# "URINARY VITAMIN D-BINDING PROTEIN LEVELS IN IDIOPATHIC NEPHROTIC SYNDROME CHILDREN."-A CROSS SECTIONAL STUDY.

**By**

**REGISTRATION NO: BM0120002**

# Dissertation

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## M. D. (DOCTOR OF MEDICINE)

## IN

## PEDIATRICS

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**BELAGAVI, KARNATAKA**

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KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,

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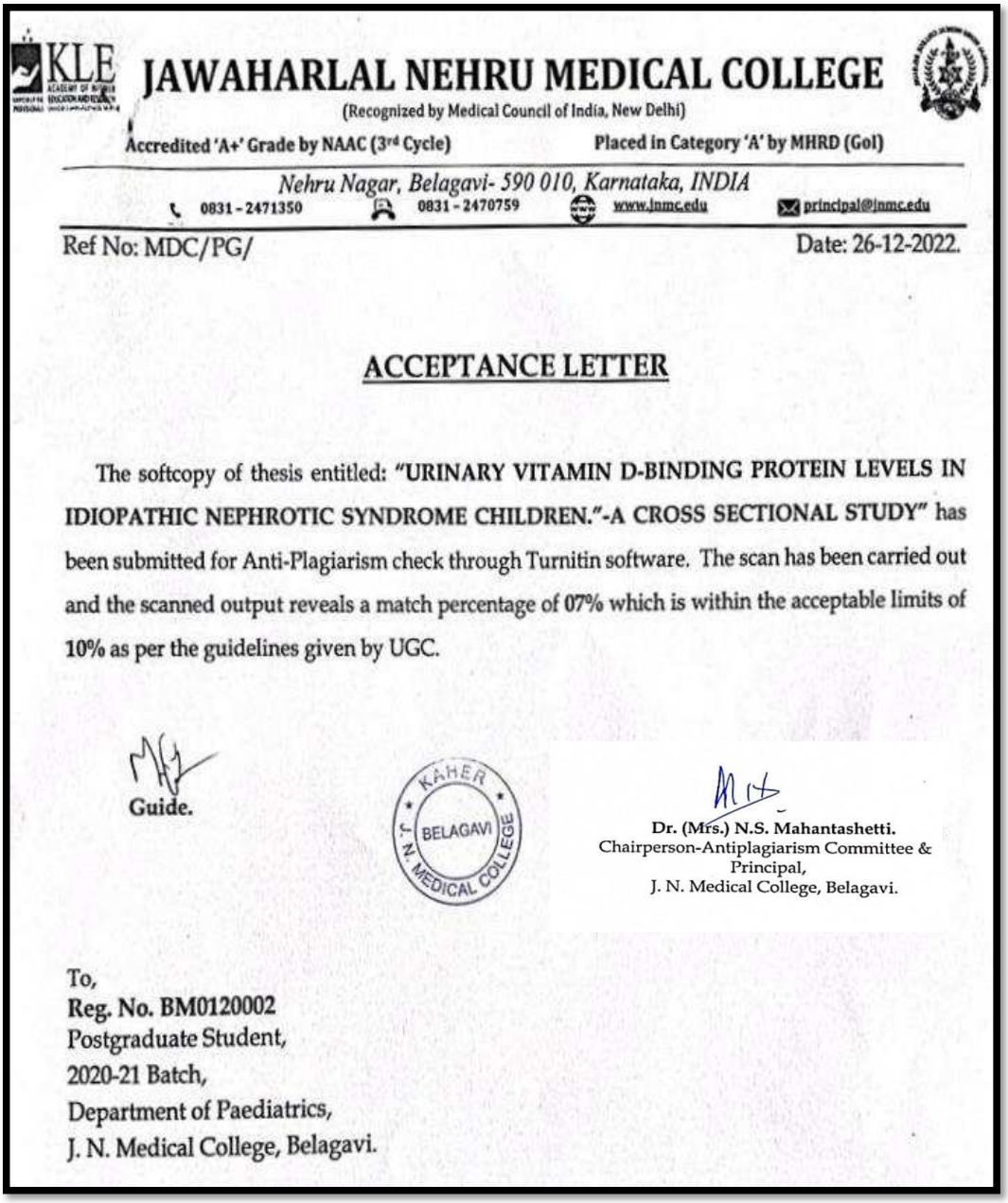

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With reference to the above, we wish to inform you that your proposed research project titled **"URINARY VITAMIN D-BINDING PROTEIN LEVELS IN IDIOPATHIC NEPHROTIC SYNDROME CHILDREN- A CROSS SECTIONAL STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

**(Dr. Smita Sonoli)**
Member Secretary
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on Human Subjects Research,
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**(Dr. Harsha Hegde)**
Chairman,
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## LIST OF ABBREVIATIONS USED

| | |
|---|---|
| NS | Nephrotic Syndrome |
| MCD | Minimal Change Disease |
| FSGS | Focal Segmental Glomerulosclerosis |
| SSNS | Steroid Sensitive Nephrotic Syndrome |
| SRNS | Steroid Resistant Nephrotic Syndrome |
| uVDBP | Urinary Vitamin-D Bindng Protein |
| CKD | Chronic Kidney Disease |
| RBC | Red Blood Cell |
| ESRD | End Stage Renal Disease |
| ISKDC | International study of kidney disease in children |
| MPGN | Membranoproliferative Glomerulonephritis |
| AKF | Acute kidney failure |
| PPD | Purified Protein Derivative |
| TB | Tuberculosis |
| ANA | Anti Nuclear Antibody |
| ACTH | Adrenocorticotropic Hormone |
| SLE | Systemic Lupus Erythematousus |

# ABSTRACT

**"Urinary vitamin D binding protein levels in idiopathic nephrotic syndrome children – a cross sectional study"**

**Background:** Nephrotic syndrome (NS) is a common chronic kidney disease in childhood and it is mostly idiopathic. Idiopathic nephrotic syndrome (NS) associated with increased urinary vitamin D-binding protein (uVDBP) excretion. we conducted this study to evaluate the early and non-invasive method to diagnose steroid resistant nephrotic syndrome children by urinary vitamin D-binding protein levels and to co-relate urinary vitamin D-binding protein in biopsy proven FSGS children.

**Objectives:**

- **Primary Objective:** Early and non-invasive method to diagnose steroid resistant nephrotic syndrome children by urinary vitamin D-binding protein levels.
- **Secondary objective:** To co-relate urinary vitamin D-binding protein in biopsy proven FSGS children.

**Settings and Design:** It is a cross-sectional study carried out in 61 patients who had idiopathic nephrotic syndrome presented to Pediatric department of Dr Prabhakar Kore Hospital & MRC Belagavi, Karnataka, India. Urine and clinical data were collected from patients. Measurements of UVDBP were performed with a commercially available ELISA kit and normalized to urine creatinine.

**Results:** The mean age (SD) of the studied population was 6.82 ±4.4 years. Majority of the nephrotic patients were aged less than 5 years. There were 17 (27.9%) in the 6-10 years age group, 10 (16.4%) in the age group 11-15 years, 3 (4.9%) in the age

group 16-18 years. There were 40 (65.6%) males and 21 (34.4%) females with a M: F ration of 1.9:1. Majority of our study subjects were steroid sensitive (72.1%) and 27.9% patients had SRNS. Majority of the patients had not undergone kidney biopsy (52.4%). Among 29 of kidney biopsy pathology reports, there were 12 (41.4%) each subject with MPGN and FSGS, 3 (10.3%) subjects with MCD and 1 (3.4%) patient each with IgM nephropathy and acute glomerulonephritis. We found that GFR was significantly less and urine VDBP was significantly more in SRNS compared to SSNS when independent sample t-test used and applied to see difference of means of 2 groups ($p<0.05$) . We performed a ROC curve study to assess the ability of urine V-DBP to diffrentiate between patient with SR-NS and patient with SS-NS. VDBP's ability to distinguish between SRNS and SSNS had an area under the curve (AUC) of 0.883 (p 0.001; 95% CI= 0.795-0.971).

**Conclusion:** Urinary vitamin D binding protein levels can be useful for early detection of steroid resistant nephrotic syndrome and also be helpful as a non invasive method to distinguish steroid resistant and sensitive Nephrotic syndrome.

**Keywords:** Steroid resistance, Nephrotic Syndrome, Urinary Vitamin D-binding protein.

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# INTRODUCTION

The existence of nephrotic-level protinuria, oedema, hyperlipidimia, and hypo-albuminemia in a child is termed as nephrotic syndrome, or nephrosis.

One of the most frequent glomerular illnesses in children is nephrotic syndrome (NS). Proteinuria in the nephrotic range (≥40 mg/m2 /hour or urine protein/creatinine ratio ≥ 2 mg/mg or 3 + protein on urine dipstick), hypoalbuminemia (<2.5 g/L), widespread edema, and hyperlipidemia are the characteristic symptoms.[1] Recurrent episodes of edema, proteinuria, and hypoalbuminemia characterize the condition.

The global incidence of NS is estimated to be 20–40 per million people, but it is expected to be 90–100 per million people on the Indian subcontinent.[2] Idiopathic nephrotic syndrome (NS) affects 1.15–16.9% of children, with rates depending by ethnicity and area.[3] In children, nephrotic syndrome is a prevalent glomerular illness with considerable differences in incidence and steroid responsiveness across ethnic groups. Understanding ethnic heterogeneity may point to factors that cause nephrotic syndrome, which is yet unknown, as well as factors that explain for disparities in drug response. Within an ethnic group, the emerging importance of genetic variables linked with steroid responsive and steroid-resistant variants of nephrotic syndrome can provide insight into putative disease-causing biological pathways.[4] The ideas of "immune dysregulation," "increased glomerular permeability," and "podocytopathy" have all been used to develop hypotheses about the syndrome's pathophysiology.[2,5-7]

The illness courses and prognosis of different NS subtypes are quite varied. Invasive biopsy is still the sole way to get a sense of the disease's prognosis and progression. Minimal change disease (MCD) and focal segmental glomerulosclerosis are the two most prevalent histological findings on invasive biopsy (FSGS). Children with MCD are more likely to have a steroid-responsive course, but those with FocalSGS are more likely to have a steroid-resistant course. When compared to steroid-sensitive nephrotic syndrome, steroid-resistent NS is accompanied with a poor prognosis.

Due to the lower core size and localized character of FSGS in children, a single biopsy is frequently underdiagnosed, and the biopsy's usefulness in bringing out an outcome is debatable.

When remission of NS is achieved after 4 weeks of proper therapy with prednisolone 60 mg/m2/day, maximum 80 mg/day, the diagnosis of steroid-sensitive NS (SSNS) is made. When proteinuria persists after 4 weeks of sufficient prednisolone 60 mg/m2/day treatment, the diagnosis of steroid-resistant NS (SRNS) is made.[8] Vitamin D insufficiency is linked to both SSNS and SRNS, owing to its carier protein loss, vit D-binding protein (V-DBP), in the urinne. It was demonstrated that urine vit D is un-conjugated & is probably excreeted with V-DBP, whic has the comparable molecular wt to albumin (66 k Da vs. 58 k Da) & iso-electric point (pI 4.8. They also discovered a link between urine VDBP (uVDBP) excretion and albumin excretion. Vitamin D insufficiency is also linked to SRNS to a greater extent than SSNS.[9]

There are currently no confirmed diagnostic markers to differentiate steroid-resistant nephrotic syndrome from steroid-sensitive nephrotic syndrome. As a result, we are in desperate need of a non-invasive approach to detect steroid resistant nephrotic syndrome, as well as a non-invasive method to expel side effects of high (dose) corticosteroid treatment & to begin alternative treatment for steroid resistant nephrotic syndrome early. VDBP excretion in the urine has lately shown to be possible measure of renal inter-stitial insult and fibrosiis. Urinary VDBP levels are higher in people having micro-albuminura ,as well as in children with Chronic KD of various causative factors who have overt proteinuria.[10]

Looking at this background, we conducted this study to evaluate the early and non-invasive method to diagnose steroid resistant nephrotic syndrome children by urinary vitamin D-binding protein levels and to co-relate urinary vitamin D-binding protein in biopsy proven FSGS children.

