"SINGLE DOSE RITUXIMAB IN STEROID DEPENDENT NEPHROTIC SYNDROME AND FREQUENTLY RELAPSING NEPHROTIC SYNDROME IN CHILDREN 2-18 YEARS OF AGE IN A TERTIARY CARE HOSPITAL DURING A PERIOD OF 1 YEAR - A PRE POST STUDY"

BY

REG NO: BM0122018

Dissertation

Submitted to the KLE Academy of Higher Education and Research, Belagavi, Karnataka

In Partial Fulfilment

of the Requirements for the Degree of

M.D. (Doctor of Medicine)

In

PAEDIATRICS

DEPARTMENT OF PAEDIATRICS
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELAGAVI, KARNATAKA

SEPTEMBER /OCTOBER 2025

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"SINGLE DOSE RITUXIMAB IN STEROID DEPENDENT NEPHROTIC SYNDROME AND FREQUENTLY RELAPSING NEPHROTIC SYNDROME IN CHILDREN 2-18 YEARS OF AGE IN A TERTIARY CARE HOSPITAL DURING A PERIOD OF 1 YEAR – A PRE POST STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee.

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LIST OF ABBREVATIONS

ABBREVATION	EXPANSION OF ABBREVATION
NS	Nephrotic syndrome
RTX	Rituximab
SDNS	Steroid dependent nephrotic syndrome
FRNS	Frequently relapsing nephrotic syndrome
SSNS	Steroid sensitive nephrotic syndrome
INS	Idiopathic nephrotic syndrome
SRNS	Steroid resistant nephrotic syndrome
MCD	Minimal change disease
FSGS	Focal segmental glomerulosclerosis
GBM	Glomerular basement membrane
SLE	Systemic lupus erythematosus
UPCR	Urine protein creatinine ratio
CNI	Calcineurin inhibitor
CyA	Cyclosporin
AIPGN	Acute infectious post glomerulonephritis
ESRD	End stage renal disease
ISKDC	The International Society of Kidney Disease in Children
KDIGO	Kidney Disease: Improving Global Outcomes

NPV	Negative predictive value
PPV	Positive predictive value
RCT	Randomised controlled trial

ABSTRACT

Background: Nephrotic syndrome (NS) is a common pediatric kidney disorder, with many children developing frequently relapsing (FRNS) or steroid-dependent nephrotic syndrome (SDNS), leading to prolonged steroid use and associated complications. Rituximab, an anti-CD20 monoclonal antibody, offers a potential steroid-sparing alternative. This study evaluates the effectiveness of a single-dose rituximab regimen in children aged 2–18 years with FRNS and SDNS, focusing on improving remission rates, reducing steroid dependence, and enhancing cost-effectiveness.

Objectives:

Primary objective: To assess the sustained remission at 3 months post single dose rituximab in patients with steroid dependent nephrotic syndrome and frequently relapsing nephrotic syndrome

Secondary objective:

- To assess B-cell suppression by measuring CD 19 levels prior to rituximab then at 3 weeks and 3 months post single dose Rituximab.
- To predict relapse by assessing for recovery of B cell at 3 weeks and 3 months post single dose Rituximab.

Methodology: This pre-post study evaluated the effectiveness of a single-dose Rituximab regimen in 21 children aged 2–18 years with FRNS and SDNS at Dr. KLES, Dr. Prabhakar Kore hospital Belagavi, over one year. Patients were recruited from the pediatric nephrology outpatient department, and detailed clinical histories,

physical examinations, and baseline CD19 levels were recorded. A single intravenous Rituximab dose (357 mg/m², max 500 mg) was administered with premedication, followed by monitoring for adverse effects. CD19 levels were re-evaluated at three weeks and three months post-infusion. Oral mycophenolate mofetil was introduced, and steroids were tapered. Remission was assessed at three months based on urine albumin, absence of edema, and CD19 suppression. Statistical analysis included paired t-tests or Wilcoxon matched pairs tests, with significance set at $p<0.05$.

Results: The present study included 21 participants (mean age: 8.81 ± 3.78 years), with 15 males (71.43%) and 6 females (28.57%). Among them, 6 (28.57%) had FRNS and 15 (71.43%) had SDNS. Baseline CD19% was 20.97 ± 8.6, reducing significantly to 0.55 ± 1.33 at 3 weeks ($p < 0.001$) and slightly increasing to 3.74 ± 5 at 3 months ($p < 0.001$). B cell suppression (<1%) was observed in 90.48% at 3 weeks and 57.14% at 3 months. Remission was achieved in 95.24% at both time points. A significant association existed between B cell suppression at 3 weeks and 3 months ($p = 0.016$). All participants who achieved remission at 3 weeks remained in remission at 3 months. Among those with B cell recovery ($n = 9$), 1 relapsed, while none relapsed in the suppression group ($n = 12$), though the association was not statistically significant ($p = 0.237$). B cell recovery had 100% sensitivity and 60% specificity for predicting relapse.

Conclusion: This study demonstrates that a single-dose rituximab regimen is highly effective in achieving remission and reducing steroid dependence in children with SDNS and FRNS. Significant and sustained B cell suppression was observed, correlating with high remission rates at three months. While B cell recovery was associated with relapse, its predictive value remains limited, highlighting the need for

additional biomarkers to refine relapse prediction. Overall, rituximab presents a promising steroid-sparing therapeutic option for children with nephrotic syndrome, potentially improving long-term disease control and reducing treatment-related toxicities. Further studies with larger cohorts and longer follow-ups are necessary to optimize treatment protocols and enhance relapse prevention strategies.

Keywords: *Frequently Relapsing Nephrotic Syndrome, Steroid-Dependent Nephrotic Syndrome, Rituximab.*

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INTRODUCTION

Nephrotic syndrome (NS) is a prevalent renal disorder in children which is marked by edema, heavy proteinuria (greater than 1 g/m² daily or 40 mg/m²/hour), and hypoalbuminemia. [1] INS is frequently occurring glomerular disorder in children, accounting for around seventy two to eighty five percentage of all cases of NS in this age group. Approximately ninety percentage of children experiencing their 1st episode of minimal change idiopathic nephrotic syndrome attain remission with corticosteroid therapy. Although, relapses occur in 70% to 80% of these children, and 30% to 50% face frequent relapses, requiring repeated courses of corticosteroids.

According to estimations, there are two to seven new cases of NS for every 100,000 children below age of eighteen in healthy youngsters each year. It is highly prevalent in males than females at younger age, however by teenage, the gender disparity becomes negligible. Additionally, in American, Hispanic & African populations, this disease tends to be more severe with high prevalence. [4]

Children with FR and SDNS often endure significant complications from prolonged steroid use. These adverse effects include Cushingoid features, high BP , abnormal lipid and glucose levels, an increased risk of severe infections, growth delays stemming from both the disease and its treatment—and reduced bone density, among other complications. [5]

Previous research indicates that around 50% of children with this condition that means they either relapse frequently or require ongoing steroid treatment to sustain remission. Studies report the prevalence of FR/SD cases within SSNS populations ranges from 22% to 61%. [6]

Children with SDNS & FRNS are often managed with additional immunosuppressive medications, which can adversely affect their quality of life. Identifying cases of SSNS early in children who are likely to develop FRNS or SDNS remains a significant challenge for pediatric nephrologists.[7] Oral corticosteroids serve as the primary treatment, achieving disease activity remission in around ninety percent of cases. However, within five years, up to eighty five percent of patients experience relapses, with many becoming dependent on steroids. In these cases, the condition recurs within 2 weeks after stopping corticosteroids, requiring ongoing treatment.[8]

Clinical guidelines suggest that maintaining remission in steroid-dependent patients can be achieved with low-dose prednisone. For children who experience steroid-related adverse effects, corticosteroid-sparing options such as calcineurin inhibitors are advised. Given the toxicity associated with these treatments, exploring alternative therapeutic strategies is essential. [9]

Observational studies provide supporting evidence that underscores the advantages of these treatments. One clinical trial demonstrated more favorable outcomes in young patients with NS who were managed by combining steroids and calcineurin inhibitors.[10] Conversely, a recent study reported improved outcomes in cases of FRNS and SDNS when managed with a combination of steroids and immunosuppressive treatments.[11] Additionally, CNIs like tacrolimus & cyclosporin are known to cause significant side effects, most notably nephrotoxicity (kidney damage). As a result, rituximab is often considered a less nephrotoxic alternative in certain cases, as it primarily targets B-cells without directly affecting kidney function. [12]

RTX, a chimeric monoclonal antibody targeting CD20, is gaining recognition as an effective alternative for minimizing steroid usage in management of children with INS. CD-20 is a trans-membrane protein found on B cells from early stages of differentiation up until the plasma cell phase. When RTX adheres to CD-20+ cells, it induces cell apoptosis via multiple mechanisms, like 'antibody-dependent cell-mediated cytotoxicity', 'complement-dependent cytotoxicity', 'antibody-dependent phagocytosis'[13], & direct effects arising from CD-20 engagement. In autoimmune diseases, RTX works by disrupting B cell functions within the immune system or by decreasing plasma cell production. This targeted disruption of B-cells has proved to be an efficient approach in sustaining remission for patients with steroid dependent NS.[13]

Overall, majority of studies highlight the temporary nature of RTX's effects. However, the ideal frequency of multiple infusions required to maximize gains while reducing possible complications has yet to be determined. Long term follow up research indicate that children treated with RTX alongside other therapies experience prolonged oral drug-free remission, especially those who were initially steroid-dependent with a shorter duration of the disease. However, the usage of RTX in the initial stages of uncomplicated SDNS has not yet been investigated in clinical trials.[14] Moreover, recent studies show use of multiple doses of rituximab in SDNS and FRNS children.

Therefore, this study seeks to fill a gap in the existing research by evaluating effectiveness of single dose of RTX in children these conditions at a tertiary care hospital over a one-year period. By adopting a single-dose strategy, the research seeks to improve cost-effectiveness and enhance accessibility for patients facing financial constraints compared to protocols that require multiple injections.

AIMS AND OBJECTIVES

AIM:

To study the effect of single dose Rituximab in SDNS and FRNS in children 2-18 years of age in a tertiary care hospital during a period of 1 year.

OBJECTIVES:

Primary objective:

- To assess sustained remission in patients with steroid dependent nephrotic syndrome and frequently relapsing nephrotic syndrome- 3 months post single dose rituximab

Secondary objective:

- To assess B-cell suppression by measuring CD 19 levels prior to rituximab then at 3 weeks and 3 months post single dose Rituximab.
- To predict relapse by assessing for recovery of B cell at 3 weeks and 3 months post single dose Rituximab.

REVIEW OF LITERATURE

➢ NEPHROTIC SYNDROME

BACKGROUND:

NS is marked by excessive proteinuria, which results in edema, hypoalbuminemia, hyperlipidemia, & multiple related complication. (Figure 1) This condition arises from increased permeability of renal GBM, which is often triggered by intrinsic renal disease factors such as diabetes, congenital infections, SLE, malignancies, or some medications. The condition predisposes patients to complications like infections and thromboembolism. [15-17]

A spot UPCR equal to or exceeding 2 grams or a urinary protein loss of 3 grams or more during a 24-hour period, are considered indicators of nephrotic-range proteinuria. Additionally, systemic diseases like amyloidosis also can contribute in the development of significant proteinuria. [18]

NS can occur in individuals of all ages, affecting both children & adults irrespective of sex or race. It may present as a typical nephrotic syndrome or alongside nephritic syndrome, which involves inflammation of the glomeruli, presence of blood in the urine (hematuria), and reduced kidney function

In children, facial swelling is frequently the earliest indicator of nephrotic syndrome which may eventually extend to the entire body. In adults, the condition commonly manifests as dependent edema. Additional symptoms include fatigue and reduced appetite. [19]

