

Rituximab Use in Nephrotic Syndrome

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Abstract

Background: Nephrotic syndrome may be caused by primary (idiopathic) renal disease or by a variety of secondary causes. Patients present with marked edema, proteinuria, hypoalbuminemia, and often hyperlipidemia. Treatment of most patients should include fluid and sodium restriction, oral or intravenous diuretics, and angiotensin-converting enzyme inhibitors. Adults with nephrotic syndrome may benefit from corticosteroid treatment. The treatment of patients with the steroid-resistant nephrotic syndrome (SRNS) and steroid-dependent nephrotic syndrome (SDNS) is challenging. On the basis of suggestions that B lymphocytes are crucial in the pathogenesis of the nephrotic syndrome, rituximab (a monoclonal antibody against CD20 antigen) is used in treatment of these patients.

Aim of study: To evaluate the role of rituximab and mycophenolic acid in treatment of patients with steroid-resistant (SRNS) and steroid-dependent nephrotic syndrome (SDNS), whom not respond or relapse after calcineurin inhibitor (CNI) (tacrolimus or cyclosporine) had been used.

Patients and methods: Case series study was done between 2012 - 2015 in AL-Sadder Teaching Hospital Nephrology Center and record 40 patients with different age groups, males and females with different histopathological types (Minimal Change Glomerulonephritis, Focal Segmental Glomerulosclerosis, Mesangio Proliferative Glomerulonephritis). These patients were taking prednisilone and/or calcineurin inhibitor (tacrolimus "prograf") or (cyclosporine "sandimmune"), and they get either Steroid Dependent Nephrotic Syndrome or Steroid Resistant Nephrotic Syndrome with frequent admission more than four time per year. To these patients we start rituximab intravenous infusion monthly for at least six months with the use of steroid and mycophenolate mofetil during these six months. The patients followed up for 3-12 months after initiation of rituximab by different investigations and the patients were classified according to their response into complete, partial and no response. After one year stop rituximab treatment, follow the patients clinically and by investigations for (1-2) years to determine which patients get relapse.

Results: Majority (80%) of patients with nephrotic syndrome who had good response to rituximab were younger age group < 15 years. Better response to rituximab associated with Minimal Change Glomerulonephritis. There was significant reduction in blood urea, serum creatinine, urine (protein/creatinine) ratio and serum cholesterol. Serum albumin was significant elevated. Response to rituximab was not significantly associated with gender or steroid response. Majority of patients with good response not relapse and need more time for follow up. Relapsing after stopping rituximab not significantly associated with age, gender, histopathological type and steroid response.

Conclusion: Rituximab and mycophenolate mofetil used in steroid-resistant nephrotic syndrome to get ride from side effects of calcineurine inhibitor (tacrolimus or cyclosporine). Rituximab and mycophenolate mofetil used in steroid-dependent nephrotic syndrome after calcineurine inhibitor to get ride from side effects of steroid. Improvement in renal function is result from stopping of calcineurine inhibitor (nephrotoxic drugs) and/or from rituximab and mycophenolate mofetil. Cost of rituximab is less than the cost that needed if the patients had frequent admissions to the hospital or developed renal failure and ended with dialysis.

Abbreviations

ANP- Atrial Natriuretic Peptide
Anti-GBMab- Anti-Glomerular Basement Membrane antibody
CNI- Calcineurin Inhibitor
FSGS- Focal Segmental Glomerulosclerosis
c-GMP- Cyclic Guanosine Monophosphat

GN- Glomerulonephritis
HBV- Hepatitis B Virus
HCV- Hepatitis C Virus
HIV- Human Immunodeficiency Virus
MCGN- Minimal Change Glomerulonephritis
MesPGN- MesangioProliferative Glomerulonephritis

MMF- Mycophenolate Mofetil
Na-K-ATPase- Sodium-Potassium Adenosine Triphosphatase
NS- Nephrotic Syndrome
SDNS- Steroid Dependent Nephrotic Syndrome
SRNS- Steroid Resistant Nephrotic Syndrome
U(P/C)- Urine protein/creatinine ratio
WBC- White Blood Cell

Introduction

Nephrotic syndrome (NS) is a disorder that indicates damaged kidney's filtering system (the glomeruli). Normally person loses less than 150 mg of protein in urine in 24 hr. period [1]. Nephrotic range proteinuria: the urination of more than 3.5 g of protein during 24 hr. period, or 25 times the normal amount is the primary indicator of nephrotic syndrome. About 2 in every 10,000 people experience NS in children, it is diagnosed in males more than females usually between 2nd and 3rd years of age [2]. In addition to protein urea, there are three main features of NS:

Hypoalbuminemia

In a healthy individual, less than 0.1% of plasma albumin may traverse the glomerular filtration barrier. The glomerular structural changes that may cause proteinuria are damage to the endothelial surface, the glomerular basement membrane, or the podocytes. One or more of these mechanisms may be seen in any one type of nephrotic syndrome. Albuminuria alone may occur, or, with greater injury, leakage of all plasma proteins, (i.e., proteinuria) may take place [3].

