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SERUM IMMUNOGLOBULIN G, M AND A LEVELS IN CHILDREN WITH NEPHROTIC SYNDROME AND ITS CORRELATION WITH BIOCHEMICAL PARAMETERS

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ABSTRACT

Nephrotic syndrome (NS) is an immune-mediated renal disease that linked with T-cells dysfunction and secondary disturbance of B-cells with changes in concentration of immunoglobulin (Ig). The aim of this study is to compare the levels of IgG, IgM, and IgA in children with nephrotic syndrome and healthy control children. Fifty children with NS were divided into four groups: fourteen steroids sensitive infrequent relapse (SSIFR), twelve steroid sensitive frequent relapse (SSFR), nine steroid resistance (SR) and fifteen newly diagnosed nephrotic syndrome (ND) compared to twenty-five unrelated healthy children. Results of IgG, IgM and IgA serum levels determined by RID showed a significant decrease (P=0.0001) in IgG mean level (464.72 ± 50.34 mg/dl) of NS children in compared with healthy control mean level (1209.34 ± 75.51 mg/dl), and a highly significant increase (P=0.0001) in IgM mean level (201.37 ± 10.12 mg/dl) of NS patients in compared with control mean level (113.08 ± 8.42 mg/dl), there is no significant difference (P=0.4387) between NS patients and healthy control in IgA serum levels. In all children with NS, in comparison to healthy control, the serum level of IgG is reduced and the serum level of IgM is elevated. There is a close relation between albuminuria, hypoalbuminemia and hyperlipidemia and serum IgG and IgM levels. There is no significant difference between IgA level in patients and control.

Keyword: Nephrotic Syndrome, Immunoglobulin, Cholesterol, Albumin, IgG, IgM, IgA.

INTRODUCTION

Childhood nephrotic syndrome (NS) is an immune-mediated renal illness. It is described by huge hypoalbuminemia, proteinuria, hypercholesterolemia and edema. Nephrotic syndrome can influence children of any age period, from babyhood to puberty, and mostly seen within school-aged children and adolescents. The prevalence worldwide is approximately sixteen affected children per 100,000 with an incidence of two to seven per 100,000 children. Males show to be affected more than females at a ratio of 2:1 in children, but this predominance flops to persist in adolescence (Andolino and Adam, 2015). The podocyte has become the chosen elect for comprising the greater portion of the glomerular filtration barrier. Most cases of NS are steroid sensitive nephrotic syndrome (SSNS). Fifty percent of the latter repeat frequently and necessitate a relapses prevention by non-steroid medication. On the obverse to SSNS, steroid-resistant (SRNS) leads predominantly to end-stage renal failure. Thirteen to fourteen percent of the latter is connected with mutations of genes coding for podocyte proteins. The remnant is because of one or several different circulating factors (Davin and Rutjes, 2011).

It is related with error in the function of T cell and secondary disorder of B cell that leads to changes in scales of immunoglobulins level (Roy *et al.*, 2009; Bahbah *et al.*, 2015). This systemic disturbance of function of T cell results in the production of humoral factors or lymphokines that responsible for the rise of permeability of glomerular basement membrane (Sahali *et al.*, 2002; Bagga *et al.*, 2005). The useful treatment by rituximab and B cell depleting antibody, directed against CD20 (an antigen expressed on B-cell) suggest a role for B cells in the pathogenicity of nephrotic syndrome (Colucci *et al.*, 2016). Through their ontogeny, the bone marrow immature B-lymphocytes precursors migrate in the periphery as transitional B lymphocytes arrive the spleen, where they earn functions and mature phenotype and can recirculate into lymph nodes. In the lymph node B cells are activated by encountered antigens in the existence of T helper cells and develop into plasma cells that produced antibody or memory cells, which can produce immunoglobulin M (IgM) before isotype switching to immunoglobulin G (IgG) or immunoglobulin A (IgA), depending on the kind of the antigen (Colucci *et al.*, 2016).

MATERIALS AND METHODS

This study included fifty children with nephrotic syndrome (thirty-five males and fifteen females) during the period from September 2017 to January 2018 from the Welfare Teaching Hospital and Al-Emamain Al-Kadhemain Medical City. Family unrelated, healthy children twenty-five (thirteen males and twelve females) individuals were selected to represent the control group. According to the responses to the steroid treatment, patients were

divided into three groups: newly diagnosis group, steroid sensitive group which divided into two subgroups: Frequent relapse and Infrequent relapse and steroid resistance group. The studied factors in these groups were compared with the healthy control group in addition to comparison with the cases groups mentioned.

