A STUDY TO EVALUATE TROUGH CONCENTRATION OF TACROLIMUS IN CHILDREN WITH STEROID RESISTANT NEPHROTIC SYNDROME

A Thesis Submitted

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
CLINICAL RESEARCH

Division of Clinical Research, Department of Biosciences
School of Basic and Applied Sciences

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CANDIDATE'S DECLARATION

I, hereby certify that the work which is being presented in the thesis, entitled **"A Study to Evaluate Trough Concentration of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome"** in fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Clinical Research and submitted in Galgotias University, Greater Noida is an authentic record of my own work carried out during a period from 2017 to 2022 under the supervision of Prof. **(Dr) Ranjana Patnaik** and Co. Supervision of **Dr Abhijeet Saha** & **Dr Preeti Chauhan**.

The matter embodied in this thesis has not been submitted by me for the award of any other degree of this or any other University/Institute.

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This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

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CERTIFICATE

This is to certify that the thesis titled **"A Study To Evaluate Trough Concentration Of Tacrolimus In Children With Steroid Resistant Nephrotic Syndrome"** is a bonafide work of **Mr. Kaptan Singh Sehrawat** undertaken in the Department of Biochemistry & Department of Paediatrics, LHMC & Associated **Kalawati Saran Children's Hospital, New Delhi** under our supervision and guidance. This thesis fulfils all the requirements stipulated by the UGC.

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STATEMENT OF THESIS PREPARATION

A Study to Evaluate Trough Concentration of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome

1. Submitted for the degree of Doctor of Philosophy in Clinical Research under the guidance of Prof. (Dr.) Ranjana Patnaik (Supervisor) and Dr. Abhijeet Saha & Dr. Preeti Chauhan (Co-Supervisors)

2. Specifications regarding thesis format have been closely followed.

3. The contents of the thesis have been organized based on the guidelines.

4. The thesis has been prepared without resorting to plagiarism.

5. All sources used have been cited appropriately.

6. The thesis has not been submitted elsewhere for a degree.

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APPROVAL SHEET

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ABSTRACT

Nephrotic Syndrome (NS) is a common kidney disorder in adults and children. It is responsible for significant morbidity and mortality among children globally. The prevalence of childhood NS varies in different population from 12–16/100,000 children affecting all ages and ethnic background. In western countries, the incidence of Nephrotic Syndrome is a 2-3/100,000 child which is slightly higher in population with south Asian origin i.e. 2- 7/100,000. In United States, 2-7 cases per 100,000 children younger than 16 years are reported annually. However, in India the exact incidence and prevalence of this disease are still unknown.

The corticosteroids are used as first line treatment of NS. The most of the children with NS initially respond to steroids and achieve remission of proteinuria following 4-6 weeks of treatment with steroids. However, 10-15% patients do not achieve complete remission and are categorised as steroid resistant Nephrotic Syndrome (SRNS) patients. Recent studies have indicated significant increase in the number of steroid resistant Nephrotic Syndrome particularly in Southeast Asia.

SRNS is predominantly treated with calcineurin inhibitors (CNI) drugs like Cyclosporine and Tacrolimus. The Tacrolimus is an immunosuppressive drug that is being prescribed mainly to avoid rejection of organ transplantation. A rigorous literature review revealed that despite its significant clinical use, the pharmacokinetics data and its correlation with therapeutic efficacy is very limited in relation to the Indian paediatric population. It is pertinent to mention that in India, no major studies have been published to find out the trough concentration of Tacrolimus in children with SRNS. The therapeutic dose of Tacrolimus for the treatment of SRNS is extrapolated from kidney transplant patients which appear to be relatively high in the treatment of SRNS. Further, the Tacrolimus has a very narrow therapeutic range which means that a minor decrease in drug dose may affect the treatment and on the other side a minor increase in drug dose may cause nephrotoxicity. The clinical management of SRNS remains a challenging task for nephrologist due to various side effects related to immunosuppression such as infections, nephrotoxicity, cytopenia neurotoxicity and malignancies. Further, the hypoalbuminemia in SRNS may lead to reduced protein binding, and gut edema can lead to uneven absorption of the drug

which may also be a cause of an altered volume of drug distribution or clearance. Hence, regular therapeutics monitoring of drug is utmost important for the safety and efficacy during the treatment. As the AUC of drug requires multiple samplings, so due to these ethical and procedural limitations the Trough concentrations monitoring of drug has become the popular method in children for monitoring the toxicity and efficacy in clinical practice today.

The present study was undertaken to evaluate the trough level of Tacrolimus with SRNS and its correlation with treatment outcomes in 60 children. Trough level of Tacrolimus was measured after a minimum of 12 weeks of treatment using Particle-Enhanced Turbidimetric Immunoassay (PETIA). Haematological and biochemistry tests were also done on blood and urine samples of 60 pediatric patients of SRNS at the diagnosis and after 12 weeks of treatment. The initial outcome of treatment in patients was decided after minimum 12 weeks as per ISPN guidelines. [1]

The results of laboratory investigations presented that 81% (49/60) of patients had complete remission while 7% (4/60) were in partial remission and 12% (7/60) were in non-remission state after minimum 12 weeks of initial treatment with Tacrolimus. The mean trough level of Tacrolimus of (n=60 patients) after a 12 weeks of Tacrolimus treatment was found 6.6±2.2 ng/mL. The mean C_0 of Tacrolimus was higher in the remission group than in the non-remission group (7.0 ng/ml, versus 4.18 ng/ml, (p=0.004). The mean C_0 of Tacrolimus in partial remission group was 5.8 ng/ml. The mean drug dose of Tacrolimus in patients with remission (n=49) was 0.11 mg/kg/daily and the mean dose of Tacrolimus in partial remission and non-remission patients was found 0.10 mg/kg/daily.

A significant correlation between three important laboratory parameters i.e. C_0 of Tacrolimus (p=0.004) UP: UC ratio (p=<0.001), and serum albumin level (p=<0.001) was observed in remission, partial remission, and non-remission groups. 63% patients (n=38/60) were late resistant to steroid treatment while 38% (n=22/60) were initial resistant. The mean age at onset of diseases was 67.08 months. The male children were higher at 63% (n=38/60) while female children were 37% (n=22/60). The Kidney biopsy was done in 43.3% (n=26/60 patients).

The results of kidney Biopsy presented that minimal change disease (MCD n=17/26) was the most common histological diagnosis, followed by Focal Segmental Glomerulosclerosis (FSGS n=9/26). The edema was noted in the majority of patients at the onset of the disease.

Significant correlation was found between Serum albumin and Serum cholesterol between different groups. Many related clinical conditions like Edema 43.3% (n=26/60), Hypertension 16.6% (n=10/60), Hematuria 17.6% (n=9/60), Oliguria 6.6% (n=4/60), Fever 11.6% (n=7/60), Joint pain 5% (n=3/60), rashes 3.3% (n=2/60) were noted in patients during SRNS treatment. The major side effect of Tacrolimus noted during treatment were heartburn/acidity in 28.3% (n=17/60), loss of appetite, 23.3% (n=14/60), Nausea 13.3% (n=9/60), Hypertension 16.6% (n=10/60). No patient had hypertrichosis. The QC results of Particle-enhanced Turbidimetric Immunoassay (PETIA) were consistent and reported within 2±SD.

We could demonstrate that children with SRNS in remission had higher trough level of Tacrolimus compare to children with partial and non-remission patients. Trough level of Tacrolimus negatively correlated with protein excretion and positively correlated with serum albumin levels. It is concluded that regular monitoring of trough level of Tacrolimus in SRNS may play an important role in treatment outcomes. The PETIA can be an alternative to LCMS in diagnostic settings. However, a pharmacokinetic study with large sample size and a long term follow-up with more patients is required to substantiate our findings.

Dedication

Every challenging work needs consistent hard work and sincere efforts as well as the guidance of the family and friends especially those who are very close to our heart.

I dedicate this work to my sweet & loving family and inspiring friends who always stood by me in all possible ways.

And especially

To my loving mother

Whose affection, love, encouragement and her prayers & blessings make me able to get such success & honour

Along with my hardworking and respected Teachers.

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"That a man is successful who has lived well, laughed often, and loved much, who has gained the respect of the intelligent men and the love of children; who has filled his niche and accomplished his task; who leaves the world better than he found it, whether by an improved poppy, a perfect poem, or a rescued soul; who never lacked appreciation of earth's beauty or failed to express it; who looked for the best in others and gave the best he had." - Robert Louis Stevenson

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Publication-2

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LIST OF PRESENTATION

Sr. No.	Name of Conference	Title of Presentation
1.	35th World Congress of International Federation of Biomedical Laboratory Science (IFBLS-2022) 5-9th October, 2022 Seoul South Korea.	Trough Level of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome
2	International Conference of Forensic & Criminology (AGORA-2021), 5-7 May, 2021 Galgotias University, Noida	Clinical Profile and Therapeutic Level of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome-A Single Centre Study
3	International Conference of Forensic & Criminology (AGORA-2021), 5-7 May, 2021 Galgotias University, Noida	Quality Assurance in therapeutic Drug monitoring in children using Particle enhanced turbidimetric immunoassay

LIST OF ABBREVIATIONS AND SYMBOLS USED

Abbreviations	**Full Form**
µ	Micron
µg	Microgram
µl	Microliter
AUC	Area under curve
BP	Blood Pressure
Co	Trough Concentration
Conc.	Concentration
CVD	Cardiovascular Diseases
DBP	Diastolic Blood Pressure
FRNS	Frequent Relapsing Nephrotic Syndrome
FSGS	Focal Segmental, glomerulonephritis
GC-MS	Gas Chromatography-Mass Spectrometry
Hb	Haemoglobin
Hg	Mercury
HPLC	High Performance Liquid Chromatography
Hr	Hour
HR	Heart Rate
HTN	Hypertension
KFT	Kidney function Test
LC-MS/MS	Liquid Chromatography- Tandem Mass
LFT	Liver Function Test
Max C	Maximum Concentration
MCD	Minimal change disease
mg	Milligram

Abbreviations	Full Form
Min	Minutes
ml	Milliliter
ng:	Nanogram
NS	Nephrotic Syndrome
PETIA	Particle-enhanced Turbidimetric Immunoassay
pH	Hydrogen ion concentration
PR	Pulse Rate
RBC	Red Blood Cell
RPM	Revolutions per minute
S.D	Standard deviation
SBP	Systolic Blood Pressure
SD	Standard Deviation
SRNS	Steroid Resistant Nephrotic Syndrome
SSNS	Steroid Sensitive Nephrotic Syndrome
Std.	Standard
UP:UC	Urine Protein-Creatinine Ratio
WBC	White Blood Cell
WHO	World Health Organization

GLOSSARY OF DEFINITIONS

Nephrotic Syndrome	Nephrotic range proteinuria (40 mg/m^2/hr or >1000 mg/m^2/day; spot Up/Uc >2 mg/mg; 3-4+ by dipstick); hypoalbuminemia (albumin <3.0g/dL); and edema
Steroid Sensitive Nephrotic Syndrome	Complete remission within 6-weeks' treatment with prednisolone at a dose of 60 mg/m^2/ day (2 mg/kg/day; maximum 60 mg/day)
Initial Steroid-Resistance	Failure to achieve complete remission after 6-weeks initial therapy with prednisolone (as defined above)
Late (Secondary) Steroid-Resistance*	Initially steroid-sensitive; steroid resistance in a subsequent relapse (Patients with steroid toxicity may receive daily Prednisolone for 4 weeks, followed by alternate-day therapy for 2 weeks)
Complete Remission:	Urine protein nil-trace by dipstick for 3 consecutive days, Up/Uc<0.2, or 24-hr protein <100 mg/m2/day
Partial Remission	Urine protein 1+/2+ (dipstick), Up/Uc between 0.2-2, or 24-hr urine protein 100-1000 mg/m2/day; serum albumin ≥3.0 g/dL; and absence of edema
Non-response	Urine protein 3+/4+ (dipstick), Up/Uc >2, or 24-hr urine protein >1000 mg/m2/day; albumin <3.0 g/dl or edema
Relapse	Urine albumin 3+/4+ for 3 consecutive days, Up/Uc>2, or 24-hr protein >1000 mg/m2/day, in a patient previously in partial or complete remission
Monogenic Disease	Pathogenic or likely pathogenic variation, defined by American College of Medical Genetics and Genomics, in a gene associated with Nephrotic Syndrome.
CNI-resistant Disease	Non-response to Cyclosporine or Tacrolimus, given in adequate doses and titrated to blood levels, for 6-months
CNI	Calcineurin inhibitor
UP: UC	Urine protein to Urinary Creatinine ratio (mg/mg)

CHAPTER-1

INTRODUCTION

1. INTRODUCTION

1.1 Nephrotic Syndrome:

The Nephrotic Syndrome (NS) is a common renal disorder in children. It is a clinical manifestation of glomerular diseases. The glomeruli (tiny blood vessels) are filtering units of the kidney and the damage of glomeruli causes kidney to release too much protein into urine. The common characteristics of Nephrotic Syndrome are nephrotic range proteinuria: (40 mg/m^2/hr or >1000 mg/m^2/day; spot UP/UC >2 mg/mg; 3-4+ by dipstick); hypoalbuminemia (albumin <3 g/dL; and edema [1].

1.2 Incidence & Prevalence:

The prevalence of childhood Nephrotic Syndrome (NS) varies in different population from 12–16/100,000 children. It affects all ages and ethnic background. The reported incidence of Nephrotic Syndrome is 2-3/100,000 children in western countries, slightly higher in 2- 7/100,000 in south Asian origin [2]. In the United States, the reported annual incidence rate of Nephrotic Syndrome is 2-7 cases per 100,000 children younger than 16 years. The cumulative prevalence rate is approximately 16 cases per 100,000 individuals. The incidence of Nephrotic Syndrome (NS) is 1·15–16·9 per 100, 000 children, varying by ethnicity and region [3]

1.3 Pathophysiology of Nephrotic Syndrome:

The common characteristics of Nephrotic Syndrome are massive proteinuria, hypoalbuminemia, hyperlipidemia and edema. The albumin helps to carry various substances including hormones, vitamins and enzymes throughout body and also helps to pull extra fluids to kidneys which is released into urine. The loss of protein (albumin) causes body to retain extra fluids which causes swelling (edema) in ankles, face, eyelids, legs and feet.

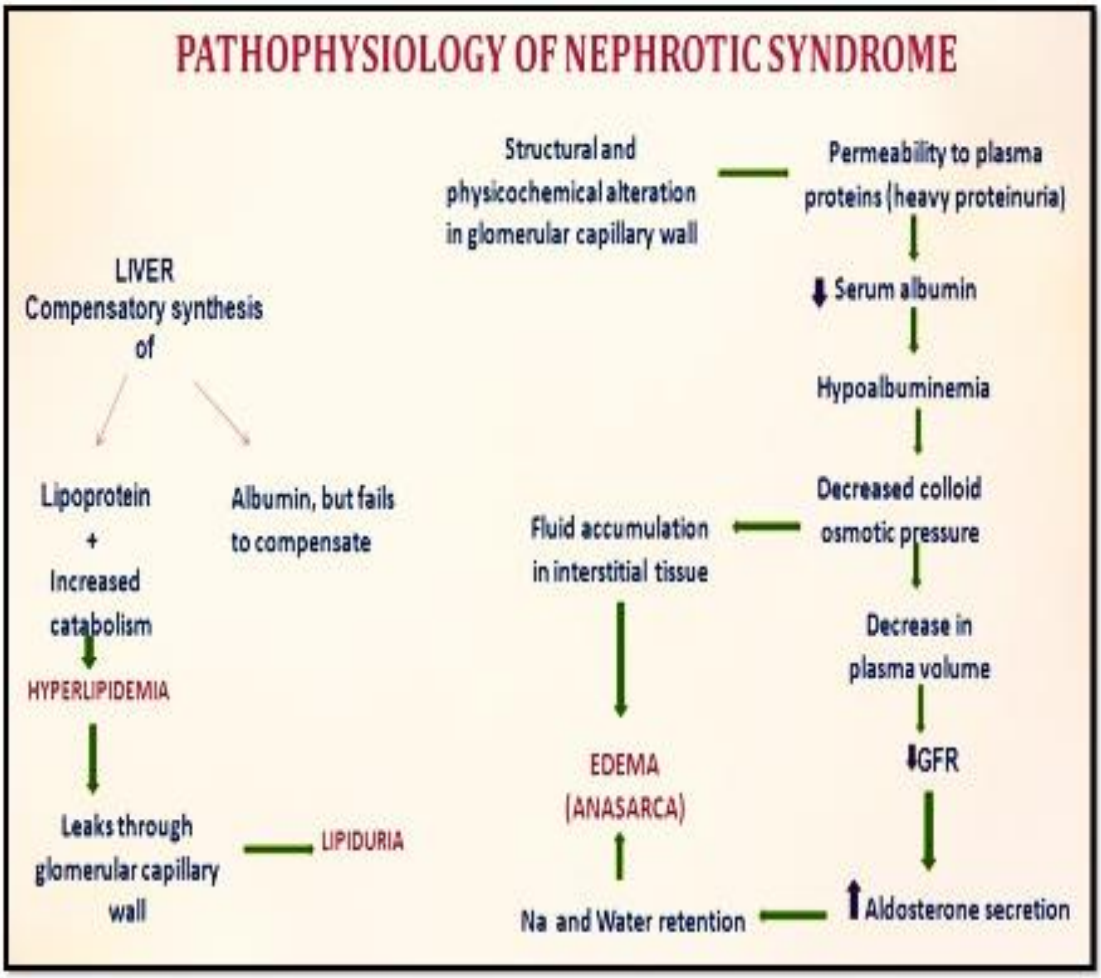
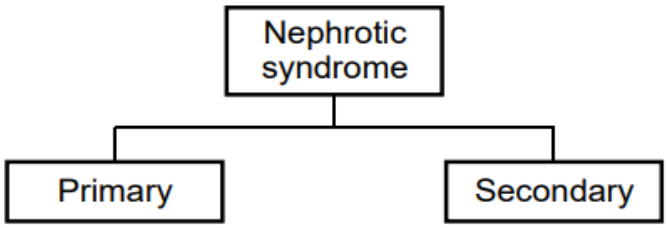


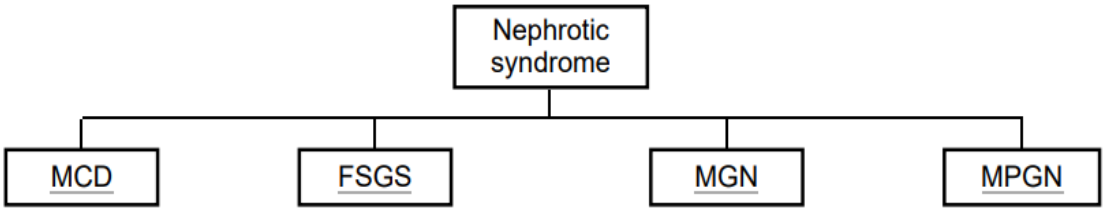
Figure No.1-Flow Chart showing Pathophysiology of Nephrotic Syndrome

1.4 Classification of Nephrotic Syndrome:

a) **General classification**: A broad classification of Nephrotic Syndrome based on underlying causes



b) Histopathological Classification



1.5 Etiology of Nephrotic Syndrome:

1.5.1 Primary Nephrotic Syndrome: There are few conditions when the only kidneys are affected due to nephrotic syndrome. These conditions are categorised primary causes of Nephrotic Syndrome. The following are major conditions of primary Nephrotic Syndrome.

a) Focal Segmental Glomerulosclerosis (FSGS): This is the condition when glomeruli are damaged due to genetic defect or an unknown reason.

b) Minimal change disease (MCD): the kidney tissue appear normal in a biopsy report under microscope in this condition, yet it does not filter properly due to some unknown reason.

c) Membranous nephropathy: In this condition, the membranes in the glomeruli thicken. However, the causes of thickening are not known, but sometimes it may occur due to lupus, hepatitis B, Cancer or Malaria.

d) Renal Vein thrombosis: In this disorder a vein that drains blood out of the kidney is blocked due to blood clot.

1.5.2 Secondary Nephrotic Syndrome: There are many other secondary reasons of Nephrotic Syndrome which affects entire body and causes due to many diseases like Diabetes, Lupus, and amyloidosis etc.

a) Diabetes: In this disease, the uncontrolled blood sugar may damage blood vessels in the kidneys.

b) Lupus: The Auto-immune disease like Lupus can causes inflammation in the joints, kidney and other organs.

c) Amyloidosis: This is a rare disease caused due to build-up of the protein amyloid in organs that can damage kidneys also.

d) Medication: Some drugs including non-steroidal anti-inflammatory drugs (NSAID) have also been linked to NS.