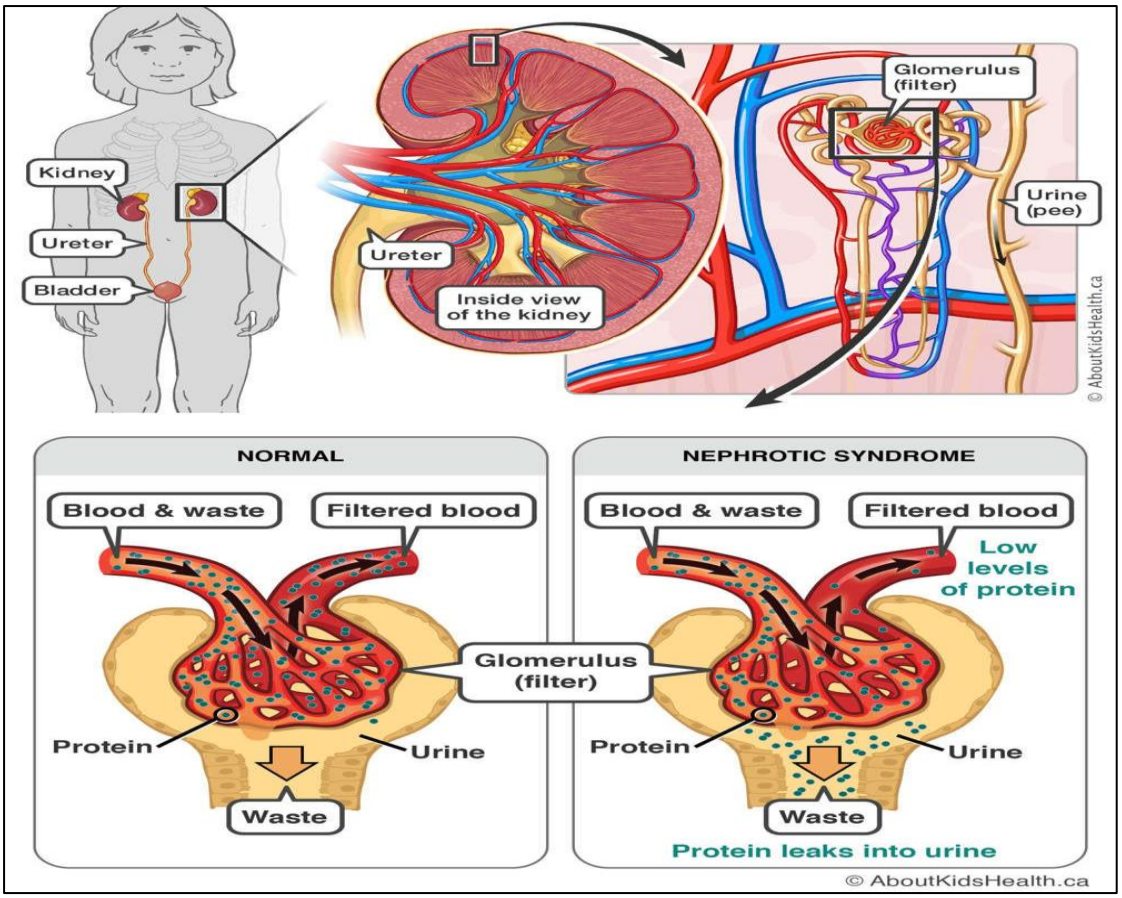


Figure 1: Nephrotic Syndrome

EPIDEMIOLOGY:

In the US, incidence of INS annually in children is estimated to be around '2.7 new cases per 100,000,[20] with a cumulative prevalence of 16 per 100,000'[20]. During childhood, condition is more commonly observed in boys than in girls, but this difference tends to disappear by adolescence. Familial cases, especially among siblings, are more frequently reported. The average age of onset is recorded as '3.4 years in Asian children[20] & 4.2 years in European children'[20]. African American and Hispanic children not only show a higher incidence but also often present with more severe forms of the disease and have less favourable prognosis. [20]

In both India and Turkey, kidney biopsy findings in children with NS reveal histological patterns that are consistent with those reported in Western countries. [20, 21] Likewise, similar observations have been made in Pakistani adults, where biopsy results align closely with the patterns seen in Western populations. [22]

Urogenital schistosomiasis infections have been linked to glomerular disorders in a number of Middle Eastern and African regions. [23] However, it is uncommon to see tropical nephrotic syndrome brought on by parasite infections.

DEMOGRAPHICS ASSOCIATED WITH AGE, RACE, AND GENDER:

Diabetes mellitus is a major contributor to NS, and as a result, incidence of NS is more among American Indians, Hispanics & African Americans compared to White individuals. HIV-associated nephropathy, a condition linked to HIV infection, is uncommon in White populations but frequently occurs more in African Americans secondary to greater prevalence of ApoL1 alleles in this group. [24] Focal glomerulosclerosis is a frequent reason for NS in African American children juxtaposed to White children. NS typically shows a higher prevalence in males, a trend also noted in other chronic kidney diseases, including paraneoplastic membranous nephropathy. [25]

PATHOPHYSIOLOGY:

Proteinuria and Hypoalbuminemia-

Proteinuria results from damage to glomerular filtration barrier , which comprised of 3 sections: fenestrated endothelium, GBM, and visceral glomerular epithelium. The visceral epithelium consists of podocytes, special cells with foot like extensions known as foot processes that reach into urinary space. In NS, foot

processes undergo effacement, while the remainder of the podocyte typically remains intact. [26]

Endothelial cells in the glomerulus are characterized by numerous fenestrae—tiny openings measuring 70 to 100 nm—that contribute to the selective filtration of blood. These fenestrae allow the passage of small solutes while restricting larger macromolecules. The glomerular basement membrane (GBM) enhances this physical barrier with its negatively charged elements, particularly heparan sulfate proteoglycans. These negatively charged molecules play an imperative role in the filtration process by repelling similarly charged plasma proteins such as albumin, thereby preventing their leakage into the renal tubule. Experimental studies have demonstrated that when these negative charges are enzymatically removed using heparinase, the GBM loses its charge-selective properties, leading to proteinuria—a condition where protein, particularly albumin, abnormally appears in the urine.

Podocytes were once thought to enact only a secondary function in glomerular filtration. However, this view shifted drastically after identification of mutations in nephrin, a vital protein of slit diaphragm, are accountable for congenital NS of Finnish type. Further research have shown that adaptor proteins like podocin and CD2AP are essential for securing the fragile slit diaphragm—which spans the filtration gaps between neighbouring podocytes—to the cell's cytoskeleton. Beyond serving as a structural link, podocytes actively contribute to the survival and proper functioning of glomerular endothelial cells. As a result, disruptions in the proteins that form the slit diaphragm complex have been associated with a range of inherited and acquired renal diseases. (Table 1)

Table 1 : Genetic types of NS.

Gene/Protein	Location	Phenotype	Inheritance
NPHS/nephrin	Slit diaphragm	Congenital nephrotic syndrome (CNF)	Autosomal recessive (AR)
NPHS2/podocin	Slit diaphragm	FSGS	Autosomal recessive (AR)
CD2AP/CD2AP	Near slit diaphragm	FSGS	-
TRPC6/TRPC6	Podocyte	FSGS	Autosomal dominant (AD)
WT1	Podocyte	FSGS	Autosomal recessive (AR)
ACTIN4	Foot process	FSGS	Autosomal dominant (AD)
TRNALeu	Podocyte	FSGS	-
COQ2	Podocyte	FSGS	-

Edema-

Traditionally, edema in NS is thought to occur because the loss of serum albumin lowers the plasma's oncotic pressure, prompting fluid to move into the interstitial spaces. This fluid shift lowers the blood volume within vessels, resulting in reduced kidney perfusion & activating renin-angiotensin aldosterone system.

Sodium re-absorption is subsequently increased by Aldosterone , primarily in distal nephron segments. However, clinical evidence does not fully support this hypothesis. Plasma volume reduction is observed in few children with MCNS , predominantly during early stages of a relapse, and is seldom seen in adults with nephrotic syndrome. Furthermore, studies have not consistently shown increased levels of renin-angiotensin aldosterone hormones & sodium re-absorption persists even after albumin infusion or suppression of renin activity with angiotensin-converting enzyme (ACE) inhibitors. This has led to the suggestion of an intrinsic renal abnormality causing sodium retention. Additionally, excess vasopressin may contribute to water retention. [26]

Hyperlipidemia-

Nephrotic syndrome is marked by abnormal lipid metabolism, where levels of VLDL, IDL, and LDL are elevated. This imbalance gives rise to increase levels of triglycerides & cholesterol in bloodstream, while the high-density lipoprotein (HDL) fraction usually remains unchanged, resulting in a higher LDL/HDL cholesterol ratio. The dyslipidaemia seen in nephrotic syndrome is linked to several causes, such as increased production of lipoproteins secondary to decreased albumin levels in plasma and reduced oncotic pressure, as well as reduced degradation of apolipoprotein B, VLDL, and chylomicrons. [26]

ETIOLOGY:

MCD, FSGS , and membranous nephropathy are frequently seen etiology of NS. Secondary NS can result from conditions such as diabetes, amyloidosis, and lupus.[27] In approximately half of the cases, infectious diseases, especially those involving the upper respiratory tract, act as triggering factors. Allergic reactions account for about one-third of cases, while insect bites or vaccinations are less common triggers. Additionally, nephrotic syndrome can be induced by the use of drugs of abuse, such as heroin. [28]

Secondary causes:

Diabetes Mellitus

- **Immune-related causes**: Immune-related factors include conditions such SLE, antibody mediated vasculitis, Berger's disease, and APIGN, as well as ANCA-associated vasculitis, Goodpasture syndrome, membranous and membranoproliferative glomerulonephritis, and thrombotic microangiopathy.

Additionally, immune reactions from enzyme replacement therapy (resulting in alloantibodies) and nephrotoxicity associated with NSAIDs or gold salts are also considered immune-mediated causes.

- **Infections**: Hep B, Hep C, HIV, CMV, parvo virus B19, pre-eclampsia, paraproteinemias, amyloidosis & toxoplasmosis

Minimal-change glomerulonephritis is a highly prevalent etiology of NS. In Caucasian adults, membranous nephropathy tends to be the leading cause, whereas in individuals of African descent, focal-segmental glomerulo-sclerosis is more commonly observed.

Nephrotic range proteinuria is often a marker of preeclampsia and may also occur during the third trimester of pregnancy. In certain instances, it may arise from scratch or be a component of pre-existing chronic kidney disease, such as proteinuria that worsens during pregnancy.

Certain medications can also lead to nephrotic syndrome, including:

- Minimal-change disease associated with NSAIDs. [29]
- Membranous glomerulonephritis caused by Bucillamine, gold & penicillamine are drugs commonly adviced for the treatment of rheumatic conditions. [30]
- FSGS linked to bisphosphonates. [31]
- Interferon therapy & lithium have been implicated in development of FSGS. [32]

- **SDNS & FRNS**

Prognosis for children with INS largely depends on histopathological findings, which provide insights into disease progression and possible complications. Biopsy results indicating FSGS and SRNS are closely linked to less favourable outcomes, including the advancement to ESRD. [33] In two weeks of starting steroid medication, most children with minimal change disease experience remission. However, disease relapses in more than 75% of cases, with nearly half of these patients experiencing frequent relapses or steroid dependency. [34] Many studies have explored the association between steroid dependency and features like male gender, early age at disease onset, low levels of serum protein, delayed response to initial steroid therapy, & the presence of hematuria. While some research indicates that early onset and prolonged time to remission may predispose children to steroid dependency, other studies have found these associations to be inconsistent. [35] While Arab children have a higher reported incidence of NS, [36] their response to steroid therapy remains poorly understood. Identifying children who are likely to develop steroid dependency will help in selecting patients who would benefit from early introduction of steroid-sparing agents (SSAs) and in minimizing probable complications of long term steroid use.

- **ROLE OF RITUXIMAB IN FR/SDNS**

A prevalent chronic glomerular disease in children is INS, taking place at a rate of 2/100,000 annually in Western countries[37] & 5 per 100,000 annually in Japan. Around 80% of these cases are identified as MCNS, the majority of which have a good response to corticosteroid therapy, also known as SSNS. [38]

Although the most children with steroid sensitive NS initially show a positive response to steroid therapy, nearly half have a frequently relapsing pattern. This pattern, referred to as FRNS, is generally characterized by a minimum of 4 relapses within a year or 2 relapses within the first six months following diagnosis. This recurrent behavior indicates that the disease is not fully controlled, leading to repeated episodes that require additional treatment. Moreover, a subset of SSNS patients may evolve into a steroid-dependent form, where the disease recurs either during the steroid tapering process or within 14 days after the discontinuation of treatment.