The ages of patients ranged from two to fourteen years and ages of controls range from five to fourteen years. Data collected from patients as well as control were taken which included age, residence, an onset of disease, family history. The study was conducted in the laboratories of Biotechnology College, Al-Nahrain University. For diagnosis biochemical test was done to determine the levels of serum cholesterol, serum albumin and proteinuria in patients and healthy control. Serum IgG, IgM and IgA levels were detected for NS patients and healthy control by using radial immunodiffusion (RID) method.

Statistical analysis: The Statistical Analysis System SAS (2012) program was used to study the effect of different factors in study parameters. Chi-square test was used for significantly compare between percentage and least significant difference –LSD test (ANOVA) or T-Test was used to significantly compare between means. Correlation coef-ficient was estimated between variables in this study.

RESULTS AND DISCUSSION

The statistical analysis for results of biochemical markers levels in children with nephrotic syndrome and healthy control were shown in Table 1.

Table 1: Levels of biochemical markers of patients and healthy controls

Group	Mean ± SE		
	Albumin (g/l)	Cholesterol (mg/dl)	Urine albumin (mg/dl)
Patients	25.43 ± 1.66	354.37 ± 19.79	131.40 ± 15.84
Control	45.13 ± 4.25	181.31± 4.25	0.00 ± 0.00
T-Test	4.947 **	56.292 **	44.801 **
P-value	0.0001	0.0001	0.0001
* (P<0.05), ** (P<0.01).			

Table 1 shows that a significant decrease in serum albumin mean levels in children with nephrotic syndrome (25.43 ± 1.66 g/l) than healthy control (45.13 ± 4.25 g/l) this results agreed with Nishi, *et al.*, (2016), in which nephrotic syndrome in children characterized by hypoalbuminemia (serum albumin ≤ 25 g/l). Hypoalbuminemia outcomes from urinary losses of protein during proteinuria, scanty reparations by liver synthesis, and probably, increased catabolism of albumin. The main problem in the hypoalbuminemia pathogenesis is the disability of the nephrotic liver to raise synthesis of protein (albumin) to compensate for urinary losses, although a normal liver synthesizes (12–14) gram albumin per day and can rising production three-fold in times of request (Mace and Chugh, 2014).

The most common sign of nephrotic syndrome is surplus fluid in the body because of the serum hypoalbuminemia. Depress serum oncotic pressure causes fluid to stack in the interstitial tissues. Water and sodium retention aggravate the edema (Pandey and Prasad, 2016).

Hyperlipidemia (hypercholesterolemia) in children with nephrotic syndrome was seen in comparing between the mean level of cholesterol in patients (354.37 ± 19.79 mg/dl) and the healthy children (181.31± 4.25 mg/dl). Buyukavci *et al.*, (2015) and Pandey and Prasad (2016) showed the same findings. Hyperlipidemia (hypercholesterolemia) in nephrotic syndrome patients is caused by two factors: the first one is hypoalbuminemia stimulates synthesis of protein in the liver, resulting in the lipoproteins overproduction. The second factor is lipid catabolism is reduced because of lower concentrations of lipoprotein lipase, the main enzyme involved in the breakdown of lipoprotein. Cofactors, like apolipoprotein C2 may also be missing by increased proteins filtration. apolipoprotein C2 secreted in plasma where it is component of very low-density lipoproteins (VLDL) and chylomicrons. This protein activates the enzyme lipoprotein lipase in capillaries (Pandey and Prasad, 2016).

The results of mean proteinuria in children with NS (131.40 ± 15.84 mg/dl) was significantly higher than healthy control (0.00 ± 0.00 mg/dl) Table 1. The podocyte plays an important role in preserving the integrity of the glomerular filtration barrier, and injury or loss has been associated to the development of albuminuria (White and Bilous, 2004). Patients with NS are characterized by a significant alteration in the structure of podocytes that is connected with the prolonged rise in the permeability of glomerular leading to huge proteinuria. Recent proteins identification that is specifically centralized at the slit diaphragm whose mutations and knockouts are known to outcome in loss of renal function has a significantly advanced understanding of the molecular structure of this filtration composition (Arif and Nihalani, 2013).