The Primary and secondary both types can occur in children, however primary Nephrotic Syndrome is most common. Few Children may also have congenital Nephrotic Syndrome which happens in the first 3 months of life. This can be due to inherited genetic defect or may be due to an infection just after birth. Children with this condition may eventually need a kidney transplant. Infantile Nephrotic Syndrome happens from 3 to 12 months and Childhood Nephrotic Syndrome occurs 12 months or older.

1.6 Diagnosis of Nephrotic Syndrome:

The clinical laboratory tests play an important role in timely diagnosis of Nephrotic Syndrome. The Clinical Criteria for Diagnosis of Nephrotic Syndrome was followed as per ISPN guidelines [1]. The following laboratory tests are required to diagnose Nephrotic Syndrome-

Urine Examination for Nephrotic range proteinuria : (40 $mg/m^2/hr$ or >1000 $mg/m^2/day$) a. Spot UP:UC : >2 mg/mg; b. Urinary Protein by dipstick : 3-4+ c. Serum albumin : <3.0g/dL d. Edema : Present	

Fig No. 2 Clinical Criteria for Diagnosis of Nephrotic Syndrome

Several other blood tests are performed to see the level of serum Total protein and albumin, Kidney function Tests (KFT), serum cholesterol and triglycerides levels.

1.7 Treatment & Clinical Management of Nephrotic Syndrome:

The treatment of the Nephrotic Syndrome is done to treat the conditions that causes Nephrotic Syndrome and to control the symptoms of this syndrome.

a) **Treatment of Diseases:** 6-weeks therapy with prednisolone at daily dose of 60 mg/m² is given for the treatment of Nephrotic Syndrome.

b) **Supporting treatment:** A variety of medicines are given for clinical management and control of symptoms

c) **Blood Pressure Medications:** This is required to control blood pressure and to reduce the amount of protein loss in to urine. These medicines includes angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers.

d) **Diuretics:** Diuretics cause your kidneys to release extra fluids which helps in reducing swelling.

e) **Statins:** These drugs lower cholesterol levels. Few examples includes atorvastatin, calcium, lovastatin etc.

f) **Blood Thinner:** In case any blood clot is reported in kidneys, medicines are given to reduce the blood ability to clot like heparin and warfarin.

g) **Immune System Suppressants:** These drugs are helpful for keeping immune system under control and for treating underlying condition like lupus.

1.8 Steroid Resistant Nephrotic Syndrome (SRNS):

SRNS is a clinical condition patients with Nephrotic Syndrome do not achieve complete remission after 6 weeks of treatment with steroids and categorised as steroid resistant Nephrotic Syndrome (SRNS) [4]. The Management of Steroid Resistant Nephrotic Syndrome requires combination of medicines like immunosuppressants, angiotensin converting enzyme inhibitor, low dose steroids and statin etc. Failure of immunosuppressive medications leads to a high risk of developing end stage renal

disease (ESRD). Recent studies have indicated significant rise in the number of steroid resistant Nephrotic Syndrome particularly in south-east Asia.

1.9 Diagnosis of SRNS:

The clinical criteria for diagnosis of SRNS is following:

a) **Initial steroid-resistance**: It is based on steroid treatment outcomes. Initial steroid-resistance is failure to achieve complete remission after 6-weeks' treatment with prednisolone at a dose of 60 mg/m^2/day (2 mg/kg/day; maximum 60 mg/day) [1].

b) **Late (secondary) steroid-resistance:** Such patients are initially reported as steroid-sensitive and become steroid resistance in a subsequent relapse. Patients with steroid toxicity are prescribed daily prednisolone for 4 weeks, followed by alternate-day therapy for 2 weeks [1].

1.10 Treatment and Management of SRNS:

The immunosuppressive drugs like Tacrolimus and Cyclosporine along with steroids and supporting medicines are given for the treatment and clinical management of SRNS. As a part of proper clinical management, regular therapeutic drug monitoring is also done to optimise treatment dose and avoid toxicity of drug [1].

1.11 Identification of Research Problem:

Tacrolimus is an immunosuppressive drug that is being prescribed for organ transplant patients to prevent the rejection of the organ. It is also being prescribed as an important drug for the treatment of SRNS in children, yet despite its significant clinical use, the pharmacokinetics data and its correlation with therapeutic efficacy is very limited in the paediatric population [5]. No major studies have been done to find out the trough concentration in steroid-resistant Nephrotic Syndrome (SRNS) in Indian children.The therapeutic dose of Tacrolimus for the treatment of SRNS is also extrapolated from kidney transplant patients.

Treatment and clinical management of children with SRNS remains very challenging due to the absence of a defined dose of the drug. Further, the Tacrolimus has a very

narrow therapeutic range which means that a minor decrease in drug dose may affect the treatment and on the other side a minor increase in drug dose may cause nephrotoxicity. The management of SRNS remains a challenging task for nephrologist due to various side effects related to immunosuppression such as infections, nephrotoxicity, cytopenia neurotoxicity and malignancies.

The published data also shows that the therapeutic range of Tacrolimus appears to be comparatively very high in organ transplant patients. The constant and reliable therapeutic monitoring of given drug level is utmost important to avoid toxicity effect of drug and to evaluate the safety and efficacy of treatment. Thus the need of the study was to evaluate the trough level of the Tacrolimus with treatment outcomes for better patient care and management of SRNS in Children with SRNS.

1.12 Research Questions:

Whether the clinical efficacy of Tacrolimus and treatment outcomes is related to the trough level of Tacrolimus in Children with steroid-resistant Nephrotic Syndrome (SRNS)?

What should be the desired therapeutic level of Tacrolimus to be maintained for children with steroid-resistant Nephrotic Syndrome?

Is there any major side effect of Tacrolimus in patients with SRNS?

Does the Particle-enhanced Turbidimetric Immunoassay (PETIA) can be an alternative to LC-MS in therapeutic drug monitoring?

1.13 Null Hypothesis (H0):

There is no relationship in treatment outcomes between clinical efficacy and trough level of Tacrolimus in Children with steroid-resistant Nephrotic Syndrome (SRNS).

1.14 Research Hypothesis:

There is a relationship of clinical efficacy and trough level of Tacrolimus in Children with steroid-resistant Nephrotic Syndrome (SRNS)

CHAPTER-2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 History of Nephrotic Syndrome:

Nephrotic Syndrome [NS] is a clinical manifestation of glomerular diseases that is characterized by heavy proteinuria, hypoalbuminemia and edema [6]. This concept took centuries to be established as a basis for diagnosis and treatment of NS. There is a long history of observations and interpretations. It is only in the mid of 19th century when an effective treatment and diagnosis approaches were established with the advent of latest diagnostic methods, steroids, antibiotics, diuretics and immune-modulators etc. However there is still gap in the understanding about its etiology and treatment approaches for steroid resistant form of the disease.

Nephrotic Syndrome has emerged over thousands of years. Although, the roots of diseases are available from classical Greece era as generalized oedema (dropsy) in adults has been known from classical times. One sagacious observation was made by Hippocrates during 460 B.C to 375 B.C about the Nephrotic Syndrome that 'when bubbles settle on the surface of the urine, it indicates disease of the kidneys and that the complaint will be protracted' [7].

Swelling of whole body of the child has been described previously in the book "***Liber de aegritudinibus infantium"*** written by Cornelius Roelans in 1484 as 51st disease among 52 diseases of the children. [8]

After almost 250 years, **Theodore Zwinger III of Basel** (1658-1724) in his Paedoiatreia practica of 1722 presented a remarkable and early description of the Nephrotic Syndrome [9]. This text is known to some historians of pediatrics, but has been completely ignored by nephrologists. [10]

Later in the eighteenth century several observers including **Cruikshank**, Cotugno, Wells, and Brande noted coagulability of urine in the patients [11]

Richard Bright (1827) described Nephrotic Syndrome as triad of generalized edema, proteinuria, and kidney disease [12].

In 1905, Müller created the term ‘nephrosis’ and described all non-inflammatory diseases of the kidney [13].

The renal biopsy was introduced in clinical nephrology practice in 1950 which improved the knowledge and understanding of this disorder further. [14]

The Study by Bhandari, *et al* was one of the original published work on NS from India which evaluated the effect of treatment with long term oral steroid therapy. [15]

Indian Expert group on Paediatric Nephrology framed treatment guidelines on steroid sensitive Nephrotic Syndrome in 2001 which were revised in 2008 [6]. The consensus guidelines were formulated by Indian Paediatrics experts on SRNS in 2009 and which were subsequently revised in 2021 [16].

Similar, evidenced based guidelines were also formulated by an international group the Kidney Diseases; improving global outcomes (KDIGO) [17]

In the majority of patients, the cause is idiopathic and in less than 10% patient’s secondary causes are SLE, Hepatitis-B, hepatitis-C, HIV, HSP and amyloidosis etc. The renal histology of the Nephrotic Syndrome includes Focal segmental glomerulosclerosis (FSGS) 40-50%, minimal change disease (MCD) in 25-40%, and mesangioproliferative glomerulonephritis in 5-8%. [18]

As per published data, there are many other secondary reasons of Nephrotic Syndrome which affects entire body and causes due to many diseases like Diabetes, Lupus, and amyloidosis etc. The hypertension, steroid induced obesity, hypoalbumnemia due to protein loss, oxidative stress, recurrent infections and calicineurin inhibitor therapy contribute to endothelial dysfunction. [19, 20].

2.2 Incidence and prevalence of Nephrotic Syndrome:

The incidence of NS varies in different region and ethnic groups and it affects all ages and ethnic background. The prevalence of childhood Nephrotic Syndrome (NS) varies in different population from 12–16/ 100,000 children [21]. The reported incidence of Nephrotic Syndrome is 2-3/100,000 children in western countries, slightly higher in 2- 7/100,000 in south Asian origin [22]. The majority of patients of Nephrotic

Syndrome are idiopathic while a small proportion of cases may be secondary or congenital [23].

2.3 Histology of Nephrotic Syndrome:

The common causes of Nephrotic Syndrome in children consist of minimal change disease (MCD), Focal segmental glomerulosclerosis (FSGS), IgA nephropathy and membranous nephropathy. The patients of Nephrotic Syndrome are treated with multiple immunosuppressive therapies which include calcineurin inhibitors (cyclosporine and tacrolimus), cyclophosphamide, Mycophenolate mofetil and Rituximab [24, 25]. Bhutani et al. reported FSGS as a common histopathological finding in 50-60% children with SRNS [26]

2.4 Steroid Resistant Nephrotic Syndrome:

Most of the children with Nephrotic Syndrome initially respond to steroids and achieve remission of proteinuria following 4-6 weeks of treatment with prednisolone. However, 10-15% patients do not achieve complete remission and are termed as steroid resistant Nephrotic Syndrome (SRNS) [27]. This clinical condition is challenging from management perspective. As these patients have persistent proteinuria which can be progress to renal failure. Recent studies have indicated significant increase in the number of steroid resistant Nephrotic Syndrome particularly in Southeast Asia.

2.5 Diagnosis and Management of Steroid Resistant Nephrotic Syndrome:

As per ISPN Guidelines 2021 [1] patients who failed to show complete remission of protein urea despite six weeks therapy with predinisolone at daily dose of 60 mg/m^2 are to be classified as SRNS. Patient with Steroid adverse effects may receive daily predinisolone for four weeks followed by alternate-day therapy for next two weeks. Similar definition for treatment of initial and late (secondary) SRNS has been recommended. As per above mentioned ISPN guidelines, 2021, all patients were evaluated properly. Since 24 hours collection of urine was difficult in children to implement, UP:UC was preferred for urinary protein estimation.

It requires combination of immunosuppressants, angiotensin converting enzyme inhibitor, low dose steroids and statin etc. Failure of immunosuppressive medications leads to a high risk of developing end stage renal disease (ESRD). The management of SRNS is a challenging task for nephrologists due to various side effects related to immunosuppression such as infections, nephrotoxicity, cytopenia neurotoxicity and malignancies. Dyslipidemia is a common biochemical abnormality found in almost all children diagnosed as SRNS predisposing them to cardiovascular complications associated with hyperlipidaemia [28]

Lipid abnormality in nephrotic syndrome may occur due to both, increased biosynthesis and decreased clearance but the deceased clearance being the predominant factor as the level of total cholesterol, LDL, VLDL, Triglycerides are increased. [29]

Persistent hyperlipidaemia in SRNS may results in to endothelial damage and can be the initial step in atherosclerosis. Endothelial dysfunction may start even at first episode of NS (FENS) and remain present in SRNS as well and result in adverse cardiovascular outcomes in such patients. There are evidences of adverse cardiovascular outcomes in children with idiopathic nephrotic syndrome [30]

Life style modification including dietary restrictions and physical activity are he first step for prevention of dyslipidemia and other cardiovascular disease risk factors. However, Kidney Diseases Improving Global Outcomes (KDIGO) guidelines suggests that dietary restrictions have only a modest effect and recommend use of drug therapy. [31]

Further, as per ISPN guidelines, (2009) in case life style modification does not work, Statins are the first line of medicine for the treatment of dyslipidemia [32].

Persistent endothelial dysfunction in children with SRNS put them at higher risk of cardiovascular co-morbidities. In patients with SRNS, hypertension, steroid induced obesity, hypoalbuminemia due to protein loss, oxidative stress, recurrent infections [33], calcineurin inhibitor therapy [34] are responsible for endothelial dysfunction.

Clinical consequences of dyslipidemia in Nephrotic syndrome results in both in cardiovascular as well as renal complications. In renal system, it results in glomerulosclerosis as well as proximal tubular cell injury. [35] Regular therapeutic monitoring of calcineurin inhibitor drugs are important and the choice of immunosuppressant primarily depends upon their efficacy and safety of drugs.

2.6 Therapeutic Drug Monitoring of Tacrolimus:

Therapeutic drug monitoring (TDM) is a branch of clinical chemistry and clinical pharmacology that is related to the measurement of drug levels in blood. TDM is helpful for improving patient care by adjusting the required dose of drugs to avoid toxicity and also for optimisation drug dose. There are multiple factors that influence the TDM. The following are important factors that may influence the results like time of blood sampling and time, route and dose of drug given etc.

The handling and storage conditions are also very important. The precision and accuracy of the analytical method, co-medications and the clinical status of the patient may also influence the TDM.

Tacrolimus has gained acceptance for treatment of SRNS yet it was found that pharmacokinetics date and correlation is very limited regarding therapeutic efficacy. There is serious dearth of target concentration for children with SRNS and treatment of SRNS is done on the basis of therapeutic concentration applied in organ transplantation.

The desirable C_0 of tacrolimus in pediatric transplant immediately after transplant are 10-12 ng/mL and 5-10 ng/mL subsequently to prevent rejection of organ.[36] On the basis of transplant data, suitable target concentration of tacrolimus in SRNS is considered from 5ng/mL to 10 ng/mL in most of the studies [37].

In recent times trials for treatment of childhood Nephrotic Syndrome are being carried out consistently throughout the globe [38]

In absence of non-effective treatment, the patient suffers from persistent proteinuria leading to progression to chronic kidney disease [39]

Calcineurin inhibitors (CNi) are now considered as preferred drugs for the treatment of childhood steroid resistant Nephrotic Syndrome. Efficacy and safety of cyclosporine versus Tacrolimus in SRNS has also been studied but again with limited being the small study group [40]

Tacrolimus has some advantage over cyclosporine in the treatment of SRNS like less cosmetic side effects [41]

As tacrolimus is bound to plasma protein it exhibits considerable inter and intra individual pharmacokinetics, hence Tacrolimus pharmacokinetics is important in children with SRNS [42].

Nowadays, the Tacrolimus is being used in the management of steroid-resistant Nephrotic Syndrome (SRNS) in children, but still the therapeutic range is extrapolated from kidney transplant recipients. It has further suggested that studies with adequate sample size are required to confirm the findings [43]

Tacrolimus is used in multiple clinical conditions like organ transplantation, autoimmune diseases, malignancies, and for treatment of Nephrotic Syndrome [44] It has a very narrow therapeutic index and even at a low trough level (4-6 ng/mL) has been linked to nephrotoxicity [45]. It is a calcineurin inhibitor that inhibits the production of IL-2 and discourages the proliferation of T cells [46, 47].

It is metabolized in the liver, mainly via CYP3A [48]. Common interactions of tacrolimus are with grapefruit, antimicrobials, and anti-fungal which increase the levels. It has high inter-individual and intra-individual pharmacokinetic variability. A Trough level of tacrolimus is essential in patients to prevent rejection of kidney, heart, or liver transplants [43]. Tacrolimus toxicity is mostly seen in children when plasma levels exceed 15 to 20 ng/mL which may include life-threatening complications [49].

2.7 Drug Profile of Tacrolimus:

Tacrolimus is an important antibiotic of fungal origin, *Streptomyces tsukubaensis* with a potent immunosuppressive function. This drug was discovered in 1984 from the fermentation broth of a Japanese soil sample that contained the bacteria Streptomyces

tsukubaensis. Tacrolimus is chemically known as a macrolide. The brief profile of Tacrolimus [50, 51, 52] is following

2.7.1 Generic Name: Tacrolimus

2.7.2 Brand Names: Advagraf, Astagraf, Envarsus, Modigraf, Prograf, Protopic

2.7.3 Drug Bank Accession Number: DB00864

2.7.4 Chemical Formula: $C_{44}H_{69}NO_{12}$

2.7.5 Metabolism: The Metabolism of tacrolimus is predominantly mediated by CYP3A4 and secondarily by CYP3A5.

2.7.6 Protein Binding: As per publishes data, ~99% is bound to human plasma protein, primarily to albumin and alpha-1-acid glycoprotein.

2.7.7 Half-life: As per available data, the elimination half-life in adult healthy volunteers, kidney transplant patients, liver transplants patients and heart transplant patients are approximately 35, 19, 12, 24 hours, respectively. The elimination half-life in pediatric live transplant patients was 11.5±3.8 hours, in pediatric kidney transplant patients is 10.2±5.0 (range 3.4-25) hours.

2.7.8 Toxicity: Side effects can be severe and include blurred vision, liver and kidney problems (it is nephrotoxic), seizures, tremors, hypertension, hypomagnesemia, diabetes mellitus, hyperkalemia, itching, insomnia, confusion.