# AIM & OBJECTIVES

**OBJECTIVES:**

**Primary Objective:** Early and non-invasive method to diagnose steroid resistant nephrotic syndrome children by urinary vitamin D-binding protein levels.

**Secondary objective:** To co-relate urinary vitamin D-binding protein in biopsy proven FSGS children.

# REVIEW OF LITERATURE

## PEDIATRIC NEPHROTIC SYNDROME

## BACKGROUND

The characteristics of paediatric nephrotic syndrome, also called nephrosis, include nephrotic- (range) proteinura, oedema, hyperrlipidemia, and hypoalbuminmia. Adults with nephrotic-(range) proteinuria excrete 3.5 g or more of protein per day or more. However, the paediatric definition of nephrotic-range proteinuria is more complicated due to the wide range of body sizes in children.

Children with nephrotic-range proteinuria excrete more than 40 mg/m2/h of protein. Many paediatric nephrologists depend upon a singular, first-morrning urine collection instead of 24-hour urine collections because they may be unreliable and time-consuming, especially in young children, and they can be difficult to collect. This method allows them to measure proteiin excretion by the difference of protein to creat.

Using a urine sample taken in the earlymorning prevents the possibility of Non-pathologicall ortho-static proteinuriia, which could or else unnecessarily boost the levels of protein in sample of a urine procured when patient is engaged in daily activities. Nephrotic range proteinuria is indicated by a urine prot/creatnine value of more than two-three mg/ mg, which is constant with 24-hour urine collection data.

Nephrotic syndrome causes the podocytes in the kidneys to have tiny pores that are large enough to allow proteinuria but too small to allow cells to pass through. This results in hypo-albuminmia as prot albumiin in some has

transferred from the bloo.d to the uriine. Nephrotic syndrome, in contrast, results in hematuria as a result of RBCs passing through the pores.

**HISTORY**

It took some time for the notion of nephrotic syndrome to be created. It is currently understood to be a mix of proteinuria, hypoalbuminemia, hyperlipidemia, and edoema. It's interesting to note that the development of steroids, antibiotics, diuretics, and other immunomodulators in the middle of the 20th century made the effective treatments finally accessible. Better medicines are still needed for the more difficult-to-treat kinds of nephrotic syndrome since we still don't fully understand its etiology(s).

The glomerulus is affected by a range of pathologic lesions, and these pathologic lesions together make up the nephrotic syndrome. Since the time of Hippocrates, generalised edoema, also known as dropsy, has been documented[11,12], though differentiating between different causes (heart, liver, nutritional issues, and renal abnormalities) wasn't done to investigate.

Hippocrates noted that the presence of bubbles in the urine indicates renal disease."[11] In 1484, Cornelus Roelans of Belgium wrote about a youngster who had "whole body edoema" and nephrotic condition. "TMake a poultice using the high of eldeer and danesworth plants, simmer it in whitecoloured winee, and cover baby into warm clothing to heal him," he said in his recommendation for a course of therapy.[13]

Dropsy was treated as a single disease from the fifteenth through the eighteenth centuries, without distinguishing between its various causes. [14] Theodore Zwinger of Basel provided earliest precise depiction of the nephrottic condition in child in 1722. [15] He also observed decreased urine production and explained it as the result of "obstructiion and compresion of renal tubule's," putting the renal organ at centre of the disaese because it was known at the time that heart and liver disease rarely appear in paediatric practice. His significant result had little impact on the scientific world, and for the next 200 years, no one cited

It is noteworthy that these observation's were done much before Morgaagni was able to prove the sickness must originate due to particular organ's. [16] William Heberden, a student of Morgagni, added, "Dropsy is often a symptom of some other which is too often incurable." Dropsy is extremely rarely an original illness. [17] A broad kind of dropsy, purportedly inflammatory, and those depending on diseased viscera (liver and heart) were two categories of the disease later in the eighteenth century.

Bloodletting was a frequent technique and a commonly prescribed treatment for these so-called "inflammatory disorders" under humoralism, a notion that was popular at the time in Europe. At about the same time, Cotugno[18], Cruikshank[19], Wells[20], and Brande[19] all reported the coagulability of the patients' urine. Finaly, in eighteen twenty seven, Richarrd Brightt (seventeen eighty nine to eighteen fifty eight) tried to link alltogether three presenting symptoms to this illness: widespread edoema, proteinuria, and kidney dysfunction. [21] Bright's coworker John Bostock also observed that protein levels in the serum were lowest

when those in the urine were highest.[21] These findings were validated by Christison in 1829. [22] Nephrotic syndrome, which includes significant albuminuria, hypoalbuminemia, and edoema brought on by damaged kidneys, was so established by 1830. Following the postmortem examination, these kidneys showed signs of illness. Bright recorded three different kidney postmortem appearances, along with the "speckniere," / kidney (bacon), which was further identified to be amyloiidosis, in works by Chritison, Pierre Rayer, and Carl Rokitansky between 1840 and 1846.[19]

The different abnormalities of lipid in NS rose to the front of arguement over the following few years. It was known that the nephrotic condition was accompanied by latescent or milky serum appearance (seen along with blood-letting sesions). Christisan demonstrated that it is ether--soluble fat. [23] Johnsan noted the faty character of the kidney's on examination(gross) in most of these patient's in 1846, as well as globule's(fat) and cast's in the tubule's.[24]

The cellulaar and tubulaar componants of the kidney's received increased focus since the techniques available for histology at the period made them more evident. The term "parenchymatous nephritis," coined by Virchow, describes a pathohistological picture with main tubules involvement. [25] Glomerular involvement and parenchymal involvement both became more obvious with improvements in microscopy. The occurrence of pale exsanguinated glomeruli in acute nephritis has long been recognised. In order to describe the exudative glomerular alterations visible under a microscope, Klebs created the term "glomerulonephritis" in 1872. [26] Müller introduced the term "nephrosis" in 1905 to replace the term "parenchymatous nephritis" for all "non-inflammatory" kidney

disorders, in contrast to the term "nephritis" for exudative and inflammatory diseases.[27] F. Volhard, T. Fahr, and C. Munk contributed to the further popularisation of the idea of "nephritis in contrast to nephrosis."

**EPIDEMIOLOGY**

Nephrotic syndrome (NS) is thought to affect 2–7 instances out of every 100,000 kids each year, with a cumulative prevalence rate of 16 cases out of every 100,000 kids under the sixteen age. Nephrotc syndrrome is fifteen times common in child than in adults. 90% of childrens with Nephrotic:syndrome are believed to have idiopathiic N-S, with secondary NS—which is connected to the systemic diseases,infections, cancer, and other diseases related to glomeruli—being the condition in the remaining 10% of cases.

In kids and teenagers, the idiopathic nephrotic syndrome is rather uncommon. It affects roughly 2.2 out of every 100,000 kids (Srivastava et.al., 1999). 85% of IdiopathicN.S are caused by MCNS, and more than 95% of them are treated with steroids without the need for a kidney biopsie. Childrens with SSNS have a better prognosis and acceptable longer term maintenance of renal function's. Chronic renal illness connected with a higher chance of steroid resistance. The main cause of SRNS and ten- twenty percent of paediatric ESRD is focal segmental glomerulosclerosis (FSGS). Previous research has emphasised the significant impact that racial and geographic characteristics have on steroid responsiveness, histological pattern, and INS result. Additionally, some data suggest that childhood INS is evolving with time. Recent research indicates that in several regions of the world, the prevelance of FSGS in childrens has drastically hiked up during the last twenty years.

Nephrotic syndrome affects 20 instances per million people in India. According to the ISKDC, MCNS was detected in sevent six % of children with Idiopathic NS who had biopsy results, and FSGS was detected in 7% of cases.

**SEX, ETHNICITY AND AGE DEMOGRAPHICS**

Children who are dark coloured or Hispanic appear to be at higher prevalance for FSGS and steroid-resistant nephrotic syndrome. Asian children have a higher INS incidence than other youngsters (six times seen the rate in European children). Children from Southwest Asia, India, and Japan are also more likely to develop INS.

In Afriica, where NS is more frequently secondaryto or steroid-resisstant, primary SSNS is uncommon. These differences in the ethnicity and regional spread of Idiopathic NS highlight roles played by environmental and genetics factors

Male to female ratios in children under the age of eight years at onset range two:one to three:two. In kids, teenagers, adult's, the chances of males and females is roughly equal. ISKDC data show that 65% of MPGN patients are female while sixty six% of children with minimal change NS or focal segmental GS are male .70% MCNS patients under five year of age. Only twenty–thirty% of teenagers with Idiopathic NS develop Minimal change NS according to biopsys results. In the 1$^{st}$ year of life, congenital infection-related secondary nephrotic syndrome and hereditary types of INS are more common.

## CAUSES

**Table 545.1** Causes of Childhood Nephrotic Syndrome

**IDIOPATHIC NEPHROTIC SYNDROME**
Minimal change disease
Focal segmental glomerulosclerosis
Membranous nephropathy
Glomerulonephritis associated with nephrotic syndrome–membranoproliferative glomerulonephritis, crescentic glomerulonephritis, immunoglobulin A nephropathy

**GENETIC DISORDERS ASSOCIATED WITH PROTEINURIA OR NEPHROTIC SYNDROME (see also Table 545.3)**
***Nephrotic Syndrome (Typical)***
Finnish-type congenital nephrotic syndrome (absence of nephrin)
Focal segmental glomerulosclerosis (mutations in nephrin, podocin, *MYO1E*, α-actinin 4, TRPC6)
Diffuse mesangial sclerosis (mutations in laminin $\beta_2$ chain)
Denys-Drash syndrome (mutations in WT1 transcription factor)
Congenital nephrotic syndrome with lung and skin involvement (integrin α-3 mutation)
Mitochondrial disorders
***Proteinuria With or Without Nephrotic Syndrome***
Nail–patella syndrome (mutation in LMX1B transcription factor)
Alport syndrome (mutation in collagen biosynthesis genes)
***Multisystem Syndromes With or Without Nephrotic Syndrome***
Galloway-Mowat syndrome
Charcot-Marie-Tooth disease
Jeune syndrome
Cockayne syndrome
Laurence-Moon-Biedl-Bardet syndrome
***Metabolic Disorders With or Without Nephrotic Syndrome***
Alagille syndrome
$\alpha_1$-Antitrypsin deficiency
Fabry disease
Glutaric acidemia
Glycogen storage disease
Hurler syndrome
Partial lipodystrophy
Mitochondrial cytopathies
Sickle cell disease

**SECONDARY CAUSES OF NEPHROTIC SYNDROME**
***Infections***
Endocarditis
Hepatitides B, C
HIV-1
Infectious mononucleosis
Cytomegalovirus
Malaria
Syphilis (congenital and secondary)
Toxoplasmosis
Tuberculosis
Schistosomiasis
Filariasis
***Drugs***
Captopril
Penicillamine
Gold
Nonsteroidal antiinflammatory drugs
Pamidronate, other bisphosphonates
Interferon
Mercury
Heroin
Lithium
Rifampicin
Sulfasalazine
***Immunologic or Allergic Disorders***
Vasculitis syndromes
Castleman disease
Kimura disease
Bee sting
Snake venom
Food allergens
Serum sickness
Poison ivy, poison oak
***Associated With Malignant Disease***
***Wilms Tumor***
Lymphoma
Pheochromocytoma
Leukemia
Thymoma
Solid tumors
***Glomerular Hyperfiltration***
Oligomeganephronia
Morbid obesity
Adaptation to nephron reduction

## PROGNOSIS

Since corticosteroids were introduced, the total mortality of Idiopathic NS has dropped significantly more than fifty% to only two-five%. Even though survival rates have increased, Most INS kids went through some level of morbiidity, along with the following, and the disease is typically chronic and relapsing:

- Chances for developing in to end-stage KF and CKF;
- In certain cases, hospitalisation;
- Regular monitoring by parents and doctors;
- Administration of drugs linked to serious complicatios;
- A increaased rate of recurence (relapses in >sixty% of patients); (ESKD)

Acute kidney failure, hyperlipidemia, thrombosis, edema, infections, infections, thrombosis, and possible increased risk of cardiovascular disease are additional complications of INS.