Edema

The cause of edema formation in the nephrotic syndrome has been an area of intense interest by nephrologists for decades. Klisic et al. demonstrate that apical albumin directly stimulates NHE3 (sodium hydrogen exchanger 3), the major apical transporter responsible for proximal tubule sodium re absorption [4]. Classically, the edema formation of nephrotic syndrome was considered to be secondary to increased sodium retention from intravascular volume depletion from low plasma oncotic pressure increased sodium re absorption by the collecting duct in proteinuria kidneys occurred due to increased sodium transport and Na-K-ATPase in collecting ducts [4]. Other studies have shown that cGMP phosphodiesterase activity in the collecting ducts (and glomeruli) of protein uria is increased, leading to resistance to ANP actions Valentin et al [5].

Hyperlipidemia

Hyperlipidemia is a characteristic of the nephrotic syndrome. The mechanism for its occurrence is complex and involves a combination of reduced clearance of lipoproteins from the circulation and increased hepatic synthesis of lipoproteins [6]. Hyperlipidemia so commonly complicates heavy proteinuria that it has come to be regarded as an integral feature of the nephrotic syndrome (NS). Characteristically, total plasma cholesterol and triglyceride levels are elevated, as are very-low-density lipoprotein and low-density lipoprotein cholesterol. Although high-density lipoprotein concentrations may be normal, HDL subtypes are abnormally distributed, with a reduction of HDL₂ and an increase in HDL₃. In addition, lipoprotein (a) levels may be elevated. The mechanisms underlying these abnormalities are multifactorial, involving both increased rates of lipoprotein synthesis and defective clearance and catabolism of circulating particles. The precise stimulus for enhanced hepatic lipoprotein synthesis in NS is unknown. It may be related directly to hypoalbuminemia, also a decrease in plasma oncotic pressure may be a more important trigger

to increased lipoprotein production by the liver [7].

There are many causes of nephrotic syndrome, these include kidney disease such as:

Primary glomerulonephritis (GN) which classify according to their histopathology into:

Minimal change glomerulonephritis (MCGN)

Nephrotic syndrome secondary to minimal change disease (MCNS) is the most common cause of nephrotic syndrome in children. The pathophysiological process of MCNS remains poorly understood; however, experimental and clinical data suggest that this nephropathy is the consequence of an immune disorder in which a permeability factor causes albuminuria by inducing podocyte foot-process retraction [8]. Abnormal T-cell function is the keystone of this glomerular disease.

This concept was reinforced later by the observation that systemic infusion of supernatants of T lymphocytes from patients with MCNS induced proteinuria in rats. To date, despite extensive research, the permeability factors are still unknown, and the pathophysiological process of MCNS remains unclear [9].

Focal segmental glomerulosclerosis (FSGS)

Focal segmental glomerulosclerosis (FSGS) is a pattern of injury defined by a segmental scar, which involves some but not all glomeruli. When all of the secondary causes of this pattern of injury are eliminated as heroin, HIV nephropathy, morbid obesity, vasculitis, toxins (pamidron), the remaining patients receive a diagnosis of primary FSGS. Although patients with primary FSGS may present with any level of proteinuria, clinical concern is greatest for those who present with nephrotic-range proteinuria because without treatment, they have an extremely poor prognosis, progressing to end stage renal disease (ESRD) over the course of 3 to 6 year. However, it is widely recognized that the prognosis in nephrotic patients with primary FSGS is significantly improved when remission of proteinuria is achieved because 50% of nephrotic adult patients with FSGS respond to an aggressive course of steroids [10].

Mesangio proliferative glomerulonephritis (MesPGN)

The glomerular mesangium contains mesangial cells and extra cellular matrix. It plays a crucial role in maintaining structure and function of glomerular capillary tuft.

(MesPGN) consists 10% of the total renal biopsy of glomerulonephritis. It is characterized by proliferations of mesangial cells with increase mesangial matrix. MesPGN was seen mostly in young adults with mean age of 28 years for males and 26 years for females. There are two types of Mes PGN diffuse and focal. There is no significant difference was found in clinical features of diffuse and focal MesPGN. Microscopic comparison between diffuse and focal variety showed that significant increase of focal glomerular basement membrane thickening, focal endothelial cell proliferation, focal smooth muscle hyperplasia, hyaline sclerosis and vasculitis was more common in diffuse variety. In focal variety, capillary loop congestion, (Periglomerulitis) cloudy swelling and vacuolar degeneration in tubules were significantly more as compared to diffuse variety [11].

Membranous glomerulonephritis (MGN)

Membranous nephropathy is an immunologically mediated disease in which immune complexes deposit in the sub epithelial space. The

antigens associated with primary membranous nephropathy are not known. They may be located in the sub epithelial space. Antigen-antibody complexes can develop by the production of immune complexes in situ or by deposition. In the experimental Heymann nephritis model of membranous nephropathy, the intrinsic antigen is a glycoprotein, megalin, synthesized by the glomerular visceral epithelial cells.

Neutral endopeptidase, a podocyte antigen that can digest biologically active peptides, was recently identified as the target antigen of antibodies deposited in the sub epithelial space of glomeruli in a subset of patients with antenatal membranous nephropathy. Many of the antigens associated with secondary membranous nephropathy are also not known. However, hepatitis B surface antigens and hepatitis C antigens have been identified in immune deposits, as have thyroid antigens in patients with thyroiditis [12].