2.8 Laboratory Methods:

There are several laboratory methods which are available for therapeutic drug monitoring like Liquid Chromatography-Mass Spectrometry (LC-MS/MS), High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), Particle Enhanced Turbidimetric Immunoassay (PETIA), and Dried-blood-spot analysis etc. [53]

a) **Particle Enhanced Turbidimetric Immunoassay (PETIA):** The QMS Tacrolimus Immunoassay is a homogeneous particle-enhanced turbidimetric immunoassay. The assay is based on competition between the drug in the sample

and the drug-coated onto a microparticle for antibody binding sites of the tacrolimus antibody reagent. The tacrolimus-coated microparticle reagent is rapidly agglutinated in the presence of the anti-tacrolimus antibody reagent and the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically at 700 nm. When a sample containing tacrolimus is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained with the maximum rate of agglutination at the lowest tacrolimus concentration and the lowest agglutination rate at the highest tacrolimus concentration. [54, 55]

b) **Liquid Chromatography-Mass Spectrometry (LC-MS):** LC-MS is now a commonly used technique with the development of electrospray ionization (ESI) providing a simple and robust interface [56]. LC-MS is mostly preferred in labs due to its high specificity and reduced run time. This technique uses molecular fragmentation for the separation of particles. Single quadrupoles, triple quadrupoles, and quadrupole ion-trap instruments are the most used mass analyzers in routine laboratories, in the fields of forensic toxicology and therapeutic drug monitoring [57]. It further helped in the quantification of lower drug concentrations in the blood samples. The advantages of LC-MS are high sensitivity, specificity, small sample requirements, minimal sample preparation, rapid throughput, and simultaneous measurement [58]. The disadvantage of this method is that it required high upfront costs and full validation for use. It is also requires a high degree of technical ability and extensive training is required.

c) **High-Pressure Liquid Chromatography (HPLC):** It is the sensitive and specific method used for measuring the tacrolimus. The disadvantages of HPLC-MS are its high cost of equipment and the availability of suitably skilled scientific staff. The advantages of HPLC-mass spectrometry were high sensitivity, specificity, small sample requirements, minimal sample preparation, rapid throughput, and simultaneous measurement [59].

d) **Gas Chromatography-Mass Spectrometry (GC-MS):** This is a method that uses very high temperature for causing sample vaporization. Vaporized fractions are then passed through the electric field where they get separated based on their

molecular weight. The pattern of separation is unique for each drug, therefore establishes a fingerprint for identification [60]. GC MS has limited use as it is preferable only for volatile substances. GC MS is not the preferred method these days.

e) **Radio Immuno Assay (RIA):** It generally, uses radioactivity for the detection of the presence of the analyte. In RIA the sample is incubated with an antibody and a radiolabeled drug. The amount of radioactivity measured is compared to the radioactivity present in the known standards which are included in each run and results are quantitated. Mostly used of determination of drugs of abuse. The advantages of RIA were high specificity and sensitivity. The side effects mostly included radiation hazards, the use of radio labelled reagents, the requirement of specially trained persons. Labs also require a special license to handle radioactive material. Further a special arrangement for storage, and waste disposal of radioactive materials is also a big challenge [61].

f) **Particle Enhanced Turbidimetric-Inhibition Immunoassay (PETINIA):** It is also an immune turbidimetric method. It mostly uses the creation of light scattering particles to measure drug levels**.** The free drug in the sample competes for the antibody fragment, thereby decreasing the rate of particle aggregation. The rate of aggregation is inversely proportional to the concentration of a drug in the sample. It's a developing technique and not much literature is available.

g) **Cloned Enzyme Donor Immunoassay (CEDIA):** It is a Competitive homogenous enzyme immunoassay. It uses a genetically formulated enzyme βgalactosidase. This assay has two component fragments of an enzyme: enzyme acceptor and an enzyme donor which are generally inactive, but in solution, they become activated and reassemble. As a single subunit, they can react with the substrate. Drug bound to the enzyme donor competes with the drug or with metabolite in the sample for antibody binding site. If the drug bound to the enzyme donor binds to the antibody, it is prevented from reassembling with the enzyme acceptor and activating the enzyme. If the drug is present the unbound enzyme donor reassembles with the enzyme acceptor and reacts with the substrate to produce a change of absorbance. Linearity generally ranges from 0-30 ng/ml.

h) **Chemiluminescent Microparticle Enzyme Immunoassay (CMIA):** Before the initiation of the procedure, a manual pre-treatment step is performed in which the whole blood sample is extracted with a precipitation reagent and centrifuged. The supernatant is transferred into a Transplant Pretreatment Tube, which is placed onto the System. Sample, assay diluent, and anti-tacrolimus coated paramagnetic microparticles are combined to create a reaction mixture. Tacrolimus present in the sample binds to the anti-tacrolimus coated microparticles. After a delay, tacrolimus acridinium-labeled conjugate is added to the reaction mixture. The tacrolimus on the acridinium-labeled conjugate competes for the available binding sites on the microparticles. Following incubation, the microparticles are washed and pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). An indirect relationship exists between the amount of tacrolimus in the sample and the RLUs detected by the system optics. A study by Marubashi, *et al* had compared the method with MEIA and found that the correlation between the two methods was highly significant (r = 0.941). They found that CMIA was much superior to MEIA in detecting the low levels [62].

i) **Dried Blood Spot (DBS):** In this method sampling using a finger prick is an emerging alternative to venous sampling. Important advantages of DBS sampling include the less amount of blood is needed, the possibility of home-based sampling after which the sample can be sent to the laboratory, easier sampling at desirable sampling times (eg, trough concentrations), and the quick results being available to the patient before the next outpatient visit[63]. This process of TDM with DBS sampling has recently been demonstrated to be cost-effective, with lifelong concentration monitoring [64]. There has been an increase in the development of DBS assays for TDM for a wide range of drug therapies, including immunosuppressants [65, 66]. The challenge is to increase the applicability of DBS sampling as adequate clinical validation is required. Second, the acceptability of DBS sampling needs to be evaluated to assess the possible bottlenecks for implementation at an early stage. Only then can widespread home-based sampling for TDM can be implemented.

CHAPTER-3

AIMS & OBJECTIVES

3. AIMS & OBJECTIVES OF STUDY

3.1 AIM:

A Study to Evaluate Trough Concentration of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome.

a. Primary Objective:

To measure and evaluate the trough level of Tacrolimus in children with steroid resistant Nephrotic Syndrome (SRNS) by particle enhanced turbidimetric immunoassay.

b. Secondary objectives:

To study the correlation of tacrolimus levels with clinical remission in children with SRNS and to study to side effect profile of Tacrolimus in children with SRNS.

CHAPTER-4

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1 Place of Study:

The study was conducted in Department of Biochemistry and Department of Pediatrics, Kalawati Saran Children's Hospital and Associated Lady Hardinge Medical College, New Delhi.

4.2 Study Design:

This is a cross sectional prospective study.

4.3 Period of Study:

January 2021 to January 2022

4.4 Study Approval:

The Study was approved by the Institutional Ethics Committee for Human Resources of Lady Hardinge Medical College and Associated Hospitals, New Delhi.

4.5 Study Population:

The Children in age group from 1 year to 16 years receiving treatment of Steroid Resistant Nephrotic Syndrome in OPD of Department of Pediatrics, Kalawati Saran Children Hospital were recruited for the study. All consecutive, consenting patients meeting the inclusion and exclusion criteria of the study were included.

4.6 Inclusion Criteria:

All Children with SRNS, receiving treatment were included. SRNS was defined as per ISPN guidelines [1]. Children with Nephrotic Syndrome who do not attain complete remission after 6 weeks of daily dose of prednisolone at 60 mg/m² were categorised as SRNS patients. Patients with steroid adverse effects were given daily prednisolone only for 4-weeks, followed by alternate-day therapy for the next 2-weeks. Standard treatment guidelines were followed for diagnosis and clinical management of children with SRNS in our hospital. The Clinical details, biochemical and hematological

investigations, and side effects if any were noted in the prescribed proforma. The Tacrolimus levels and other required blood and urine chemistry parameters were done in Biochemistry Department of our hospital for patient care.

4.7 Exclusion Criteria:

Children with secondary Nephrotic Syndrome were excluded.

4.8 Instruments of the Study:

- **Diagnosis & treatment criterion**:

    a. Consensus Guidelines on Management of Steroid Resistant Nephrotic Syndrome Approved by the Expert Group of the Indian Society of Pediatric Nephrology [1] was the clinical criteria for deciding the categories of Remission, Partial-Remission and No-Remission.

- **Important Laboratory Equipment's, Kits and Protocols**

    a. Indiko Thermofisher Drug Analyser

    b. Thermofisher Tacrolimus Test Procedure Protocol

    c. QMS Tacrolimus Immunoassay kit

4.9 Sample Size:

A total of 60 patients of SRNS receiving treatment from said hospital were included in the study. Their age range was between 1-16 years. The sample size was calculated and validated statistically with the help of a statistician.

4.10 Sample Size Calculation & validation: The detailed calculation is as under:

Difference between two independent group means or median:

A study advocating a significant difference of 59% in median trough concentration (C_0) of Tacrolimus between remission (median C_0=2.95 ng/ml, n=14) and relapse (median C_0=1.20 ng/ml, n=11) steroid-resistant nephritic syndrome (SRNS) children

was referred. Expecting, mean or median difference at least of 0.41% (effect size: d) between two SRNS children group (remission and relapse) of the present study and considering 5.0% margin of error (*α=0.05 i.e. type I error*) and 80.0% power (*1-β=0.80 i.e. type II error*), then minimum 49 samples need to be sampled (n) for the study, evaluated by G*Power 3.1.9.4 software as

Input parameters	**Output parameters**
Tails: Two sided (*α*=2)	Non-centrality parameter (б)=2.8700000
Effect size (d)=0.41	Critical t value=2.0106348
α error probability=0.05	Df=48
Power (1-β error probability)=0.80	Total sample size=49
	Actual power=0.8029445

Df: degree of freedom

Reference study [5]

Thus, minimum 49 children were required for the study.

4.11 Methodology:

Consecutive patients from Pediatric Nephrology OPD and wards fulfilling the eligibility criteria were enrolled. Nephrotic Syndrome was defined, investigated as per ISPN guidelines [1]. Definitions and protocol as mentioned below were applied. Demographic parameters and clinical details including detailed clinical history, examination were done at enrolment in a structured performa. All patients were assessed for height, weight, BMI, hypertension, baseline characteristics, cumulative dose and sign and symptoms of toxicity. Clinical practice guidelines of American Academy of Paediatrics was used for screening and management of high blood pressure in children. All patients were subject to the following investigations at the enrolment.

1) Haemogram
2) Kidney Function Test
3) Serum Electrolytes
4) Lipid Profile
5) Serum Calcium
6) Phosphorous
7) ALP
8) Total Protein, Albumin
9) UP: UC
10) Urinary Creatinine etc.

After confirmed diagnosis of NS, the patients were put on steroid for standard treatment of nephrotic syndrome by Pediatric Nephrologist. The same treatment protocol were followed for all cases of nephrotic syndrome who were receiving treatment from KSCH. The regular monitoring UP:UC was done to evaluate the treatment outcome of nephrotic syndrome. The patients with steroid adverse effects were given prednisolone daily for 4-weeks, followed by alternate-day therapy for the next 2-weeks. Standard treatment guidelines were followed for diagnosis and clinical management of children with SRNS. Children with Nephrotic Syndrome who failed to attain complete remission after 6 weeks of daily dose of prednisolone at 60 mg/m² were defined as SRNS patient in terms of ISPN guidelines [1].

The diagnosis criteria was as under- Urine protein 3+/4+ (dipstick), UP: UC >2, or 24-hr urine protein >1000 mg/m2/day; albumin. All patients were examined, diagnosed and treated by a qualified Pediatric Nephrologist.

4.12 Treatment Protocol: the patients diagnosed with SRNS were put on Tacrolimus treatment with tapering dose of alternate day steroids. The purpose of treatment was to induce remission with minimum drug related side effects.

Supplementary treatment was also given like lipid lowering medicine, ACE inhibitors etc. The clinical details, biochemical and hematological investigations, and side effects if any were noted in the prescribed proforma. The Tacrolimus levels and other required blood and urine chemistry parameters were done in Biochemistry Department of our hospital.

The daily dose of Tacrolimus was given from 0.1 to 0.2 mg/kg/daily. All patients of SRNS were followed for a minimum period of 12 weeks. The weight, height and BP were also recorded at the onset and after the 12 weeks of the treatment. The following laboratory parameters were done after 12 weeks of the tacrolimus treatment. Tacrolimus level, Haemogram, Kidney Function Test, Serum Electrolytes, Lipid Profile, Serum Calcium, Phosphorous, ALP, Total Protein, Albumin, UP: UC, Urinary Creatinine etc.

4.13 Therapeutic Drug Monitoring of Tacrolimus:

a) **Laboratory Method:** The measurement of trough level of Tacrolimus was done using particle-enhanced turbidimetric immunoassay (PETIA)

b) **Sampling Technique:** Patients were advised to take drugs at fixed time during morning and evening. One ml of venous blood was obtained in EDTA vial, 12 hours post intake of tacrolimus dose after 12 weeks from the starting of the drug. Sample were taken only once duly following all safety precautions. Routine Biochemical and haematological investigations and UP: UC was also performed at the time of diagnosis and after 12 weeks of treatment.

Fig. No-3 Sample collection from SRNS Patient

c) **Sample Storage & Handling:** Sample was stored at -20° C in Elanpro® Deep Freezer and processed within a week.

Fig. No. 4 Elanpro® Deep Freezer for storage of samples

d) **Summary & Principle of Procedure:** The QMS Tacrolimus is an Immunoassay. This assay is based on competition between drug in the sample and drug coated onto a micro-particle for antibody binding sites of the tacrolimus antibody reagent. The tacrolimus-coated micro-particle reagent is rapidly agglutinated in the presence of the anti-tacrolimus antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically at 700 nm. When a sample containing Tacrolimus is added, the agglutination reaction is partially inhibited, which slow down the rate of absorbance. A concentration-dependent classic agglutination inhibition curve is produced. The maximum rate of agglutination means the lowest tacrolimus concentration and the lowest agglutination rate is the highest tacrolimus concentration.

e) **Sample Preparation:** Calibrators, Controls and patient specimens were equilibrated to room temperature before extraction. Calibrators, Controls and patient specimens were thoroughly mixed at room temperature prior to use by gentle inversion avoiding the formation of bubbles.

Fig. No. 5 Blood Samples for Tacrolimus Testing

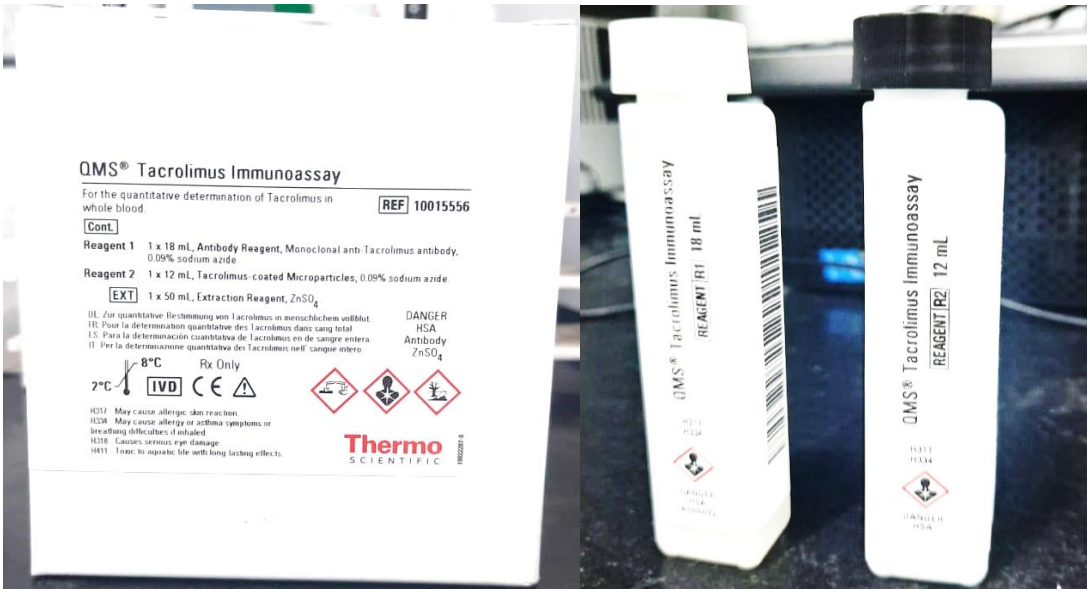


Fig. No. 6 Tacrolimus Reagent Kit

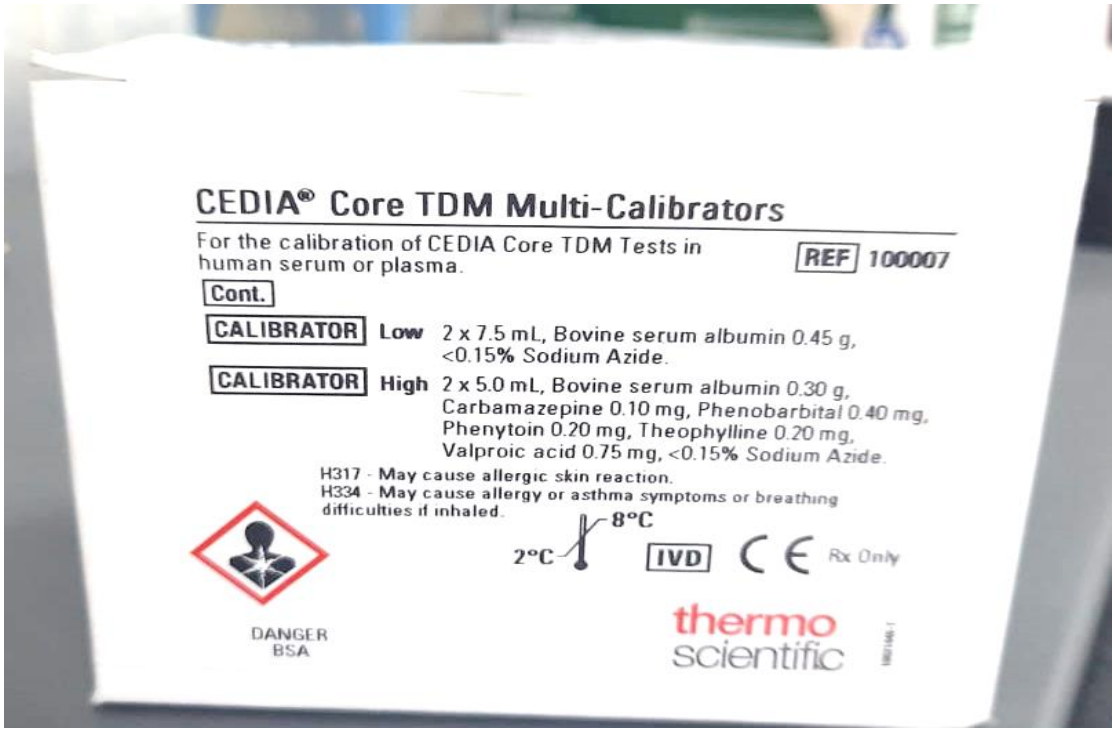


Fig. No. 7 Tacrolimus Calibrators

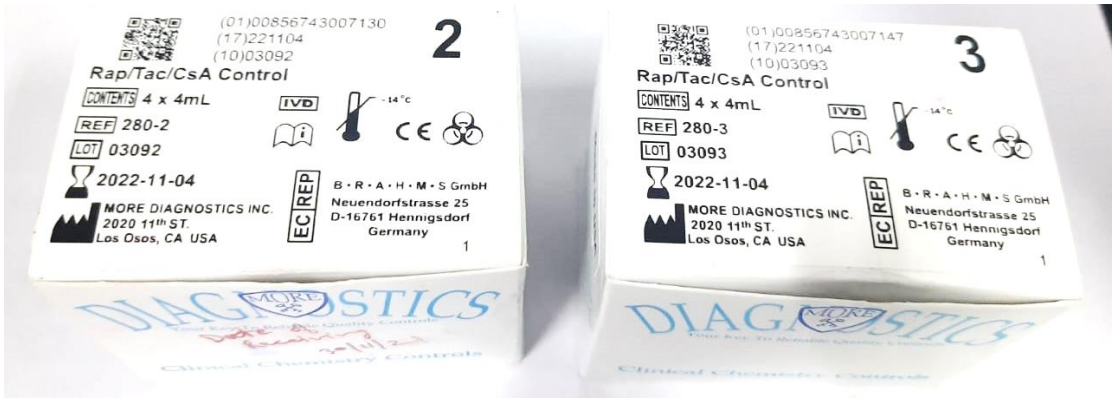


Fig. No. 8 Tacrolimus Quality Controls

Fig. No. 9 Bar-coded Sample Rack with sample cups

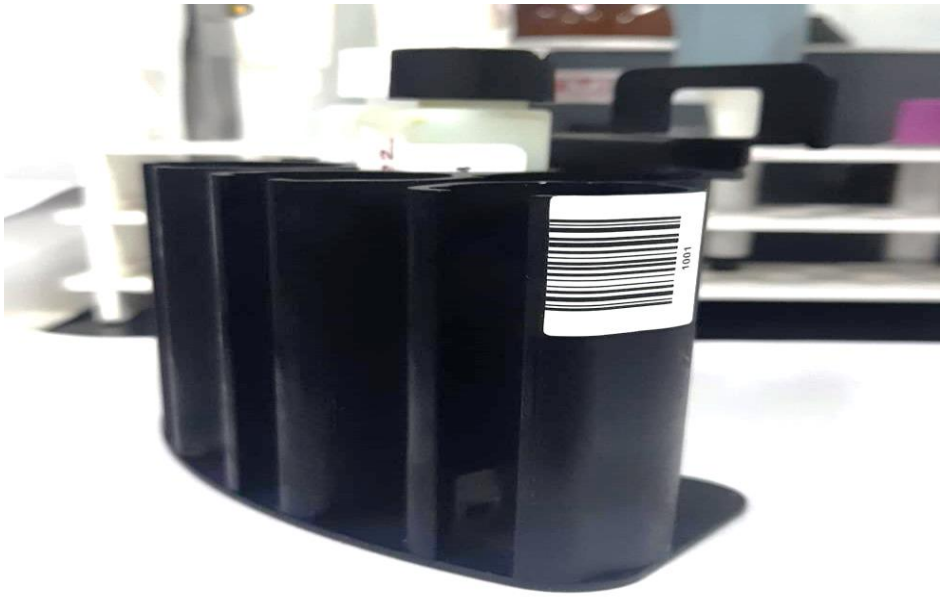


Fig. No. 10 Bar-coded Reagent Rack

Fig. No. 11 Vortex Mixer

f) Extraction Solution Preparation

1. To prepare "Tacrolimus Working Extraction Solution" 1 part of Extraction Reagent was mixed to 4 part of HPLC Grade Methanol (≥ 99.8% purity) in a clean, dry, airtight bottle.