Approximately 50–60% of children with FRNS also fulfill the criteria for SDNS, according to the guidelines established by the ISKDC. This overlap underscores that a considerable number of patients with recurrent relapses also face relapses during steroid tapering or soon after stopping steroid treatment, reflecting a difficult treatment journey. Moreover, around 10–20% of cases of INS are classified as SRNS. [39]

Worldwide, immunosuppressive medications like cyclophosphamide, chlorambucil, CyA, levamisole & tacrolimus are commonly employed as standard treatments for SDNS & FRNS in children.[40] CyA remains the preferred therapy for treating SRNS in children. [41] The Japanese Society for Pediatric Nephrology (TJSPN) 2013 Guidelines for Pediatric Nephrotic Syndrome state that CyA is the recommended treatment for SRNS and cyclophosphamide, mizoribine, or CyA are suggested for FRNS/SDNS. [42] While the majority of patients benefit from these treatments, some have difficult clinical outcomes. For example, 10–20% of children with this condition treated with CyA still experience relapses frequently. [43, 44] Additionally, almost 30% of children develop relapses following complete remission. [45] Furthermore, CyA use is associated with side effects, particularly chronic

nephrotoxicity [46] emphasizing the need to discontinue CyA after long-term use to minimize these risks.

Stopping cyclosporine (CyA) frequently results in recurrent relapses or the development of steroid dependence, necessitating extended use of steroid therapy, which carries considerable risks. Approximately, 20 % of children with INS have complex SDNS/FRNS, while 1–3% are resistant to both steroids and immunosuppressants, risking advancement to end stage renal failure (refractory SRNS). These challenges highlight the urgent need for new treatment options.

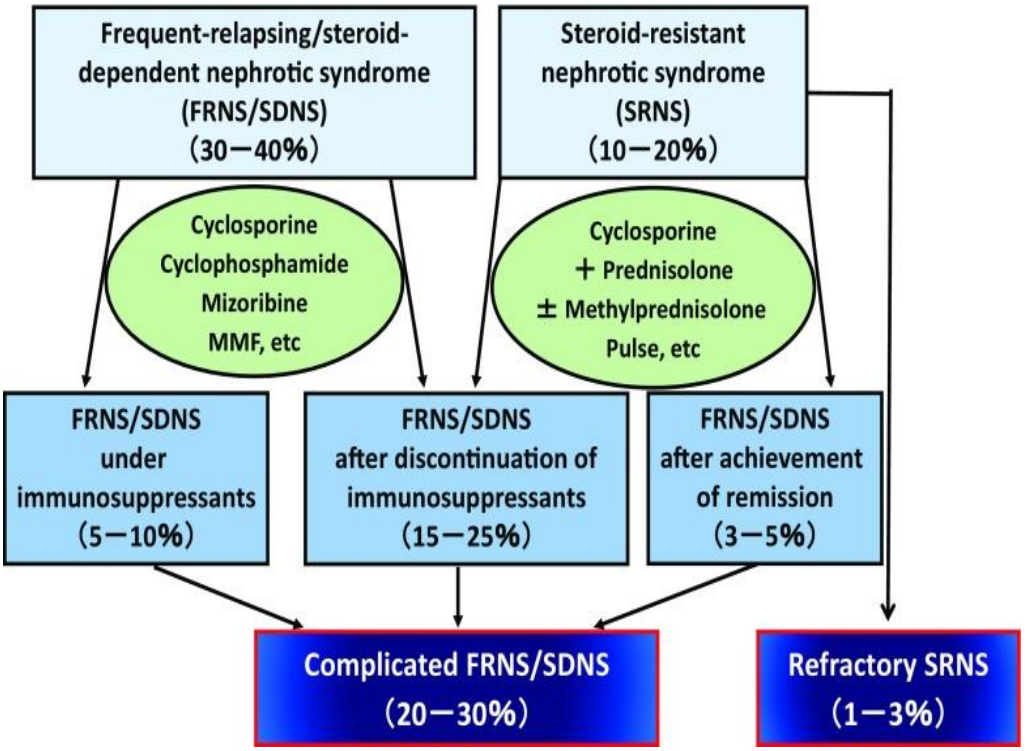


Figure 2: Complicated FR/SDNS and SRNS

RTX, a chimeric monoclonal Ab targeting CD-20, was earlier designed for treating B-cell NHL. Now, it is widely utilized in managing various auto-immune diseases. As shown in Table 2, numerous studies over the past ten years have shown efficacy of RTX in managing challenging cases of FRNS, SDNS [47] & SRNS. [48]

Table 2: Definitions of complicated FRNS/SDNS

Term	Definition
Complicated FRNS/SDNS	Patients were diagnosed with complicated FRNS/SDN if they met one of the following criteria: 1. Frequent relapses or steroid dependence aft completing treatment with immunosuppressive agen such as cyclosporine, cyclophosphamide, mizoribine, mycophenolate mofetil. 2. Frequent relapse or steroid dependence duri immunosuppressive drug therapy. 3. A history of steroid resistance, with frequent relap or steroid dependence during or after completion immunosuppressive therapy.

MODE OF ACTION OF RITUXIMAB :

The precise mechanism underlying the development of NS is unclear. Over four decades ago, it was initially proposed to involve T cell dysfunction. [49] B-cells are known to contribute to autoimmune damage through antibody-independent pathways, as well as by releasing cytokines and costimulatory molecules that promote sustained T-cell activation in autoimmune disorders. Rituximab (RTX) suppresses connections amid B & T -cells by inducing B cell depletion through processes like phagocytosis, antibody dependent cellular cytotoxicity, or B cell death. This may help avoid relapses in NS.

Research has indicated that patients with minimal-change NS exhibit impaired Treg cell function. It has been illustrated that T-reg cells play a crucial role in inducing remission in these patients. [50, 51] Additionally, play a crucial role in maintaining remission in NS involving the restoration of Treg cell function. It is also possible that nephrotic syndrome is driven by B cell-derived factors. [52]

Through the action of acid sphingomyelinase (ASMase), sphingomyelin is converted to ceramide by acid sphingomyelinase like phosphodiesterase 3b (SMPDL-3b). Patients with recurrent FSGS were found to have reduced levels of SMPDL-3b.

Moreover, podocytes exposed to sera from these patients are more susceptible to damage due to lower SMPDL-3b expression. Rituximab (RTX) was found to interact one to one with SMPDL-3b on surface of podocytes, influence ASMase activity & modulate ceramide production. By stabilizing podocyte shape and function, these measures help in the recurrence of FSGS. [53] Further research is essential to gain a clearer understanding of RTX's role in managing SDNS & FRNS. (Figure 3)

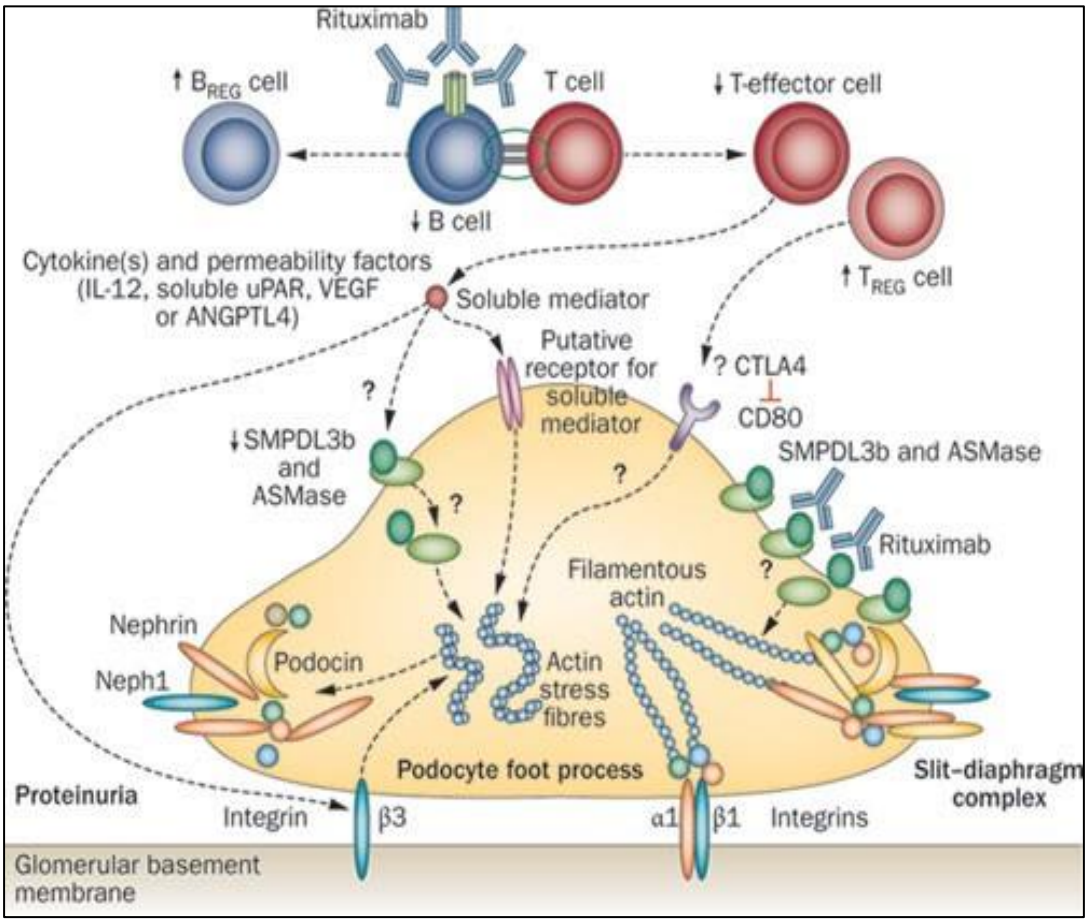


Figure 3 : Mechanism of action of RTX

PHARMACOKINETICS:

RTX adheres to a linear pharmacokinetic profile **and achieves nearly 100% bioavailability when administered intravenously. It has a volume of distribution of approximately 3.1 litres, as stated in the FDA-approved package insert.** The drug undergoes processing by proteases throughout body & hepatic CYP450 enzyme system. It's elimination involves formation of immune complexes between the drug and monoclonal antibodies, which are removed by the reticuloendothelial system, predominantly through Fcγ-mediated endocytosis. **Rituximab has a half-life ranging from 18 to 32 days. [54]**

- **EFFICACY OF RTX IN SDNS & FRNS**

During the early 2000s, initial evidence of rituximab's benefits in idiopathic nephrotic syndrome (INS) emerged through case series and case reports involving 1–24 patients with FRNS & SDNS. These studies showed that rituximab produced notable effects, frequently leading to long-lasting remission and allowing many children to stop taking other immunosuppressive drugs. [55, 56] However, because no reliable randomized studies or clinical trials were available at the time to evaluate rituximab's efficacy, the 2012 KDIGO Glomerulonephritis Guidelines did not highly suggest it for FRNS or SDNS.

Recent research has offered more robust evidence highlighting the benefits of rituximab in the treatment of SDNS & FRNS. [57, 58] Analyzing the outcomes of these studies is complex due to differences in rituximab dosages, the number of administered doses, and the timing of its use. However, these trials report that 80–100% of patients exhibited favorable clinical responses to rituximab, with many witnessing substantial reduction in relapse rates within year after treatment.

Numerous well-executed clinical trials have produced impressive and encouraging outcomes. A RCT involved 30 children with SDNS who needed heavy doses of corticosteroids to sustain remission.[59] These children had uncomplicated SDNS, no prior exposure to calcineurin inhibitors (CNIs), and were followed up for at least one year. In treatment group, participants were given a single dose of rituximab. Results showed that this group had significantly reduced prednisone doses at three months ($p < 0.001$) & a substantially lesser likelihood of relapses at six months, one year, and two years post-treatment ($p < 0.01$), although proteinuria only showed a slight reduction after 3 months. [59]

In India, an open label randomized controlled trial (RCT) was done involving 120 children. Participants in the study received tacrolimus for 12 months alongside tapering doses of prednisolone. Rituximab demonstrated superior outcomes, including higher twelve month relapse free survival rates, prolonged remission, and mitigated corticosteroid exposure. Rate of infections was 2 times higher in tacrolimus group (43% vs 21%). [60] Additionally, RTX has shown potential in managing complex, treatment resistant NS in children with FSGS, SDNS & FRNS.

A study on NS involved 54 children, divided into two groups. Group 2 followed standard treatment with prednisone and calcineurin inhibitors (CNI), while Group 1 received rituximab in 1–2 doses of 375 mg/m². After three months, Group 1 showed a 70% reduction in proteinuria. Children treated with rituximab experienced fewer relapses and had a significantly higher likelihood of being drug free (62.9 % vs 3.7 % & $p < 0.001$).