Regarding group of patients there is the lowest mean concentration of albumin was seen in ND (22.49 ± 2.27g/l) and SR (22.99 ± 3.31 g/l) and the highest mean level of albumin was seen in SSFR (31.59 ± 4.96 g/l) and the highest mean concentration of cholesterol was seen in ND (361.60 ± 32.87mg/dl) and there is an insignificant difference between the groups Table 2.

Table 2: Comparison of biomarkers among different types of NS patients and healthy control

Type	Mean ± SE		
	Albumin (g/d)	Cholesterol (mg/dl)	Urine albumin (mg/dl)
ND	22.49 ± 2.27 c	361.60 ± 32.87 a	177.33 ± 31.62 a
SR	22.99 ± 3.31 c	343.43 ± 44.04 a	147.78 ± 39.85 ab
SSFR	31.59 ± 4.96 b	358.34 ± 53.76 a	87.50 ± 30.47 b
SSIR	24.87 ± 2.35 bc	350.24 ± 33.91 a	109.28 ± 23.86 ab
Control	45.13 ± 1.09 a	181.31 ± 4.25 b	0.00 ± 0.00 c
LSD value	7.618 **	90.553 **	68.292 **
P-Value	0.0001	0.0001	0.0001
** (P<0.01).			

ND: Newly diagnosis, SR: steroid resistance, SSFR: Steroid sensitive frequent relapse, SSIR: Steroid sensitive infrequent relapse Different letters mean significant differences between means at P<0.01

The new cases patients showed the highest mean levels of albumin in urine (177.33±31.62mg/dl) compared with other types of NS, then steroid resistance (147.78 ± 39.85 mg/dl) and steroid sensitive infrequent relapse (109.28±23.86mg/dl) and the low-est level of mean albumin in urine was seen in steroid sensitive frequent relapse (87.50±30.47 mg/dl) About eighty percent of children with idiopathic nephrotic syndrome display remission of proteinuria after therapy with corticosteroids (steroid-sensitive). Most affected children have numerous relapses, placing them at risk for toxicity of steroid, systemic infections and another intricacy. A little ratio of patients (steroid-resistant) are also at risk for comparable complications and kidney failure (Bagga, A. 2008).

Concentration values of serum IgG, IgM and IgA corresponding to immune-complex precipitating ring diameter was read in the reference tables.

IgG precipitating ring diameter in patients with nephrotic syndrome was reduced in comparison with healthy control children (Figure 1).

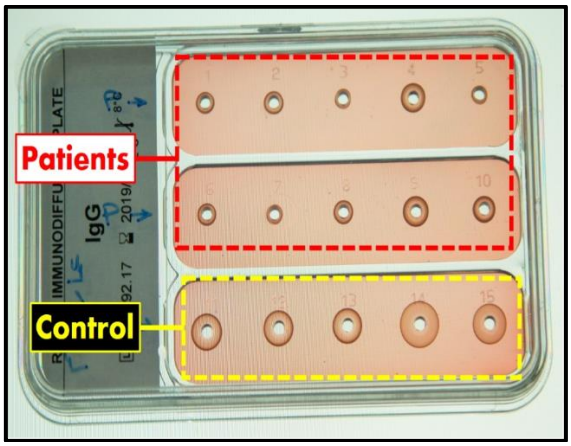

Figure 1: Radial immunodiffusion plate shows the differences between the patients with NS and controls in level of serum IgG

IgM precipitating ring diameter increased in children with NS in comparison with healthy control children (Figure 2).

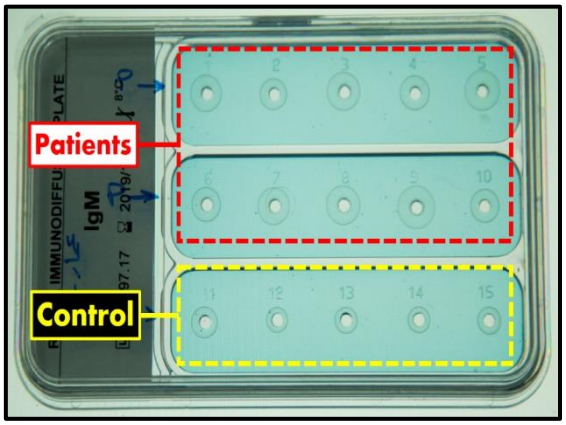

Figure 2: Radial immunodiffusion plate shows the differences between the patients with NS and controls in level of serum IgM

IgA precipitating ring diameter shows no change in the children with NS than healthy control children (Figure 3).