**RESPONSIVE TO STERIOD: NEPHROTIC SYNDROME**

Pateint those continiously respond to steroid and experience repeated relapses while their proteinuria is in remission typically have a favourable prognosis. According to the ISKDC, kidney biopsy results showed MCNS in 93% of INS-affected kids who reacted to steroids. In contrast, MCNS histology was not present in 75% of individuals who did not react to steroids at first.

Following the initial round of steroid therapy, Ninty % of children having MCNS, as only Twenty % of childrens with FSGS, experience remission.

Although patients who react to steroids typically have a good prognosis, the ISKDC observed a 60% probability of future relapses, which might cause difficulties, higher morbidity, and a lower quality of life. The probability of further relapse may be reduced to thirty six% with longer initial steroid treatment regimen (12 weeks as opposed to the original ISKDC protocol's 8 weeks), but this still represents a sizable patient population undergoing repeated

immunosuppression with the potential for hospitalizations, edoema, infections, medication side effects, and other comorbidities.

**STEROID-RESISTANT NEPHROTIC SYNDROME**

A preliminary trial of steroids is unsuccessful in 10% of people with INS overall (compared to 2% of patients with MCNS). Furthermore, roughly 1 to 3% who initially respond positively to steroids later develop treatment resistance ("late non-responders").

The majority of patients whose proteinuria does not improve for steroid had biopsy (kidney) results other to Minimal change NS. FSGS is the typical for such patients.

The prevalence of Focal Segmental GS and nephrotic syndrome is greater than 60% who receive no relief from treatment eventually develop end-stage kidney disease (ESKD). In comparison, patient who have Focal Segmental GS who attain remission through treatment only 15% advance to ESKD. Patients with INS who reached remission had a 90% decreased risk of developing ESKD, according to Gipson et al.

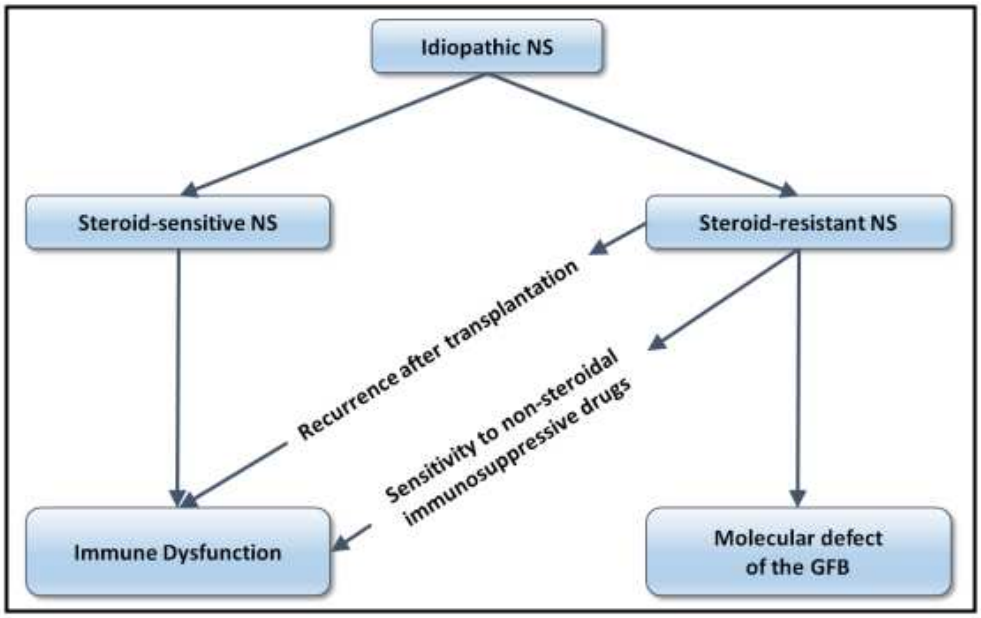
As a result, patients with SR Idiopathic NS has a fair prognosis if other drugs successfully treat proteinuria. Poor outcome and progression to ESKD are predicted by treatment failure (i.e., if the patient fail to attain remission) & renal in-sufficiency at the begining.

## PATHOPHYSIOLOGY

Protein loss in the kidney is the main flaw in NS. Proteinuria may develop from a lack of tubular reabsorption, but NS range proteinuria typically indicates glomerular membrane permeability problems that cause this excessive protein loss. As a result, albuminuria develops, which causes the two primary symptoms of NS—edema and concomitant hypoalbuminemia.

One of the consequences of NS, increased platelet aggregation and thrombosis, may result from the hyperlipidemia, which is often caused by the enhanced lipoprotein synthesis induced by the hypoalbuminemia. Malnutrition and infections may be made more likely by the proteinuria's loss of more proteins, minerals, and vitamins. In the field of podocyte biology and structure of the slit diaphragm, the most notable developments in our understanding of the pathophysiology of NS have taken place. (3-5)

The BM( basement memnbrane) extracellular , the podocytee foot proceses, and the fenestrated capillary endothelium make up the glomerular filtration barrier. NS is connected to the biopsy’s finding of podocyte foot proceses effacement. Podocyte flattening, foot processes retracting, and sporadically microvillous transformation are the characteristics of effacement. It is also known that MCNS and FSGS are podocytopathies, which can cause proteinuria and glomerular disease by disrupting normal podocyte activity and the slit diaphragm. (3–5).




**DIAGNOSTIC CONSIDERATIONS**

A rare consequence of idiopathic nephrotic syndrome is acute kidney failure (AKF). Acute interstitial nephritis is typically characterised by a "bland" urinalysis (minimum cellular components), fever, rash, arthralgia, and eosinophilia in the presence of AKF. Except for the AKF and an unremarkable urine, clear clinical signs can be lacking. The presence of thrombocytopenia, flank discomfort, and gross hematuria could indicate renal vein thrombosis. When a patient has anasarca, hemoconcentration could signify intravascular volume depletion.

**GENETIC TESTING**

Patients who have diffuse mesangial sclerosis, gonadoblastoma, Wilms tumor, or pseudohermaphroditism should undergo WT1 testing. Patients who have biopsy and clinical results that are compatible with Finnish-type nephrotic syndrome should have NPHS1 testing done. Analysis of the NPHS2 gene should

also be taken into consideration because congenital NS has been associated with NPHS2 mutations. Younger kids with NS and neuro or vision issues ought to taken into consideration as LAMB2 testing candidates (Pierson syndrome).

In addition to kidney biopsy, testing for podocin (NPHS2), ACTN4 and TRPC6 mutations should be taken into consideration in patient who don't respond to steroid sooner or later.

**KIDNEYs ULTRASONOGRAPHY**

MCNS and other chronic kidney diseases may be distinguished by renal ultrasonography, however results are typically nonspecific. The kidneys are typically enlarged in all cases of nephrotic syndrome as a result of tissue edoema. Other than MCNS, where echogenicity is typically normal, increased echogenicity is typically a sign of chronic renal illness. Small kidneys are a sign of chronic kidney disease other than MCNS, and increased serum creatinine levels are frequently present in conjunction with this observation.

**CHEST RADIOGRAPHY**

When a youngster has respiratory problems, chest radiography is recommended. Although pulmonary edoema is uncommon, pleural effusions are frequently seen.

Prior to starting steroid therapy, chest radiography should be taken into consideration in order to rule out tuberculosis (TB) infection, particularly in children who have had a positive Mantoux test or who have previously received treatment for TB.

**MANTOUX TEST**

Prior to starting steroid therapy, the Mantox test ( PPD) to be carried out so that we can exclude out TB infection.

Mantoux testing can be done concurrently with beginning steroid therapy because using steroid for forty eight hours before interpreting the PPD will not hide a result that is positive and taking steroids for two days has a very low risk (finding the test reports to be positive, steroid to be stopped).

Chest radiography should be done on kids who have a positive PPD, a previous positive PPD, or who have had TB treatment in the past.

**KIDNEY BIOPSY**

When PNS first manifests in a child between the ages of 1 and 8 years old, a kidney biopsy is not recommended unless the history, physical examination, or laboratory data suggest $2^0$ NS / a $1^0$ NS different to Minimal Change NS. Patients should get a kidney biopsy if they are older than eight years, when chronic glomerular disorders like FSGS are more prevalent, and younger than one yr, when congenital NS related to genetics are common.

Empirical steroid therapy can be considered in a few preadolescent adolescents older than 8 years prior to kidney biopsy, however this should only take place under the supervision of a paediatric nephrologist with experience in nephrotic syndrome. Undertaking a biopsy of kidney in patient >12 yrs has been advised by certain authors.

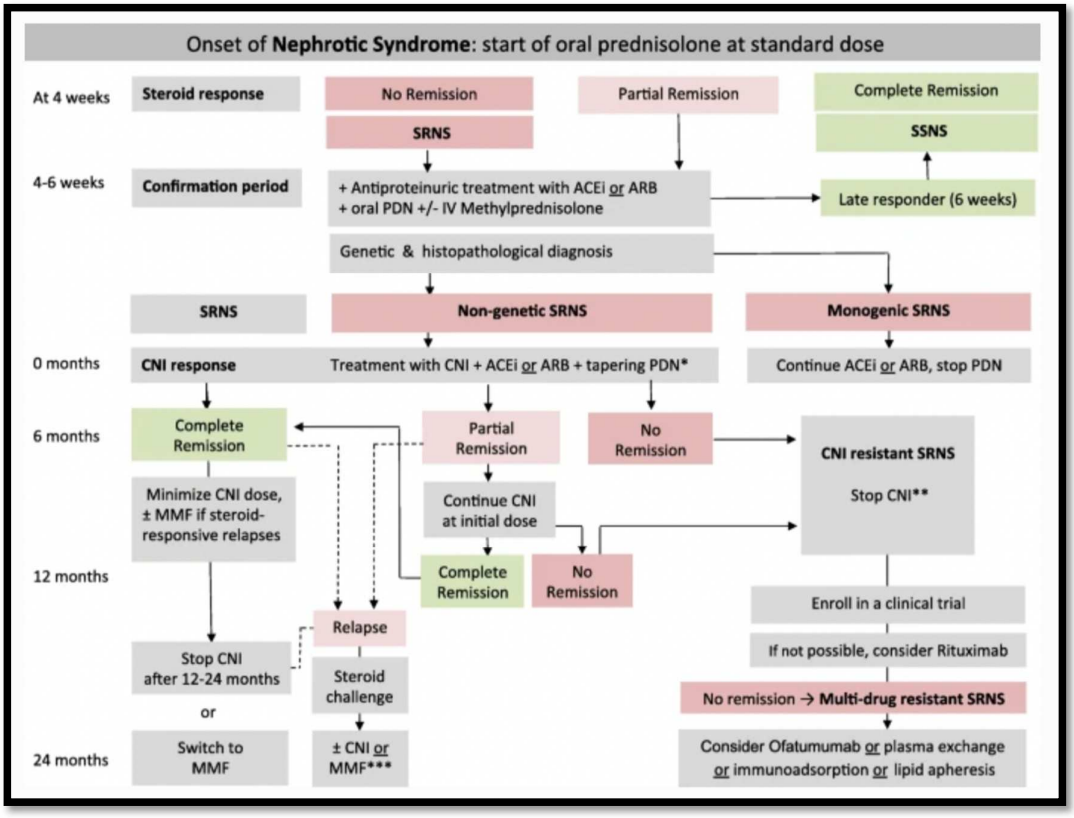
When a history, physical exam, or lab results point to 2º NS / disease related to kidney other than Minimal Change NS, a biopsy of kidney should be also done. Consequently, a kidney biopsy is advised for patients who experience any of the following:

- Systemic illness symptoms
- Results of tests suggesting $2^0$ nephrotic syndrome (eg, positive ANA report, positive anti–ds DNA antibody, low complemant level)
- Increased levels of creatinine that don't go down when the intravascular volume is reduced
- Relevant kidney illness in the family history

Finally, a kidney biopsy should be done on patients who initially or later become refractory to steroid treatment because this condition is highly correlated with prognostic wise unfavourable findings on histology like Focal Segmental GS or Minimal GN.

All patients with a diagnosis of primary SRNS should undergo a renal biopsy, WITH the exception of those with secondary causes (infection/malignancy).