Based on these studies, rituximab has proven to be an effective treatment for childhood FRNS & SDNS. It helps manage condition, reduces relapse frequency, and supports reduced or stoppage of steroids & other immunosuppressive drugs use. Moreover, RTX might offer a cost effective alternative to day to day medications that require ongoing monitoring, while also contributing to improved growth outcomes in children. While other immunosuppressive drugs like MMF and calcineurin inhibitors (CNIs) have proven effective for FRNS and SDNS, they require twice-daily dosing to maintain remission. Therefore, rituximab, as an intravenous medication, may offer added value for children who may struggle with adherence to daily oral medications. [61]

- **ADVERSE EFFECTS OF RITUXIMAB**

Previous studies have indicated that RTX is commonly well tolerated in children with NS. Commonly noticed side-effects are mild infusion-related reactions, occurring in 5–53% of cases, that generally resolve with administration of anti-histaminics, & anti-pyretics, or by reducing the infusion rate. Although rare, severe reactions such as anaphylaxis or hypotension can occur rarely, [62] and rituximab may sometimes cause more serious adverse effects.

Acute lung injury (less than 1%), arthritis, serum sickness, severe and protracted neutropenia that can occur up to six months post infusion, inflammatory bowel disease (IBD), and acute demyelinating neuropathy (ADN) are among the serious side effects linked to rituximab. Furthermore, rituximab raises the risk of infections, particularly severe ones including sepsis, viral myocarditis, Pneumocystis jiroveci pneumonia, deadly hepatitis B infection, and severe or protracted hypogammaglobulinemia. It has been reported that 5 out of 98 NS patients (~5%) died within a year after receiving rituximab.

Numerous rituximab courses over a number of years have been used in studies during relapses or following CD count recovery, with no notable adverse effects noted. However, the safe number of doses for children with NS has not yet been established. Furthermore, likelihood of developing anti-rituximab antibodies might get amplified with repeated treatments with RTX which have been linked with severe infusion related reactions. Thus, more investigation is necessary to establish the proper dosage and frequency of rituximab for kids with NS. [63]

- **PREVIOUS STUDIES**

Ravani et al., [59] performed a open labelled, noninferiority randomized-controlled trial in Italy to know efficacy of RTX compared to steroids in sustaining remission in children with steroid dependent NS. It included children aged one to sixteen years with recent SDNS, who were either maintained on prednisone or given a single infusion of rituximab.

After 1 month, prednisone was tapered. The primary outcome was 3-month proteinuria, with rituximab showing a 42% reduction in proteinuria, within the noninferiority margin. In RTX group, median time duration to relapse was eighteen months , compared to six months in control group. Side-effects noted were nausea, skin rash, and a single case of transient acute arthritis. Rituximab was determined to be noninferior to steroids.

A multicenter trial conducted by **Ruggenenti et al.** [64] involved ten children and twenty adults to evaluate RTX therapy. Patients with ≥2 relapses in the previous year received 1 or 2 doses of rituximab. After one year of follow-up, all patients achieved remission, with 18 being treatment free & 15 never experiencing a relapse.. Total count of relapses decreased significantly, from eighty eight to twenty two, while

median number of relapses per patient was reduced from 2.5 to 0.5. There was also a notable reduction in steroid use and cumulative steroid doses. Study summarized that RTX is safe, well tolerated & was effective, contributing to fewer relapses & reduced dependence on steroids.

Lijima et al. (2014) [65] organized a RCT in Japan to evaluate efficacy of RTX in children with SDNS & FRNS. RCT included children experiencing NS recurrence. Findings revealed that RTX group had a prolonged duration of median relapse free period compared to placebo group. Both groups reported similar adverse effects. Study concluded that rituximab is a harmless and well tested treatment for these conditions.

Sinha et al. (2013) [66] identified rituximab as a potential alternative for managing challenging cases of nephrotic syndrome, particularly in steroid-dependent patients unresponsive to multiple treatments. They noted that rituximab is less effective for SDNS. However, both rituximab and plasma exchange have demonstrated effectiveness in treating recurrent FSGS. Rituximab is beneficial in younger population and those serum albumin levels in normal range during recurrence. Additionally, it has been observed to induce remission in idiopathic membranous nephropathy by reducing proteinuria and phospholipase A2 receptor autoantibodies.

Kamei et al., (2009) [67] carried out a multi-center trial to examine pharmacokinetics, safety of RTX & efficacy of single dose of RTX in children with refractory steroid-dependent NS. Study involved 12 participants, all of whom discontinued steroid use within a median of 74 days following treatment. However, 75% of patients witnessed relapses at a median of 129 days, which coincided with recovery of B cells. Interestingly, twenty five percent of participants remained in remission for over a year

without experiencing any relapses. No life-threatening adverse events were observed. The findings indicate that while RTX may be beneficial for managing refractory steroid dependent NS, it's ability to prevent relapses appears to be temporary in majority of cases.

MATERIALS AND METHODS

Study Design:

Pre Post study was chosen to meet objectives of study. A pre post study is a research design where a group of participants are measured on a specific outcome variable before an intervention is implemented, and then measured again after the intervention is introduced. In this study pre and post outcomes was observed after single dose of RTX in children with SDNS & FRNS.

Study Population:

Children with SD/FRNS of 2 to 18 years of age admitted from Paediatric Nephrology out patient department at KLE's Dr. Prabhakar Kore hospital, Belagavi

Study Period:

Present study was carried over a duration of 1 year.

Sampling procedure:

The process of selecting a number of subjects from a specific group or universe is known as sampling. Only the population sampled may be held responsible for a conclusion drawn from sample results.

In this study we considered all eligible patients aged 2-18 years of age coming to pediatric nephrology out-patient department at Dr. Prabhakar Kore Hospital and MRC Belagavi.

Inclusion criteria:

- Children aged 2-18 years with steroid dependent nephrotic syndrome and frequently relapsing nephrotic syndrome

Exclusion criteria:

- Children with steroid resistant nephrotic syndrome
- Children with chronic kidney disease stage 2 (eGFR <90ml /min /1.73m^2).
- Children who have already received Rituximab within last 1 year.
- Children who are on Cyclosporin and on other calcineurin inhibitors.

Sample Size: 21

- Single Mean: paired t test
- Pre-test mean: 33.24
- Post-test mean: 39.31
- Standard deviation in pre-test: 7.87
- Standard deviation in post-test: 8.61
- Effect size: 0.736650485436893
- Power (%): 85
- Alpha Error (%): 5
- Slided: 2

Required sample size = 19+10% extra for dropouts should be taken

Formula

$$n_{pairs} = \frac{(Z_{1-\alpha} + Z_{1-\beta})2}{\Delta^2} + \frac{Z_{1-\alpha}^2}{2}$$

$$\text{Where } \Delta = \frac{\overline{x_2} - \overline{x_1}}{SD} \quad SD = \frac{S1+S2}{2}$$

$x_{1 \text{ and }} x_2$ are mean of pretest and post test

S1 and S2 are SD of pretest and posttest

Δ: Delta is effect size

Method of data collection

In this pre-post interventional study, total of 21 patients were enrolled. The patients were recruited from pediatric nephrology outpatient department at Dr. Prabhakar Kore KLES hospital, Belagavi following a file review to assess the details of nephrotic syndrome and determine eligibility. A detailed explanation of procedure and nature of study was provided to parents or guardians of the patients. Additionally, written informed consent was obtained.

Clinical course of nephrotic syndrome, including age of onset, frequency of relapses, steroid dependence, and biopsy details was recorded. A complete physical examination of the child was performed. Baseline CD19 levels were measured prior to Rituximab administration using flow cytometry, with a 2.5 ml venous blood sample collected in an EDTA tube. The child was admitted for RTX infusion after meeting the eligibility criteria. Upon admission, the vital signs of child were recorded. Investigations were done to ensure child was in remission.

Before RTX infusion, a complete blood count, renal function tests (including electrolytes), serum albumin, serum cholesterol, urine albumin, and urine protein to creatinine ratio were performed. A single intravenous dose of 375mg/m^2 was administered along with premedication, which included IV Diphenhydramine (1 mg/kg/dose), oral paracetamol (10-15 mg/kg/dose), & IV hydrocortisone, all given 30 minutes prior to RTX infusion. RTX was infused over 4 to 6 hours, starting at a rate of 1 mg/kg/hour and progressively increasing to 0.5 mg/kg/hour every 30 minutes, provided the patient tolerated the infusion well, with maximum infusion rate of 5 mg/kg/hour. During this time, the child was monitored for immediate adverse events like chills, fever, and tachycardia. A second CD19 level sample was obtained 3 weeks after Rituximab administration via flow cytometry to assess B cell depletion, defined as CD-19+ cells < 1% or an absolute count less than 5 cells/µL.

Figure 4 : Flowcytometry machine

Patients were followed up at three months after receiving RTX. They were started on oral mycophenolate mofetil tablets (600-800 mg/m²) following RTX infusion. Steroids were gradually tapered and discontinued over 1-2 months. ACE inhibitors/ARBs were continued. At the 3-month follow-up, patients were tested again for CD19 levels to assess sustained B-cell suppression, which helps in evaluating remission. The recovery of B cells, if observed, could predict the probability of relapse. Patients were considered to be in remission if, after a biochemical and physical examination (absence of edema) at 3 months, their urine albumin was absent or trace, and or their UPCR was below 0.2.

STATISTICAL ANALYSIS:

Statistical methods:

For quantitative variables, descriptive analysis was done using mean & SD for categorical variables, it was done using frequency and proportion. Additionally, data was showed using suitable graphics, such as bar diagrams.

To observe the association between Cross-tabulation and percentage comparisons were used and McNemar test was used to evaluate statistical significance. Significance of paired quantitative explanatory factors was evaluated using paired sample t-test. *P*-values below 0.05 were regarded as statistically significant. IBM SPSS version 22 was used for all statistical analyses, and bar graphs and other suitable visual aids were used to show the results. [1]

IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

RESULTS

Table 3: Descriptive analysis of age in years in study population (N=21)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Age (years)	8.81 ± 3.78	8.0	3.0	14.0	7.1	10.5

Study population (N=21) had a mean age of 8.81 years (SD = 3.78). Median age was 8 years, ranging from 3 to 14 years. The 95% confidence interval for mean age was 7.1 to 10.5 years.

Table 4: Determination according to gender (N=21)

Gender	Frequency	Percentages
Male	15	71.43%
Female	6	28.57%

In the present study, 15 (71.43%) were male, while 6 (28.57%) were female. This indicates a higher proportion of males in the study sample.

Figure 5: Pie chart according to the gender distribution (N=21)

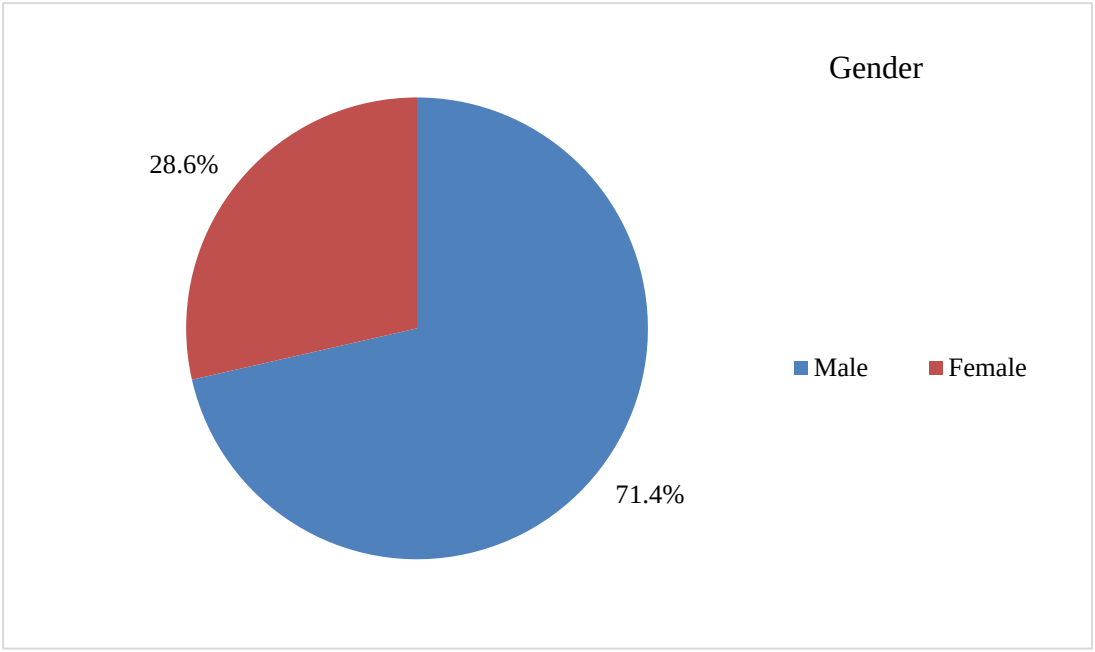


Table 5: Descriptive analysis of diagnosis in study population (N=21)

Diagnosis	Frequency	Percentages
FRNS	6	28.57%
SDNS	15	71.43%

The study participants was categorized into two diagnostic groups: FRNS 6 (28.57%) and SDNS 15 (71.43%). Most of the participants were diagnosed with SDNS, indicating a higher prevalence of this condition within the study sample.