2. Date of preparation was mentioned on the vial and was stored at room temperature.

Fig No. 12 Tacrolimus Extraction Solution

Fig No. 13 Methanol (HPLC Grade)

g) Sample Extraction & Processing (for Calibrators, Controls and Samples):

1. One micro-centrifuge tube was prepared for extraction of each samples, calibrators and controls.

2. 200 µL of control, calibrator and sample was Pipetted into the properly labeled micro-centrifuge tubes. The sample was taken carefully to ensure that no air bubble are there in tip which might be a potential source of analytical error. The pipette tip was wiped to remove any access sample.

3. 200 µL of extraction solution was taken into the micro-centrifuge tube. Prior to dispensing the extraction solution in micro-centrifuge tube, the air bubbles if any, were removed from the pipette tip.

4. Micro-centrifuge tubes were properly caped and vortexed immediately for 20-30 seconds. It was ensured that each tube had a homogeneous mixture.

Fig. No. 14 Sample Processing on Vortex Mixer

5. The mixture was allowed to rest in the micro-centrifuge tube at Room Temperature for 5-7 minutes.

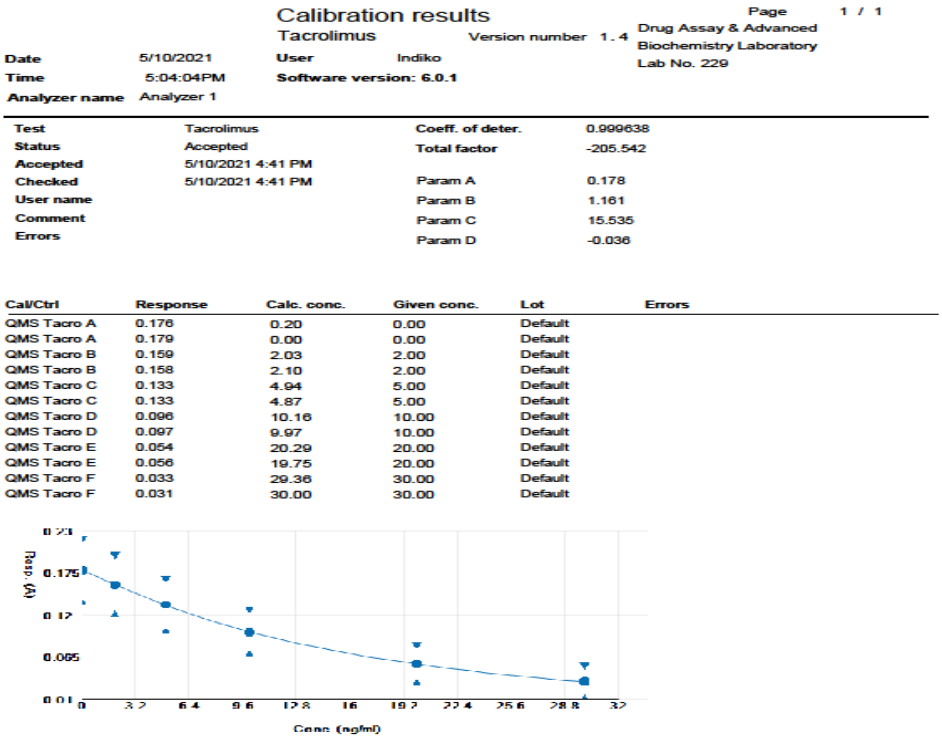
6. Then the mixer was centrifuged for 10 minutes at 4,000 - 5,000 RPM.

Fig. No. 15 Centrifugation of Samples for extraction

7. The supernatant was taken into a sample cup and was run immediately on Drug Analyser to minimize sample evaporation.

Fig. No. 16 Sample Processing on Drug Analyser

Analyzer Calibration Results Sheet



Calibration results

Tacrolimus Version number 1.4

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Drug Assay & Advanced Biochemistry Laboratory
Lab No. 229

Date	5/10/2021	User	Indiko
Time	5:04:04PM	Software version: 6.0.1	
Analyzer name	Analyzer 1		

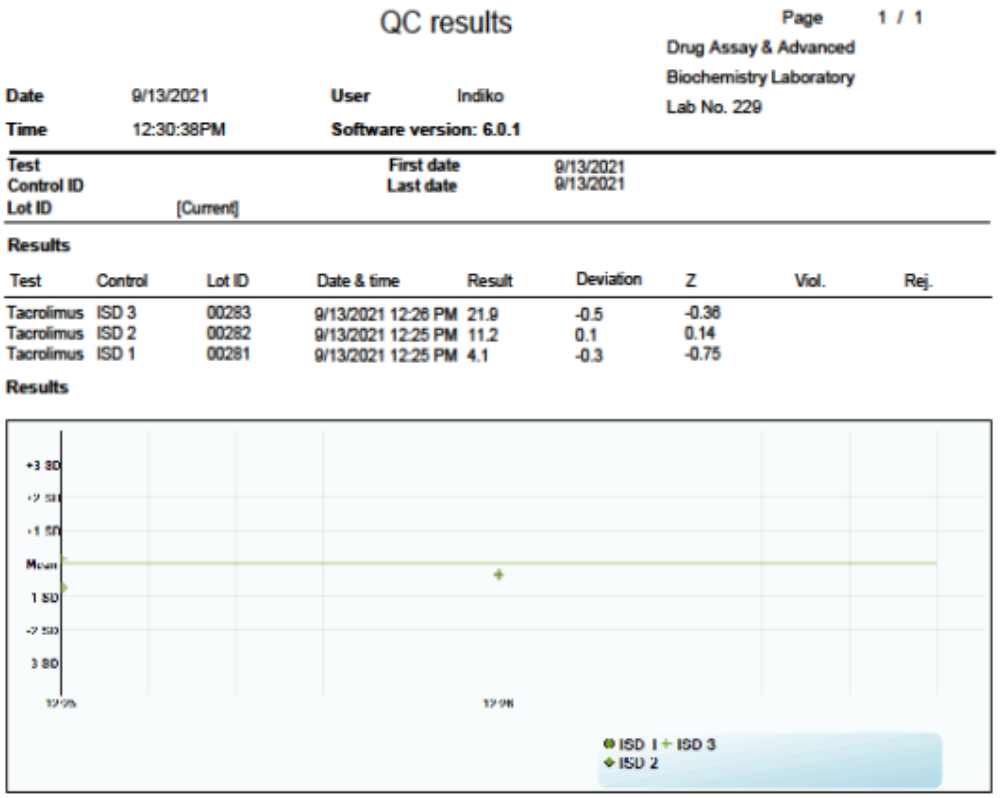
Test	Tacrolimus	Coeff. of deter.	0.999638
Status	Accepted	Total factor	-205.542
Accepted	5/10/2021 4:41 PM		
Checked	5/10/2021 4:41 PM	Param A	0.178
User name		Param B	1.161
Comment		Param C	15.535
Errors		Param D	-0.036

Cal/Ctrl	Response	Calc. conc.	Given conc.	Lot	Errors
QMS Tacro A	0.176	0.20	0.00	Default	
QMS Tacro A	0.179	0.00	0.00	Default	
QMS Tacro B	0.159	2.03	2.00	Default	
QMS Tacro B	0.158	2.10	2.00	Default	
QMS Tacro C	0.133	4.94	5.00	Default	
QMS Tacro C	0.133	4.87	5.00	Default	
QMS Tacro D	0.096	10.16	10.00	Default	
QMS Tacro D	0.097	9.97	10.00	Default	
QMS Tacro E	0.054	20.29	20.00	Default	
QMS Tacro E	0.056	19.75	20.00	Default	
QMS Tacro F	0.033	29.36	30.00	Default	
QMS Tacro F	0.031	30.00	30.00	Default	

Fig. No. 17 Calibration Results

Quality Control Result Sheet:

Quality Controls were performed every time before sample processing.



QC results

Page 1 / 1

Drug Assay & Advanced Biochemistry Laboratory
Lab No. 229

Date 9/13/2021 User Indiko
Time 12:30:38PM Software version: 6.0.1

Test
Control ID
Lot ID [Current]

First date 9/13/2021
Last date 9/13/2021

Results

Test	Control	Lot ID	Date & time	Result	Deviation	Z	Viol.	Rej.
Tacrolimus	ISD 3	00283	9/13/2021 12:26 PM	21.9	-0.5	-0.36		
Tacrolimus	ISD 2	00282	9/13/2021 12:25 PM	11.2	0.1	0.14		
Tacrolimus	ISD 1	00281	9/13/2021 12:25 PM	4.1	-0.3	-0.75		

Results

Fig. No. 18 Quality Control Results

h) Unit of Results:

The result for the QMS Tacrolimus assay were expressed in ng/mL. The Results were validated and released with authorized signatures

i) Reportable Range

The reportable range for the test was 1 ng/mL to 30 ng/mL.

4.14 Measurement of Biochemical and Hematological Investigations:

The following biochemical and haematological investigations were also performed at the time of diagnosis and after 12 weeks of treatment.

1) Haemogram
2) Kidney Function Test
3) Serum Electrolytes
4) Lipid Profile
5) Serum Calcium
6) Phosphorous
7) ALP
8) Total Protein, Albumin
9) UP: UC
10) Urinary Creatinine etc.

The laboratory tests were performed on following laboratory analysers in Department of Biochemistry & 24 hours Emergency Lab Services at Kalawati Saran Children's Hospital, New Delhi.

As part of regular treatment & clinical management following Biochemical, Hematological & Urine Chemistry Parameters were also performed at the onset and during the treatment:

- KFT
- LFT
- Lipid Profile
- CBC
- UP:UC

Fig. No. 19 Showing Laboratory Analysers utilised for performing various Biochemical and Haematological test in Department of Biochemistry, KSCH

The results of Biochemical & Hematological Laboratory Parameters were also documented and correlated with the outcomes of the Tacrolimus treatment of SRNS in Children

4.15 Quality Control of Biochemical Investigations

- All tests were performed following standards operating procedures and required quality control procedures.
- Drug Test were performed on Thermo fisher Drug Assay Auto Analyzer (Indiko)
- Blood and Urine Biochemical Tests were performed on XL-640 Fully Auto Analyzer (Erba-Transasia) and Diatron Pictus-700.
- Hematological Tests were performed on DxH 500 CBC Analyzer.
- Serum Electrolytes were performed on Diestro 103AP and Horiba Yumizen E100 electrolyte analysers.

QC Statistics

Instrument SN 801011002 08-07-2022 10:16

From 02-10-2021

To 02-10-2021

Test	Control	Lot Number	Units	Assigned Mean	Assigned Range	Last Result	Time	Cumulative Mean	Cumulative Low - High	CV	SD	WR
ALB	Med L1	1440	g/dL	4.2	3.4 - 5.0	4.0	Sat 02-10 03:32	4.0	4.0 - 4.0	0.00%	0.00	-
ALB	Med L2	1161	g/dL	3.0	2.4 - 3.6	2.9	Sat 02-10 03:36	2.9	2.9 - 2.9	0.00%	0.00	-
ALP	Med L1	1440	U/L	195	156 - 234	185	Sat 02-10 03:54	185	185 - 185	0.00%	0.0	-
ALP	Med L2	1161	U/L	346	277 - 415	369	Sat 02-10 03:54	326	284 - 369	18.41%	60.1	-
ALT	Med L1	1440	U/L	40	32 - 48	44	Sat 02-10 03:33	44	44 - 44	0.00%	0.0	-
ALT	Med L2	1161	U/L	141	113 - 169	145	Sat 02-10 03:37	145	145 - 145	0.00%	0.0	-
AMY	Med L1	1440	U/L	82	66 - 99	75	Sat 02-10 03:36	75	75 - 75	0.00%	0.0	-
AMY	Med L2	1161	U/L	287	230 - 344	277	Sat 02-10 03:39	277	277 - 277	0.00%	0.0	-
AST	Med L1	1440	U/L	34	27 - 41	31	Sat 02-10 03:34	31	31 - 31	0.00%	0.0	-
AST	Med L2	1161	U/L	153	122 - 184	156	Sat 02-10 03:37	156	156 - 156	0.00%	0.0	-
BiD	Med L1	1440	mg/dL	1.11	0.89 - 1.33	0.98	Sat 02-10 03:35	0.98	0.98 - 0.98	0.00%	0.000	-
BiD	Med L2	1161	mg/dL	1.65	1.30 - 2.00	1.78	Sat 02-10 03:39	1.78	1.78 - 1.78	0.00%	0.000	-
BiT	Med L1	1440	mg/dL	1.51	1.21 - 1.81	1.36	Sat 02-10 03:37	1.36	1.36 - 1.36	0.00%	0.000	-
BiT	Med L2	1161	mg/dL	4.90	3.90 - 5.90	5.03	Sat 02-10 03:39	5.03	5.03 - 5.03	0.00%	0.000	-
CKMB	CK MB L1	62125	U/L	30	22 - 38	32	Sat 02-10 03:39	32	32 - 32	0.00%	0.0	-
CKMB	CK MB L2	52025	U/L	86	64 - 108	85	Sat 02-10 03:39	85	85 - 85	0.00%	0.0	-
CPK	Med L1	1440	U/L	190	152 - 228	165	Sat 02-10 03:35	165	165 - 165	0.00%	0.0	-
CPK	Med L2	1161	U/L	517	414 - 620	487	Sat 02-10 03:38	487	487 - 487	0.00%	0.0	-
CRT	Med L1	1440	mg/dL	1.34	1.07 - 1.61	1.41	Sat 02-10 03:45	1.25	1.10 - 1.41	17.47%	0.219	-
CRT	Med L2	1161	mg/dL	4.22	3.38 - 5.06	4.41	Sat 02-10 03:45	4.41	4.41 - 4.41	0.00%	0.000	-
Ca	Med L1	1440	mg/dL	8.8	7.0 - 10.5	8.0	Sat 02-10 03:36	8.0	8.0 - 8.0	0.00%	0.00	-
Ca	Med L2	1161	mg/dL	13.0	10.4 - 15.6	12.8	Sat 02-10 03:38	12.8	12.8 - 12.8	0.00%	0.00	-
Chol	Med L1	1440	mg/dL	157	126 - 188	148	Sat 02-10 03:35	148	148 - 148	0.00%	0.0	-
Chol	Med L2	1161	mg/dL	286	229 - 343	285	Sat 02-10 03:38	285	285 - 285	0.00%	0.0	-
GGT	Med L1	1440	U/L	57	45 - 68	52	Sat 02-10 03:36	52	52 - 52	0.00%	0.0	-
GGT	Med L2	1161	U/L	190	152 - 228	170	Sat 02-10 03:40	170	170 - 170	0.00%	0.0	-
LDL	Med L1	1440	mg/dL	80	64 - 96	82	Sat 02-10 03:40	82	82 - 82	0.00%	0.0	-
LDL	Med L2	1161	mg/dL	149	119 - 179	157	Sat 02-10 03:42	157	157 - 157	0.00%	0.0	-
Mg	Med L1	1440	mg/dL	2.00	1.60 - 2.40	1.89	Sat 02-10 03:55	1.78	1.67 - 1.89	8.74%	0.156	-
Mg	Med L2	1161	mg/dL	4.18	3.34 - 5.02	3.90	Sat 02-10 03:55	3.90	3.90 - 3.90	0.00%	0.000	-
PO4	Med L1	1440	mg/dL	4.5	3.6 - 5.4	4.3	Sat 02-10 03:37	4.3	4.3 - 4.3	0.00%	0.00	-
PO4	Med L2	1161	mg/dL	7.1	5.7 - 8.5	6.8	Sat 02-10 03:40	6.8	6.8 - 6.8	0.00%	0.00	-
SUG	Med L1	1440	mg/dL	108	86 - 130	97	Sat 02-10 03:43	97	97 - 97	0.00%	0.0	-

Fig. No. 20 Showing QC Results of Biochemistry Analyser

4.16 Outcome Measures of the Study

a) Primary Outcome Measures

i. Mean±2SD tough levels of Tacrolimus in children with SRNS in complete remission, partial & non remission.

ii. Proportion of children with SRNS showing complete remission partial & non remission

b) Secondary Outcome Measures of the Study:

i. Correlation of tacrolimus level in patient with complete remission

ii. Proportion of children showing side effects like hypertrichosis, hypertension and rise in creatinine after starting the tacrolimus drug.

4.17 Statistical Analysis

The Data of the study was coded properly and recorded in MS Excel sheet. SPSS v26 Statistical tool was applied for data analysis.

1. Descriptive statistics were explained in the form of Mean± standard deviations.

2. Medians/IQRs for was provided for continuous variables, and percentage was calculated for categorical variables.

3. ANOVA, Chi-square test was used to check the association between the categorical data. Non-parametric test such as Mann–Whitney U test was used where required.

4.18 Content and Ethical Consideration:

- Clearance was taken from Institutional Ethics Committee.

- Witten & informed consent was taken from parents/guardians in a language they understand, assent was taken from patient older than 8 Years of age.

CHAPTER-5

RESULT & DISCUSSION

5. RESULTS AND DISCUSSION

The Study comprised of 60 patients diagnosed as SRNS who were prospectively followed up and examined for a period of 12 weeks under the supervision of Paediatric Nephrologist. The Baseline characteristics of the patients were recorded at the time of diagnosis. Various Laboratory markers like Haemogram, KFT, LFT, Lipid Profile, Total Protein, Serum Albumin, UP:UC, were measured at the onset of the disease and after 12 weeks of standard treatment with Tacrolimus. The Trough level of Tacrolimus was also measured after 12 weeks of treatment. The outcome of treatment was decided as per the revised guidelines published by the Indian Society for Paediatric Nephrology (ISPN) guidelines published in 2021 [1]. As part of the treatment & follow-up process, following clinical assessment and laboratory parameters were performed to evaluate treatment outcomes.

a) Demographic Parameters

b) Age, Gender, Height Weight and BMI monitoring.

c) Major characteristics in NS

d) Baseline Biochemical and Haematological Parameter in SRNS Patients

e) Urinary Protein and Creatinine ratio.

f) Routine follow-up and clinical examination

g) BP Monitoring and BP Centiles.

h) Side effects Profile during Treatment with Tacrolimus

i) Trough level of Tacrolimus after minimum 12 Weeks of treatment with Tacrolimus

j) Biochemical and Haematological Parameter in SRNS Patients after minimum 12 Weeks of treatment with Tacrolimus

k) UP:UC Ratio

l) Height Weight and BMI after minimum 12 Weeks of treatment with Tacrolimus.

The outcome measures were:

(a) Mean± 2SD levels of Tacrolimus in children with SRNS in complete remission

(b) Proportion of children with SRNS showing complete remission, partial remission and non-remission

(c) Correlation of tacrolimus level in patient with complete remission

(d) Proportion of children showing side effects like hypertrichosis, hypertension and rise in creatinine after starting the drug.

Table-1 Baseline Characteristics of Patients with Nephrotic Syndrome

Parameter (n=60)	**Mean (SD)/Frequency n (%)**
Age	
Age at onset of Nephrotic Syndrome (months)	67.08 ± 41.20
Age at Diagnosis of SRNS (months)	79.4 ± 47.81
Gender	
Male	38 (n=38/60) (63 %)
Female	22 (n=22/60) (37 %)
Type of Resistance	
Initial	22 (n=22/60) (37 %)
Late	38 (n=38/60) (63 %)
Steroid dose (mg/kg/day)	2 (mg/kg/day)

On the evaluation of basic characteristics as shown in Table 5.1, the mean age at diagnosis of SRNS was 79.4 months and the mean age at onset of Nephrotic Syndrome was 67.08. The male children were higher at 63% (n=38/60) while female children were 37% (n=22/60). The gender distribution ratio was 1.72:1. 63% patients (n=38/60) were late resistant to steroid treatment while 37% (n=22/60) were initial resistant. The cumulative steroid dose was given as 2 (mg/kg/day). 22/60 patients were initially resistant to Steroid Treatment while 38 patients were reported late resistant.