**TREATMENT**




Knowing the findings histologically in NS was expanded when renal biopsy was introduced into the main clinical practise of nephro in the nineteen fifties and sixtys . A case series from 1958 summarises these histologic clasification, which was dependent on the appearance in light microscopy along with nephropathy(diabetic) with nodules (hyaline) , FSGS, mixed membranous and proliferative glomerulonephritis, and membranous glomerulonephritis (18). The existence of immune-fluorescent localisation of the protein and electron microscopy was a happy coincidence. These innovative methods revolutionised the concepts of morphology and aetiology and established clear connections between the pathological image and the clinical presentation. In these regards, the nintetenn sixty one Cibaa Foundation symposum on application of biopsy of kidney marked a turning point (19).

The mortality rate for infections caused by nephrotic syndrome was significantly lowered by the use of antibiotics, going from two thirds to only 35%. (20). It is crucial to note that numerous desperate treatments were tried before steroids were available. A Boston research listed the different nephrotic syndrome therapies that were tried between 1926 and 1948. The most effective therapies at the time were probably dietary changes and a low-sodium diet. There were a few ineffective mercurial diuretics that had little to no effect. Other severe tactics were implemented, including the introduction of measles and vaccinia. Many of the vaccinated kids experienced diuresis after infection, a decreased proteinuria (eleven out of fourteen), but these symptoms would later return.Transfusions (blood),extract therapy(thyroid), kidney de-capsulation, testosterone, a number of vitamin, horse as, and para-thyroid hormone was additional supportive measures.

In 1936, steroid hormones were first extracted and recognised (21, 22). By 1946, bile acids were used to partially synthesise cortisone for the first time (23). similarly, in the 1940s, ACTH was removed by isoelectric precipitation from the pituitary glands of sheep and pigs (22, 24). One form of ACTH was marketed as sterile powder that could be injected intramuscularly after being reconstituted with isotonic saline. Arneil and Wilson from Glasgow employed cortisone injections intramuscularly (25) and ACTH (26) for the 1$^{st}$ time to treat paediatric nephrotic syndrome in the early 1950s. In the first research, these were relatively brief courses lasting 5 days (daily intramuscular cortisone doses of 100–300 mg) and 12 days (daily intramuscular ACTH doses of 40–80 mg) (25, 26). Prednisone was initially created later by oxidising cortisone (27, 28). Prednisolone and prednisone swiftly replaced ACTH and cortisone since they could be taken orally and didn't require daily injections (29, 30). The mortality rate from nephrotic syndrome substantially fell to

3% with the introduction of steroid therapy (31).

ISKDC :Inetrntional study of kidney disease in children , founded in nineteen sixty five with subjects from America (north) , east-Europe, and south-Asia, heralded a significant continuity of research to understand the treatment of paediatric NS in era of renal biopsy and steroids. The ISKDC developed criteria, clinicopathological correlations, and treatment guidelines that served as a foundation to diagnose and manage patients of paediatric NS , which is still in use today. From the 24 participating clinics, children with the NS who were older than twelve weeks and <sixteen yrs old were participated in the clinical trial between January 1967 and June 1974. (32–34). 75% of the 521 participants had membranoproliferative glomerulonephritis, and seventy six point six % had minimalchange NS and six point nine % had focal segmental GS(35). Prior to beginning prednisone-based steroid medication, all individuals had biopsies. The first four weeks of treatment consisted of divided doses of 60 mg/24 h/m2 (80 mg/24 h at the maximum), followed by divided doses of 40 mg/24 h/m2 given three days out of the week for the next four weeks. Patients with lesions that were non minimal showed a variable and constrained reply to steroid, according to seminal finding (36).

This team also examined the purine synthesis inhibitor azathioprine in a randomised placebo-controlled experiment (37). Prednisone with azathioprine was given every other day to patients who were thought to be often relapsing patients or those who were not responding to to steroid (no response to the fiest eight week treatment) (control group). In comparison to the control group, neither proteinuria nor the relapses significantly decreased in group of test (37).

Nitrogen mustard was the first alkylating drug used in the resistant to steroid group of NS as early as 1958. (38). In order to clarify the function of cyclophosphamide in kids with frequently relapsing nephrotic syndrome and early non-responders, the ISKDC conducted a randomised control experiment in the 1960s. This was extended to other alkylating drugs, including chlorambucil and cyclophosphamide. It has been demonstrated that cyclophosphamide can reduce the frequency of nephrotic syndrome relapses and reverse proteinuria in early non-responders (39). Although it led to gonadal failure in post-pubertal boys, it nonetheless proved to be a crucial agent in reducing steroid use and preventing steroid toxicity (39). Despite being as efficient as cyclophosphamide, chorambucil has kidney cancer and acute leukaemia as significant adverse effects (41).

Later, in the late 1980s, A longer steroid treatment time was compared to the first eight week steroid plan (four week everyday followed by four week alternate day) (42).The first eight-week steroids treatment was suggested to expand at twelve week due to monting evidenc that a duration(longer) of steroid helps reduce the relapse and dependence over steroid (43, 44).

Levamisole, a drug with anthelmintic properties, has been utilised as a agent that spares steroid in several nations since it was 1$^{st}$ seen to be used in childrens with NS in nineteen eighty (45). Hans Peter Frey (46) originally discovered the calcineurin inhibitor cyclosporine A (CsA) in the fungus Tolypcladium inflatam in a sample from soil from Norway in nineteen sixty nine. In 1978, it was first applied to transplanting human kidneys, and since then, the field of transplantation has seen significant change (47). CsA was first used in individuals having NS who where difficult to manage in 1986. (48). By keeping goal levels of Cs-A between fifty and two hundred

nanogram/ml, there were reports of its successful usage by the late 1980s in kids with SRNS or SDNSe who had not responded well to alkylating drugs (49,, 50).

Another inhibitor (calcineurin) , tacrolimus, was 1st isolated from Streptomycees tsukubainis by a Japaneese team in 1987 (51.) was first applied as medication in organ transplantation in human . Beginning in the early 1990s (52–54), adults were the first to utilise it for nephrotic syndrome; by the early 2000s, reports indicated that it was also being used on children (55–57). It was demonstrated to be comparable to CsA in terms of effectiveness and kidney toxicity, but without the hirsutism and gingival hypertrophy aesthetic side effects (58).

Mycophinolic acid, another puriine syntheses inhibitor discovered in Penicilium speciies, comes in prodrug forms such as MMF (cell-cept®) and, myco-phenolate -sodium (my-fortic®). After successfully being used utilised in further illnesses (glomerular) (60) and kidney transplantations , it was first reported as used for paediatric nephrotic syndrome in 2000 (59). It has been demonstrated to be beneficial as a first-line treatment for steroid-dependence and frequent relapsing steroid-reasponsive NS, while data suggests that this is lesser effective than calcineuren inhibitors. (43,,61–63).

Rituximab is a MAB that fights the CD20 B lymphocyte antigen. Case studies documenting accidental discovery of rituximab's favourable effect on paediatric nephrotic syndrome led to its inclusion in the arsenal (64). Rituximab was originally utilised by Benz et al. to treat ITP in a sixteen year old child who had steroiddependent NS and was resistant to steroids and immunoglobulin treatment. Rituximab alleviated the nephrotic syndrome in addition to successfully treating the ITP, causing a relapse-free duration of more than twelve months on a lower dose

CsA. (65). Rituximab has been used in the treatment of PTLD:post transplant lymphoproliferative disease in the other two cases the boys those also experienced FSGS reappearance in their transplant (66,, 67), with favourable results on protein-uria. In a multiple-center, double-blinded, randomised, and placebo to controlled trial (68) headed by Iijiima et al., its effectiveness as a drug that spares steroid in juvenile development of frequently relapsing and steroid dependent NS was proven. Despite being taken off additional immunosuppressive medications like mycophenolate or cyclosporine, patients in the group of rituximab treated remained free of relapse for a considerable peroid. There are few cases report of its effectiveness to treating primary refractory Focal segmental GS, but controlled trials still required to pinpoint the precise function (69,.70). Its been demonstrated for being efficient to preventing the post-transplant recurrence of FSGS when combined with plasmapheresis (70, 71). Its direct impact on the podocyte may play a role in mediating some of this effect (72).

**FUTURE TREATMENTS**

It's interesting to note that ACTH is once again being considered as a nephrotic syndrome treatment. It can be purchased from swine or bovine sources in United States : Acthar® gel, a high level purified formula, and as a synthetec depot formula (Synacthen. ®) Europe (73). Focal Segmental GS, MCD , mesangial GN, idiopathic membranous nephropathy, and other types of nephrotic syndrome have all been reported to be effective in treating it, with response rates ranging from twenty nine to hundred %. (73.–79). Its recently demonstrated to be especially helpful in IMN. Even though the precise mode of action of Adrenocortico TH is unknown, its believed that it operates straught on podocytes through the podocytes' melanocortinn receptors.

As more nephrotic syndrome-related gene abnormalities have been identified, our understanding of the numerous podocyte signalling pathways involved in the disease's pathophysiology is growing (80). To create future tailored therapies, a deeper comprehension of these pathways is required. Gene-wide association studies (GWAS) have discovered that the high-risk variant genotype of the APOL1 gene (which codes for the apolipoprotein 1 protein, known for its trypanolytic characteristics) increases the risk of kidney disease (81–83). Its relationship to the nephrotic syndrome and its function in podocyte biology are still unknown (80).

Future therapies for steroid-resistant nephrotic syndrome will include biologics like adaliimumab (-anti-TNF-) and abtacept (-anti-CD80), as well as novel drugs like losmapimod (a p38(thirty eight)- MAPK- inhibitor), sparsenten (an type 1A antagonist of endothelin receptor), & sparsentan (a p38 MAPK inhibitor) (79). In conclusion, the ISKDC made important contributions that demonstrated the importance of international collaborations in carrying out significant multicenter trials meant to successfully treat children with NS, halt renal deterioration , and enable every child to attain full potential. Now that sophisticated genetics & molecular application to customised medicines are available, new prospects on this hopeful road are emerging.

# METHODOLOGY

**Study design:** Cross sectional study design was chosen to meet the objectives of the study. Cross-sectional studies are carried out either at one time point or over a short period. They're frequently used to figure out how common a particular outcome is in a given population. Individual characteristics, such as risk factor exposure, can also be collected, as well as information on the outcome. In this way cross-sectional studies provide a snapshot of the outcome and the characteristics associated with it, at a specific point in time.[121] Hence, this study design was considered appropriate for the present study.

**Study population:** Children with idiopathic nephrotic syndrome admitted in the department of Pediatrics at Dr Prabhakar Kore Hospital & MRC Belagavi, Karnataka, India.

**Study Time**: Research study was conducted for 12 months from January 2021 to December 2021.

**Sampling procedure:** Sampling is defined as the process of selecting a number of subjects from all the subjects available in a particular group or universe. A conclusion based on sample results may be attributed only to the population sampled.[80]

In this study we considered all eligible patients consecutively admitted in the department of Pediatrics at Dr Prabhakar Kore Hospital & MRC Belagavi with idiopathic nephrotic syndrome till we meet the sample size.

**Inclusion criteria:** All children aged from 1 to 18 years diagnosed with idiopathic nephrotic syndrome.

**Exclusion criteria**

- Nephrotic syndrome secondary to systemic diseases:
- SLE
- Sarcoidosis
- Rheumatoid arthritis
- Wagener's granulomatosis
- Goof pasture syndrome
- Amyloidosis
- Myxedema
- Diabetes mellitus

**Sample size:** Sample size was estimated by using Open Epi software version 2.31. We assumed that the prevalence of steroid resistance in nephrotic syndrome to be 20%. At confidence level 95%, Absolute precision 10% and 80% power of the study, the sample size estimated was 61.

Formula used:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where;

Z = 1.96

P = 0.02

(1-P) = 0.80

d=0.1

**Method of data collection:** Informed written consent was obtained from all the parents of study subjects. Cases are those patients with idiopathic nephrotic syndrome admitted in the department of Pediatrics at Dr Prabhakar Kore Hospital & MRC Belagavi.