Figure 6 : Pie chart showing study population's diagnoses (N=21)

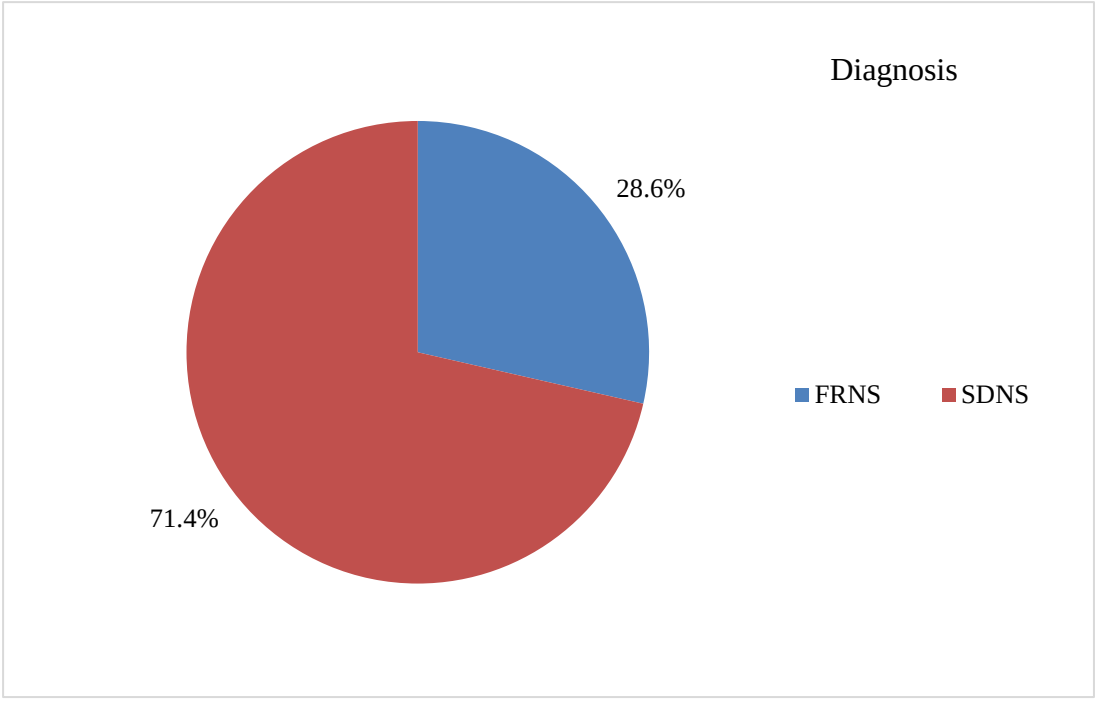


Table 6: Descriptive analysis of CD- 19 % in study population (N=21)

CD 19 %	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Baseline	20.97 ± 8.6	23.5	1.1	33.7	17.1	24.9
3 Weeks	0.55 ± 1.33	0.0	0.0	5.2	-0.1	1.2
3 Months	3.74 ± 5	0.8	0.0	14.3	1.5	6.0

At baseline, the mean CD19% was 20.97% (SD=8.6). A significant reduction was observed at 3 weeks, with a mean value of 0.55% (SD=1.33). By 3 months, the mean CD19% increased to 3.74% (SD=5). The confidence intervals further confirm a significant drop at 3 weeks, followed by a slight increase at 3 months**.**

Table 7: Descriptive analysis of B cell suppression at 3 weeks and 3 months in the study population (N=21)

B Cell Suppression At 3 Weeks (<1 %)	Frequency	Percentages
Present	19	90.48%
Absent	2	9.52%
B Cell Suppression At 3 Months		
Present	12	57.14%
Absent	9	42.86%

At 3 weeks, 19 (90.48%) participants exhibited B cell suppression (<1%), while 2 (9.52%) did not. By 3 months, 12 (57.14%) participants still had B cell suppression, whereas 9 (42.86%) had recovered. This shows a trend toward immune recovery over time.

Figure 7: Pie chart of b cell suppression at 3 weeks in the study population (N=21)

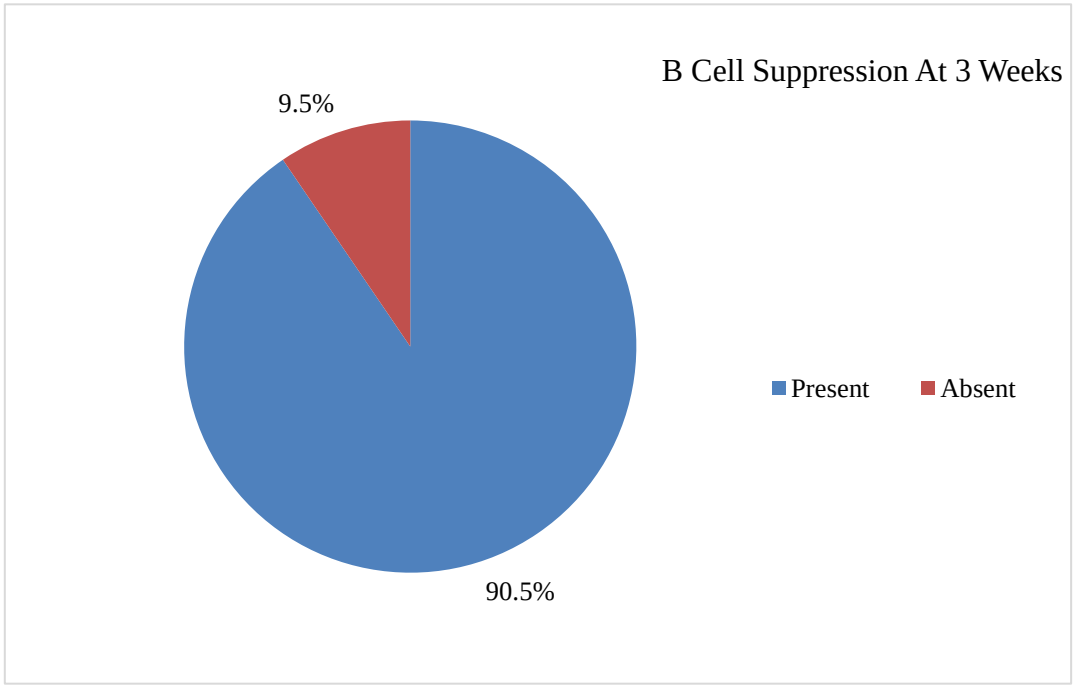


Figure 8: Pie chart of b cell suppression at 3 months in the study population (N=21)

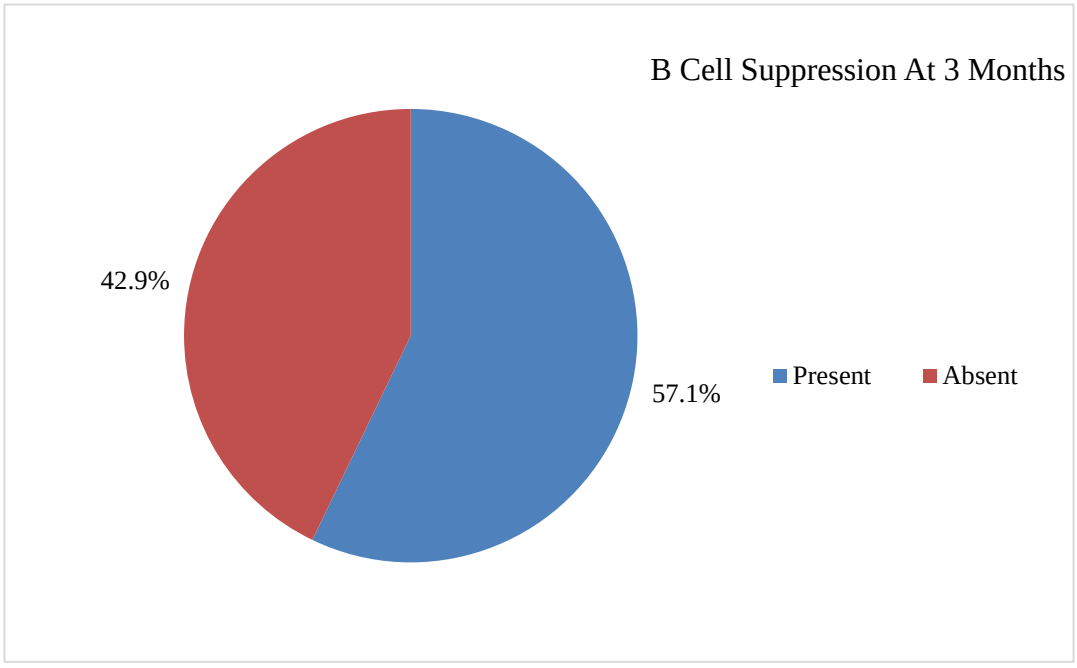


Table 8: Descriptive analysis of remission at 3 weeks & 3 months in the study population (N=21)

Remission At 3 Weeks	Frequency	Percentages
Yes	20	95.24%
No	1	4.76%
Remission At 3 Months		
Yes	20	95.24%
No	1	4.76%

Remission was observed in 20 (95.24%) participants at both 3 weeks and 3 months, with only 1 (4.76%) participant not achieving remission.

Figure 9: Bar chart of remission at 3 weeks in study population (N=21)

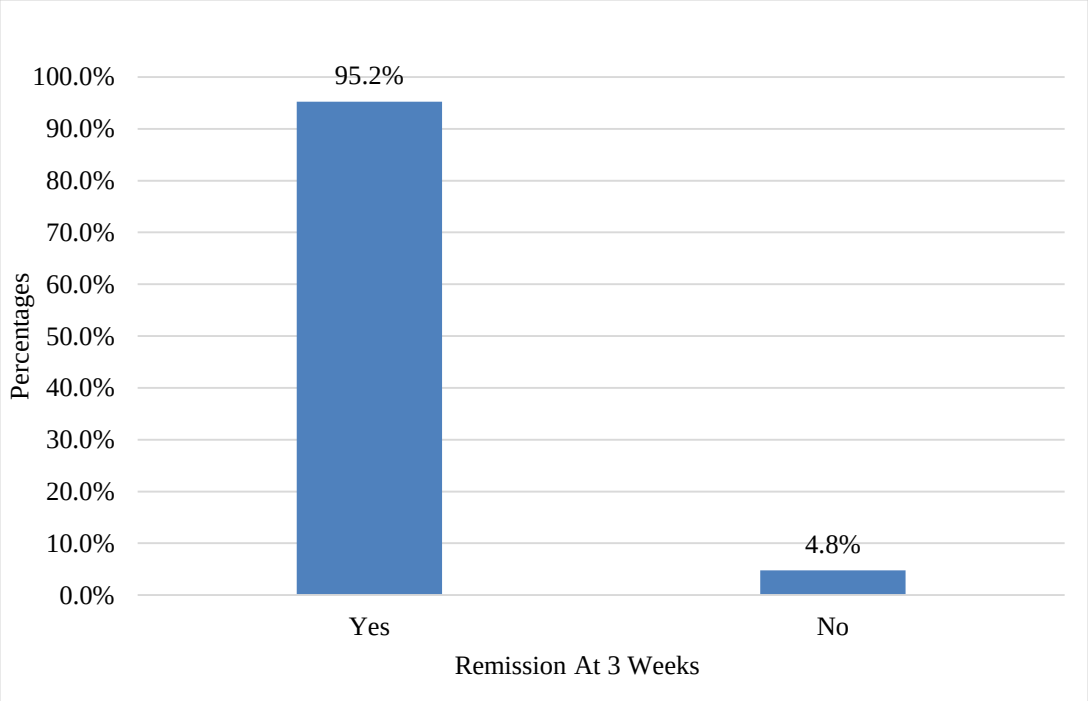


Figure 10: Bar chart of remission at 3 months in study population (N=21)