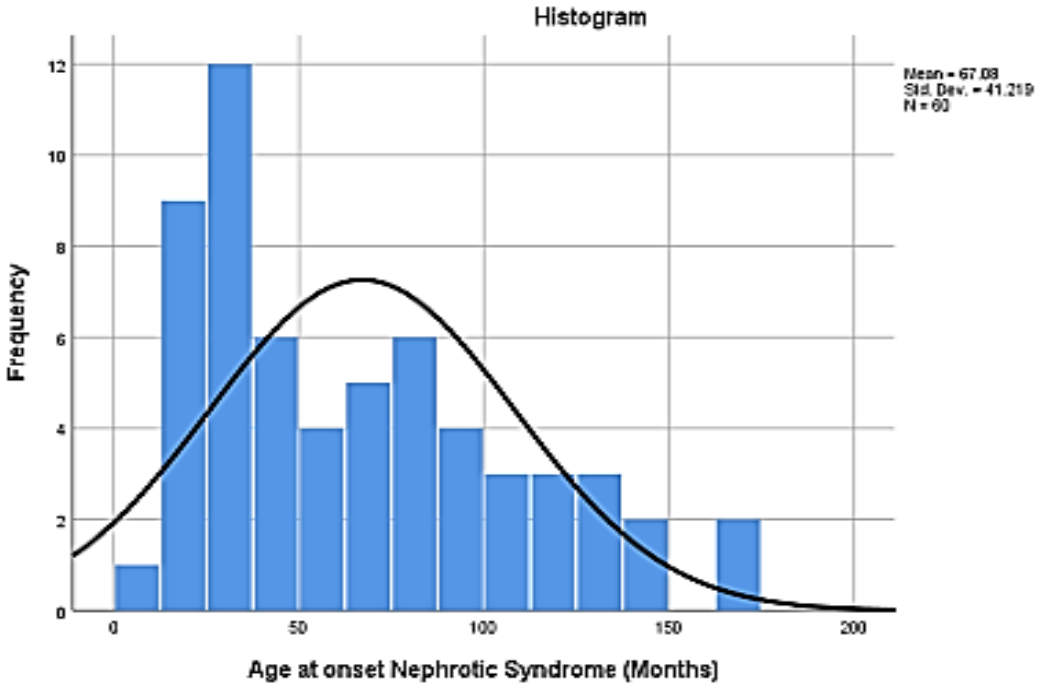


Fig. No. 21 Age at onset of Nephrotic Syndrome.

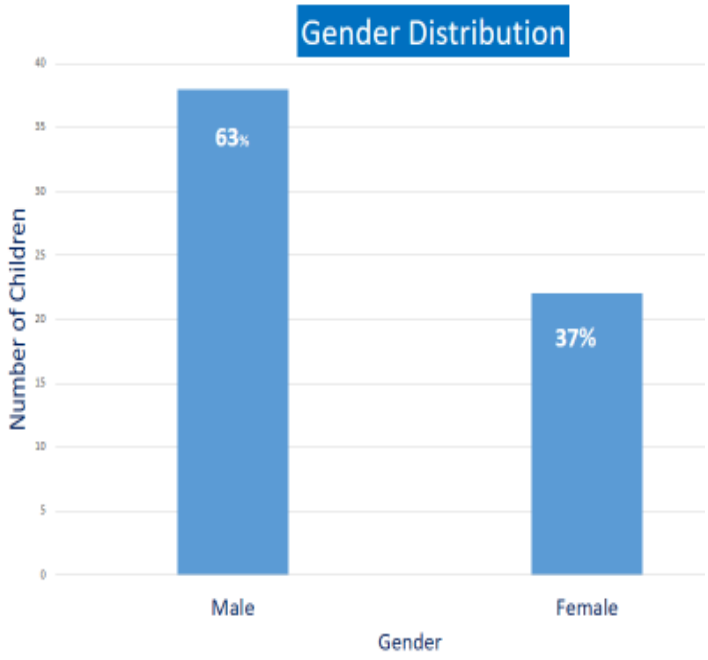


Fig. No. 22 Gender Distribution of SRNS Patients.

In our study the mean age of onset of nephrotic syndrome was 67.08 months (5.7 years) which is slightly higher than the study done by Mutalik *et al*, in which the mean age of onset of nephrotic syndrome was found to be 4.6 years. [67], by Arumugam et al, in which it was 4.5 years [68] and by Kaddah et al in which it was 4.4 years [69].The mean age in our study at diagnosis of SRNS was 79.4 months (6.7 years) which is similar to the study by done by Beins *et al*, in which it was found to be 6.9 years. [70]. As per the study done by Franke I *et al*, the average age of the 346 patients was 5.5 ± 3.7 years. [71]

In our study initial resistant patients were 37% while 63% were in Late Resistant category. The gender distribution ratio was 1.72:1. The male children were higher at 63% (n=38/60) while female children were 37% (n=22/60) which is very similar to a study by Arumugam *et al*, which has reported 64% male patients and by Kikunaga which also reported 67% male patients. Most of the other studies also indicated that male patients higher than female.

The published literature shows that the incidence of NS in children varies and is commonly reported between 2 and 7/100,000 children. The incidence is dependent on the country and ethnic origins of the population. For example, in parts of Nigeria the reported incidence is 0.56/ 100 000 whereas in Asian children B16 years in Birmingham the reported incidence is 16.9/100 000 [72]

The diverse range of international examples demonstrates that NS incidence varies greatly across continents and ethnic groups. Whilst the reason for the difference between different ethnic groups remains unclear, it could be attributed to genetic variations. In the study undertaken by Franke 1 et al, [71] the average age of NS onset occurred at 5.5 years. Similarly, Simpson et al. have reported an average age of onset of 5.4 years in New Zealand [73]. The average age of onset is reported as 4.6–4.9 years in Turkey [74, 75], 4.8 years in Israel [76] and 6.6 years in the USA [77].

Table-2-Characterstics reported at onset of Nephrotic Syndrome

Type of Characteristics	**Frequency n & (%)**
Haematuria (Gross)	15% (n=09/60)
Oliguria	6.6% (n=04/60)
Hypertension	16.6% (n=10/60)
Fever	11.6% (n=07/60)
Rash	3.3% (n=2/60)
Joint Pain	5% (n=3/60)
Edema	43.3% (n=26/60)
Ascites	8.33% (n=5/60)
Family History of Hypertension	Nil
Family History of Idiopathic Nephrotic Syndrome	Nil
Family History of CVD	Nil

Many clinical conditions were reported at onset of Nephrotic Syndrome like Edema 43.3% (n=26/60), Hypertension 16.6% (n=10/60), Hematuria 15% (n=9/60), Oliguria 6.6% (n=4/60), Fever 11.6% (n=7/60), Joint pain 5% (n=3/60), rashes 3.3% (n=2/60) in patients during SRNS treatment. The family history of Idiopathic Nephrotic Syndrome was not found in any patient. Hypertension and gross Haematuria and Edema were found major characteristics.

Table-3: Histopathological Report of Paediatric Patients with SRNS

Type	Frequency n (%)
MCD	28% (n=17/60)
FSGS	15% (n=9/60)
Renal Biopsy not available	56% (n=34/60)

The Kidney biopsy was done in 43.3% (n = 26 / 60 patients). The results of kidney Biopsy presented that minimal change disease ((MCD n = 17 / 26) was the most common histological diagnosis, followed by Focal Segmental Glomerulosclerosis (FSGS n=9/26).

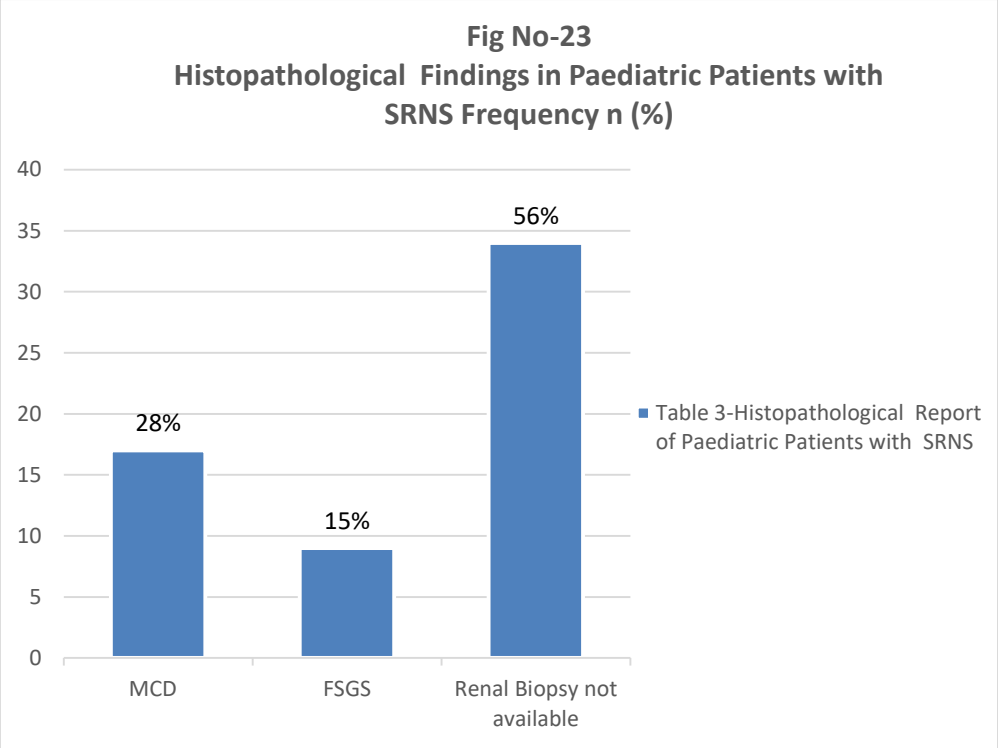


Table 4-Drug given during the Treatment of SRNS Patients	
Therapy	**(%) n**
Immuno-modulator (Tacrolimus)	100% (n=60/60)
Steroids	100% (n=60/60)
ACei	100% (n=60/60)
Atorvastatin	33.3% (n=20/60
Anti-Hypertensive	16.6% (n=10/60)

n = number

The drug shown in Table-4 were given for treatment and supportive management in SRNS Patients

Table 5- Treatment compliance status in SRNS Patients	
Indicator	(%) n
Fill Count	100% (n=60/60)
Compliance	97% (n=58/60)
Immuno Suppression (Duration)	12 Weeks to 16 Weeks
Daily dose of Tacrolimus	0.1-0.2 mg/kg/Daily

n=number

The majority of patients have followed the fill counts for proper compliance of treatment. The follow up monitoring was done at regular interval during the treatment. The daily cumulative drug dose of Tacrolimus in most of the patients at the starting of treatment was 0.1 mg/kg/daily.

Table 6-Complication of Steroid Therapy	
Type of Complications	n (%)
Acanthosis	0 (0.0%)
Caushing's Syndrome	8 (13.3%)
Cataract	0 (0.0%)
Striae	2 (3.3%)

n=number

Complication in Steroid Therapy shown in Table 7 indicated that Caushing's Syndrome was in 13.3% patient and Striae was found in 3.3%. Patients

Table-7 Anthropometric Parameters at diagnosis and after 12 Weeks of treatment in SRNS Patients				
Parameter	Type of variable	Mean, SD & Median Value (at Diagnosis of SRNS)	Mean, SD & Median Value (at 12 Weeks of SRNS treatment with Tacrolimus)	*p*-value
Weight (Kg)	Mean (SD)	22.65 ± 11.06	22.42 ± 10.83	<.001

	Median	19.65	19.65	
Height (Cm)	Mean (SD) Median	117.1 ± 21.77 109.5	117.43 ± 21.87 110	<.001
BMI (kg/m2)	Mean (SD) Median	15.68 ± 2.88 15.58	15.46 ± 2.79 15.50	.013

Table 7 shows Anthropometric parameters of SRNS patients at diagnosis of SRNS and at 12 weeks of Treatment with Tacrolimus. There was significant decrease in weight of SRNS patient from 22.65 to 22.42 after 12 weeks of treatment. The Mean BMI significantly decrease from 15.68 to 15.46 after 12 weeks of treatment. There was no significant difference in height of patients

Table No. 8 Trough Concentration of Tacrolimus Remission, Partial Remission, and Non Remission groups after 12 weeks of treatment

	Category	N	Mean	Std. Deviation	Minimum	Maximum
Trough Concentration of Tacrolimus (ng/ml	Remission (UP:UC Ratio <0.2)	49	7.016	2.1733	4.2	13.0
	Partial Remission (UP:UC Ratio >0.2- 2)	4	5.825	1.3099	4.3	7.5
	Non Remission (UP:UC Ratio >2)	7	4.186	1.1067	3.0	5.7
	Total	**60**	**6.607**	**2.2205**	**3.0**	**13.0**

The Mean C_0 of remission patients of this study was higher than in the non-remission group (7.0 ng/ml, versus 4.18 ng/ml, p=0.004). There was no significant correlation of trough level of tacrolimus and drug doses in different groups. The mean C_0 of Tacrolimus in patients showing complete remission was higher than in children with non-remission. The mean trough concentration found in our study was 6.6 ng/ml.

Table No. 9 Showing UP:UC Ratio & Serum Albumin Level in different groups after 12 weeks of initial treatment						
	Category	N	Mean	Std. Deviation	Minimum	Maximum
UP:UC Ratio	Remission (UP:UC Ratio <0.2)	49	.13067	.076366	.012	.520
	Partial Remission (UP:UC Ratio >0.2-2)	4	1.61250	.151079	1.430	1.800
	Non Remission (UP:UC Ratio >2)	7	5.35000	3.561456	2.740	12.810
	Total	**60**	**.83838**	**2.041410**	**.012**	**12.810**
Serum Albumin	Remission (UP:UC Ratio <0.2)	49	3.6533	.45897	2.20	4.70
	Partial Remission (UP:UC Ratio >0.2-2)	4	3.2500	.23805	3.00	3.50
	Non Remission (UP:UC Ratio >2)	7	2.5286	.48206	1.50	2.90
	Total	**60**	**3.4952**	**.57756**	**1.50**	**4.70**

There was a significant correlation of three important laboratory parameters Tacrolimus C_0, UP: UC ratio, and serum albumin and Cholesterol level in remission, partial remission, and non-remission groups. Significant correlation was found between Serum albumin and Serum cholesterol between different groups.

Table No-10. Correlation of Trough Level of Tacrolimus, UP:UC and Serum Albumin between groups and within groups in SRNS Patients						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Trough Concentration of Tacrolimus (ng/ml)	Between Groups	51.694	2	25.847	6.159	**.004**
	Within Groups	239.203	57	4.197		
	Total	290.897	59			
UP:UC Ratio	Between Groups	169.422	2	84.711	63.157	**<.001**
	Within Groups	76.452	57	1.341		
	Total	245.874	59			
Serum Albumin	Between Groups	8.005	2	4.003	19.541	**<.001**
	Within Groups	11.676	57	.205		
	Total	19.681	59			

The mean drug dose of tacrolimus in patients with remission (n=49) was 0.11 mg/kg/daily and the mean dose of tacrolimus in partial remission and non-remission patients was found 0.10 mg/kg/daily which shows that there was no significant relation of drug dose found with treatment outcomes of different groups. However, there was a significant correlation of three important laboratory parameters C_0 of Tacrolimus (p=0.004) UP: UC ratio (p=<.001), and serum albumin level (p=<.001) in remission, partial remission, and non-remission groups.

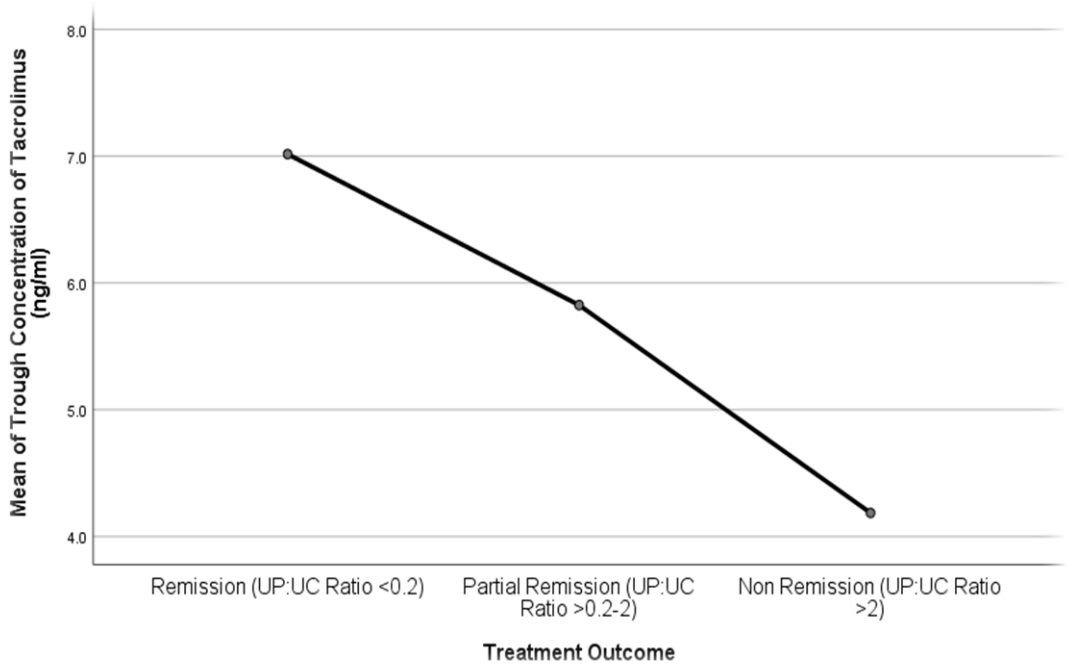


Fig. No. 24- Showing Trough level of Tacrolimus in different Groups after 12 Weeks of initial Treatment with Tacrolimus

The mean C_0 of Tacrolimus in patients showing complete remission was higher than in children with non-remission. The mean trough concentration found in our study was 6.6 ng/ml which is significantly low in comparison with the therapeutic range of Tacrolimus noted in kidney transplant patients. However it was higher than the similar published study by Jahan Afsana, Pediatr Nephrol [5] which has reported the mean trough level of Tacrolimus between 2.1 to 4.8 ng/ml in remission. The Mean C_0 of remission patients of this study was higher than in the non-remission group (7.0 ng/ml, versus 4.18 ng/ml, p=0.004).

There was no significant correlation of trough level of tacrolimus and drug doses in different groups. There was a significant correlation of three important laboratory parameters Tacrolimus C_0, UP: UC ratio, and serum albumin and Cholesterol level in remission, partial remission, and non-remission groups.

As per study [78-81] Desirable C_0 in pediatric transplants are 10–12 ng/ml in the immediate post-transplant and 5–10 ng/ml thereafter to prevent rejection. Following the the transplant data doses, most studies target tacrolimus levels between 5 and 10 ng/ml in children with SRNS [82, 83]. Our data suggests that aiming at a C_0

(7.0±2.17 ng/ml) may be appropriate, which may be possible, as these diseases are diverse in the pathogenic mechanism. The mechanism of action of tacrolimus in SRNS appears to be due to its immunosupressive effects and direct action on podocyte cytoskeleton.