Rapid progressive glomerulonephritis (RPGN)

Rapid progressive glomerulonephritis (RPGN) can result from glomerular deposition of anti-GBM antibody, immune complexes, or from some as yet undefined mechanism that does not involve glomerular antibody deposition. The latter process may be cell mediated and resembles a small vessel vasculitis. Most cases of idiopathic RPGN are not accompanied by pathogenic glomerular immunoglobulin deposition. Recent experimental studies of immune mechanisms of glomerular injury have identified several new processes that can induce damage to the capillary wall sufficient to result in crescentic glomerulonephritis (GN). These include direct effects of anti-GBM antibody alone and of the complement C5b-9 (membrane attack) complex, nephrotogenic effects of inflammatory effector cells that involve reactive oxygen species and glomerular halogenation, and injury mediated by sensitized lymphocytes independently of antibody deposition. Macrophages have been shown to participate in both intracapillary and extra capillary fibrin deposition and crescent formation as well as to mediate capillary wall damage. The role of resident glomerular cells and cell-cell interactions in glomerulonephritis is still under active investigation [13].

Nephrotic syndrome can also result from systemic disease that affects other organ in addition to kidneys as: [14]

- Diabetes mellitus
- Amyloidosis
- Systemic lupus erythematosus
- Rheumatoid arthritis
- Viral (HIV, HCV, HBV)
- Preeclampsia
- Cancer
- Genetic disorder

Complication of nephrotic syndrome: [3]

Infection

Infection is a major concern in nephrotic syndrome. Both gram positive and gram negative bacterial infect. The most common infectious complications are bacterial sepsis, cellulitis, pneumonia, and peritonitis. Proposed explanations for the increased infection risk include the following:

- Urinary immunoglobulin losses
- Edema fluid acting as a culture medium
- Protein deficiency
- Decreased bactericidal activity of the leukocytes
- Immunosuppressive therapy

- Decreased perfusion of the spleen caused by hypovolemia
- Urinary loss of a complement factor Properdin factor B) that opsonizes certain bacteria

Hypocalcemia

Hypocalcemia is common in the nephrotic syndrome, but rather than being a true hypocalcemia, it is usually caused by a low serum albumin level. Nonetheless, low bone density and abnormal bone histology are reported in association with nephrotic syndrome. This could be caused by urinary losses of vitamin D-binding proteins, with consequent hypovitaminosis D and, as a result, reduced intestinal calcium absorption. Low bone mass may be found in relation to cumulative steroid dose. It is possible that long duration of either the nephrotic syndrome or treatments for it are the important risk factors for bone disease in these patients.

Hypercoagulability

Venous thrombosis and pulmonary embolism are well-known complications of the nephrotic syndrome. Hypercoagulability in these cases appears to derive from urinary loss of anticoagulant proteins, such as antithrombin III and plasminogen, along with the simultaneous increase in clotting factors, especially factors I, VII, VIII, and X.

Diagnosis of nephrotic syndrome

There are many measurements usually needed to diagnose nephrotic syndrome: [1]

- Serum albumin
- Blood urea
- Serum creatinine
- Urinary protein measurement
- Urine protein/creatinine ratio
- Renal ultrasonography
- Renal biopsy needed to confirm the diagnosis.

The goals of treatment are to relieve symptoms, prevent complication and delay kidney damage. The treatment includes: [14]

- Fluid and sodium restriction
- Diuretics
- Angiotensin converting enzyme inhibitor
- Albumin
- Lipid lowering agents
- Antibiotics
- Anticoagulants
- Corticosteroid

The patients classify according to their response to steroid into: [15]

- Steroid sensitive patients (response in first eight weeks of treatment).
- Steroid resistant patients (SRNS) (lack of response; persist protein urea after eight weeks of treatment).
- Steroid dependent patients (SDNS) (protein urea appears when decrease the dose or complete treatment after two weeks).

Management of SRNS and SDNS remains challenging and patients with steroid resistant who do not achieve remission will develop end stage renal failure. The exact pathogenesis of SRNS and SDNS has not been fully elaborated but immunological factor might play a vital role and the use of immune suppressing agents appear to achieve promising results. The immune suppressants agents include cyclophosphamide, levamisole, chlorambucil, cyclosporine and

mycophenolate mofetil (MMF). Now a day treatment of SRNS and SDNS with rituximab yield encouraging results by maintaining remission of the disease and decrease dose of steroid [15].

Rituximab

Rituximab is a monoclonal antibody that acts directly against CD20 expressed on B lymphocytes. It is widely used to treat lymphoma and rheumatoid arthritis. Rituximab administration results in rapid and sustained B cell depletion. Several reports have proposed rituximab as a new treatment strategy for patients with SDNS or SRNS [16].

Rituximab should not be considered as a first line treatment for a patient with nephrotic syndrome. However, it does appear to have a definite role in patients failing to achieve satisfactory responses with standard therapies, such as steroids, calcineurin inhibitors, leflunomide and mycophenolate mofetil. The primary indications include: [16]

- Steroid-dependent nephrotic syndrome (SDNS) with excessive steroid side effects as osteoporosis, cataracts, obesity.
- SDNS requiring maintenance therapy with 2 or more immunosuppressive and still experiencing break-through relapses.
- Steroid-resistant nephrotic syndrome (SRNS): most likely to have a benefit from rituximab if the patient can be brought into remission with immunosuppression first.

Contra-indications to rituximab therapy

- Allergy to rituximab.
- Pneumonitis- due to risk RALI (Rituximab associated lung injury).

