to what **Rhuma et al.** [6] found; INS is a condition that mostly affects preschool-aged children, with a peak age incidence of 2-3 yrs.

In this study, as regard gender, the majority of patients in all groups were males, with proportions ranging from 60% to 73.3%. Regarding sex, there were no discernible variations between the groups under study (P = 0.848). Therefore, it is possible to rule out the influence of age and sex in explaining the variations in their biochemical and medical traits.

Males were more impacted by INS than females, according to **Rhuma et al.** [6] **and Ephraim et al.** [7], which corroborated our findings.

However, **El hamshary et al.** [8] sought to ascertain the urine level of uMCP-1 and its correlation with steroid sensitivity in individuals with idiopathic NS. There are around 54% more women than men in their research.

In our study, serum IgM showed a significant difference between the studied groups (P < 0.001). Pairwise analysis revealed that it was significantly lower in the control group (median = 0.71 mg/ml) than in groups A (median = 1.48 mg/ml), B (median = 1.27 mg/ml), and C (median = 1.62 mg/ml).

Numerous causes, including their low molecular weight, which results in excess loss in urine, and their isotype switching defect from IgM-secreting cells to IgG-secreting cells, might be responsible for the low amount of IgG in NS as described in our work.

Roy et al. [9] found that there was a significant difference (P<0.05) in the IgG:IgM ratio, IgM level, and IgG level when compared to healthy youngsters. Children with SRNS and frequent relapse (FRNS) paired with SDNS had substantially lower blood IgG levels and IgG:IgM ratios than children with IFRNS (P<0.05, respectively).

El Mashad et al. [10] found that whereas serum IgM was higher in nephrotic patients than in healthy children (p<0.05), serum IgG levels were lower in the former group. They discovered a strong relationship between the first presentation levels of IgG and IgM serum levels, the rate of recurrence, and the responsiveness to therapy after a year of follow-up.

Our findings concur with research by **Azat** [11] that suggested increased albumin excretion in the urine or T cell malfunction as the cause of variations in serum IG levels. Accordingly, she discovered that when comparing the sick group to the healthy controls, there was a noticeably lower IgG level and a higher IgM level.

Youssef et al. [12], while comparing the mean age of 20 healthy children (mean age 12.1 years) to 27 patients with active NS (split into two subgroups: the mean age of patients was 12.3 years in the steroid-resistant group and 11.6 years in the steroid-sensitive group), they examined the serum levels of IgA, IgM, and IgG. The results revealed that the patients in the NS group had lower serum IgA and IgG levels (both subgroups) than those of the control group (P < 0.05). Additionally, there was no discernible difference in blood IgM levels between the healthy group and the individuals with idiopathic NS, according to this study.

Studies have shown that variations in serum Ig levels may be related to either increased albumin excretion in the urine or T-cell malfunction [9,11].

According to **Roy et al.** [9], the incidence of relapse increases with greater blood IgM and lower serum IgG levels at presentation.

ROC analyses were conducted to assess the discriminatory potential of serum IgG and IgM levels among the different study groups (Group A, Group B, and Group C) compared to controls. For serum IgG, the analysis for all groups demonstrated significant AUC values, ranging from 0.938 to 0.958, with confidence intervals indicating excellent discrimination. The best cutoff values for differentiation were provided for each group, with sensitivity and specificity levels varying, but generally showing strong discriminatory power. In the case of serum IgM, AUC values ranged from 0.869 to 0.993, indicating very good to excellent discrimination. The best cutoff values were specified for each group, with sensitivity and specificity metrics reflecting the diagnostic capability of IgM levels in distinguishing the groups from controls. These findings highlight the potential of serum IgG and IgM as valuable biomarkers for discriminating between the studied groups and the control group.

According to **Mohammed et al.** [13], compared to youngsters in good health, all NS patients had lower IgG and higher IgM serum levels. Ig levels are a significant serological marker that can be utilised to forecast treatment response in various NS types. In order to quickly diagnose and treat the illness, serological markers are crucial.

Serum IgM levels were not a reliable indicator of steroid resistance in children with primary NS,

according to **Le Viet et al.** [3], but serum IgG levels and the IgG/IgM ratio were. Since blood IgM levels in NS patients remain unchanged, this conclusion appears to be fair. However, serum IgG levels in NS children were much lower than in healthy children.

Twenty healthy volunteers and forty patients with NS—24 with steroid sensitivity and 19 with steroid resistance—participated in research by **Roy et al.** [9]. Every patient had their serum IgG and IgM levels as well as their IgG/IgM ratio measured. Their study's findings supported the idea that declining IgG levels and the IgG/IgM ratio are prognostic indicators of a poor prognosis in kids with NS.

Youssef et al. [12], in addition, verified that the children with NS and poor steroid responsiveness had lower blood IgG levels and an IgG/IgM ratio than the children with steroid sensitivity. T-cell dysfunction, increased albumin excretion in the urine, increased IgG catabolism, and reduced IgG production can all cause a reduction in serum IG levels. Moreover, IgG is more likely than IgM to be eliminated in urine because to its smaller molecular weight.

CONCLUSION

Our findings highlight the potential of serum IgG and IgM levels as diagnostic markers for differentiating NS cases and provide valuable insights into the pathophysiology and treatment response in these patients.

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