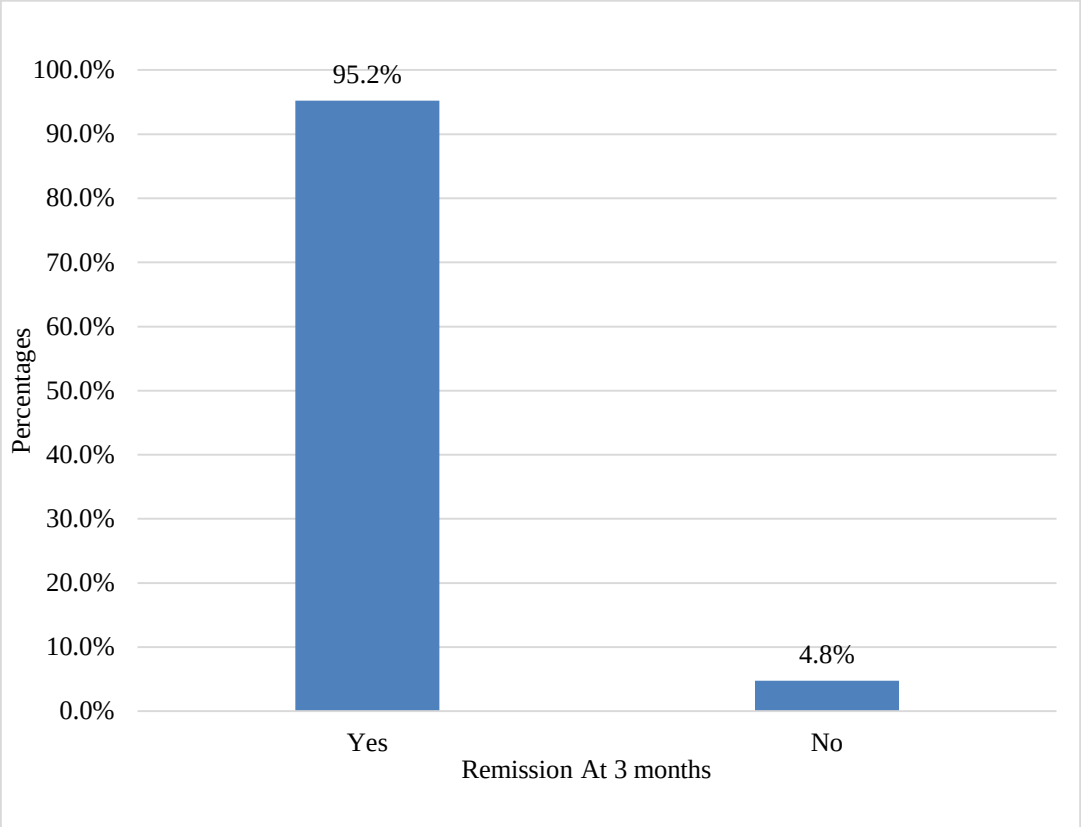


Table 9: Comparison of mean of CD 19 % between different follow-ups (N=21)

Follow-up periods (CD 19 %)	**(Mean± STD)**	**Mean Difference**	**P-value**
Baseline	20.97 ± 8.6	20.41	<0.001
3 Weeks	0.55 ± 1.33		
Baseline	20.97 ± 8.6	17.22	<0.001
3 months	3.74 ± 5		

A statistically significant reduction in CD19% was observed over time. The mean CD19% at baseline was 20.97 ± 8.6. At 3 weeks, it dropped significantly to 0.55 ± 1.33, with a mean difference of 20.41 ($p<0.001$). By 3 months, the mean CD19% slightly increased to 3.74 ± 5, but it remained significantly lower than baseline, with a mean difference of 17.22 ($p<0.001$).

Table 10: Comparison of urine albumin at 3 weeks and 3 months between urine albumin at baseline (N=42)

Urine Albumin At 3 Weeks	**Urine Albumin Baseline**		**Chi square**	**P value**
	Nil (N=21)	**-**		
4^+	1 (4.76%)	-	-	-
Nil	20 (95.24%)	-		
Urine Albumin At 3 Month	**Urine Albumin Baseline**		**Chi square**	**P value**
	Nil (N=21)	**-**		
3^+	1 (4.76%)	-	-	-
Nil	20 (95.24%)	-		

At both 3 weeks and 3 months, 20 (95.24%) participants had nil urine albumin, while 1 (4.76%) participant continued to show albuminuria (4+ at 3 weeks and 3+ at 3 months). This suggests a significant reduction in proteinuria, with most participants achieving complete remission.

Table 11: Comparison of relapse between B- cell recovery (N=21)

B-Cell Recovery	Relapsed		Chi square	P value
	Present (N=1)	Absent (N=20)		
Present	1 (100%)	8 (40%)	1.400	0.237
Absent	0 (0%)	12 (60%)		

Among participants who experienced B cell recovery (n=9), 1 (100%) relapsed, while 8 (40%) did not. In contrast, none (0%) of the participants without B cell recovery (n=12) relapsed, and all (100%) remained in remission. However, the association between B cell recovery and relapse was not statistically significant (Chi-square = 1.400, p = 0.237). While relapse was observed exclusively in those with B cell recovery, the lack of statistical significance suggests that B cell recovery alone may not be a strong predictor of relapse, and other factors may contribute to disease recurrence.

Figure 11: Cluster bar chart of comparison of relapse between B- cell recovery (N=21)

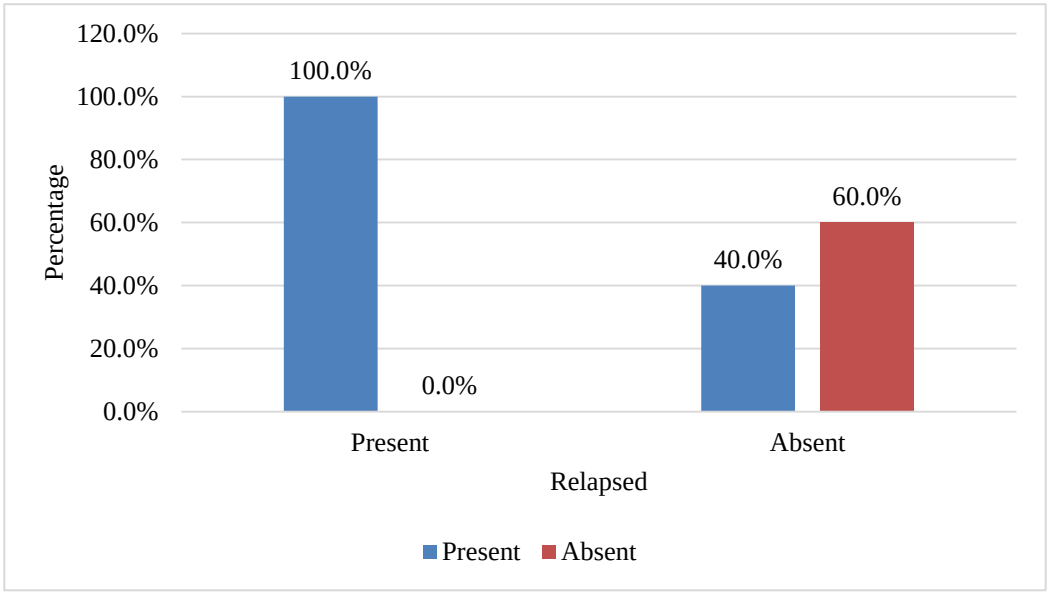


Table 12: Predictive validity of B Cell recovery in predicting Relapse (N=21)

Parameter	Value	95% CI	
		Lower	Upper
Sensitivity	100.00%	2.50%	100.00%
Specificity	60.00%	36.05%	80.88%
False positive rate	40.00%	19.12%	63.95%
False negative rate	0.00%	-	97.50%
PPV	11.11%	0.28%	48.25%
NPV	100.00%	73.54%	100.00%
Diagnostic accuracy	61.90%	38.44%	81.89%

This analysis evaluated the ability of B cell recovery at 3 months to predict relapse. Sensitivity was 100.00% (95% CI: 2.50%–100.00%), indicating that all relapsed cases had B cell recovery. Specificity was 60.00% (95% CI: 36.05%–80.88%), meaning a significant proportion of non-relapsed participants also exhibited B cell recovery. The false positive rate was 40.00% (95% CI: 19.12%–63.95%), suggesting that many individuals with B cell recovery did not relapse, while the false negative rate was 0.00% , indicating that no relapse cases were missed.

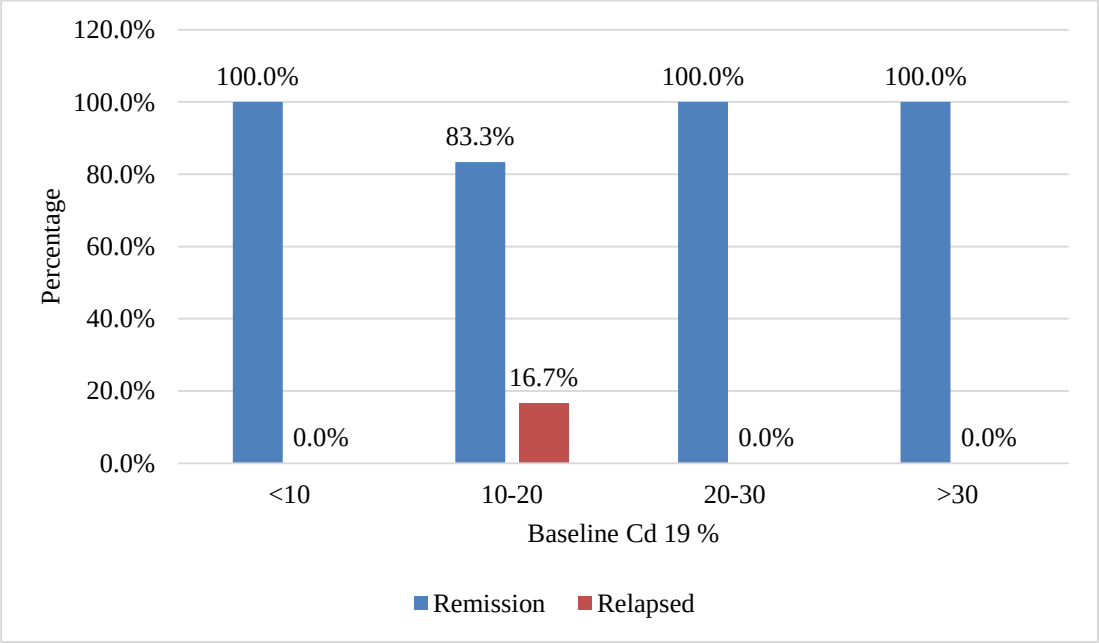
The PPV was low at 11.11% (95% CI: 0.28%–48.25%), meaning only a small fraction of participants with B cell recovery actually relapsed. However, the NPV was 100.00% (95% CI: 73.54%–100.00%), implying that the absence of B cell recovery reliably predicted non-relapse. With a diagnostic accuracy of 61.90% (95% CI: 38.44%–81.89%), these results suggest that while B cell recovery is associated with relapse, it alone is not a strong predictor. Additional clinical markers may be required for better relapse prediction

Table 13: Comparison of remission and relapse at 3 months across baseline CD 19 % (N=21)

Remission At 3 Months	Baseline CD 19 %			
	<10 (N=3)	**10-20 (N=6)**	**20-30 (N=10)**	**>30 (N=2)**
Remission	3 (100%)	5 (83.33%)	10 (100%)	2 (100%)
Relapsed	0 (0%)	1 (16.67%)	0 (0%)	0 (0%)

Among patients with baseline CD19% <10% (N=3), all (100%) achieved remission at 3 months, with no cases of relapse. Similarly, in the 20–30% (N=10) and >30% (N=2) baseline CD19% groups, all patients (100%) achieved remission with no relapse. However, in the 10–20% baseline CD19% group (N=6), while the majority (83.33%) achieved remission, one patient (16.67%) experienced relapse at 3 months. These findings suggest that remission rates were generally high across all baseline CD19% categories, irrespective of their levels

Figure 12: Cluster bar chart of comparison of remission and relapse at 3 months across baseline CD 19 % (N=21)



DISCUSSION

SDNS refers to cases where relapses occur either while tapering steroids or within two weeks after discontinuing them, whereas FRNS is characterized by more than equal to 2 relapses within six months or four relapses within a year. SDNS & FRNS are difficult forms of NS in children, marked by ongoing or frequent relapses despite standard corticosteroid treatments. Managing these conditions often necessitates the use of additional immunosuppressive therapies to regulate disease activity and avoid complications.