Side effect of rituximab

Serious adverse events, which can cause death and disability, include: [16]

- Severe infusion reaction; it typically developed within 30 minutes to 2 hour after initiation of drug infusion, although symptoms may be delay up to 24 hour. Majority of reactions occur after first or second exposure to the agents but between 10-30 % of reaction occur during subsequent treatment. The main features are fever, chills, nausea, headache, skin rash and pruritus. Small but significant percentages of the patients develop bronchospasm, hypotension, urticaria and or cardiac arrest. The mechanism by which induce infusion reaction unclear but monoclonal antibodies interact with their molecule target (CD20) will promote release of inflammatory cytokines.
- Cytokine release syndrome; it is common immediate complication characterized by fever, rigor and hypotension .it is type of systemic inflammatory response occur because antibodies activate T-cells to release cytokines before they are destroyed, this can prevented by slow infusion of the drug and give chlorphenamine and hydrocortisone .
- Progressive multifocal leukoencephalopathy (PML); fatal viral disease lead to inflammation and damage of white matter of the brain at multiple locations caused by JC (John Cunningham) virus, it is harmless virus except in immune compromised patients lead to activation of the virus.
- Hepatitis B and other virus reactivation.
- depletion of B cells in 70% to 80% of lymphoma patients
- Pulmonary toxicity

Patients and methods

Case series study had been done between 2012 - 2015 in AL-Sadder

Teaching Hospital Nephrology Center and record 40 patients with different age groups (≤ 15 , 16 - 30 and >30) years their numbers were (16, 16 and 8) respectively. The study group included 25 males and 15 females have primary GN. The main histopathological types of the studied groups are (MCGN, FSGS, Mes PGN).

These patients were taking prednisolone 2.5 mg/kg/day and/or calcineurin inhibitor (tacrolimus "prograf" 0.1-0.2 mg/kg/day) or (cyclosporine "sandimmune" 4-6 mg/kg/day), and they get either (SDNS) or (SRNS) with frequent admission more than four time per year.

To these patients we start rituximab in a dose of 375 mg/m² intravenous infusion monthly for six months with close observation by dividing the dose as follow: 1/5th in the first hour and 4/5th in the remaining six hours with close monitoring of vital signs. When using rituximab, the calcineurin inhibitor was stopped, the dose of prednisolone for patients was tapered to 1mg/kg every other day and use mycophenolate mofetil (MMF) "cellcept" in a dose 15 mg/kg/day (halving the dose of cellcept (MMF) because concomitant use of these drugs with rituximab lead to more B-cell depletion).

The patients followed up for 3-12 months after initiation of rituximab by:

Base line investigations which include: [14]

- Complete blood count (for monitoring white blood count). WBC count must ≥ 5000 cell per microliter and neutrophil should have kept more than 500 cells.
- Blood urea. (7-20 mg/dl).
- Serum creatinine. (0.7-1.3 mg/dl).
- Serum albumin. (35-50 g/l).
- Serum cholesterol less than 200 mg/dl.
- Urine protein / creatinine ratio (UP/UC).

Then the above points were followed at 3, 6, 12 months and after 1 year from stopping rituximab.

The patients were classified according to their response after last dose of rituximab into: [17]

- Complete response (good response) if (UP/UC) < 0.2 with no edema.
- Partial response if (UP/UC) 0.2 – 2 with no edema.
- No response (poor response) if (UP/UC) > 2 with edema.

Relapse if urine albumin +3 (300 mg/dl) to +4 (>1000 mg/dl) for three consecutive days in patients with previous remission or recurrent of edema with hypercholesteremia and hypoalbuminemia, then send for protein creatinine ratio or 24-hour urine protein test to determine the degree of relapse.

After last dose of rituximab continue on MMF 30mg/kg/day with prednisolone 0.5 mg/kg/day every other day for another 6 months after that stop all treatment mentioned above for another 6 months and during these period follow up the patients by investigations mentioned previously every 3 months and by clinical features. For patients with good response with no relapse further 2 years of follow up recommended, and for patients with relapse or with partial response give them 2 booster dose of rituximab 375 mg/m² intravenous infusion, 6 months between the doses in addition to MMF 30 mg/kg/day with prednisolone 0.5 mg/kg/day every other day and follow up these patients every 3 months.

The patients with poor response, they complained from frequent admission to hospital and ended with renal failure and dialysis.

- For child bearing age female we advise the patients to contraceptive by using either male contraception or intra uterine contraceptive device.
- All patients included in this study were Bacilli Chalmette Guerin (BCG) vaccine positive.
- We record any infections that developed during the time of study especially chest infection, and closed followed up for the respond of those patients to the types of antibiotics used and if not respond to first line, we send the sputum for acid fast bacilli, chest x\ray and follow the patients respond to 2 or 3 line of antibiotics with complete blood count and erythrocytes sedimentation rate.
- For urinary tract infection we depend on culture and sensitivity to continue on antibiotics for 21 days and if recurrent, continue for 3 months with half the dose.
- For all patients, we get agreement for the risk of the drug even the death during the infusion or later from complication of the drug.
- All the patients had been advised to take influenza virus vaccine yearly.
- All patients had histological record.
- In this study we exclude:

Patients with secondary causes of nephritic syndrome, by making the following investigations that excludes that diseases:

- Antinuclear antibody
- Rheumatoid factor
- Glucose tolerance test
- HBV Ag, HCVab, HIV tests
- Anti-cyclic citrullinated peptide antibody
- Protein electrophoresis
- Antiphospholipid anti body
- Anti-double strand anti body

Any patients who had positive family history to exclude cases with Alport syndrome.

Cases less than 2 years of age because the high risk and need more facilities for following.