For the diagnosis of NS, all patients met the criteria set forth by ISKDC:

- Nephrotic proteinuria, known as an urine spot protein to creatinine ratio of two or higher.
- Hypoalbuminemia (less than 2.5 g/dl of serum albumin).
- Edema and
- Hyperlipidemia (blood cholesterol level

Age, sex, the presence of symptoms, the CNBC, urine alysis, examined under microscope, twentt four hour protein excretionin urine, clearance of creatinine, electrolytes in serum, urea and creatinine levels in serum, the course of mangement, and the result were study parameters.

The various forms of nephrotic syndrome were identified based on the results of the kidney biopsy reports. These ISKDC definitions were used to classify the treatment response:

a) Within eight weeks of starting prednisolone therapy, proteinuria completely resolves in those who are steroid sensitive.
b) Steroid resistance, or failing to improve after receiving prednisolone at a dose of 2 mg/kg/day for eight weeks in a row.
c) Repeated relapses, defined as four or more episodes of nephrosis within a year or two episodes within six months following the first response.

d) Remission: Excretion of Urinary protein is less than 4 mg/m2/h; a dipstick spot sample for 3 days in a row shows no protein at all or only a little amount.

Prednisolone (60)sixty miligram/metre2/day for four-six weeks was given to patients who did not have a need for a kidney biopsy, and then prednisolone (40)fourty miligram/metre2 on alternate day for an additional four weeks. Over following two to three months, the prednisolone dose was decreased and then stopped. Other different drugs such as cyclo-phosphamide (two–three miligramg/kg/day for eight–twelve mnts) and cyclosporine (three-six miligramg/kg/day) were used to treat steroid resistance and frequent relapses. We collected information on sex,age , characteristics (presenting), lab findings, pattern of response, mangement, and results of biopsy using a standardised data-sheet.

**Measured parameters:**

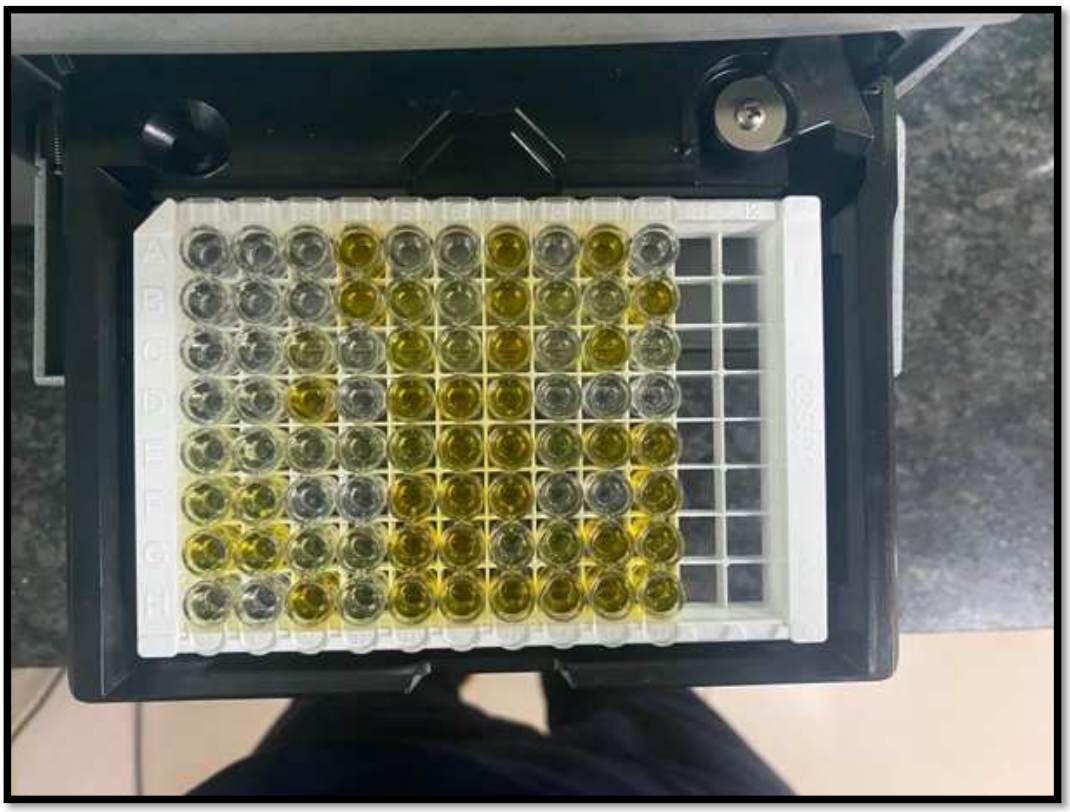
The various parameters studied are

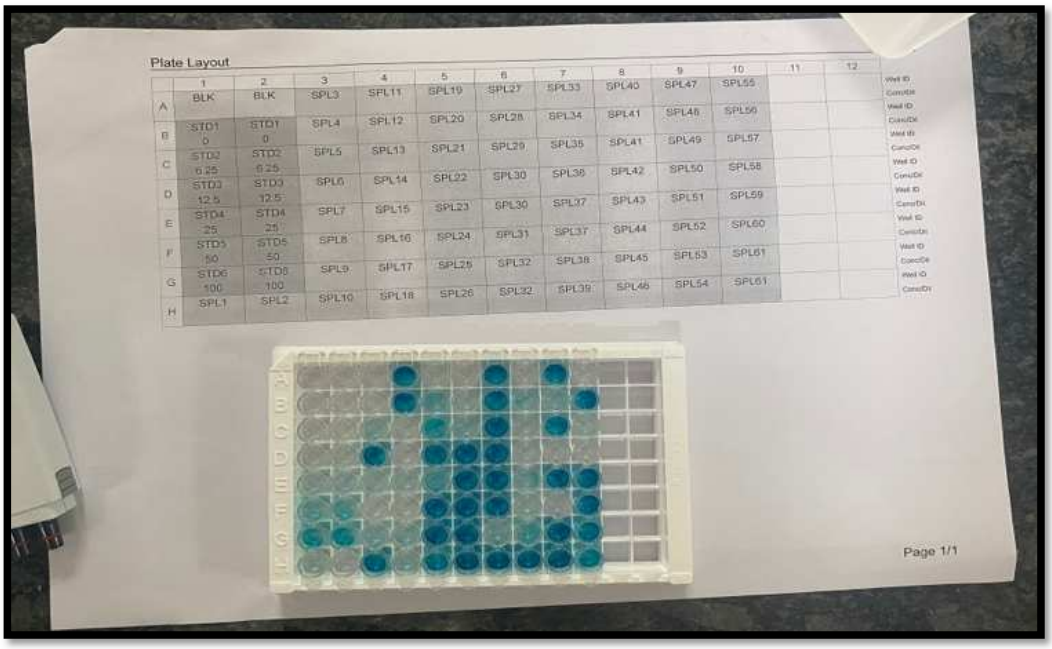
- ✓ Hemoglobin
- ✓ Erythrocyte-sedimentation-rate
- ✓ Glucose in blood
- ✓ Urea in plasma
- ✓ Creatinine in plasma
- ✓ Cholesterol in plasma
- ✓ Total Protein of plasma
- ✓ Albumin in plasma
- ✓ Protein to Creatinine ratio
- ✓ Pus or Epithelial cells
- ✓ Red blood counts

**Ethical Consideration:** Ethical clearance was taken from Ethical Committee of Dr Prabhakar Kore Hospital & MRC Belagavi before conducting the study.

**Statistical Analysis:** Data was tabulated in a sheet od excel and analyzed using package of statisticse for the Social Sciences twenty(SPSS Inc. Chicago). Results were recorded in tabular and graphical forms Mean, median, SD and ranges were accounted for quantitative data.

Chi square analysis was useful in testing for major differences between proportions and frequencies. T test was useful in testing for majot differences between two means. The confidence interval was set to be at ninty five % limit, with level of the significance to be at p: < 0.05.

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# RESULTS

This is the cross-sectional study with 61 children aged from 1 to 18 years diagnosed with idiopathic nephrotic syndrome.

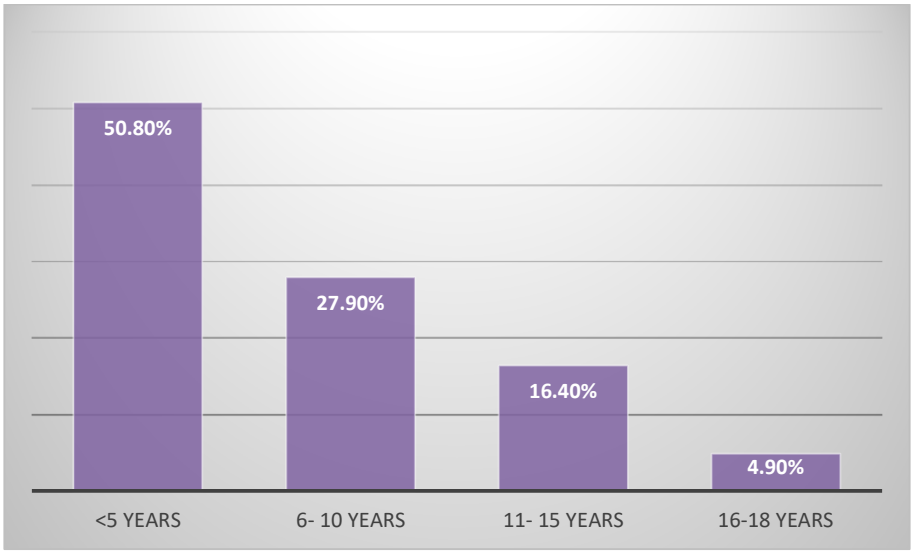
**Age distribution**

The mean age (SD) of the studied population was 6.82 ±4.4 years. Majority of the nephrotic patients were aged less than 5 years. There were 17 (27.9%) in the 6-10 years age group, 10 (16.4%) in the age group 11-15 years, 3 (4.9%) in the age group 16-18 years. (Table 1 and figure 1)

**Table1: Age distribution of study subjects**

| Age Group | Frequency | Percentage |
|---|---|---|
| <5 Years | 31 | 50.8% |
| 6- 10 Years | 17 | 27.9% |
| 11- 15 Years | 10 | 16.4% |
| 16-18 Years | 3 | 4.9% |
| **Total** | **61** | **100%** |

**Figure 1: Bar chart showing age distribution of study subjects**



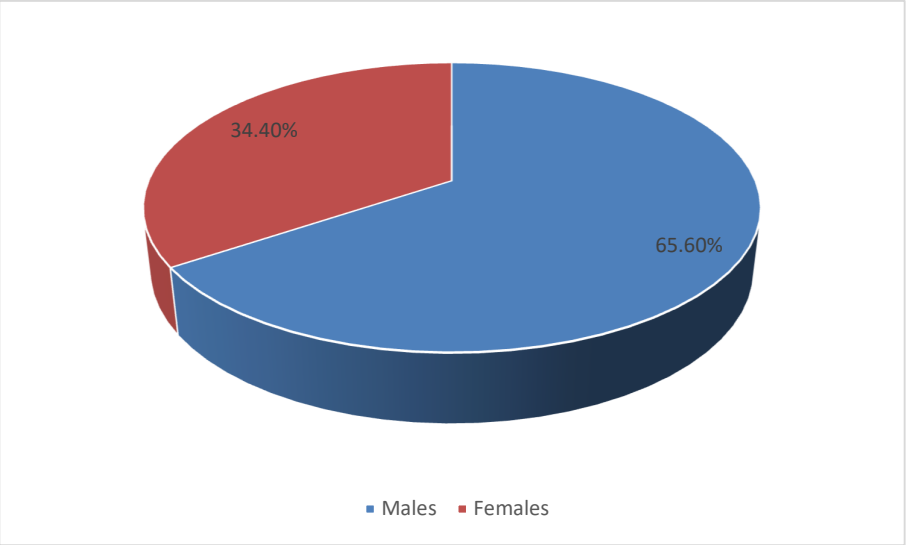

**Gender distribution**

There were 40 (65.6%) males and 21 (34.4%) females with a M: F ration of 1.9:1. (Table 2 and figure 2)