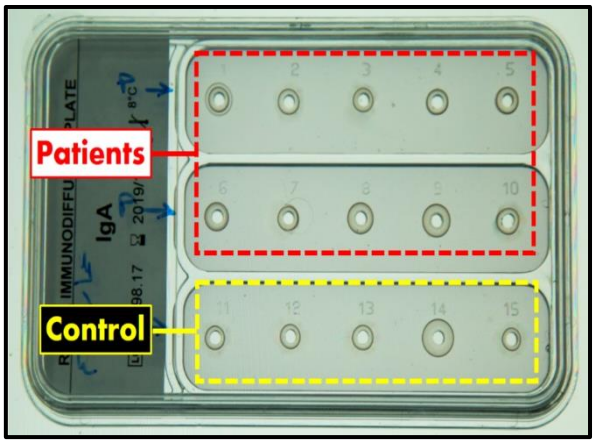

Figure 3: Radial immunodiffusion plate shows the differences between the patients with NS and controls in level of serum IgA

The serum IgG means level in patients with NS (464.72 ± 50.34 mg/dl) was significantly lower than healthy control children (1209.34 ± 75.51 mg/dl). The serum IgM mean level in patients with NS (201.37 ± 10.12 mg/dl) was significant higher than healthy control children (113.08 ± 8.42 mg/dl). The serum IgA mean level in patients with NS (175.47 ± 12.93 mg/dl) showed no significantly difference with healthy control children (193.57 ± 20.30 mg/dl) Table 3.

Table 3: Comparison between patients and control in serum levels of IgG, IgM and IgA

Group	Mean ± SE		
	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)
Patients	464.72 ± 50.34	201.37 ± 10.12	175.47 ± 12.93
Control	1209.34 ± 75.51	113.08 ± 8.42	193.57 ± 20.30
T-Test	177.32 **	30.977 **	46.324 NS
P-value	0.0001	0.0001	0.4387
** (P<0.01).			

The decrease of IgG level and increase of IgM level in Patients comparing with healthy control was agreed with the studies done by Azat (2012), Bahbah *et al.* (2015) and El-Mashad *et al.* (2017) and disagreed with Youssef *et al.* (2011) who observed that no significant difference between IgM levels in Patients with NS and healthy control.

There is no significant difference between IgA level in NS patients and healthy control children this result was in agreement with the reported by Youssef *et al.* (2011), and disagreed with the studies reported by El-Mashad *et al.* (2017), which showed that decreased serum IgG and IgA levels and increased serum IgM level in children with NS. Children with NS had an unusual encapsulated bacterial infection; there are alterations in the levels of serum IgG, IgM correlated with proteinuria in nephrotic patients (Bahbah *et al.* 2015). T-cell dysfunction is responsible for defeat to transform surface IgM-bearing B-cell (plasma cell) into next IgG and IgA secreting plasma cell apart from loss of immunoglobulin in urine (Roy *et al.* 2009).

Bahbah *et al.*, (2015) reported that reduce of IgG level in nephrotic syndrome could be attributed to numerous factors such as their small molecular weight give rise to their spare loss in urine and isotype switching disorder from IgM secreting cells to IgG secreting cells. C-maf (proto-oncogene) boosts T-helper 2 and weakens T-helper1 differentiation. Downregulation of the IL-12 receptor β2 subunit during relapse attenuates T-helper1 raised production of IL-13 prompts T-helper 2 cytokine during relapse. Decrease in concentration of IL-2 and INF-γ lead to reduction of IgG levels, increase in concentration of IL-4 and IL-5 lead to elevate of IgM levels.

The immunoglobulin levels abnormalities in children with nephrotic syndrome compared with healthy control children, corroboration the evidence that functional glomerular changes in NS may outcome from T-cell disturbance and B-cell involvement (Azat, 2012).