Statistical analysis

Data of the patients were transformed into computerized data form and analyzed using the statistical package for social sciences (SPSS) version 22, IBM, Chicago, US, 2013. Variables were expressed as mean, standard deviation (SD), frequencies (No.) and proportions (%).

Chi square test was used to compare and assess the significance of association between categorical variables, Fisher's exact test was as an alternative when chi square was inapplicable.

Analysis of variances (ANOVA) test was used to compare means across the subsequent visits. Level of significance, of ≤ 0.05 , considered as significant difference or association. Finally, results presented in tables and figures with an explanatory paragraph for each.

Results

Table 1 shows the distribution of age and gender of the 40 patients enrolled in this prospective study. The median age of the studied group was 17 (IQR: 10 - 29) years, (40%) of the studied group aged ≤ 15 years and equal proportion aged 16 - 30 years while (20%)

aged > 30 years. The study group included 25 males (62.5%) and 15 females (37.5%) with Male: female ratio of 1.67 to one.

Table 1: Age and gender distribution of the studied group (N=40)

	Variable	N	%
Age (year)	≤ 15	16	40.0
	16 - 30	16	40.0
	> 30	8	20.0
	Median (IQR)	17 (10-29)	-
Gender	Male	25	62.5
	Female	15	37.5
Male: female ratio		1.67: 1	-

IQR: inter-quartile range

As it shown in figure 1, the histopathological typing of the studied group revealed that 25 patients (62.5%) had minimal change glomerulonephritis(MCGN), 11 (27.5%) with focal segmental glomerulosclerosis (FSGS) and only 4 patients (10%) had mesangioproliferative glomerulonephritis(MesPGN).

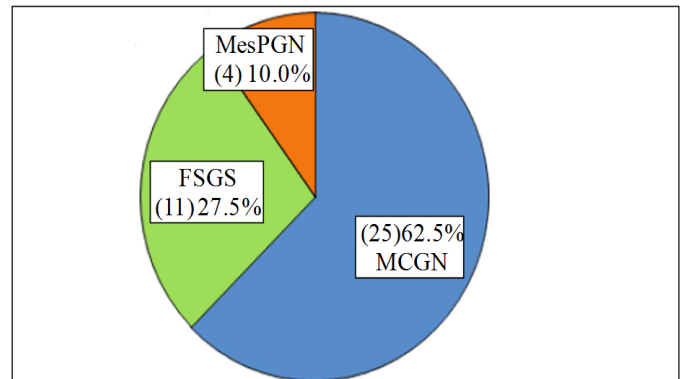


Figure 1: Distribution of histopathological types (N =40)

Regarding the response to steroid, majority of the patients, 33/40 (82.5%) were steroid resistant and the remaining 7 patients (17.5%) were steroid dependent, (Fig. 2).

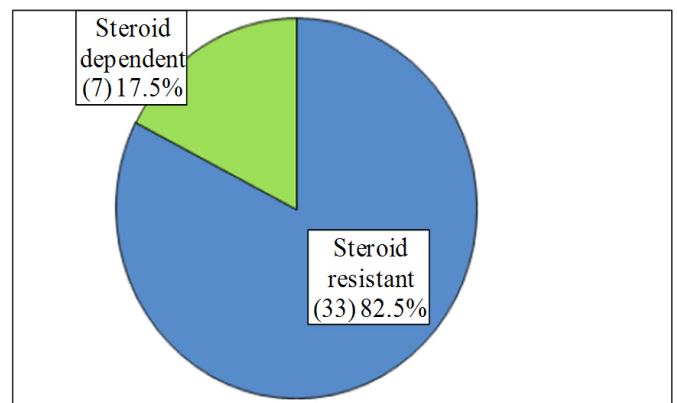


Figure 2: Distribution of the studied group according to the response to steroids (N=40)

Table 2 and figure 3 summarize the response to Rituximab, where 21 patients (52.5%) had good response, 14 (35%) partial response and 3 patients (7.5%) had poor response. On the other hand, 2 patients developed adverse reaction to Rituximab and the medication was ceased. Therefore, only 38 patients were completed the follow up period to the end of the study.

Table 2: Distribution of the studied group according to their response to Rituximab (N = 40)

Response to Rituximab	No.	%
Good	21	52.5
Partial	14	35.0
Poor	3	7.5
Stop Treatment (allergy)	2	5.0
Total	40	100.0

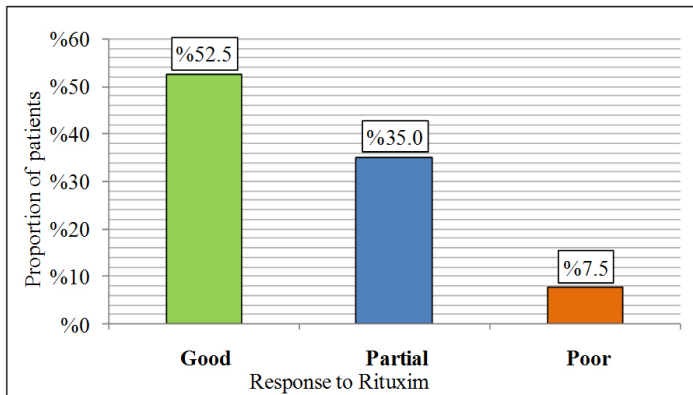


Figure 3: Proportional distribution of the studied group according to their response to Rituximab (N= 38)