**Table 2: Gender distribution of study subjects**

| Gender | Frequency | Percentage |
|---|---|---|
| **Males** | 40 | 65.6% |
| **Females** | 21 | 34.4% |
| **Total** | **61** | **100%** |

**Figure 2: Pie chart showing gender distribution**



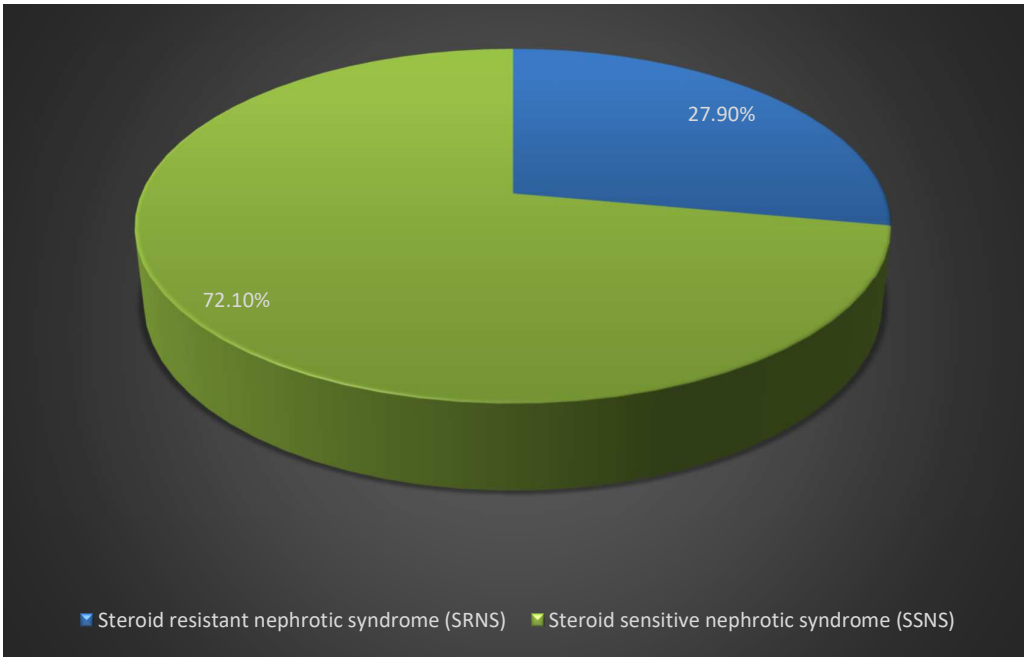

**Response to steroid treatment**

Table 3 shows the response to steroid treatment in studied population. Majority of our study subjects were steroid sensitive (72.1%) and 27.9% patients had SRNS (Figure 3).

**Table 3: Steroid responsiveness in nephrotic syndrome patients**

| Steroid responsiveness | Frequency (%) |
|---|---|
| SRNS | 17 (27.9%) |
| SSNS | 44 (72.1%) |
| **Total** | **61 (100%)** |

**Figure 3: Pie chart showing Steroid responsiveness in nephrotic syndrome patients**



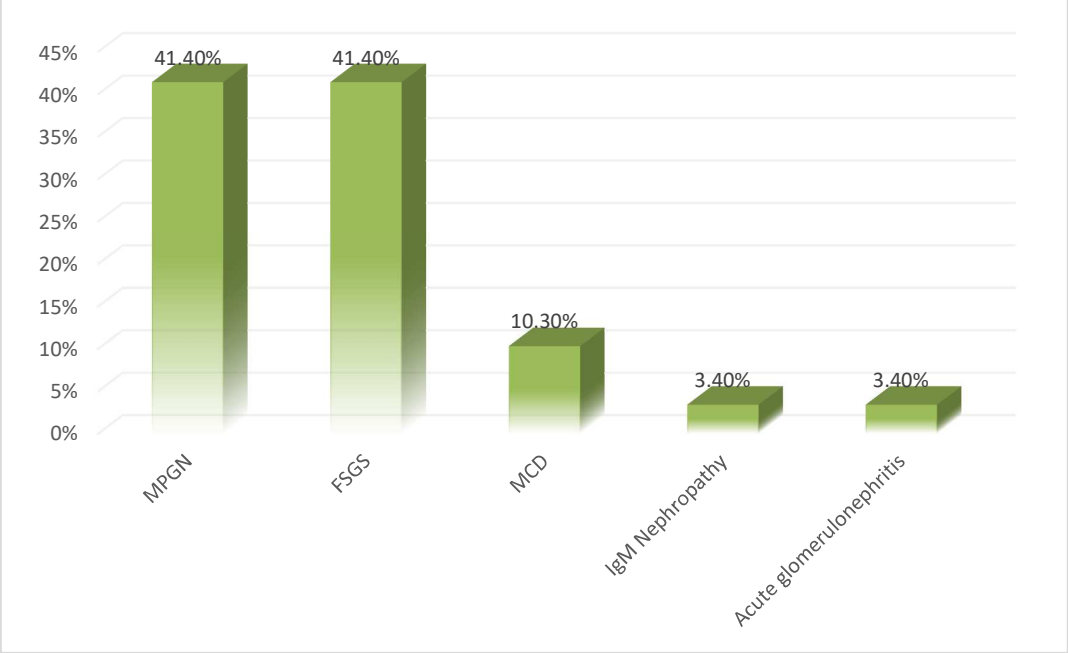

**Pathology of kidney biopsy in nephrotic syndrome**

Table 4 shows the results of histopathology of study population. Majority of the patients had not undergone kidney biopsy (52.4%). Among 29 of kidney biopsy pathology reports, there were 12 (41.4%) each subject with MPGN and FSGS, 3 (10.3%) subjects with MCD and 1 (3.4%) patient each with IgM nephropathy and acute glomerulonephritis (figure 4).

**Table 4: Frequency distribution of kidney biopsy pathology**

| Pathology | Frequency (%) |
|---|---|
| Membranoproliferative glomerulonephritis (MPGN) | 12 (41.4%) |
| Focal Segmental Glomerulosclerosis (FSGS) | 12 (41.4%) |
| Minimal Change Disease (MCD) | 3 (10.3%) |
| IgM Nephropathy | 01 (3.4%) |
| Acute glomerulonephritis | 01 (3.4%) |
| **Total** | **29 (100%)** |

**Figure 4: Bar chart showing kidney biopsy pathology**



**Investigations in nephrotic syndrome**

Table 5a shows the urine examination in NS patients. In our study, majority of NS patients had 2+ urine albumin (57.4%) and normal urine routine (67.2%). Table 5b shows the average values of various investigations in NS patients.

**Table 5a: Urine examination of NS patients**

| **Urine Examination** | | **Frequency (%)** |
|---|---|---|
| Urine albumin | 1+ | 14 (23%) |
| | 2+ | 35 (57.4%) |
| | 3+ | 12 (19.7%) |
| Urine Routine | Normal | 41 (67.2%) |
| | 1-2 epithelial cells | 19 (31.1%) |
| | 8 WBCs | 1 (1.6%) |

**Table 5b: Average levels of various investigations in NS patients**

| **Investigations** | **Mean±SD** |
|---|---|
| GFR (ml/min/1.73m$^2$) | 115.5 ± 21.6 |
| Urine VDBP (ng/mL) | 1338.8 ±1915.3 |
| Serum Creatinine (mg/dl) | 0.322 ±0.22 |
| Serum Albumin (mg/g) | 2.22 ±0.965 |
| Serum cholesterol (md/dl) | 183.9±48.3 |

**Comparison between SRNS and SSNS**

Table 6a and table 6b shows the comparison of SRNS and SSNS patients with respect to patient characteristics and various investigations. We found that GFR was significantly less and urine VDBP was significantly more in SRNS compared to SSNS when independent sample t-test used and applied to see difference of means of 2 groups ($p<0.05$)

**Table 6a: Comparison of patient's characteristics.**

| Variables | | SRNS | SSNS | P value |
|---|---|---|---|---|
| **Mean Age in years** | | 6.82±5.4 | 6.82±4 | 0.997 |
| **Age Group** | <5 Years | 8 (47.1%) | 23 (52.3%) | 0.831 |
| | 6- 10 Years | 4 (23.5%) | 13 (29.5%) | |
| | 11- 15 Years | 4 (23.5%) | 6 (13.6%) | |
| | 16-18 Years | 1 (5.9%) | 2 (4.5%) | |
| **Gender** | Males | 9 (52.9%) | 31 (70.5%) | 0.237 |
| | Females | 8 (47.1%) | 13 (29.5%) | |
| **Pathology** | Normal | 8 (47.1%) | 24 (54.5%) | 0.948 |
| | MPGN | 4 (23.5%) | 8 (18.2%) | |
| | FSGS | 4 (23.5%) | 8 (18.2%) | |
| | MCD | 1 (5.9%) | 2 (4.5%) | |
| | IgM Nephropathy | 0 | 1 (2.3%) | |
| | Acute glomerulonephritis | 0 | 1 (2.3%) | |

**Table 6b: Comparison of various investigations in SRNS and SSNS patients**

| Variables | | SRNS | SSNS | P value |
|---|---|---|---|---|
| Urine albumin | 1+ | 2 (11.8%) | 12 (27.3%) | **0.05** |
| | 2+ | 14 (82.4%) | 21 (47.7%) | |
| | 3+ | 1 (5.9%) | 11 (25%) | |
| Urine Routine | Normal | 15 (88.2%) | 26 (59.1%) | 0.077 |
| | 1-2 epithelial cells | 2 (11.8%) | 17 (38.6%) | |
| | 8 WBCs | 0 | 1 (2.3%) | |
| GFR (ml/min/1.73m$^2$) | | 81.71±4.98 | 128.5±4.34 | **<0.001** |
| Urine VDBP (ng/mL) | | 3128.3±1777.7 | 647.43±1478.9 | **<0.001** |
| Serum Creatinine (mg/dl) | | 0.23±0.12 | 0.36±0.24 | **0.043** |
| Serum Albumin (mg/g) | | 2.11±0.83 | 2.26±1.01 | 0.597 |
| Serum cholesterol (md/dl) | | 196±47.2 | 179.3±48.4 | 0.229 |

**Correlation between urine VDBP and other investigations in NS patients**

Table 7 shows the correlation between urine VDBP and other investigations levels in NS patients. We found that there was significant very high negative correlation between GFR and Urine VDBP levels in NS patients ($p<0.001$).

**Table 7: Correlation between urine VDBP and other investigations in NS patients**

| Urine VDBP | Correlation (r) | P value |
|---|---|---|
| **GFR** | -0.552 | **<0.001** |
| **Serum Cholesterol** | 0.044 | 0.737 |
| **Serum Albumin** | 0.061 | 0.639 |
| **Serum Creatinine** | -0.224 | 0.082 |

**ROC analysis**

We performed a ROC curve study to assess the ability of urine V-DBP to diffrentiate between patient with SR-NS and patient with SS-NS (Figure 8). VDBP's ability to distinguish between SRNS and SSNS had an area under the curve (AUC) of 0.883 (p 0.001; 95% CI= 0.795-0.971).