Low levels of IgG and high levels IgM are seen in hyper IgM syndrome, a situation in which hypogamma globulinemia happens with elevated IgM and reduced IgG levels in the absence of characteristics of nephrotic syndrome. Thus, reduced IgG and elevated IgM levels related with other characteristics of nephrotic syndrome may be of diagnostic value (Roy *et al.* 2009).

The results of this study showed that no significant difference among the clinical types of nephrotic syndrome patients Table 4.

Table 4. Comparison between difference type in IgG, IgM and IgA

Type	Mean ± SE		
	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)
ND	432.73 ± 118.90 b	184.30 ± 18.74 a	182.03 ± 25.09 a
SR	397.40 ± 101.40 b	209.29 ± 25.17 a	163.48 ± 34.21 a
SSFR	485.71 ± 77.12 b	197.68 ± 10.68 a	153.47 ± 26.04 a
SSIR	524.28 ± 93.87 b	217.72 ± 24.36 a	195.01 ± 22.33 a
Control	1209.34 ± 75.51 a	113.08 ± 8.42 b	193.57 ± 20.30 a
LSD value	283.92 **	49.133 **	73.858 NS
P-value	0.0001	0.0001	0.732
* (P<0.05), ** (P<0.01).			

ND: Newly diagnosis, SR: steroid resistance, SSFR: Steroid sensitive frequent relapse, SSIR: Steroid sensitive infrequent relapse. Different letters mean significant differences between means at P<0.01

These results were in agreement with Kemper *et al.* (2002) which they found that no difference in IgM level elevated more in frequent relapse NS than in infrequent relapse NS group and was the highest in steroid resistance NS.

Statistical analysis showed a highly significant correlation between the immunoglobulin G and M levels in our study and the laboratory finding used in diagnosis of patients Table 5.

Table 5: Correlation between Immunoglobulin G, M and A with laboratory finding of patients with NS

Laboratory parameters for diagnosis NS	IgG	IgM	IgA
Serum albumin	0.60 **	-0.47 **	-0.07 NS
Cholesterol	-0.62 **	0.46 **	-0.08 NS
Urine albumin	-0.54 **	0.43 **	-0.09 NS
** (P<0.01), NS: Non-Significant.			

There was a highly significant relationship (P< 0.01) between the serum albumin concentration and mean levels of serum IgG and IgM of children with NS. Reduce albumin levels is accompanied by lowering in mean levels of serum IgG and elevate IgM levels. There was highly significant relationship (P<0.01) between the degree of hypercholestremia and the mean level of IgG and IgM. Rise in concentration of serum cholesterol is associated with reduce in serum IgG and elevate in IgM levels. There was a highly significant (P<0.01) correlation between protein in urine and mean concentration of IgG and IgM. Increase albumin in urine associated with decrease of IgG and increase IgM concentration.

Our study detected that elevate in albuminuria is accompanied by a decrease in the level serum IgG and these results were in identity with that reported El-Mashad *et al.*, (2017) who pronounced that in NS there is an elevated albumin urinary excretion and IgG accompanied by a reduce in their serum level. Children with NS overproduce rise molecular weight proteins, such as lipoproteins, which lead to hyperlipidemia and are kept from urinary loss for preserved the oncotic pressure. Proteins with large molecular weight such as lipoproteins are overproduced by children with NS, which can lead to hyperlipidemia, and are conserved from urinary loss which maintains the oncotic pressure. Our study showed a highly significant correlation between cholesterol concentration and serum IgG concentration of the patients with NS. These results were in identity with that reported by other studies (Azat, 2012; Bahbah *et al.*, 2015 and El-Mashad *et al.*, 2017).

High molecular weight immunoglobulin (IgM) elevate to defend level of serum protein and oncotic pressure. Marked proteinuria linked with NS is thus likely to affect the concentrations of serum Immunoglobulin (Bahbah *et al.*, 2015)

A direct relationship was found between the degree of albuminuria and the mean serum IgM level when there is an elevate in albuminuria it is linked with rising of serum IgM concentrations. These results were in agreement with those reported by (Azat, 2012).

We concluded that in all children with NS, in comparison to healthy control, the serum concentration of IgG is reduced, and the serum level of IgM is elevated. There is close relation between albuminuria, hypoalbuminemia and hyperlipidemia and serum IgG and IgM levels. There is no significant difference between IgA level in patients and control.

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