As it shown table 3 there was there was statistically significant reduction in the mean blood urea, at each subsequent visit; at baseline the mean blood urea was 83.0 ± 12.7 mg/dL reduced to 66.2 at the three month, then to 47.7 ± 11.3 mg/dL after 6 months to reach 32.2 ± 15.3 mg/dL at the one year checking, the mean difference between the baseline value and after one year was 50.8 ± 14.7 mg/dL, ($P < 0.001$), (Fig. 4)

Table 3: Changes in Blood urea during the subsequent visits of the patients (N=38)

Variable	Blood urea (mg/dL)	
	Mean	SD
Baseline	83.0	12.7
Three months	66.2	11.3
Six months	47.7	13.3
One year	32.2	15.3
mean difference	50.8	14.7

ANOVA test **P. value < 0.001**

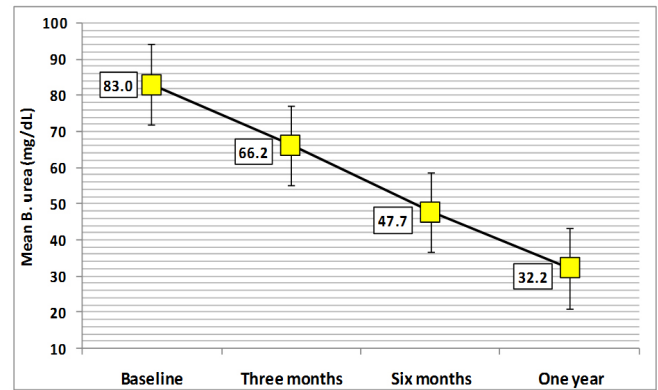


Figure 4: Changes in Blood urea during the follow up period

Similar trend of the change in mean blood urea is reported in each of serum creatinine, urine protein/ creatinine ratio and serum cholesterol, the changes were statistically significant (P .value < 0.001), (Tables 4, 5&6, figures 5, 6&7).

Table 4. Changes in Serum creatinine during the subsequent visits of the patients (N=38)

Variable	Serum creatinine (mg/dL)	
	Mean	SD
Baseline	2.29	0.50
Three months	1.97	0.42
Six months	1.68	0.39
One year	1.24	0.49
mean difference	1.05	0.31

ANOVA test **P. value < 0.001**

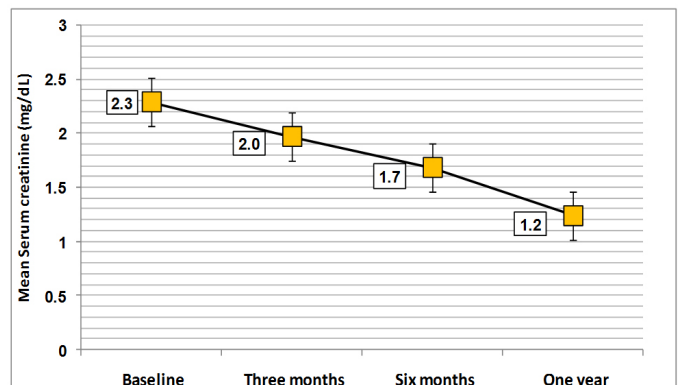


Table 5: Changes in Urine protein/ Creatinine ratio during the subsequent visits of the patients (N=38)

Variable	Urine protein/ Creatinine ratio	
	Mean	SD
Baseline	3.21	0.66
Three months	2.57	0.61
Six months	1.75	0.57
One year	0.94	1.05
mean difference	2.26	0.74

ANOVA test **P. value < 0.001**

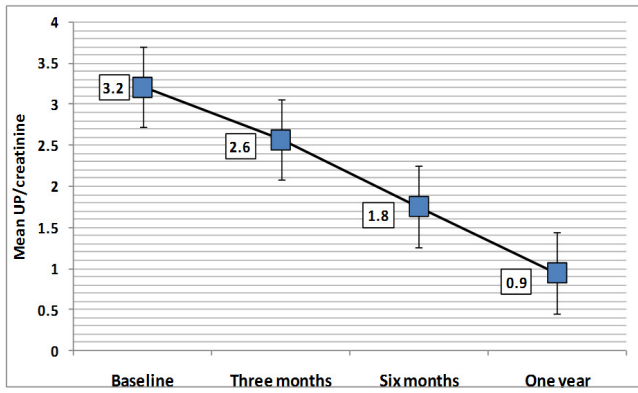


Table 6: Changes in Serum Cholesterol during the subsequent visits of the patients (N=38)

Variable	Serum Cholesterol (mg/dL)	
	Mean	SD
Baseline	291.4	20.2
Three months	268.8	15.4
Six months	238.8	25.5
One year	195.6	45.4
mean difference	95.8	29.7

ANOVA test P. value < 0.001

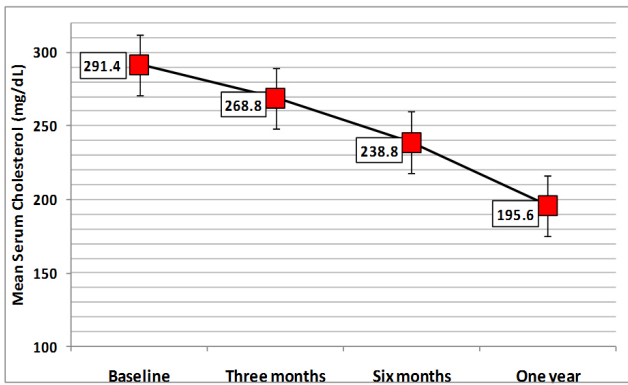


Figure 7: Changes in Serum Cholesterol during the follow up period

Table 8: Relationship between response to Rituximab and demographic variables of the patients (N = 38)