**Figure 8: ROC curve for Urine VDBP**

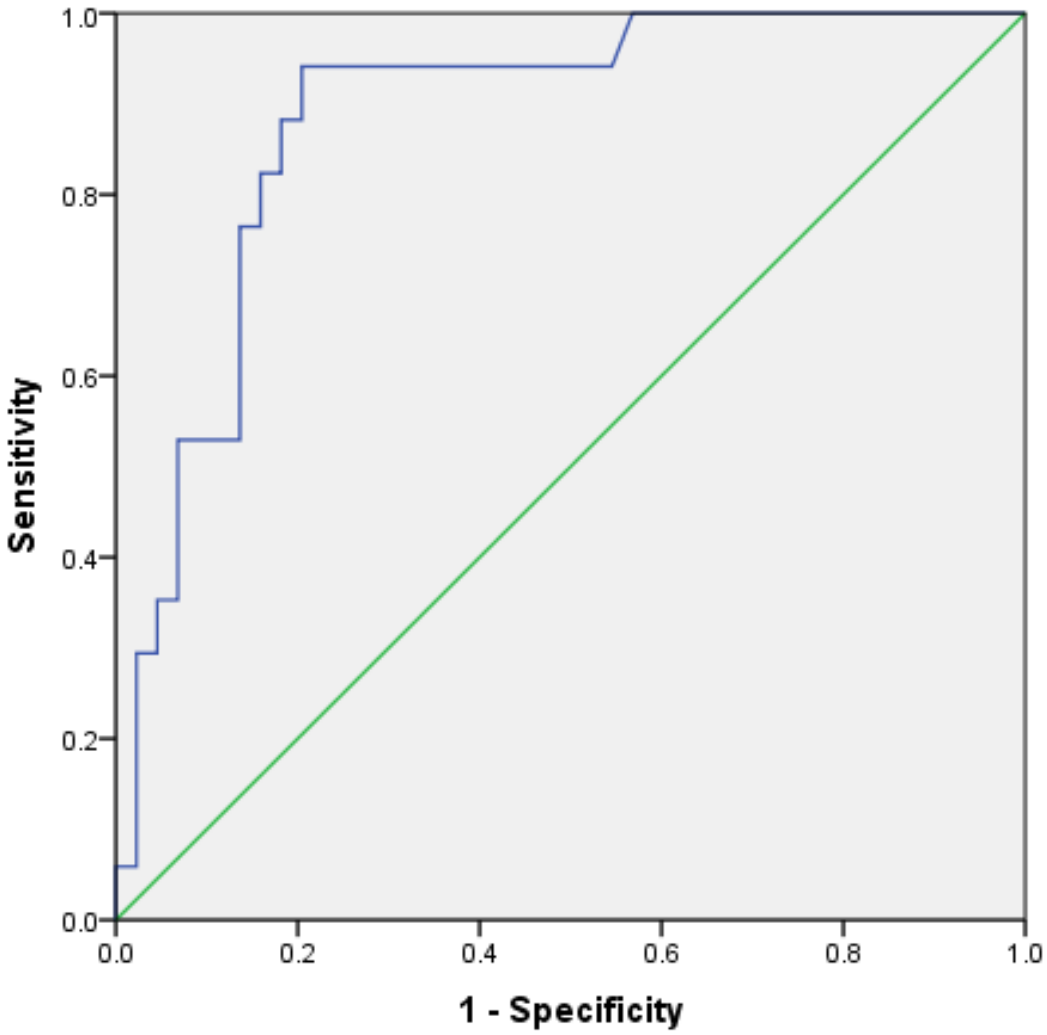


Table 8 shows the mean UVDBP values among different pathologies of nephrotic syndrome patients. There was no statistically significant difference in mean UVDBP among different pathologies when one-way ANOVA was applied (p>0.05)

**Table 8: Comparison of mean UVDBP among various pathologies**

| **Pathology** | **UVDBP (Mean ± SD)** | **p-value** |
|---|---|---|
| Membranoproliferative glomerulonephritis (MPGN) | 1388.1±2021.1 | 0.825 |
| Focal Segmental Glomerulosclerosis (FSGS) | 1714.5±2107.03 | |
| Minimal Change Disease (MCD) | 25.5±9.2 | |
| IgM Nephropathy | 2096.5±2945.1 | |
| Acute glomerulonephritis | 17±0 | |

# DISCUSSIONS

Steroid-resistance in INS is highly correlated to unfavourable prognosis, such as the development of ESRD. SSNS and SRNS cannot currently be distinguished by validated diagnostic markers, and patients frequently need to undergo therapy using corticosteroid of high dose before invasive biopsy of the kidney and patho-histologic diagnosis. Our goal for conducting these studies was able to see if uV-DBP measures might get useful as non-invasive bio-marker to differentiate between SR-NS, that typically has a prognosis that is poor and progression with a lot of high risk , and SS-NS beinf more benign.

The present study showed that 65.5% of cases were males and 34.4% were females with their mean age 6.82 ±4.4 years. From the above results, male predominance among nephrotic patients is clear. This was in accordance with Marzouk et al.[1] who reported that the mean age of nephrotic children was 5.30 ± 2.45 years. Jayesh R Solanki et.al.[2] also reported majority were males (fifty five point nine %) and 44.1% were females. The mean age was 5.7±2.7 years (ninty five % confidence interval: 4.79–6.68), and the range was two –twelve years. In a study by Sahana KS[3], children presented between the ages of two to fifteen years with mean age at presentation being seven and half years and seventy six % of the cases were male while as twenty four % of cases were female with male to female ratio of 3.27:1 suggesting male preponderance. Familiar observations were seen by Chahar OP et al.[4] and Shastri NG et al.[5] Siegal NJ et al.[6]

Our study showed that 72.1% of cases were steroid sensitive (SSNS) and 27.9% of cases were steroid resistant (SRNS). In a study by Mohammad Abdelmonaem Sharaf et.al.[7] reported that 53.3% of cases were steroid sensitive

(SSNS) and 46.7% of cases were steroid resistant (SRNS). This agrees with Bennett et al.[8] who reported that 46% of patients had SRNS, 54% of patients were SSNS. Sahana KS[3] reported that majority of cases (ninty seven %) were responders to therapy with steroid and 3% had SRNS. Madani et al,[9] also noted that ninety six % of children with minimal change NS were responding to steroid therapy.

VDBP is a liver-primarily generated circulating plasma protein in the alpha globulin region. Although some physiological events, such as pregnancy, can raise its levels, its production is generally steady in both children and adults. All varieties of NS frequently exhibit vitamin D insufficiency, which is primarily brought on by the excessive excretion of VDBP in urine. [10] Patients with NS excrete VDBP, which has a similar molecular weight and isoelectric point to albumin.[11]

In our study, majority of NS patients had 2+ (100 to 300 mg/dL) urine albumin (57.4%) and 3+ proteinuria (300 to 1000 mg/dL) in 19.7% patients. According to Mohammad Abdelmonaem Sharaf et.al.[7] study the mean 24hrs urinary proteins was 6200.9+ 3912.8 mg/24h ranging between 958.4 and 18840.0 mg/24h. Mean serum albumin was 1.7+0.4g/dl ranging between 0.9 and 2.5 g/dl. In contrast to this, Marzouk et al.[1] stated that the mean 24hrs urinary proteins of NS children was 1270+1860 mg/24h ranged between 20and 8500 mg/24h. Moneim et al.[12] reported that mean 24h proteinuria was 2880±1120 mg/24h in SRNS and 2520±800 mg/24h in SSNS. Aggarwal et al.[13] reported that mean 24h proteinuria was 5510 ± 3240 mg/24h in nephrotic syndrome adult patients, ranging between 1700 and 19000 mg/24h.

In the present study, the mean uVDBP level of idiopathic nephrotic sundrome patients was1338.8 ±1915.3 ng/ml and Mohammad Abdelmonaem Sharaf et.al.[7] reported the mean uVDBP level of cases 433.3+ 200.3 ng/ml while the mean uVDBP

level of controls was 49.2+ 18.0 ng/ml. It was clear that levels of uVDBP were significantly higher in cases than in healthy control children (p=0.000). Our results agreed with the results of Bennett et al.[8] who found that uVDBP concentrations were markedly increased in patients than in controls.

Our study reported that the mean uVDBP among SRNS was 3128.3±1777.7ng/mL and among SSNS was 647.43±1478.9ng/mL. There was significantly increased uVDBP among SRNS patients compared to SSNS. Bennett et al.[8] also reported higher urinary VDBP level in SRNS versus SSNS patients. According to our findings, patients with SRNS had significantly higher uVDBP concentrations than those with SSNS (P 0.001). Previous studies looking into VDBP in NS revealed significant associations between proteinuria and uVDBP. [14,15] However, these earlier investigations didn't look into the distinctions between SRNS and SSNS. It is possible to have a process that is specific to a disease causing elevated urineV-DBP in patient with SteroidResistant NS, as evidenced by the uVDBP's high discriminatory power (AUC = 0.883, P0.001) between patients with SRNS and SSNS. One probable explanation is that the integration of receptors of meglin and cublin in the pct is necessary for the reabsorption of any filtered VDBP. Therefore, increased uVDBP excretion could occur as a result of any type of chronic damage to tubule, which can be expected in SteroidResistant NS.

We conducted a ROC curve study to evaluate the urine VDBP's ability that can didderentiate between patients with Steroid Resistant NS and patients with Steroid Sensitive NS. For VDBP to discriminate SRNS from SSNS, the area under the curve (AUC) was 0.883 (95% CI= 0.795-0.971; p 0.001) In order to assess the potential of various urine indicators to predict steroid responsiveness in kids with

idiopathic NS, Choudhary et al.[16] published ROC curve analysis. According to their results, uVDBP showed an area under the curve (AUC) of 0.897 indicating that uVDBP had a significant predicting power with cutoff value of 303.8ng/ml. They concluded that uVDBP can be used to predict the steroid responsiveness accurately in NS children. In the present study, there was no significant correlation between values of 24h urinary proteins and uVDBP levels (P> 0.05). Also, there was a nonsignificant correlation between serum albumin and uVDBP levels.

In addition to the results of the present study, Grymonprez et al.[14] and Doorenbos et al.[15] investigated urineV-DBP in NS children and showed strong similarities between uV-DBP and protein levels in urine . In addition, Bennett et al.[8] reported positive relation of urineV-DBP excretion and levels of protein in urine (r equals 0.66, P less than 0.001).

In fact, it has recently been shown that uVDBP excretion may serve as a marker for fibrosis and interstitial injury to the kidneys. 17 In Adriamycin nephrosis of a rat model, this study showed enhanced urine excretion of uVDBP extremely early, before the beginning of inflammatory renal interstitial injury and fibrosis. In human beings , urineV-DBP has been elevated in pateint those have albuminuria and also in CKD patient along with overt proteinuria than a variety of causative factors(etiology). Urine VDBP levels of CKD patient decreased to some extent with tretament using reno-protective therapy, but stayed hundred-times higher at the time of maximum therapy compared to control (P= 0.001), showing the level remain same even in the absence of proteinuria.

This would be in line with our findings that uVDBP levels are substantially higher in those patient with Steroid Resistant NS which could be accounted for by the levels of protein in urine alone, even along patient with Steroid Resistant NS and Steroid Sensitive NS with high-grade active levels of protein in urine . If the relationship between uVDBP and these histological findings can be further validated, it may make uVDBP a useful potent distinguisher to see fibrosis and T1 damage in patient with Nephrotic Syndrome. There have been failed attempts previously to identify something that might mark characteristics fibrotically in the NS. Beta- 2 and NAG, tubular damage indicators, were measured in the urine of FSGS patients by Valles et al.18 throughout disease that is active and remission over follow-up period of three years. Although urinary NAG levels in patients with FSGS were found to be higher than those in patient with Steroid Sensitive NS and steroiddependent nephritis, no correlation was observed betwen these patients' levels of beta 2M or NAG with signs of tubulointerstitial (TI) damage histologically.

# CONCLUSION

- Hence Urinary Vitamin D Binding protein levels are significantly higher in steroid resistant nephrotic syndrome as to steroid sensitive nephrotic syndrome.
- uVDBP levels can be a early biomarker and also a non invasive method for early diagnosis of steroid reistant nephrotic syndrome.
- Study did not show any significant correlation between uVDBP and the pathologies.
- As its single center study, more multi-centered and larger number of studies are required to make out findings more concrete.

# SUMMARY

The study was conducted over one year from 2021 to 2022 at KLEs Prabhakar Kore Hospital. All the samples collected were a spot sample among OPD/IPD Nephrotic syndrome patients. The collected samples were stored at the BSRC lab(KLE) at $-20^0$, all the samples were run using commercially available ELSIA kit to detect the urinary vitamin D protein levels in nephrotic syndrome patient.

Results were recorded in tabular and graphical forms Mean, median, SD and ranges were accounted for quantitative data. Chi square analysis was useful in testing for major differences between proportions and frequencies. T test was useful in testing for majot differences between two means. The confidence interval was set to be at ninty five % limit, with level of the significance to be at p: < 0.05.