Tacrolimus is a more potent drug compared to cyclosporine and suppresses cytokine production, interleukin-18 (IL-18) and IL-12, decreases mRNA levels of granulocyte macrophage colony stimulating factor, tumor necrosis factor alpha, interferon and c-myc in activated T cells. Tacrolimus affects growth and differentiation of T and B lymphocytes resulting in potent immunosuppression

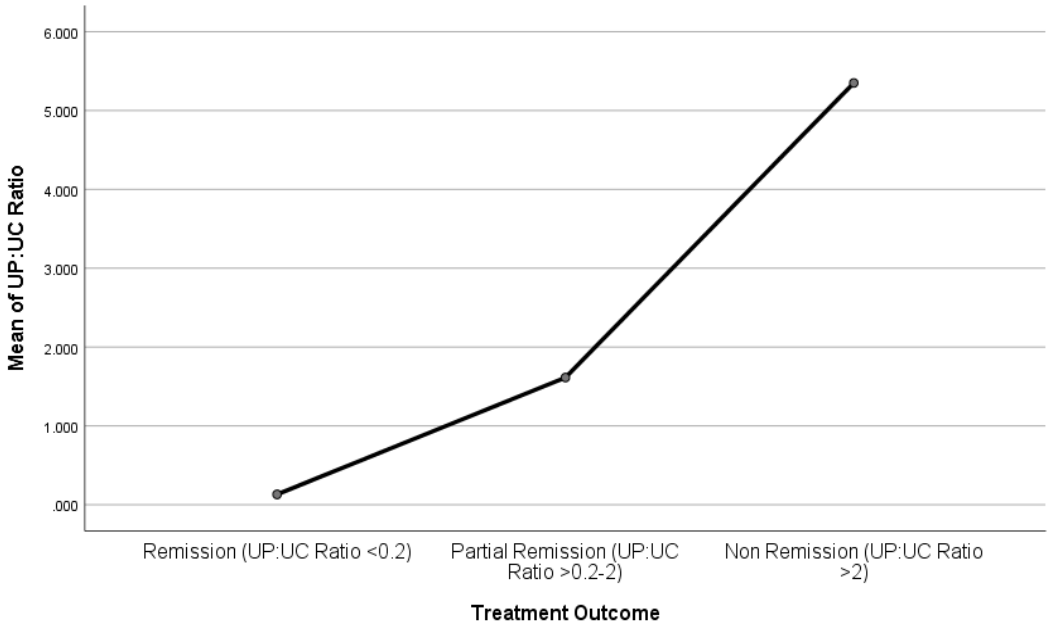


Fig. No 25- UP: UC in different Groups after 12 Weeks of initial Treatment with Tacrolimus

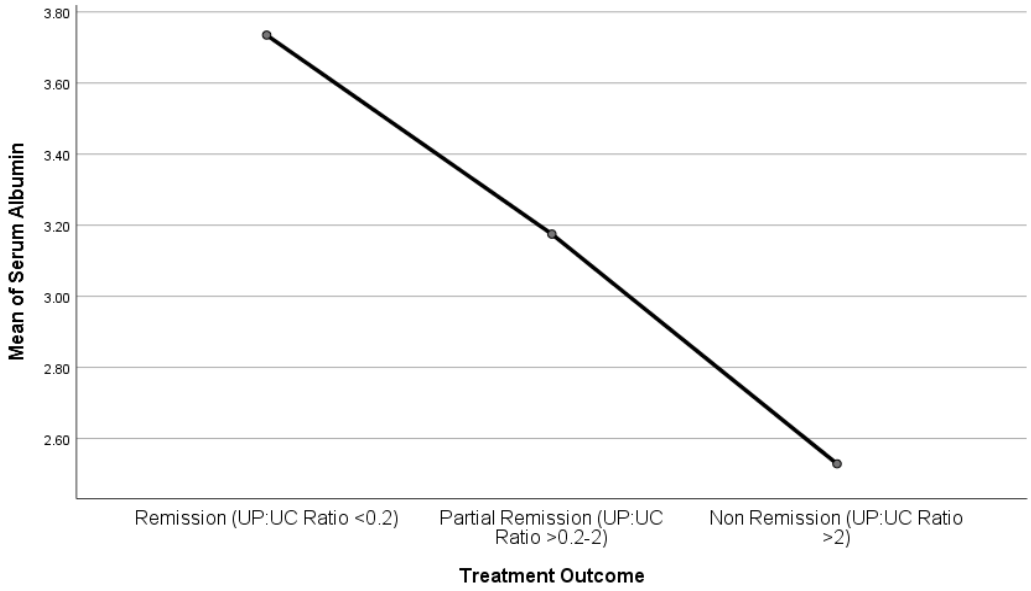


Fig. No-26 Serum Albumin in different Groups after 12 Weeks of initial Treatment with Tacrolimus

Table No. 11 Descriptive Analysis of Haematological Tests

Parameter	**Mean (SD)** **Median (IQR)**	**At Diagnosis**	**At 12 Weeks**
Haemogram			
Hb	Mean (SD) Median (IQR)	10.93 (1.67) 11.15 (2.63)	11.96 (1.38) 12.25 (1.25)
WBC	Mean (SD) Median (IQR)	9609 (2181) 9405 (2592)	8577 (1704) 8310 (1945)
DLC			
Neutrophil	Mean (SD) Median (IQR)	50.52 (8.60) 51 (12.0)	50.69 (6.47) 50.0 (5.5)
Lymphocyte	Mean (SD) Median (IQR)	43.45 (8.69) 41 (13)	41.94 (7.24) 43.00 (6.50)
Monocyte	Mean (SD) Median (IQR)	5.23 (2.92) 4.0 (3.25)	6.06 (3.26) 5.0 (3.00)
Eosinophil	Mean (SD) Median (IQR)	0.58 (0.96) 0 (1.0)	1.15 (2.72) 0.0 (2.0)
Platelets	Mean (SD) Median (IQR)	3.66 (1.46) 3.215 (1.05)	2.58 (0.53) 2.63 (0.77)

Table No. 12 Descriptive Analysis of Biochemical Tests

Kidney Function Test	Mean (SD) Median (IQR)	At Diagnosis	At 12 Weeks
Urea	Mean (SD) Median (IQR)	32.56 (18.89) 29 (8)	22.23 (9.43) 19.0 (9.0)
Creatinine	Mean (SD) Median (IQR)	0.41 (0.14) 0.395 (0.13)	0.40 (0.14) 0.390
Uric acid	Mean (SD) Median (IQR)	4.25 (1.11) 4.15 (1.13)	4.11 (0.72) 4.1 (0.58)
Serum Electrolytes			
Na	Mean (SD) Median (IQR)	140.34 (3.04) 140.0 (4.0)	140.72 (2.30) 141 (2.0)
K	Mean (SD) Median (IQR)	4.29 (0.50) 4.3 (0.63)	4.09 (0.27) 4.1 (0.23)
Calcium	Mean (SD) Median (IQR)	8.66 (0.71) 8.7 (0.70)	9.31 (0.41) 9.3 (0.53)
Phosphorous	Mean (SD) Median (IQR)	4.12 (0.56) 4.15 (0.80)	4.09 (0.40) 4.1 (0.40)
ALP	Mean (SD) Median (IQR)	125.02 (47.53) 120 (27.75)	126.42 (33.52) 123.00 (46.50)
TP	Mean (SD) Median (IQR)	5.79 (0.93) 6.0 (0.93)	6.72 (0.76) 6.7 (1.20)
ALB	Mean (SD) Median (IQR)	2.47 (0.75) 2.65 (1.30)	3.56 (0.54) 3.6 (0.53)
Lipid Profile			
Total Chl.	Mean (SD) Median (IQR)	228.93 (72.87) 215.5 (75.50)	155.83 (35.07) 156.0 (50.25)
HDL	Mean (SD) Median (IQR)	52.17 (15.42) 49.0 (13.50)	43.36 (8.17) 43.0 (6.25)
LDL	Mean (SD) Median (IQR)	148.10 (54.38) 128.5 (64.50)	94.36
TG	Mean (SD) Median (IQR)	206.98 (70.65) 195.0 (88.75)	132.13 (32.71) 126.0 (51.75)

The results of Laboratory investigations presented that 81% (49/60) of patients had complete remission while 7% (4/60) were in partial remission and 12% (7/60) were in Non-remission status after minimum 12 weeks of initial treatment with Tacrolimus. The mean trough level of tacrolimus of (n=60 patients) at the follow-up after a minimum of 12 weeks of Tacrolimus treatment was found 6.6±2.2 ng/mL. The mean C_0 of Tacrolimus was higher in the remission group than in the non-remission group (7.0 ng/ml, versus 4.18 ng/ml, p=0.004).

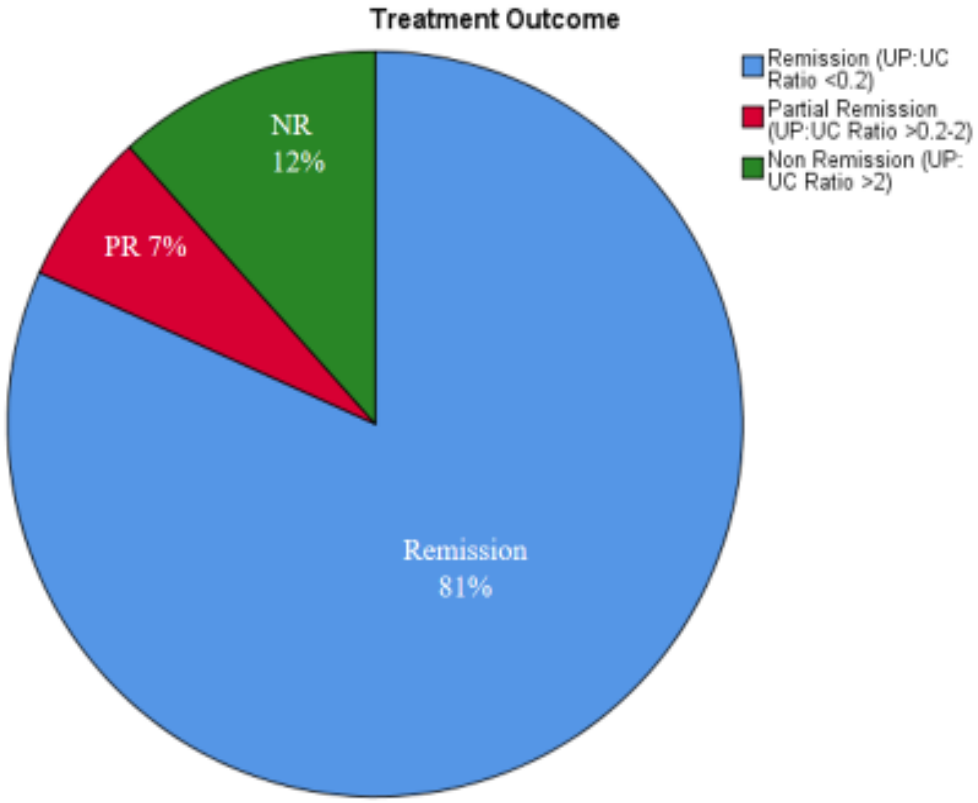
Table No. 13 - Side effects Profile of Tacrolimus Treatment

Parameter	**(n)**
Headache	7
Diarrhoea	4
Constipation	3
Nausea	12
Vomiting	7
Heartburn	14
Stomach pain	2
Loss of appetite	11

The major side effect of Tacrolimus noted during treatment were heartburn/acidity in 28.3% (n=17/60), loss of appetite, 23.3% (n=14/60), Nausea 13.3% (n=9/60), however, no other major side effect was reported in patients.

Table No. 14 - BP Centiles in SRNS Patients

	PR	RR	BP_Systolic	BP_Diastolic	BP_Centiles_50th_SBP	BP_Centiles_50th_DBP	BP_Centiles90th_SBP	BP_Centiles90th_DBP	BP_Centiles95th_SBP	BP_Centiles95th_DBP	BP_Centiles95_12SBP	BP_Centiles95_12DBP
N	60	60	60	60	60	60	60	60	60	60	60	60
	0	0	0	0	0	0	0	0	0	0	0	0
Mean	86.63	22.75	97.82	63.45	94.87	54.48	107.63	66.92	111.62	70.20	123.45	82.20
Median	86.00	22.00	99.00	63.00	92.50	54.00	105.50	66.50	109.00	69.50	121.00	81.50
Std. Deviation	10.268	3.245	13.324	11.003	6.448	6.331	6.468	6.006	6.592	5.954	6.833	5.954
Range	63	18	56	50	25	36	26	24	27	25	31	25
Minimum	72	16	66	38	84	40	97	52	101	54	109	66
Maximum	135	34	122	88	109	76	123	76	128	79	140	91



PR: Partial Remission

NR: Non Remission

Fig. No. 27 Treatment outcome status after 12 weeks of treatment with Tacolimus in 60 SRNS Patients.

Table No. 15 -Tacrolimus level in different groups (Initial Resistant and Late Resistant)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Initial Resistant	22	6.3909	2.33909	.49870	5.3538	7.4280	3.00	10.90
Late Resistant	38	6.7316	2.17095	.35217	6.0180	7.4452	3.10	13.00
Total	60	6.6067	2.22046	.28666	6.0331	7.1803	3.00	13.00

Table No. 16 Descriptive Analysis of Biochemical Tests in different groups

Initial Resistant and Late Resistant

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Spot_UPUC	Initial	22	6.85545	4.904990	1.045747	4.68070	9.03021	1.800	17.110
	Late	38	.13695	.040744	.006610	.12356	.15034	.012	.190
	Total	60	2.60040	4.384538	.566041	1.46775	3.73305	.012	17.110
TP_gmdl	Initial	22	5.4986	.82026	.17488	5.1350	5.8623	3.60	7.28
	Late	38	6.0750	.60641	.09837	5.8757	6.2743	3.94	6.80
	Total	60	5.8637	.74064	.09562	5.6723	6.0550	3.60	7.28
Albumin	Initial	22	1.9536	.76964	.16409	1.6124	2.2949	.90	4.23
	Late	38	2.7239	.64636	.10485	2.5115	2.9364	1.32	3.60
	Total	60	2.4415	.78292	.10107	2.2393	2.6437	.90	4.23
Cholesterol	Initial	22	234.91	69.976	14.919	203.88	265.93	141	436
	Late	38	178.92	42.502	6.895	164.95	192.89	112	283
	Total	60	199.45	60.133	7.763	183.92	214.98	112	436

The results of Laboratory investigations presented that 81% (49/60) of patients had complete remission while 7% (4/60) were in partial remission and 12% (7/60) were in non-remission status after minimum 12 weeks of initial treatment with Tacrolimus. The mean trough level of tacrolimus of (n=60 patients) at the follow-up after a minimum of 12 weeks of Tacrolimus treatment was found 6.6±2.2 ng/mL. The clinical profile and trough level of tacrolimus showed a better treatment outcome with tacrolimus therapy. The mean C_0 of Tacrolimus was higher in the remission group than in the non-remission group (7.0 ng/ml, versus 4.18 ng/ml, p=0.004).

Detection of tacrolimus by immunoassay is also a preferred method nowadays. These methods mostly include the use of anti-tacrolimus antibodies conjugated with specific antigens. Antibodies used in immunoassay are well known to cause cross-reactivity with a variety of metabolites. Advances in immunoassay measurement involve automated specimen pre-treatment, enhanced reagent stabilities to lower potential matrix effect, and new anti-Tac antibodies that provide more sensitivity and affinity to the target drug. Immunoassay continues to be used in many laboratories across the country because of its ease of use and lower costs associated with services. Many laboratories find this option appealing because it does not involve a high level of technical skill from staff; the equipment can be leased; and the manufacturer often provides training, support, and maintenance for these systems. A study held in Japan had mentioned that the Microparticle enzyme immunoassay was the most widely used method for more than 20 years.

Although there has been literature suggesting LC-MS as the gold standard assay due to its high specificity it has a major disadvantage of a high-ended and costly setup, moreover requires extremely trained professionals. Ultimately labs now prefer immunoassays as they are more cost-effective and do not require specialized training. Although the major disadvantage the immunoassays exhibit is cross-reactivity with different metabolites, with advances in technology newer methods like PETIA, CEDIA, and Dried blood spot analysis have been implemented. Studies have exhibited that these techniques significantly correlated with the gold standard techniques and they were found to be cost-effective. CEDIA correlated well with LC-MS (r= 0.924) and could detect TAC concentration between1-30ng/ml. It further followed 2 point calibration throughout standard curve assay. On the other hand,

PETIA is emerging as the most cost-effective procedure nowadays. Correlation studies also established a highly significant association between test specimens with PETIA to that of LC-MS (r = 0.972) and the Abbott Architect assay (r=0.937). PETIA can detect as low as 1ng/ml with linearity of 0-30ng/ml. It also follows 6 point calibration which makes this technique highly sensitive. Dried spot analysis using finger prick is usually a less invasive, painless method for clinical analysis but this technique needs proper validation for implementation.

LC-MS and Immunoassay are two important techniques that are used for the therapeutic monitoring of Tacrolimus. The LC-MS is a gold standard method which requires high costs and full validation for use. A high degree of technical ability and extensive training is required to perform this type of testing. Turbidimetric immunoassays can be considered a better alternative to LC-MS in terms of cost and turnaround time (TAT), especially in resource-constraint laboratory facilities.

Chapter I focused on a brief introduction to Nephrotic Syndrome, Steroid Resistant Nephrotic Syndrome and brief introduction to different laboratory methods. The profiles of drugs used in research are discussed in this chapter. The literature review of Nephrotic Syndrome, Steroid Resistant Nephrotic Syndrome and drug was discussed in Chapter II. In this chapter briefly were discussed available articles on different analytical techniques. Chapter III discussed in detail the objective and plan of the research work. Chapter IV discussed the material & method used in the research work. This chapter has also discussed about the principle & summary of test used in the research. Chapter V highlighted the results & discussion. The summary& conclusion has been mentioned in Chapter-VI. The limitation and future scope of the study has been mentioned in chapter VII.

CHAPTER-6

SUMMARY AND CONCLUSION

6. SUMMARY & CONCLUSION

Summary

The present study was a cross sectional prospective study to evaluate the trough concentration of Tacrolimus in Children with steroid resistant nephrotic syndrome. 60 children between 1 to 16 years of age with steroid-resistant Nephrotic Syndrome were included. The study was conducted from January 2021 to January 2022. All clinical parameters were noted at onset and after 12 weeks of standard treatment like BP, Weight Height etc. Trough concentration of Tacrolimus was measured after a minimum of 12 weeks of treatment using Particle-Enhanced Turbidimetric Immunoassay (PETIA). Biochemical and haematological laboratory parameters were also done at onset and after 12 weeks of standard treatment with Tacrolimus. The correlation of various laboratory parameters was done in different group i.e. remission, non-remission and partial remission to evaluate the outcome of treatment.

The findings were as follows:

1. The results of Laboratory investigations presented that 81% (49/60) of patients had complete remission while 7% (4/60) were in partial remission and 12% (7/60) were in non-remission status after minimum 12 weeks of initial treatment with Tacrolimus.

2. The mean trough level of tacrolimus of (n=60 patients) at the follow-up after a minimum of 12 weeks of Tacrolimus treatment was found 6.6±2.2 ng/mL.

3. The clinical profile and trough level of tacrolimus showed a better treatment outcome with tacrolimus therapy. The mean C_0 of Tacrolimus was higher in the remission group than in the non-remission group (7.0 ng/ml, versus 4.18 ng/ml, p=0.004).

4. The mean drug dose of tacrolimus in patients with remission (n=49) was 0.11 mg/kg/daily and the mean dose of tacrolimus in partial remission and non-remission patients was found 0.10 mg/kg/daily which shows that there was no significant relation of drug dose found with treatment outcomes of different groups.

5. There was a significant correlation of three important laboratory parameters C_0 of Tacrolimus (p=0.004) UP: UC ratio (p=0.000), and serum albumin level (p=0.000) in remission, partial remission, and non-remission groups.

6. 63% patients (n=38/60) were late resistant to steroid treatment while 38% (n=22/60) were initial resistant.

7. The mean age at onset of diseases was 67.08 months.

8. The gender distribution ratio was 1.72:1. The male children were higher at 63% (n=38/60) while female children were 37% (n=22/60).

9. The Kidney biopsy was done in 43.3% (n=26/60 patients). The results of kidney Biopsy presented that minimal change disease ((MCD n=17/26) was the most common histological diagnosis, followed by Focal Segmental Glomerulosclerosis (FSGS n=9/26).

10. The edema was noted in the majority of patients at the onset of the disease.

11. Many related clinical conditions like Edema 43.3% (n=26/60), Hypertension 16.6% (n=10/60), Hematuria 15% (n=9/60), Oliguria 6.6% (n=4/60), Fever 11.6% (n=7/60), Joint pain 5% (n=3/60), rashes 3.3% (n=2/60) were noted in patients during SRNS treatment. The family history of Idiopathic Nephrotic Syndrome was found only in one patient. Good compliance was noted in almost all patients only two patients showed poor compliance to treatment.

12. Significant correlation was found between Serum albumin and Serum cholesterol between different groups.

13. The major side effect of Tacrolimus noted during treatment were heartburn/acidity in 28.3% (n=17/60), loss of appetite, 23.3% (n=14/60), Nausea 13.3% (n=9/60), however, no other major side effect was reported in patients.