Variable		Response to Rituxim						P.value
		Good (n= 21)		Partial (n = 14)		Poor (n = 3)		
		No.	%	No.	%	No.	%	
Age (year)	≤ 15	12	80.0	2	13.3	1	6.7	0.014 sig
	16 - 30	8	53.3	6	40.0	1	6.7	
	> 30	1	12.5	6	75.0	1	12.5	
Gender	Male	16	66.7	7	29.2	1	4.2	0.16 ns
	Female	5	35.7	7	50.0	2	14.3	
Steroid response	Steroid resistant	18	85.7	11	78.6	2	66.7	0.68 ns
	Steroid dependent	3	14.3	3	21.4	2	21.4	

There was a statistically significant elevation in the mean value of serum albumin level from 24.4 ± 3.6 g/L at the base line to reach 35.9 ± 4.4 g/L after one year of treatment with Rituximab, ($P < 0.001$), (Table 7 and Fig. 8).

Table 7: Changes in Serum Albumin during the subsequent visits of the patients (N=38)

Variable	Serum Albumin (g/L)	
	Mean	SD
Baseline	24.4	3.6
Three months	29.0	3.2
Six months	32.4	5.7
One year	35.9	4.4
mean difference	11.4	2.8

ANOVA test P. value < 0.001

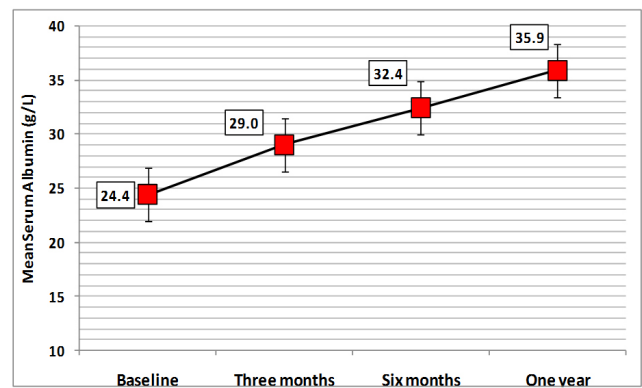


Figure 8: Changes in Serum Albumin during the follow up period

As it shown in table 8 there was a statistically significant association between young age and good response to Rituximab, ($P = 0.014$), the response seemed to be reduced with the advanced age. Response to Rituximab was not significantly associated neither with gender nor Steroid response ($P > 0.05$).

For the relationship between response to Rituximab and histopathological typing, it had been significantly found that (78.3%) of the patients with Minimal change glomerulonephritis, (27.3%) of those with Focal segmental glomerulosclerosis and none of the patients with Mesangioproliferative glomerulonephritis had good response to Rituximab, (P=0.001), this indicated that better response associated with MCGN compared to other types, (Table 9)

Table 9: Relationship between response to Rituximab and histopathological findings of the patients (N=38)

Histopathology	Response to Rituxim					
	Good (n = 21)		Partial (n = 14)		Poor (n = 3)	
	No.	%	No.	%	No.	%
Minimal change glomerulonephritis	18	78.3	3	13.0	2	8.7
Focal segmental glomerulosclerosis	3	27.3	7	63.6	1	9.1
Mesangioproliferative glomerulonephritis	0	0.0	4	100.0	0	0.0
P. value = 0.001						

Further distribution of the patients with good response according to the relapsing status is shown in (Table 10), where majority of those patients, 17/21 (81%) not relapsed while 4 patients (19%) relapsed. From other point of view, relapse was not statistically significant associated with the age, gender, Histopathology or Steroid response, in all comparisons, (P>0.05), (Table 11).

Table 10: Distribution of the 21 patients with good response to Rituximab after one year from stopping treatment

Prognosis	Good response to Rituximab (n=21)	
	No.	%
Not Relapse	17	81.0
Relapse	4	19.0

Table 11: Relationship between relapsing of the 21 patients with good response to Rituximab and the demographic and clinical characteristics

Variable		Prognosis				P.value
		Not Relapse		Relapse		
		No.	%	No.	%	
Age (year)	≤ 15	8	47.1	4	100.0	0.061 ns
	16 - 30	8	47.1	0	0.0	
	> 30	1	5.9	0	0.0	
Gender	Male	13	76.5	3	75.0	1.0 ns
	Female	4	23.5	1	25.0	
Histopathology	MCGN	15	88.2	3	75.0	0.85 ns
	FSGS	2	11.8	1	25.0	
	MesPGN	0	0.0	0	0.0	
Steroid response	Steroid resistant	14	82.4	4	100.0	0.36 ns
	Steroid dependent	3	17.6	0	0.0	

Discussion

Our study differs from other studies in that we used rituximab for the patients whom received calcineurin inhibitor “prograf” or “sandimmune” and not respond or relapse after stopped it, while the other studies used rituximab in primary nephrotic syndrome without using calcineurin inhibitor. Several case report and observational studies have created a considerable expectation about therapeutic possibilities of rituximab in nephrotic syndrome.

Several case report and uncontrolled series suggest that rituximab could be potentially effective and safe alternative for pediatric and adult patients with SDNS & SRNS. Cuignis et al. in multi center report from France examined the efficacy of rituximab in 22 patients with SDNS & SRNS, at a median follow up of 9.5 months, 19 (83.3%) of patient had beneficial effect with sustained remission and reduction of proteinuria [18].