RTX is monoclonal antibody designed to target B-cells that express the CD20 antigen. CD19 is the biomarker used to monitor effects of rituximab. Monitoring of CD19 levels rather than CD20 is done as CD19 is expressed across broader range of B cell stages as compared to CD20. Moreover, RTX can lead to downregulation or loss of CD20 hence may not be detectable on testing. Additionally, when B cells begin to regenerate after rituximab, they first express CD19 before CD20 hence, measuring CD19 allows for early detection of B cell recovery.

RTX has become an encouraging therapeutic solution for children with FRNS & SDNS. Research has explored effectiveness of single dose of RTX in minimizing relapse rates and sustaining remission in FRNS & SDNS. This study focussed on assessing safety & effectiveness of single dose of RTX in managing these conditions in children.

In this study, average age of participants (N=21) was 8.81 years, with standard deviation of 3.78 years. The median age was recorded as 8 years, ranging from 3 to 14 years. These findings suggest that the study cohort primarily consists of

school-aged children and early adolescents, which aligns with the typical age group affected by SDNS and FRNS. [Table 3]

This observation aligns with earlier research, which predominantly focused on children aged 2 to 18 years to assess rituximab's efficacy in managing SDNS and FRNS.

Tasdemir et al. [68] conducted a study involving 42 children, whose ages ranged from 1.9 to 17.3 years.

Morais et al. [69] observed median age of 2.7 years at onset of NS, with ages spanning from 2.0 to 4.9 years. RTX was administered after a median disease duration of 9.6 years, with a coverage of 2.9 to 14.8 years[69].

Similarly, **Guigonis et al.,** [70] studied a cohort with median age of 2.9 years at disease onset, with a range from 1.9 to 7.8 years.

In the present study, the gender distribution revealed a male predominance, with 15 (71.43%) male participants and 6 (28.57%) female participants (Table 4). This male predominance is in alignment with the findings of **Chan et al., [71]** who recorded a greater number of males (343 males vs. 160 females) in their study of NS.

In comparison, study conducted by **Morais et al.,**[69] revealed predominance of females, with 5 females and 3 males.

On the other hand, **Niu et al.** [72] reported male to female ratio of 1.96:1 in their cohort, emphasizing the variability in gender distribution across different studies.

In current study, participants were categorised in 2 diagnostic groups: FRNS (6 participants, 28.57%) and SDNS (15 participants, 71.43%). The majority of

participants were diagnosed with SDNS, suggesting that this condition is more prevalent in the study sample. [Table 5]

Ruggenenti et al., [64] carried out a study in which all pediatric participants were found to be steroid-dependent, whereas 5 adult participants experienced frequent relapses.

Regarding immunological markers, our study observed a significant reduction in CD19+ B cells at 3 weeks post-rituximab administration. At baseline, the mean CD19% was 20.97%, which decreased to 0.55% at 3 weeks, followed by a slight increase to 3.74% at 3 months. This finding indicates effective B cell depletion immediately following rituximab treatment, with partial recovery observed after 3 months. [Table 6]

These results align with those from **Niu et al., [72]** who reported rapid depletion of CD19+ and CD20/CD45 B-cells within 3 days of rituximab administration. The B-cells remained suppressed for up to 3 months before gradually recovering. By 12 months, median percentage of CD19+ and CD20/CD45 B-cells had recovered to baseline levels, although the recovery was statistically insignificant ($p > 0.05$).

CD19 count = 0 was similarly noted by **Guigonis et al., [70]** in all patients after rituximab treatment, regardless of proteinuric status or the total rituximab (RTX) dose given. The B-cell depletion period ranged from two to eleven months, with median period of six months. Importantly, length of this impact showed no correlation with nephrotic status or total administered dose of rituximab. These findings support the present study's observation of B cell depletion with partial recovery after a few

months, demonstrating that rituximab effectively targets B cells and can have a lasting impact on disease control.

In this study, at 3 weeks, 19 (90.48%) participants showed B cell suppression (<1%), while 2 (9.52%) did not. By 3 months, 12 (57.14%) participants still had B cell suppression, whereas 9 (42.86%) had experienced recovery. This indicates a trend of immune recovery over time. [Table 7]

Another study by **George et al., [73]** showed that suppression of CD 19 B -cell count less than one percent was observed in 40 out of 42 (95.2%) patients at day 30, in 18 out of 42 (42.9%) at day 90, and in 7 out of 42 (16.7%) at day 180.

In the present study, remission was observed in 20 (95.24%) participants at both 3 weeks and 3 months, with only 1 (4.76%) participant not achieving remission. This indicates a high rate of remission within the study population. [Table 8]

Benz et al., [74] evaluated RTX for complicated SDNS cases, reporting that its use achieved sustained remission in a child with SDNS & ITP.

In a study by **R. Sinha et al.,** [75] where 46 SDNS and FRNS children where given Rituximab 2-4 doses weekly at 375mg/m2, 78% of patients attained complete remission.

Ravani et al., [59] stated that children with SDNS & FRNS had a 48% probability of sustaining remission within six months following RTX treatment. These findings indicate that RTX might be a viable therepeutic choice for NS.

Similarly, **Basu et al.,** [60] performed a RCT comparing RTX & tacrolimus over a 1 year duration in patients with SDNS and FRNS. Study concluded that a

single course of RTX was more efficient than maintenance tacrolimus in preventing relapses.

Usui et al. [76] investigated the potential for optimizing rituximab dosing, proposing that a lower dose might still be effective in depleting CD-20+ B- cells in patients with NS. They administered a single fixed dosage of 200 mg every six months, which resulted in complete remission in three cases without the need for additional treatment with prednisolone or cyclosporine. This highlights the importance of determining the optimal dosing strategy for rituximab therapy in SDNS.

Likewise, **Takei et al.,** [77] demonstrated that rituximab substantially decreased rate of relapses and collective steroid dosage required over a 12-month period. Number of relapses was reduced from 25 (100%) to 4 (16%) ($P < 0.001$), while the total prednisolone dose dropped from 8.2 g to 3.3 g ($P < 0.001$). Furthermore, the daily maintenance dose of prednisolone significantly decreased from 26.4 mg/day at start of study to 1.1 mg/day after twelve months.

Another research by **Colucci et al., [78]** proposes that rituximab's ability to sustain remission may depend on its impact on memory B-cell populations rather than overall B-cell depletion.

This study showed a single dose of RTX led to swift reduction of B-cell followed by partial recovery of B cells at 3 months. Out of 21 patients, overall 9 showed repopulation of B cells (>1%). CD-19 level at 3 weeks showed a mean difference of 20.41 from baseline whereas at 3 months a mean difference of 17.22 from baseline. Both of which had a statistically significant p value of < 0.001 [Table 9]

A research by **Choi et al.,** [79] done on 50 patients of SDNS showed B cell recovery typically began at 5.9 months after rituximab treatment but can differ depending on factors like age, previous rituximab exposure and disease severity

The present study also compared urine albumin levels at 3 weeks and 3 months with baseline levels (N=42). At both time points, 20 participants (95.24%) had no detectable urine albumin, while 1 participant (4.76%) continued to show albuminuria (4+ at 3 weeks and 3+ at 3 months). The findings demonstrate a substantial decrease in proteinuria, with most participants attaining full remission. [Table 10]

According to a research by **Casiraghi et al. [80],** patients with SDNS/FRNS had considerably lower serum creatinine and proteinuria levels and higher serum albumin levels than SRNS patients, which is consistent with NS remission.

Webendorfer et al., [81] study showed total resolution of proteinuria at one year even in the absence of circulating B cells suggesting that other immune mechanisms potentially involving CD20+ T cells might contribute to disease activity

Moreover, a meta analysis by **Liu et al.,** [82] highlighted that RTX notably enhanced rate of total remission in children with SDNS and led to improvements in serum albumin levels and eGFR. Although the rituximab (RTX) group showed a more substantial reduction in proteinuria compared to control group, the difference was statistically insignificant.

In this study, among the participants who experienced B cell recovery (n=9), 1 (11.11%) relapsed, while 8 (88.89%) did not. In contrast, none (0%) of the participants without B cell recovery (n=12) relapsed, and all (100%) remained in

remission. However, the association between B cell recovery and relapse was not statistically significant (Chi square = 1.400, P = 0.237). Even though only those who had B cell recovery experienced relapse, the lack of statistical significance indicates that additional factors might be involved in illness recurrence and that B cell recovery alone may not be a reliable indicator of relapse. [Table 11]

A research by **Guigonis et al., [70]** found that all nineteen patients who showed response to RTX maintained their remission throughout the B cell suppression period. B cells, however, recovered to 3–7% of leukocytes in the relapsed patients.

Colucci et al., [79] suggests that pre-existing memory B cells may contribute to treatment resistance, leading to shorter remission durations. In contrast, patients with lower baseline memory B-cell levels tend to experience longer relapse-free periods, indicating that B-cell depletion depth influences rituximab's effectiveness.

Takei et al.,[78] reported full remission was seen in all patients that underwent B-cell suppression. 4 of seventeen patients with B-cell recovery had relapse.

Similarly**, Kamei et al.,** [67] observed a substantial reduction in relapse frequency & an increase in steroid-free periods in the six months following rituximab treatment. Of the patients, nine (75%) experienced a relapse with median duration of 129 days following treatment, 7 required further rituximab therapy due to steroid dependence. Interestingly, while most relapses occurred alongside B-cell recovery, three patients (25%) maintained remission for over a year despite the repopulation of B cells.

In contrast to this, **Iijima et al.,** [65] observed all patients in their study experienced a relapse within nineteen months following randomization. Their results

indicate that although rituximab is highly effective in controlling refractory SDNS, it may not be adequate for achieving sustained long term remission.

The analysis revealed that B cell recovery at 3 months demonstrated a sensitivity of 100%, meaning all relapse cases showed recovery, & specificity of 60%. PPV was limited at 11.11%, whereas NPV was considerable at 100%, indicating that the absence of B cell recovery was a reliable predictor of non-relapse. With a diagnostic accuracy of 61.90%, these findings indicate that B cell recovery alone is not a strong predictor of relapse, and additional markers may be necessary. [Table 12]

A study by **Fujjinaga et al.,** [62] reported one patient who relapsed just 2 days after receiving rituximab despite complete B cell remission suggesting that patients may still experience relapses even when their B cells are suppressed

Furthermore, according to research by **Colucci et al.** [76], tracking recovery of switched memory B- cells after RTX treatment might serve as an effective method for predicting risk of relapse in individuals with NS.

Another research by **Chan et al.,** **[71]** reported that patients undergoing repeated RTX treatments experienced progressively longer relapse-free intervals, suggesting a potential cumulative benefit of successive infusions. This observation implies that rituximab may lead to prolonged B-cell suppression or modulation of immune memory over time.

In this study it was seen that there was no association of higher baseline CD 19 levels with relapse [Table 13]

On the other hand, research by **Colucci et al.** [79] found that patients with elevated baseline CD19 levels were associated with a higher risk of relapse, attributed to the early reconstitution of memory B cells following treatment.

Overall, this study demonstrates effectiveness of single dose of RTX in sustaining remission and minimizing relapses in pediatric patients with FRNS & SDNS. Further long term studies are needed to optimize rituximab dosing strategies and to better understand factors influencing relapse and remission outcomes.

LIMITATIONS

- A longer follow up period is imperative to better understand progression of disease and the occurrence of relapses associated with B cell recovery. The short duration of follow-up limits the ability to determine long-term effectiveness & durability of RTX beyond three months, including sustained remission rates, delayed relapses, or extended effects of B cell depletion.
- A larger sample size would have provided a clearer understanding of effectiveness of single dose of RTX.

CONCLUSION

- RTX effectively induces and sustains remission by depleting B cell thereby reducing frequent relapses which will help in minimizing or discontinuing corticosteroid and CNIs use, thereby reducing their long term adverse effects.
- B cell recovery to ascertain rate of relapse cannot be completely relied upon but can be taken as a predictor to give additional doses of RTX in order to prevent relapse
- Pre treatment B cell levels did not show any contribution to treatment resistance as seen in previous studies.
- Administering single dose of RTX to patients with FRNS & SDNS is a more practical and effective approach in resource-constrained settings compared to using two or more doses.