14. The mean C_0 of Tacrolimus in patients showing complete remission was higher than in children with non-remission. The mean trough concentration found in our study was 6.6 ng/ml which is significantly low in comparison

with the therapeutic range of Tacrolimus noted in kidney transplant patients. However it was higher than the similar published study by Jahan Afsana, Pediatr Nephrol which has reported the mean trough level of Tacrolimus between 2.1 to 4.8 ng/ml in remission. The Mean C_0 of remission patients of our study was higher than in the non-remission group (7.0 ng/ml, versus 4.18 ng/ml, p=0.004). There was no significant correlation of trough level of tacrolimus and drug doses in different groups. There was a significant correlation of three important laboratory parameters Tacrolimus C_0, UP: UC ratio, and serum albumin and Cholesterol level in remission, partial remission, and non-remission groups.

15. The study was conceived with the Hypothesis that the clinical efficacy of Tacrolimus in SRNS children is related to its trough levels in Indian children. Our findings also corroborate that regular monitoring of trough level of Tacrolimus during treatment may play an important role in treatment outcomes. The trough level of the drug can be a better indicator for clinical management of SRNS. The Particle-enhanced Turbidimetric Immunoassay (PETIA) can be a an alternative to LCMS which is being utilized for drug monitoring.

To our knowledge, this was one of the largest studies of north India undertaken to evaluate the trough level of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome (SRNS). Our study has demonstrated that-

1) The regular monitoring of Tacrolimus is very important for proper treatment and clinical management of patient with SRNS.

2) Trough level of drug can be a better indicator for clinical management.

3) The mean trough concentration found in our study was 6.6 ng/ml which is significantly low in comparison with the established therapeutic range of Tacrolimus extrapolated from kidney transplant patients.

4) The Mean Concentration of Tacrolimus in patients with remission was 7.016 ng/ml, in partial remission group was 5.825 ng/ml and in non-remission patients was 4.186 ng/ml

5) Proteinuria, Hypoalbuminemia, Edema and Hypertension were prominent condition in patients at the onset of Diseases and during Steroid Treatment.

6) The major side effect of Tacrolimus noted during treatment were heartburn/acidity in 28.3% (n=17/60), loss of appetite, 23.3% (n=14/60), Nausea 13.3% (n=9/60), however, no other major side effect was reported in patients.

7) PETIA can be an alternative of LCMS for TDM of Tacrolimus, however, a pharmacokinetic study with large sample size and a long-term follow-up with more patients is required to substantiate the findings.

7. LIMITATIONS & FUTURE SCOPE

The limitations of our study are:

This study was undertaken to evaluate trough concentration of Tacrolimus in 60 Children with Steroid Resistant Nephrotic Syndrome (SRNS) and Tacrolimus level was measured after minimum 12 weeks treatment with Tacrolimus. Single sample was taken to measure trough concentration, however, further studies can be considered with multiple sampling and large sample size for pharmacokinetic study in SRNS.

This study was performed on particle enhanced turbidimetric immunoassay (PETIA) which may be considered an economic alternative of LCMS however comparative study is suggested with LCMS to substantiate the finding.

The study was done a tertiary care children hospital of north India. Similar multi center studies may be considered in future for safety, efficacy and better treatment outcomes.

BIBLIOGRAPHY

REFERENCES

1. Vasudevan A, Thergaonkar R, Mantan M, Sharma J, Khandelwal P, Hari P, Sinha A, Bagga A; Expert Group Of The Indian Society Of Pediatric Nephrology. Consensus Guidelines on Management of Steroid-Resistant Nephrotic Syndrome. Indian Pediatr. 2021 Jul 15;58(7):650-666.

2. Noone DG, Iijima K, Parekh R. Idiopathic Nephrotic Syndrome in children. Lancet. 2018 Jul 7; 392 (10141):61-74.

3. Eddy AA, Symons JM. Nephrotic Syndrome in childhood. Lancet 2003;362:629-39.

4. Tullus K, Webb H, Bagga A. Management of steroid-resistant Nephrotic Syndrome in children and adolescents. Lancet Child AdolescHealt.2018; 2:880-90.

5. Jahan A, Prabha R, Chaturvedi S, Mathew B, Fleming D, Agarwal I. Clinical efficacy and pharmacokinetics of tacrolimus in children with steroid-resistant Nephrotic Syndrome. Pediatr Nephrol. 2015 Nov;30(11):1961-7.

6. Indian Pediatric Nephrology Group, Indian Academy of Pediatrics, Bagga A, Ali U, et al. Management of steroid sensitive Nephrotic Syndrome: revised guidelines. *Indian Pediatr*. 2008;45(3):203-214.

7. Chadwick J, Mann WN. The medical works of Hippocrates. London: Oxford University Press, 1950: 228 (Section 2, No 136), 240 (Section 13, No 266), 244 (Section 7, No 34).

8. Roelans C. Liber de aegritudinibus infantium. (c 1484). Reproduced in: Sudhoff KFJ. Erstlinge der padiatrischen Literatur. Munchen: MOnchner Drucke, 1925: cxciii-cxciiii. [8]

9. Zwinger T. Paedoiatreia practica. Basel, 1722: cap 119. Anasarca puerorum. (See also: Buess H, Portman ML, Molling P. Theodor Zwinger III 1658-1724: ein Basler Arzt und Kinderarzt der Barockzeit. Basel: Helbing & Lichtenhahn, 1962: 172-174).

10. Still GF. History of paediatrics. London: Oxford University Press, 1931: 338.

11. Cameron JS. The nephrotic syndrome: A historical review. In: Cameron JS, Glassock RJ, editors. The Nephrotic Syndrome. New York: Dekker; (1988). p. 3–56.

12. Bright R. Reports of Medical Cases. London: Orme (1827).

13. Müller F. Morbus Brightii. Verh Dtsch Ges Path. 1905;9: 64-99.

14. Allen AC. The Kidney: Medical and Surgical diseases. New York: Grune and Stratton. 1951; p. 583.

15. Bhandari NR, Bhoraskar SN. Nephrotic Syndrome in children. Indian Pediatr. 1966 Dec;3(12):422-7.

16. Vasudevan A, Thergaonkar R, Mantan M, et al. Consensus Guidelines on Management of Steroid-Resistant Nephrotic Syndrome. *Indian Pediatr*. 2021;58(7):650-666.

17. BH, Caster DJ, Cattran DC, et al. Management and treatment of glomerular diseases (part 2): conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int*. 2019;95(2):281-295.

18. Gulati S, Sengupta D, Sharma RK, Sharma A, Gupta RK, Singh U, Gupta A. Steroid resistant Nephrotic Syndrome: role of histopathology. Indian Pediatr. 2006 Jan;43(1):55-60.

19. Querfeld U. Should hyperlipidemia in children with the Nephrotic Syndrome be treated? Pediatr Nephrol. 1999 Jan;13(1):77-84. doi: 10.1007/s004670050568.

20. Lamas S. Cellular mechanisms of vascular injury mediated by calcineurin inhibitors. Kidney Int. 2005 Aug;68(2):898-907.

21. Noone DG, Iijima K, Parekh R. Idiopathic Nephrotic Syndrome in children. Lancet. 2018 Jul 7; 392 (10141):61-74.

22. Eddy AA, Symons JM. Nephrotic Syndrome in childhood. Lancet 2003;362:629-39.

23. Niaudet P. Steroid sensitive Nephrotic Syndrome in children. In: Avner ED, Harmon WE, Neasden P, editors. Paediatric Nephrology. 1st ed. Philadelphia: Lippincott Williams and Wilkins; 2004. p. 543-56.

24. Niaudet P, Boyer O. Paediatric Nephrology. 6th ed. Berlin, Heidelberg:springer-verlag.2009:667-668.

25. Chua A, Yorgin P. Evidence based Nephrology. 1st ed. Chichester, west Sussex, UK wiley-blackwell.2009:787-789.

26. Butani L, Ramsamooj R. Experience with tacrolimus in children with steroid-resistant Nephrotic Syndrome. Pediatr Nephrol. 2009 Aug; 24(8):1517-23.

27. Tullus K, Webb H, Bagga A. Management of steroid-resistant Nephrotic Syndrome in children and adolescents. Lancet Child AdolescHealt.2018; 2:880-90.

28. Sinha A, Bagga A. Nephrotic syndrome. Indian J Pediatr. 2012 Aug;79(8):1045-55.

29. Hari P, Khandelwal P, Smoyer WE. Dyslipidemia and cardiovascular health in childhood nephrotic syndrome. Pediatr Nephrol. 2020 Sep;35(9):1601-1619.

30. Hopp L, Gilboa N, Kurland G, Weichler N, Orchard TJ. Acute myocardial infarction in a young boy with nephrotic syndrome: a case report and review of the literature. Pediatr Nephrol. 1994 Jun;8(3):290-4.

31. Beck L, Bomback AS, Choi MJ, *et al* KDOQI US commentary on the 2012 KDIGO clinical practice guideline for glomerulonephritis. Am J Kidney Dis. 2013 Sep;62(3):403-41.

32. Indian Society of Pediatric Nephrology, Gulati A, Bagga A, Gulati S, Mehta KP, Vijayakumar M. Management of steroid resistant nephrotic syndrome. Indian Pediatr. 2009 Jan;46(1):35-47.

33. Querfeld U. Should hyperlipidemia in children with the nephrotic syndrome be treated? Pediatr Nephrol. 1999 Jan;13(1):77-84.

34. Lamas S. Cellular mechanisms of vascular injury mediated by calcineurin inhibitors. Kidney Int. 2005 Aug;68(2):898-907.

35. Agrawal S, Zaritsky JJ, Fornoni A, Smoyer WE. Dyslipidaemia in nephrotic syndrome: mechanisms and treatment. Nat Rev Nephrol. 2018 Jan;14(1):57-70.

36. Lancia P, Jacqz-Aigrain E, Zhao W. Choosing the right dose of tacrolimus. Arch Dis Child. 2015 Apr; 100(4):406-13.

37. Lombel RM, Gipson DS, Hodson EM; Kidney Disease: Improving Global Outcomes. Treatment of steroid-sensitive Nephrotic Syndrome: new guidelines from KDIGO. Pediatr Nephrol. 2013 Mar; 28(3):415-26.

38. Filler G. Treatment of Nephrotic Syndrome in children and controlled trials. Nephrol Dial Transplant. 2003 Aug; 18 Suppl 6:vi75-8.

39. Lombel RM, Hodson EM, Gipson DS; Kidney Disease: Improving Global Outcomes. Treatment of steroid-resistant Nephrotic Syndrome in children: new guidelines from KDIGO. Pediatr Nephrol. 2013 Mar;28(3):409-14.

40. Prasad N, Manjunath R, Rangaswamy D, Jaiswal A, Agarwal V, Bhadauria D, Kaul A, Sharma R, Gupta A. Efficacy and Safety of Cyclosporine versus Tacrolimus in Steroid and Cyclophosphamide Resistant Nephrotic Syndrome: A Prospective Study. Indian J Nephrol. 2018 Jan-Feb;28(1):46-52.

41. Gulati S, Prasad N, Sharma RK, Kumar A, Gupta A, Baburaj VP. Tacrolimus: a new therapy for steroid-resistant Nephrotic Syndrome in children. Nephrol Dial Transplant. 2008 Mar;23(3):910-3.

42. Loeffler K, Gowrishankar M, Yiu V. Tacrolimus therapy in pediatric patients with treatment-resistant Nephrotic Syndrome. Pediatr Nephrol. 2004 Mar;19(3):281-7.

43. Port FK, Dykstra DM, Merion RM, Wolfe RA. Trends and results for organ donation and transplantation in the United States, 2004. Am J Transplant. 2005;5(4 Pt 2):843–849. 6. Gounden V, Soldin SJ. Tacrolimus measurement: building a better immunoassay. Clin Chem. 2014;60(4):575–576.

44. Thölking G, Gerth HU, Schuette-Nuetgen K, Reuter S. Influence of tacrolimus metabolism rate on renal function after solid organ transplantation. World J Transplant. 2017 Feb 24; 7(1):26-33.

45. Starzl T, Todo S, Fung J, et al. FK506 for human liver, kidney and pancreas transplantation. Lancet1989; 2:1000–4.

46. McCauly J, Fung J, Jain A, et al. The effects of FK506 on renal function after liver transplantation. Transplant Proc 1990; 22:17–20.

47. Tamura K, Kobayashi M, Hashimoto K, et al. A highly sensitive method to assay FK-506 levels inplasma. Transplant Proc 1987;19: 23–9.

48. Elens L, Capron A, van Schaik RH, De Meyer M, De Pauw L, Eddour DC, Latinne D, Wallemacq P, Mourad M, Haufroid V. Impact of CYP3A4*22 allele on tacrolimus pharmacokinetics in early period after renal transplantation: toward updated genotype-based dosage guidelines. Ther Drug Monit. 2013 Oct; 35(5): 608-16.

49. Scott LJ, McKeage K, Keam SJ, et al. FK506: a further update of its use in the management of organ transplantation. Drugs. 2003;63:1247-1297.

50. https://go.drugbank.com/drugs/DB00864 Tacrolimus: Uses, Interactions, Mechanism of Action | DrugBank Online.

51. Iwasaki K: Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. Drug Metab Pharmacokinet. 2007 Oct;22(5):328-35.

52. https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/213721s000lbl.pdf label (fda.gov).

53. Sehrawat K S, Patnaik R, Naik S, Chauhan P, Saha A. Measurement of Tacrolimus: A Review of Laboratory Methods. Annals of RSCB:3323–3331. https://www.annalsofrscb.ro/index.php/journal/article/view/4977.

54. https://assets.thermofisher.com/TFS-Assets/CDD/Package-Inserts/10015557-QMS-Tacrolimus-Assay-EN.pdf

55. Christians U, Pokaiyavanichkul T, Chan L, Tacrolimus, Applied Pharmacokinetics and Pharmacodynamics: Principals of Therapeutic Drug Monitoring. 4th Edition. Lippincott Williams & Wilkins, Philadelphia, PA 2005: 529-562.

56. Ho CS, Lam CWK, Chan MHM, Cheung RCK, Law LK, Lit LCW, et al. Electrospray ionisation mass spectrometry: principles and clinical applications. Clin Biochem Rev. 2003;24:3–12.

57. Jansen R, Lachatre G, Marquet P. LC-MS/MS systematic toxicological analysis: comparison of MS/MS spectra obtained with different instruments and settings. Clin Biochem. 2005;38:362–72

58. Yang Z, Wang S. Recent development in application of high performance liquid chromatography-tandem mass spectrometry in therapeutic drug monitoring of immunosuppressants. J Immunol Methods. 2008;336:98–103

59. Taylor PJ. Therapeutic drug monitoring of immunosuppressant drugs by high-performance liquid chromatography-mass spectrometry. Ther Drug Monit. 2004 Apr;26(2):215-9.

60. Kapur BM. Drug-testing methods and clinical interpretations of test results. Bull Narc. 1993;45(2):115-54.

61. Mould G.P., Aherne G.W., Morris B.A. *et al.* Radioimmunoassay of drugs and its clinical application. *European Journal of Drug Metabolism and Pharmacokinetics* 2, 171–184 (1977)

62. Marubashi S, Nagano H, Kobayashi S, Eguchi H, Takeda Y, Tanemura M, Umeshita K, Monden M, Doki Y, Mori M. Evaluation of a new immunoassay

for therapeutic drug monitoring of tacrolimus in adult liver transplant recipients. J Clin Pharmacol. 2010 Jun;50(6):705-9.

63. Demirev P.A. Dried blood spots: Analysis and applications. Anal. Chem. 2013;85:779–789.

64. Zakaria R., Allen K.J., Koplin J.J., Roche P., Greaves R.F. Advantages and Challenges of Dried Blood Spot Analysis by Mass Spectrometry Across the Total Testing Process. EJIFCC. 2016;27:288–317.

65. Hinchliffe E, Adaway JE, Keevil BG. Simultaneous measurement of cyclosporin A and tacrolimus from dried blood spots by ultra high performance liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2012; 883–884: 102–107.

66. Koop DR, Bleyle LA, Munar M, Cherala G, Al-Uzri A. Analysis of tacrolimus and creatinine from a single dried blood spot using liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2013; 926: 54–61

67. Clinico-pathological correlations of childhood glomerular disease in Eastern India Clinico-pathological correlations of childhood glomerular disease in Eastern India | available on https://www.researchgate.net/publication/276838060

68. Arumugam V, Saha A, Kaur M, Endothelin 1 and endothelial dysfunction in children with idiopathic nephrotic syndrome. Artery Research 2017;18(C):36-40.

69. Kaddah A, Sabry S, Emil E, El-Refaey M. Epidemiology of primary nephrotic syndrome in Egyptian children. J Nephrol. 2012;25(5):732-7.

70. Beins NT, Dell KM. Long Term outcomes in Children with Steroid-Resistant Nephrotic Syndrome Treated with Calcineurin Inhibitors. Front Pediatr. 2015;3:104

71. Franke I, Aydin M, Llamas Lopez CE, Kurylowicz L, Ganschow R, Lentze M, Born M. The incidence of the nephrotic syndrome in childhood in Germany. Clin Exp Nephrol. 2018 Feb;22(1):126-132.

72. Sharples PM, Poulton J, White RHR. Steroid responsive nephrotic syndrome is more common in Asians. Arch Dis Child. 1985;60:1014–7

73. Simpson AK, Wong W, Morris MC. Pediatric nephrotic syndrome in Auckland, New Zealand. J Peadiatr Child Health. 1998;34:360–2.

74. Bircan Z, Yilmaz AY, Katar S, Vitrinel A, Yildrim M. Childhood idiopathic nephrotic syndrome in Turkey. Pediatr Int. 2002;44:608–11.

75. O¨ zkaya N, C¸ akar N, Ekim M, Kara N, Akko¨k N, Yalc¸inkaya F. Primary nephrotic syndrome during childhood in Turkey. Pediatr Int. 2004;46:436–8

76. Landau D, Oved T, Geiger D, Abizov L, Shalev H, Parvari R. Familial steroid sensitive nephrotic syndrome in Southern Israel: clinical and genetic observations. Pediatr Nephrol. 2007;22:661–9.

77. Srivastava T, Simon SD, Alon US. High incidence of focal segmental glomerulosclerosis in nephrotic syndrome of childhood. Pediatr Nephrol. 1999;13:13–8.

78. Zhao W, Fakhoury M, Jacqz-Aigrain E (2010) Developmental pharmacogenetics of immunosuppressants in pediatric organ transplantation. Ther Drug Monit 32:688–699.

79. Przepiorka D, Blamble D, Hilsenbeck S, Danielson M, Krance R, Chan KW (2000) Tacrolimus clearance is age-dependent within the pediatric population. Bone Marrow Transplant 26:601–605.

80. O'Seaghdha CM, McQuillan R, Moran AM, Lavin P, Dorman A, O'Kelly P, Mohan DM, Little P, Hickey DP, Conlon PJ (2009) Higher tacrolimus trough levels on days 2–5 post-renal transplant are associated with reduced rates of acute rejection. Clin Transplant 23:462–468.

81. Filler G, Feber J, Lepage N, Weiler G, Mai I (2002) Universal approach to pharmacokinetic monitoring of immunosuppressive agents in children. Pediatr Transplant 6:411–418

82. Gulati A, Sinha A, Jordan SC, Hari P, Dinda AK, Sharma S, Srivastava RN, Moudgil A, Bagga A (2010) Efficacy and safety of treatment with rituximab for difficult steroid-resistant and - dependent nephrotic syndrome: multicentric report. Clin J Am Soc Nephrol 5:2207–2212.

83. Lombel RM, Hodson EM, Gipson DS (2013) Treatment of steroid resistant nephrotic syndrome in children: new guidelines from KDIGO. Pediatr Nephrol 28:409–414.

ANNEXURES

Annexure No.	List of Annexures
1.	NOC from Employer
2.	Permission from Department of Paediatrics, KSCH
3.	Institutional Ethical Committee Approval
4.	MOU between Galgotias University and LHMC
5.	PhD Course work Completion Certificate
6.	ISPN Guidelines on Steroid Resistant Nephrotic Syndrome
7.	Participation Information Sheet Hindi & English
8.	Informed Consent Hindi & English
9.	Assent Form Hindi & English
10.	Case Performa
11.	TDM of Tacrolimus-Report Performa
12.	Biochemistry investigations report performa
13.	Master Chart
14.	Conference Presentations & Participation (Certificates)
15.	Plagiarism Certificate
16.	Published Papers
17.	Author's CV

ANNEXURE-I

Ph. No.011-23346538
011-23408503
Fax No.011-23745627

GOVERNMENT OF INDIA
MINISTRY OF HEALTH & FAMILY WELFARE
KALAWATI SARAN CHILDREN'S HOSPITAL
BANGLA SAHIB MARG: NEW DELHI.