In Gulati et al. study, who take 33 patients with SRNS and 24 patients with SDNS, the median age group was 12 years while in present study the median age group was 17 years and we found the majority of patients had good response to rituximab were young age group and the response seemed to be reduced with the advanced age as Gulati et al. found [19].

Rituximab has evolved as efficient alternative in treatment of SDNS & SRNS. Response to rituximab was less frequent in patients with steroid resistance [19,20].

In Gulati et al. study, six month after rituximab therapy, 9 (27.2%) of patients with SRNS show complete remission, 7 (21.2%) had partial remission and 17 (51.5 %) had no response [19]. While response in our study was not significant associated with steroid response, 21 (52.5 %) with good response, 14 (35 %) with partial response and 3 (7.5 %) had poor response.

Regarding relapsing state after 12 month from stopping treatment is not significantly associated with steroid response in our study while in Gulati et al. showed tremendous improvement of proteinuria mainly in SDNS where the remission had sustained in 20 (83.3 %) of 24 patients with SDNS and 15 patient with SRNS at 12 month (i.e.) the relapse is more in steroid resistance [19].

In our study the response to rituximab administration is significantly affected by the histopathological typing of nephrotic syndrome. It had been found that 78.3 % of patients with MCGN, 27.3 % of them with FSGS and none of the patients with MesPGN had good response to rituximab (i.e.) better response associated with MCGN compared to other type as in Fernandez et al. [21]. Fernandez et al. observed sustained response in two out of eight patients with severe steroid resistance FSGS that is because more damage to glomeruli occurred in FSGS than in MCGN.

In another cohort study, Sugiura et al., found significant decline in proteinuria was achieved in patients with MCGN after single administration of rituximab from 3.8 ± 4.1 g/day at base line to 0.4 ± 1.2 g/day at follow up, while patients with FSGS had a decrease from 5.2 ± 2.4 g/day to base line to 2.3 ± 2.8 g/day six months after treatment [22].

The risk of side effects attributed to rituximab is variable. In this study rituximab had been stopped in 2 (5 %) cases because of

development of anaphylactic reaction. The metacentric French report found adverse effects in 45 % of patients including anaphylactic reaction and pneumocystis carina pneumonia [18]. While Prytula et al. report acute reaction in 27% of patients and high incidence of severe side effects including anaphylaxis and serious infection [23].

Another case series by Sellier A. et al. reported occasional cases of reversible cytokine shock and neutropenia with no risk of severe infection [24]. In this study the number of doses of rituximab used was based on WBC and neutrophil count every two weeks rather than targeting specific CD 20.

We used rituximab infusion once monthly for 6 months because it is more applicable for patients especially pediatric age group while in Bagga et al. used rituximab infusion once weekly for 3 months and same response in renal function was shown [25]. In present study we used the same dose of rituximab and same duration in patients with SDNS & SRNS while data from other studies, Cuigonis et al., used more doses in patients with SRNS than those with SDNS [18]. In those studies, 2 to 4 doses each of 375 mg/m² is associated with CD 20 depletion to < 1%.

We had multiple limitations, the main was lack of control groups, although it is unlikely that the observed impact of therapy with rituximab was fortuitous, prospective controlled trial are necessary to confirm the efficacy of this agent. Other limitation is lack of CD 20 protein marker level.

Our results show significant reduction in mean urine (p/c) ratio from base line 3.21 to 0.94 after one year from receiving rituximab, this was similar to Bagga et al. results where the mean value of urine (p/c) ratio decreases from 8.3 at base line to 0.8 after follow up [25].

The same reduction was observed for mean cholesterol level where there is significant reduction from 291.4 mg/dl at base line to 195.6 mg/dl after one year from receiving rituximab as shown in Bagga et al. where mean cholesterol level reduced from 481 mg/dl to 250 mg/dl after follow up [25].

For mean albumin level, our result shows increases the level from 24.4 g/l to 35.9 g/l after one year from receiving rituximab. Similar to the same significant improvement in mean albumin level from 1.4 to 3.4 g/dl after follow up in Bagga et al. [25].

Our results show significant reduction in the mean blood urea from 83.0 mg/dl to 32.2 mg/dl after one year follow up. The same significant reduction in serum creatinine from 2.29 mg/dl to 1.24 mg/dl.

Conclusion

- In SRNS start rituximab and mycophenolate mofetil used to get ride from side effects of calcineurin inhibitor (tacrolimus or cyclosporine)
- In SDNS start rituximab and mycophenolate mofetil used after calcineurin inhibitor (tacrolimus or cyclosporine). No more longer course of steroid to get ride from side effects of steroid and CNI.
- Good improvement in renal function either result from cessation of calcineurin inhibitor (nephrotoxic drugs) and or from our management with rituximab.
- Cost effects of rituximab is less than the cost that needed if the patients had frequent admissions to the hospital or developed

renal failure and ended with dialysis.

Recommendation

- We need another 5 years follow up for patients especially after stop rituximab and this need further study to follow if there is relapse or not and to monitor if patients developed renal impairment.
- More number of patients are required to include with different age groups .
- CD20 protein marker should be available to close monitor of rituximab effect because rituximab is anti CD20 monoclonal antibody cause B-cell depletion especially when mycophenolate mofetil used concomitantly with rituximab.

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