SUMMARY

- Effectiveness of administrating single dose of RTX in children aged 2-18 years with SDNS and FRNS at a tertiary care hospital over course of one year was evaluated
- The primary objective was to assess sustained remission 3 months post-rituximab administration, while secondary objectives focused on B-cell suppression by measuring CD19 levels before, 3 weeks after, and 3 months after treatment, as well as predicting relapse based on B-cell recovery.
- The study included 21 patients, of which 71.43% had SDNS, and 28.57% had FRNS. A significant reduction in CD19 levels was noticed at three weeks after treatment, indicating effective B cell depletion.
- At 3 months, a partial recovery of B- cells occurred in some patients, but remission was maintained in 95.24% of the participants at both 3 weeks and 3 months.
- B-cell suppression at 3 weeks was a strong predictor of sustained remission, with a high association between the two.
- B-cell recovery at 3 months did not prove to be a statistically significant indicator of relapse; however, the lack of B-cell recovery was closely linked to sustained remission.
- The findings indicate that RTX serves as a beneficial therapeutic choice for inducing remission in children with FRNS & SDNS, and the single-dose regimen offers an alternative to multiple doses, potentially improving cost-effectiveness, accessibility and risk of side effects that may occur with multiple doses.

- Overall, this research offers important insights into potential of RTX as steroid-sparing therapy for pediatric NS, emphasizing its effectiveness in achieving long-term remission while underscoring need for additional research to enhance understanding of predictive factors for relapse.

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ANNEXURES

ANNEXURE – I - INFORMED CONSENT FORM

"SINGLE DOSE RITUXIMAB IN STEROID DEPENDENT NEPHROTIC SYNDROME AND FREQUENTLY RELAPSING NEPHROTIC SYNDROME IN CHILDREN 2 TO 18 YEARS OF AGE IN A TERTIARY CARE HOSPITAL DURING A PERIOD OF 1 YEAR- A PRE POST STUDY"

Name of Student/Principal Investigator: _____

Name of Guide/Co Investigators: _____

Introduction: To see if single dose of rituximab is helping in achieving remission that is active disease free period for 3months after giving rituximab injection to your child and to check if B cell recovery can help in predicting a relapse

Explanation of procedure: Blood tests and urine tests will be done on admission just prior to rituximab injection. Child will be discharged after a single dose of rituximab after proper vital monitoring. Your child will be tested at 3 weeks for CD19 level and reassessed at 3 months to look for sustained remission that is active disease free period and to predict chances of relapse based on CD19 test.

Withdrawal from participation in the study: Participation in this study in voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will get benefits by participating in this study. Single dose of Rituximab will help your child in achieving

remission and CD19 level recovery if seen will help in predicting relapse**.** The data gathered will help population at large.

Possible risks from participating in the study: There are no life threatening risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Cost of investigations done during the course of study will be paid by the **principal investigator / Participant.**

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups. However, your identity will never be revealed.

Questions: If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights

CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"SINGLE DOSE RITUXIMAB IN STEROID DEPENDENT NEPHROTIC SYNDROME AND FREQUENTLY RELAPSING NEPHROTIC SYNDROME IN CHILDREN 2-18 YEARS OF AGE IN A TERTIARY CARE HOSPITAL DURING A PERIOD OF 1 YEAR- A PRE POST STUDY"** My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ASSENT FORM

Title : SINGLE DOSE RITUXIMAB IN STEROID DEPENDENT NEPHROTIC SYNDROME AND FREQUENTLY RELAPSING NEPHROTIC SYNDROME IN CHILDREN 2 to 18 YEARS OF AGE IN A TERTIARY CARE HOSPITAL DURING A PERIOD OF 1 YEAR- A PRE POST STUDY

Study participant information sheet

S.NO.	ROLE OF INVESTIGATOR	NAME	CONTACT NO.
1.	Principal investigator	_____	_____
2.	Guide	_____	

Dear Participant,

The purpose of this form is to inform you about the study ?

The purpose of the study is to assess sustained remission in steroid dependent nephrotic syndrome and frequently relapsing syndrome at 3 months post single dose rituximab infusion. Your parent knows about this study, and gave permission for you to be involved.

Why are you being invited to take part in this study?

Your participation will be voluntary and there is no compulsion, neither incentive to participate in this research study. Further, you are free to withdraw yourself from study at any time with or without giving any reason.

What will you do if you agree to participate?

If you agree to participate in the study, you need to read the information and details regarding the study and give consent for your participation in the study.

What happens if you say yes, but you change my mind later?

Your participation in this study is voluntary. You may decline to participate at any time, even after the study has started. If you choose not to participate or to withdraw from the study, there will be no penalty, and you will be able to keep any incentives you have earned up to the point at which you withdraw.

What are the benefits to you for being in this study?

You will not have any direct or indirect disadvantages in case you do not agree to participate. You may withdraw your consent at any time. This study will help you achieve remission and help us determine whether there one dose of rituximab can help in achieving remission at 3 months post rituximab infusion.

What happens to the information collected for the study?

Your information will be Confidentiality of your details in medical records will be maintained and will not be disclosed. Data on this study and the relevant sections will be used for research publication without disclosing name, contact details.

Is there any way being in this study could be bad for you? Is there any risk to you by being in this study? If so, how will these risks be minimized?

There is no additional risk of injury by virtue of participating in this study.

Any additional expenditure incurred ?

No additional expenditure

Whom should you contact for questions?

In case of any query / information needed you may call

Dr Kavina Shah. Ph. No. 8080740904/ 7676047185

Signing here means that you have read this form or have had it read to you and that you are willing to be in this study.

Name of the Participant (Write your name in the line): _____

Signature of the Participant (Put your signature in the line): _____

Date: ___ / ___ / ___

_____ _____

Guardian's Signature Parent/Guardian's Name

Date: ___ / ___ / ___

SIGNATURE OF PERSON CONDUCTING ASSENT DISCUSSION

I have explained the study to _____ in language he/she can understand, and the child has agreed to be in the study.

_____ Date: ___ / ___ / ___

Signature of Person Conducting Assent Discussion

_____

Name of Person Conducting Assent Discussion

PLEASE KEEP THIS INFORMATION SHEET FOR YOUR RECORDS

ANNEXURE-II PROFORMA

Name: Age/Gender: IP/OP NO:

Father/Guardian's name: Mobile No:

Diagnosis Other non-renal illness if any:

Age at onset:

No. of. Relapses in last 1 year:

Current status:

Family History:

- Kidney disease – if yes specify diagnosis
- Any other illness if present –

Clinical examination:

Vitals:

Temperature:

Heart rate:

Respiratory rate:

Blood pressure: mm of Hg

Systemic examination:

Injection Rituximab (375mg/m^2) given on-

Anthropometry:

Weight: … kg Height: ……cm BSA: …. m^2 BMI: … . kg/m^2

Baseline Investigations prior to rituximab administration:

CD-19 LEVELS	URINE ALBUMIN	URINE PROTEIN CREATININE RATIO(UPCR)	SERUM CHOLESTEROL	SERUM ALBUMIN	SERUM CREATININE

Treatment History:

Drugs	Drug Name	Duration	Current dose
1. Steroid			
2. ACE inhibitors / ARB			
3. Other if any			

CD-19 LEVEL AT 3 WEEKS: _____

POST RITUXIMAB INFUSION AT 3 MONTHS :

CD-19 LEVEL	URINE ALBUMIN	UPCR

ANNEXURE III- MASTERCHART

SR.NO	NAME	AGE/SEX	IP/PT NO	DIAGNOSIS	RITUXIMAB GIVEN ON	BASELINE CD 19 %	URINE ALBUMIN BASELINE	3 WEEKS CD 19 %	URINE ALBUMIN AT 3 WEEKS	B CELL SUPPRESSION AT 3 WEEKS (<1 %)	B CELL RECOVERY AT 3 WEEKS (>1%)	IN REMISSION AT 3 WEEKS	3 MONTHS CD19 %	URINE ALBUMIN AT 3 MONTHS	B CELL SUPPRESSION AT 3 MONTHS (<1%)	B CELL RECOVERY AT 3 MONTHS (>1%)	IN REMISSION AT 3 MONTHS
1	AJAY	14 YEARS/MALE	2592394	FRNS	01-10-2023	26.19	NIL	0.04	NIL	PRESENT	ABSENT	YES	0.07	NIL	PRESENT	ABSENT	YES
2	TANVEER	6 YEARS/MALE	7093944	SDNS	03-10-2023	18.39	NIL	0	NIL	PRESENT	ABSENT	YES	0.35	NIL	PRESENT	ABSENT	YES
3	AISHWARYA	4 YEARS/ FEMALE	7204044	FRNS	18-10-2023	16.98	NIL	0.03	NIL	PRESENT	ABSENT	YES	13.94	NIL	ABSENT	PRESENT	YES
4	YADVIKA	8 YEARS/FEMALE	5459028	SDNS	28-10-2023	23.47	NIL	0	NIL	PRESENT	ABSENT	YES	8.22	NIL	ABSENT	PRESENT	YES
5	SIDDHARTH	3 YEARS/MALE	7028315	SDNS	05-11-2023	13.65	NIL	0	NIL	PRESENT	ABSENT	YES	0.05	NIL	PRESENT	ABSENT	YES
6	OMKAR	13 YEARS/MALE	6671281	SDNS	11-01-2024	26.18	NIL	0.2	NIL	PRESENT	ABSENT	YES	6.3	NIL	ABSENT	PRESENT	YES
7	PRAJWAL	6 YEARS/MALE	7356201	SDNS	13-03-2024	26.9	NIL	0.5	NIL	PRESENT	ABSENT	YES	4	NIL	ABSENT	PRESENT	YES
8	PRITAM	11 YEARS/MALE	3752992	FRNS	29-04-2024	28.33	NIL	0	NIL	PRESENT	ABSENT	YES	0.05	NIL	PRESENT	ABSENT	YES
9	SIDDHI	13 YEARS/FEMALE	5421464	SDNS	22-06-2024	30.2	NIL	0	NIL	PRESENT	ABSENT	YES	0.41	NIL	PRESENT	ABSENT	YES
10	MAHESH	7 YEARS/MALE	4944731	SDNS	05-07-2024	1.13	NIL	0.07	NIL	PRESENT	ABSENT	YES	0.82	NIL	PRESENT	ABSENT	YES
11	GURUPADH	13 YEARS/MALE	6881031	SDNS	13-07-2024	27.9	NIL	0.05	NIL	PRESENT	ABSENT	YES	0.04	NIL	PRESENT	ABSENT	YES
12	PRATIKSHA	7 YEARS/ FEMALE	5585744	SDNS	13-07-2024	21.76	NIL	0.07	NIL	PRESENT	ABSENT	YES	0.24	NIL	PRESENT	ABSENT	YES
13	SIDDHARTH	3 YEARS/MALE	7316649	SDNS	15-07-2024	33.69	NIL	1	NIL	PRESENT	ABSENT	YES	4.5	NIL	ABSENT	PRESENT	YES
14	KARTHIK	9 YEARS/MALE	5297749	SDNS	26-07-2024	26.57	NIL	0.02	NIL	PRESENT	ABSENT	YES	0.03	NIL	PRESENT	ABSENT	YES
15	SHRAVAN	13 YEARS/MALE	2069309	FRNS	30-07-2024	17.74	NIL	0.22	4+	PRESENT	ABSENT	NO	14.3	3+	ABSENT	PRESENT	NO
16	PRANAV	4 YEARS/MALE	7523813	SDNS	31-07-2024	12	NIL	3.65	NIL	ABSENT	PRESENT	YES	7.58	NIL	ABSENT	PRESENT	YES
17	PAVAN	13 YEARS/ MALE	7382118	SDNS	01-08-2024	26.52	NIL	5.2	NIL	ABSENT	PRESENT	YES	13.48	NIL	ABSENT	PRESENT	YES
18	SURAJ	12 YEARS/MALE	7563787	SDNS	05-09-2024	28	NIL	0.04	NIL	PRESENT	ABSENT	YES	0.8	NIL	PRESENT	ABSENT	YES
19	SAMRUDDHI	8 YEARS/FEMALE	7177762	FRNS	14-09-2024	5.63	NIL	0.5	NIL	PRESENT	ABSENT	YES	3.2	NIL	ABSENT	PRESENT	YES
20	RAJVEER	6 YEARS/MALE	7561710	SDNS	25-09-2024	19.28	NIL	0.02	NIL	PRESENT	ABSENT	YES	0.17	NIL	PRESENT	ABSENT	YES
21	BHUMIKA	12 YEARS/FEMALE	4693097	FRNS	06-10-2024	9.8	NIL	0.04	NIL	PRESENT	ABSENT	YES	0.08	NIL	PRESENT	ABSENT	YES