- The study included 61 nephrotic syndrome patients, of whom a clean mid stream urine sample was collected, stored and investigated using the ELISA(R&D) kit.
- The mean age (SD) of the studied population was 6.82 ±4.4 years. Majority of the nephrotic patients were aged less than 5 years.
- There were 40 (65.6%) males and 21 (34.4%) females with a M: F ration of 1.9:1.
- Most of our study subjects were steroid sensitive (72.1%) and 27.9% patients had SRNS
- Majority of the patients had not undergone kidney biopsy (52.4%). Among 29 of kidney biopsy pathology reports, there were 12 (41.4%) each subject with MPGN and FSGS, 3 (10.3%) subjects with MCD and 1 (3.4%) patient each with IgM nephropathy and acute glomerulonephritis

- Most of NS patients had 2+ urine albumin (57.4%) and normal urine routine (67.2%)
- Our study showed GFR was significantly less and urine VDBP was significantly more in SRNS compared to SSNS when independent sample t-test was applied to compare means of two groups ($p<0.05$)
- There was significant very high negative correlation between GFR and Urine VDBP levels in NS patients ($p<0.001$).
- The ROC curve analysis showed the area under the curve (AUC) for VDBP to distinguish SRNS from SSNS was 0.883 ($p< 0.001$; 95% CI= 0.795–0.971).
- There was no statistically significant difference in mean UVDBP among different pathologies when one-way ANOVA was applied ($p>0.05$)
- Hence Urinary Vitamin D Binding protein levels are significantly higher in steroid resistant nephrotic syndrome as to steroid sensitive nephrotic syndrome.
- uVDBP levels can be a early biomarker and also a non invasive method for early diagnosis of steroid reistant nephrotic syndrome.
- Study did not show any significant correlation between uVDBP and the pathologies.
- As its single center study, more multi-centered and larger number of studies are required to make out findings more concrete.

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# ANNEXURE - I

## INFORMED CONSENT FORM

**K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH**

**"URINARY VITAMIN D-BINDING PROTEIN LEVELS IN IDIOPATHIC NEPHROTIC SYNDROME CHILDREN."-A CROSS SECTIONAL STUDY**

**Principal Investigator : REGISTRATION NO: BM0120002**

**Guide: Dr. _____**

You are hereby requested to involve yourself/your child in the above said research to be conducted at KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2020 to December 2020 by me.

**Introduction:**The study aims to investigate urinary vitamin D-binding protein levels in idiopathic nephrotic syndrome children and and attempt will be made to diagnose the steroid resistant nephrotic syndrome in an early and non inivasive way.

**Voluntary participation:** You and your child's participation in this study is your voluntary decision. Whether to participate or not to participate will not affect your current or future relationship with the KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. You are free to discontinue the participation in the study at any time for any reasons and you will not be paid any reimbursement for participation in the research.

**Risk and benefits:** There are no major risks involved

**Privacy and Confidentiality:** The only people who will know that you are a research participant are member of the research team. No information about you or provided by you, during research will be disclosed to others without your written consent. When the results of the research are published or discussed in the conferences, no information will be disclosed that would reveal your identity. Any information obtained in connections with this study and that can be identified with you remain confidential and will be disclosed only with your permission.

Queries

If you have any queries you may contact

REGISTRATION NO: BM0120002
POST GRADUATE STUDENT
DEPARTMENT OF PEDIATRICS
JNMC, BELAGAVI-590010

DR _____
CONSULTANT PEDIATRICIAN & PEDIATRIC NEPHROLOGIST
PROFESSOR AND HEAD
DEPARTMENT OF PEDIATRICS,
KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELGAUM -590010.

If you have any questions about your rights or research participation you may contact

DR. ROOPA M BELLAD,MD,DCH

PROFESSOR,

DEPARTMENT OF PEDIATRICS.

KAHER

K.L.E UNIVERSITY'S

JAWAHARLAL NEHRU MEDICAL COLLEGE, BELGAUM -590010.

You will be given a copy of this form for your information and to keep for your records

**STATEMENT OF CONSENT**

I hereby voluntarily agree for my and my childs participation in this study. I understand that even if I choose to allow my baby to take part in this study I have the liberty to withdraw at any time. My signature below indicates that I have read or have been told about this entire consent form including the risks and benefits and have had all my questions answered. I will be given a copy of this consent form.

Signature of the authorized representative/ parent: _____

Date: _____

Name: _____

Relation to the Subject: _____

Signature of the witness: _____

Date: _____

Name: _____

Signature of investigator:_____

Date: _____

Name: _____

## ANNEXURE-II

## PROFORMA

1)NAME

2)AGE

3)DATE

4)GENDER

5)HISTORY OF PRESENT ILLNSS:

6)PAST HISTORY:

7)FAMILY HISTORY:

8)BIRTH HISTORY:

9)IMMUNIZATION HISTORY:

10)ANTHROPMETRY:

11)VITALS:

12)BP CENTILE CHART:

13)INVESTIGATIONS:

| | |
|---|---|
| URINE ALBUMIN | |
| URINE ROUTINE MICROSCOPY | |
| SERUM CREATININE | |
| SERUM ALBUMIN | |
| SERUM CHOLESTEROL | |

14)GENERAL EXAMINATION:

15)SYSTEMIC EXAMINATION:

16) DIAGNOSIS:

# ANNEXURE III

## KEY TO MASTERCHART

S.Alb - Serum Albumin

S.Chol - Serum Cholesterol

UVDBP - Urinary vitamin D binding protein

AGN - Acute Glomerulonephritis

FSGS - Focal Segmental Glomerulosclerosis

MCD - Minimal Change Disease

NPGN - Non Proliferative Glomerulonephritis

SRNS - Steroid resistant nephrotic syndrome

SSNS - Steroid sensitive nephrotic syndrome

| Sl.no | age | gender | biopsy | Steriod responsiveness | U.Albumin | Urine routine | Creatinine | S. Alb | S.Chol | GFR | UVDBP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 4 | 1 | AGN | SSNS | 2+ | n | 0.18 | 2.5 | 268 | | 16.747 |
| **2** | 16 | 1 | FSGS | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 7.496 |
| **3** | 6 | 1 | nil | SSNS | 2+ | n | 0.23 | 1.2 | 154 | | 5.852 |
| **4** | 9 | 1 | nil | SSNS | 3+ | 1-2 epi cells | 0.72 | 1.1 | 190 | | 5.679 |
| **5** | 10 | 1 | nil | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 25.449 |
| **6** | 1 | 1 | nil | SRNS | 2+ | n | 0.2 | 2.1 | 220 | | 3419 |
| **7** | 3 | 2 | nil | SSNS | 2+ | n | 0.18 | 2.5 | 268 | | 19.726 |
| **8** | 4 | 1 | nil | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 6.281 |
| **9** | 17 | 1 | FSGS | SRNS | 2+ | n | 0.23 | 1.2 | 154 | | 3570 |
| **10** | 2 | 2 | nil | SSNS | 3+ | 1-2 epi cells | 0.72 | 1.1 | 198 | | 24.841 |
| **11** | 5 | 1 | MCD | SRNS | 2+ | n | 0.2 | 2.1 | 220 | | 4179 |
| **12** | 8 | 1 | nil | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 4154 |
| **13** | 3 | 1 | nil | SSNS | 2+ | n | 0.18 | 2.5 | 268 | | 7.103 |
| **14** | 3 | 1 | nil | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 5.894 |
| **15** | 14 | 1 | nil | SSNS | 3+ | 1-2 epi cells | 0.72 | 1.1 | 189 | | 20.561 |
| **16** | 11 | 1 | nil | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 5.842 |
| **17** | 3 | 1 | FSGS | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 25.737 |
| **18** | 4 | 2 | nil | SSNS | 2+ | n | 0.18 | 2.5 | 268 | | 20.93 |
| **19** | 4 | 1 | nil | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 6.732 |
| **20** | 3 | 1 | nil | SRNS | 2+ | n | 0.23 | 1.2 | 154 | | 48.726 |
| **21** | 6 | 2 | nil | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 91.925 |
| **22** | 5 | 2 | FSGS | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 4210 |
| **23** | 4 | 2 | nil | SSNS | 2+ | n | 0.18 | 2.5 | 268 | | 51.909 |
| **24** | 8 | 1 | FSGS | SRNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 4193 |
| **25** | 7 | 1 | nil | SRNS | 3+ | wbc 8 | 0.54 | 1.4 | 104 | | 4258 |
| **26** | 8 | 1 | nil | SRNS | 1+ | n | | | 146 | | 2877 |
| **27** | 3 | 2 | nil | SSNS | 1+ | n | 0.58 | 2.2 | 136 | | 5.569 |
| **28** | 7 | 1 | IGM Nephropathy | SSNS | 2+ | n | 0.77 | 1.5 | 128 | | 13.517 |
| **29** | 5 | 1 | nil | SSNS | 2+ | n | | | 198 | | 25.108 |
| **30** | 6 | 2 | nil | SRNS | 3+ | n | 0.68 | 2 | 132 | | 4207 |
| **31** | 3 | 2 | nil | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 4228 |
| **32** | 1 | 2 | nil | SRNS | 2+ | n | 0.2 | 2.1 | 220 | | 4232 |
| **33** | 6 | 1 | NPGN | SRNS | 2+ | n | 0.18 | 2.5 | 268 | | 4222 |
| **34** | 3 | 1 | NPGN | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 4027 |
| **35** | 15 | 2 | FSGS | SRNS | 2+ | n | 0.18 | 2.5 | 268 | | 4247 |
| **36** | 10 | 1 | NPGN | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 20.612 |
| **37** | 2 | 1 | FSGS | SRNS | 2+ | n | 0.23 | 1.2 | 154 | | 4245 |
| **38** | 7 | 1 | nil | SSNS | 3+ | 1-2 epi cells | 0.72 | 1.1 | 245 | | 25.669 |
| **39** | 14 | 1 | NPGN | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 31.221 |
| **40** | 12 | 2 | NPGN | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 5.957 |
| **41** | 2 | 2 | NPGN | SRNS | 2+ | n | 0.18 | 2.5 | 268 | | 33.512 |
| **42** | 17 | 1 | NPGN | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 12.69 |
| **43** | 8 | 1 | NPGN | SSNS | 2+ | n | 0.23 | 1.2 | 154 | | 9.376 |
| **44** | 1.5 | 1 | NPGN | SSNS | 3+ | 1-2 epi cells | 0.72 | 1.1 | 214 | | 37.009 |
| **45** | 12 | 2 | FSGS | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 14.972 |
| **46** | 1 | 1 | NPGN | SRNS | 2+ | n | 0.2 | 2.1 | 220 | | 4007 |
| **47** | 10 | 1 | NPGN | SSNS | 2+ | n | 0.18 | 2.5 | 268 | | 13.212 |
| **48** | 4 | 2 | FSGS | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 25.606 |
| **49** | 4 | 1 | MCD | SSNS | 1+ | n | 0.61 | 1 | 142 | | 32.123 |
| **50** | 14 | 1 | FSGS | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 7.135 |
| **51** | 9 | 1 | MCD | SSNS | 2+ | n | 0.23 | 1.2 | 154 | | 19.21 |
| **52** | 6 | 1 | FSGS | SSNS | 3+ | 1-2 epi cells | 0.72 | 1.1 | 222 | | 7.287 |
| **53** | 7 | 2 | nil | SRNS | 2+ | n | 0.2 | 2.1 | 220 | | 4269 |
| **54** | 5 | 2 | nil | SSNS | 3+ | n | 0.72 | 2 | 138 | | 9.316 |
| **55** | 6 | 1 | nil | SSNS | 2+ | n | 0.7 | 2.1 | 132 | | 5.559 |
| **56** | 5 | 1 | nil | SSNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 4124 |
| **57** | 3 | 2 | nil | SRNS | 2+ | n | 0.23 | 1.2 | 154 | | 15.44 |
| **58** | 5 | 2 | nil | SSNS | 3+ | 1-2 epi cells | 0.72 | 1.1 | 200 | | 7.726 |
| **59** | 5 | 2 | FSGS | SSNS | 2+ | n | 0.2 | 2.1 | 220 | | 21.425 |
| **60** | 8 | 2 | nil | SRNS | 1+ | 1-2 epi cells | 0.17 | 3.9 | 153 | | 3964 |
| **61** | 15 | 1 | NPGN | SRNS | 2+ | n | 0.23 | 1.2 | 154 | | 4237 |
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