KSCH/Admn/0462/Gr.C/2016-17/ 4493 Date:25/10/2016

NO OBJECTION CERTIFICATE

This is to certify that Shri Kaptan Singh Sehrawat is employed as Technical Officer in this Institution since 21.02.2002.

This Institute has no objection for pursuing Ph D in Clinical Research from Galgotia University, Greater Noida, Utter Pradesh subject to the condition that his routine duties will not hampered in the department. This institution will not bear any financial implication.

This issues with the approval of the Director, LHMC & Assoc. Hospital.

(Hukam Chand)
Administrative Officer
27/10/16

To

Shri Kaptan Singh Sehrawat
Technical Officer,
KSC Hospital, New Delhi.

ANNEXURE-II

19th September, 2020

To

Dr Virender Kumar,
Director Professor & HOD
Department of Pediatrics,
Kalawati Saran Children's Hospital, New Delhi

Sub: Request for allotment of Co. Supervisor from Department of Pediatrics and forwarding my PhD Research Proposal to Institutional Ethical Committee of LHMC & Associated Hospitals.

Respected Sir,

With due regards, this is to inform you that I am pursuing my PhD in Clinical Research from Galgotias University, Greater Noida. I had already taken the official permission from the Director LHMC for the said purpose. **(Copy Annexed as A-1)**

I proposed to carry out my PhD thesis on "***To evaluate trough level concentration of Tacrolimus in Children's with Steroid Resistant Nephrotic Syndrome*** under the supervision of Dr (Mrs) Ranjana S. Patnaik, Dean Department of Clinical Research, Galgotias University. I shall be highly thankful if you could please allot me a Co. Supervisor from the division of Nephrology, Department of Pediatrics, KSCH.

I am also seeking a permission from Institutional Ethical Committee of LHMC & Associated Hospitals to undertake my research on above topic. In this context, it is also to inform you that the Tacrolimus level is already being done in KSCH Biochemistry Labs for the treatment and therapeutic monitoring of the pediatric patients of SRNS.

You are therefore requested to kindly allot me a co. supervisor from Department of Pediatrics and forward my PhD Research Proposal to Institutional Ethical Committee of LHMC & Associated Hospitals

Thanking you.

Agreed in Principle
Dr Abhijeet Saha
19/9/2020

निदेशक आचार्य एवं विभागाध्यक्ष
Director Professor & Head
बाल रोग चिकित्सा विभाग
Department of Pediatrics
लेडी हार्डिंग मेडिकल कालेज
Lady Hardinge Medical College
कलावती सरन बाल चिकित्सालय
Kalawati Saran Children's Hospital
नई दिल्ली-110001/ New Delhi-110001

Yours Sincerely,

Sehrawat

Kaptan Singh Sehrawat
Technical Officer,
Advanced Biochemistry & Drug Assay Lab.
Department of Biochemistry
Kalawati Saran Children's Hospital, New Delhi

ANNEXURE-III

INSTITUTIONAL ETHICS COMMITTEE
LADY HARDINGE MEDICAL COLLEGE & ASSOCIATED HOSPITALS, SHAHID BHAGAT SINGH MARG, NEW DELHI-110001, INDIA.

LHMC/IEC/2021/03/30

Dated: 16 Feb. 2021

Shri. Kaptan Singh Sehrawat,
Technical Officer,
Department of Clinical Biochemistry,
Kalawati Saran Children's Hospital, New Delhi.

Sub: Research project entitled, **"A study to evaluate trough concentration of tacrolimus in children with steroid resistant nephrotic syndrome."**

Dear Shri Sehrawat,

Institutional Ethics Committee, LHMC & Associated Hospitals, New Delhi in its meeting held on 28 Jan. 2021 via Microsoft Teams, reviewed and discussed your application in respect of the research proposal **"A study to evaluate trough concentration of tacrolimus in children with steroid resistant nephrotic syndrome."**

Following members were present in the IEC meeting held on 28 Jan. 2021

1.	Dr. A.K. Dutta	External Member	Chairperson
2.	Dr. Harish K. Pemde,	Internal Member	Member Secretary
3.	Mr. Munawwar Naseem	External Member	Legal Expert
4.	Dr. J.S. Arora	External Member	Social Scientist
5.	Mr. Rajeev R. Singh	External Member	Social Scientist
6.	Dr. Sudha Chandella	External Member	Clinician
7.	Dr. Monika Bahl	External Member	Medical Scientist
8.	Dr. Aparna Agarwal	Internal Member	Clinician
9.	Dr. Shailaja Shukla	Internal Member	Basic Medical Scientist
10.	Dr. Anup Mohta	Internal Member	Clinician
11.	Dr. Ekta Debnath,	Internal Member	Basic Medical Scientist
12.	Dr.Indranil Banerjee	Internal Member	Basic Medical Scientist
13.	Dr. Manish K. Goel	Internal Member	Public Health Expert

The Committee decision is as under:

Decision: Approved.

The study should be conducted in accordance with the provisions of New Drugs and Clinical Trial Rules 2019, GCP regulations and the ICMR Guidelines for Biomedical Research on Human Participants (2017). You are required to inform IEC, LHMC about any serious adverse events or death of study participants and the amendments in Protocol/ study procedure/site/investigator and also about premature termination of study with reasons. You are also required to submit a final study report at the completion of study/termination of the study, and copies of any future publication arising out of the same. The IEC shall be conducting site visit any time before the completion of the study.

(Dr. Harish K. Pemde)
Member Secretary, IEC

Place- New Delhi

Ethics Committee for Human Research ★ NEW DELHI-110001 INDIA ★ LHMC & Assoc. Hospitals

डॉ. हरीश कुमार पेमदे / Dr. Harish K. Pemde
सदस्य सचिव / Member Secretary
मानव अनुसंधान हेतु आचारनीति समिति
Ethics Committee for Human Research
लेडी हार्डिंग मेडिकल कॉलेज एवं सह-अस्पताल
Lady Hardinge Medical College & Associated Hospitals
शहीद भगत सिंह मार्ग, नई दिल्ली-110001
Shahid Bhagat Singh Marg, New Delhi-110001

ANNEXURE-IV

9th December, 2020

To

The Director
LHMC & Associated Hospitals, New Delhi

Through the Vice Principal, Lady Hardinge Medical College, New Delhi

Sub: Request to sign a MoU with the Galgotias University, Greater Noida which is required as part of my PhD in Clinical Research as per framework ethics guidelines for promotion of multi-disciplinary research in LHMC dated 03.12.2020

Respected Sir,

With due regards, this is to inform you that I am pursuing my PhD in Clinical Research from Galgotias University, Greater Noida. I had already taken the official permission from the competent authority for the said purpose. (Copy enclosed)

I have also completed my mandatory course work from the University and passed all required examinations. Further, I proposed to carry out my PhD thesis on ***"To evaluate trough concentration of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome"*** under the supervision of Dr (Mrs) Ranjana S. Patnaik, Dean Department of Clinical Research, Galgotias University. The Head of Pediatrics, KSCH has also permitted me to carry out my Research under the co.supervision of Dr Abhijeet Saha, Professor Department of Pediatrics, KSCH

I am also seeking a permission from Institutional Ethical Committee of LHMC & Associated Hospitals to undertake my research on above topic. The said committee has asked me to provide a copy of MoU between LHMC and the Galgotias University as per framework ethics guidelines issued for promotion of multi-disciplinary research in LHMC on 03.12.2020. This is also to inform you that I am not seeking any kind of financial support and leave from my office and bearing all necessary expenses including university fee. Further the above mentioned test is already being done in KSCH for the treatment of SRNS pediatric patients.

You are therefore requested to kindly approve and sign the enclosed MoU duly signed by the Dean of Galgotias University.

I shall be highly obliged.

Thanking you.

Yours Sincerely,

Kaptan Singh Sehrawat
Technical Officer,
Biochemistry & Drug Assay Lab.
Department of Biochemistry
KSCH, New Delhi

9/12/2020
DR. ABHIJEET SAHA
MD, MAMS, FACE
Fellow, ISPN, IPNA (NUH)
Clinical Fellow, (NUH)
Fellow, ISPD (Heidelberg)
Professor, Department of Pediatrics
LHMC & KSCH, New Delhi

forwarded to director
(09/12/2020)

उप-प्रधानाचार्य
Vice Principal
लेडी हार्डिंग मेडिकल कॉलेज एवं सह-अस्पताल,
Lady Hardinge Medical College & Assoc. Hospitals,
नई दिल्ली-110001 / New Delhi-110001

MEMORANDUM OF UNDERSTANDING

Between

Department of Clinical Research,
School of Biosciences & Biomedical Engineering,
Galgotias University, Greater Noida, Uttar Pradesh 203201.

And the
Lady Hardinge Medical College and Associated Hospitals,
Shaheed Bhagat Singh Marg, Connaught Circus,
New Delhi-110001

TERMS OF UNDERSTANDING

Department of Clinical Research, School of Biosciences and Biomedical Engineering, Galgotias University and **Lady Hardinge Medical College and Associated Hospitals** (individually a party and collectively parties to this Memorandum) undertake that:

1. Mutual benefit can be derived through creating platforms for exchange of experiences and ideas to recognize similarities, differences and potential opportunities for collaborative learning to strengthen Academic & Research objectives in PhD Clinical Research Programme.

2. Further, to achieve above stated objectives, the both parties agreed to the following areas of cooperation and understanding with regard to the PhD Research Proposal of Mr. Kaptan Singh Sehrawat, Technical Officer from Department of Clinical Biochemistry, Kalawati Saran Children's Hospital who is pursuing his PhD in Clinical Research as part time candidate from Galgotias University, Greater Noida. *The details of his research proposal is as under-*

   (A) **Title of Research Proposal:** "To evaluate trough concentration of Tacrolimus in children with steroid resistant Nephrotic Syndrome"

   (B) **Principal Investigator:** Mr Kaptan Singh Sehrawat shall be as Principal Investigator (PI) and Dr Preeti Chauhan, Associate Professor of Biochemistry; LHMC shall be as Co. Investigator of said PhD research project.

   (C) **Supervisor**: Dr (Prof.) Ranjana S Patnaik, Dean, Department of Clinical Research, School of Biosciences and Biomedical Engineering, Galgotias University.

(D) **Co. Supervisor:** The Co. Supervisor shall be Dr Abhijeet Saha from Department of Pediatrics, (KSCH) LHMC & Associated Hospitals, New Delhi.

(E) **Funding**: This is non-funded PhD research project being done as part of PhD degree programme. Both parties agree that there shall be no financial obligation and funding requirements under this MOU.

(F) **Publication plan:** Publication of data and authorship of publication shall be shared mutually by both parties as stated above.

(G) **Relevance & Value**: Evaluation of trough concentration of Tacrolimus in children with Steroid Resistant Nephrotic Syndrome is very essential for the treatment of SRNS patients taking Tacrolimus. The monitoring and evaluation of drug is important to avoid toxicity of the drug in patients. Test is already being done in KSCH Lab in the interest of patient care.

(H) **Commercial use, if any:** Publication will not be used as a document for any commercial purpose.

(I) **Permission:** The official permission has already been taken by the candidate from both parties to carry out his PhD research in above collaborative manner with both parties. He will also take permission from Institutional Ethics Committee of LHMC & Associated hospitals.

3. This MoU shall remain in effect for a period of three (2) years from the date of signature or completion of his PhD project whichever is earlier. It may be amended or modified at any time by the mutual consent of the parties.

Signed for and on behalf of:

School of Biosciences
& Biomedical Engineering,
Galgotias University

Dr. Ranjana S. Patnaik
Professor & Dean
School of Biosciences & Biomedical Engineering
Galgotias University, Uttar Pradesh

(Dr.Prof.) Ranjana S Patnaik
Dean (SBBE)
Mobile No. 7897712091

Date: 8th December 2020

LHMC & Associated Hospital,
New Delhi

डॉ (आचार्य) एन एन माथुर / Dr (Prof) N N Mathur
अपर महानिदेशक स्वास्थ्य सेवायें एवं निदेशक
Addl D G H S & Director
लेडी हार्डिंग मेडिकल कॉलेज एवं सह-अस्पताल
Lady Hardinge Medical College & Assoc Hospitals
नई दिल्ली-११०००१ / New Delhi-110001

(Dr N.N. Mathur)
Director, LHMC & Associated
Hospitals, New Delhi

Date:

डाक अवस्था में / IN DAK STAGE
निदेशक का कार्यालय / OFFICE OF THE DIRECTOR
चिन्हित / MARKED TO.....
09 DEC 2020
लेडी हार्डिंग मेडिकल कॉलेज एवं सह-अस्पताल
Lady Hardinge Medical College & Associated Hospitals
नई दिल्ली- ११०००१ / New Delhi-110001

ANNEXURE-V

Sl. No. 1922537

GALGOTIAS UNIVERSITY

UTTAR PRADESH

(Under the Uttar Pradesh Private Universities Act No. 12 of 2019)

CONSOLIDATED STATEMENT OF GRADES

DOCTOR OF PHILOSOPHY
in Clinical Research

Course Work Examinations, March 2018

Name of the Student : Kaptan Singh Sehrawat Admission No : 17SCRH301012

Father's Name : Sh. Balbir Singh Mother's Name : Smt. Kitabo Devi

Name of the School / Department : School of Basic & Applied Sciences

S. No.	Course Code	Course Name	Associated Credits	Grade Obtained	Credits Earned
1	RME701	Research Methodology	3	B+	3
2	CR702	Basic in Clinical Research	3	A+	3
3	CR703	Biothics in Clinical Research	3	A+	3
4	CR704	Regulatory Aspects of Clinical Research	3	A	3

Total Credits Registered : 12

Total Credits Earned : 12

Dated : July 10, 2021

Controller of Examinations

ANNEXURE-VI

Table I Comparison Between Present and 2008 [7] Guidelines of the Indian Society of Pediatric Nephrology (ISPN), and Kidney Disease Improving Global Outcomes (KDIGO) 2021 [9]

Parameter	*ISPN 2021*	*ISPN 2008 [7]*	*KDIGO 2021 [9]*
Nephrotic syndrome	Nephrotic range proteinuria, hypoalbuminemia (albumin <3 g/dL) and edema	Nephrotic range proteinuria, hypoalbuminemia (<2.5 g/dL), cholesterol >200 mg/dL and edema	Nephrotic range proteinuria and either hypoalbuminemia (<3 g/dL) or edema
Steroid resistance	Lack of complete remission despite daily therapy with prednisolone for 6-wk	Lack of complete remission despite daily therapy with prednisolone for 4-wk	Lack of complete remission despite daily therapy with prednisone for 4-weeks[a]
Prednisolone for initial episode	6-wk daily and 6-wk AD; surface area (BSA) or weight-based dosing[b]; no indication for prolonged therapy	6-wk daily and 6-wk AD; weight-based dosing[b]; no indication for prolonged therapy	4-6 wk daily and 4-6 wk AD; BSA or weight-based dosing[b]; prolong therapy (16- 24 wk) if <4-6 yr-old, or if delayed remission
Frequent relapses	≥2 relapses in first 6-months after initial therapy; ≥3 relapses in any 6-mo; ≥4 relapses in 1 year	≥2 relapses in first 6-months after stopping initial therapy; ≥4 relapses in 1-year	≥2 relapses in 6-months; ≥4 relapses in 1-year
Prolonged AD prednisolone	Taper to 0.5-0.7 mg/kg AD for 6-12 months	Taper to 0.5-0.7 mg/kg AD, for 9-18 months	Limited role in view of risk of toxicity
Prednisolone during infections	Daily for 5-7 days, if receiving AD prednisolone	No recommendation	Daily at 0.5 mg/kg for 5-7 days, whether on/off steroids
Steroid sparing therapy: Indications, choice	Failure of AD therapy: Levamisole or MMF Steroid threshold >1 mg/kg AD, toxicity, complicated relapses: Cyclophosphamide, MMF Difficult-to-treat: CNI, then rituximab	Failure of AD therapy, steroid toxicity: Levamisole Steroid toxicity, severe relapses, poor compliance: Cyclophosphamide Failure of above therapies: CNI; MMF an option	Frequent relapses with steroid toxicity; patients with dependence Frequent relapses: Levamisole, cyclophosphamide Dependence: MMF, rituximab, cyclophosphamide, CNI
Supportive	Advice on diet, immunization, management of edema; calcium and vitamin D supplements		

AD-alternate days; CNI-calcineurin inhibitor; MMF-mycophenolate mofetil; [a]Late responder: Partial remission at 4 weeks and complete remission at 6 weeks of daily prednisone; [b]BSA-based dosing: 60 mg/m^2 daily and 40 mg/m^{2}AD; weight-based: 2 mg/kg/day and 1.5 mg/kg AD; maximum 60 mg daily and 40 mg AD.

Box I Definitions of Disease Course and Severity in Nephrotic Syndrome

Nephrotic range proteinuria	Urine protein 3+ or 4+; urine protein to creatinine ratio (Up/Uc) >2 mg/mg in first morning urine specimen; proteinuria >40 mg/m^2/hr
Remission	Urine protein nil or trace (Up/Uc <0.2 mg/mg) for 3 consecutive early morning specimens
Relapse	Urine protein ≥3+ (Up/Uc >2 mg/mg) for 3 consecutive early morning specimens, having been in remission previously
Frequent relapses	Two or more relapses in the first 6-months after stopping initial therapy[a]; ≥3 relapses in any 6-months; or ≥4 relapses in one yr
Steroid dependence	Two consecutive relapses when on alternate day steroids, or within 14 days of its discontinuation
Steroid resistance[b]	Lack of complete remission despite therapy with daily prednisolone at a dose of 2 mg/kg (or 60 mg/m^2) daily for 6 weeks
Stable remission	Sustained remission or infrequent relapses during immunosuppressive therapy
Complicated relapse	Relapse associated with life-threatening complications: *(i)* hypovolemia requiring inpatient care, *(ii)* severe infection (peritonitis, cellulitis, meningitis), or *(iii)* thrombosis
Significant steroid toxicity	Hyperglycemia (fasting glucose >100 mg/dL, post-prandial glucose >140 mg/dL, or HbA1c >5.7%) [12]; obesity (body mass index >equivalent of 27 kg/m^2 in adults [13]); short stature (height <–2 SDS for age [13]) with height velocity (< –3 SDS for age [14]); raised intraocular pressure; cataract(s); myopathy; osteonecrosis; or psychosis
Difficult-to-treat steroid sensitive disease	Both of the following: *(i)* frequent relapses, or significant steroid toxicity with infrequent relapses; and *(ii)* failure of ≥2 steroid sparing agents (including levamisole, cyclophosphamide, mycophenolate mofetil)

[a]Or during initial therapy; [b]Therapy in the last 2 weeks may be given on alternate days in patients with steroid toxicity. HbA1c-glycosylated hemoglobin; SDS-standard deviation score.

A

Table I Guidelines on Steroid-Resistant Nephrotic Syndrome (SRNS): Current Indian Society of Pediatric Nephrology(ISPN), ISPN 2009 and International Pediatric Nephrology Association (IPNA) 2020

	Current ISPN	*ISPN 2009 [5]*	*IPNA 2020 [6]*
Definition: Duration of prednisone therapy	6 weeks daily	4 weeks daily	4 weeks daily; if partial remission: 2 weeks additional therapy (confirmation period)
Kidney biopsy	All; except if monogenic SRNS identified	All patients	All; except if monogenic SRNS identified
Genetic testing	Specific subsets of initial SRNS, congenital NS; not in late SRNS	Specific forms of initial SRNS	All patients with initial SRNS; not in late SRNS
Immunosuppression in monogenic SRNS	Not advised; may continue after counseling if partial remission	Not discussed	Not advised; may continue after counseling
Estimated GFR, ml/min/1.73 m^2	At diagnosis; q 3-6 months Avoid immunosuppression if eGFR<60	At diagnosis	At diagnosis; q 3 months Prefer MMF if eGFR<30 ml/min/1.73 m^2
First line: Calcineurin inhibitors (CNI)	Duration of therapy at least 2-yr	Duration: 2-3 yr	Duration: 1-2 yr
Cyclophosphamide	IV cyclophosphamide may be used; oral not advised	IV therapy has low efficacy; oral not used	IV or oral cyclophosphamide
Indications for mycophenolate mofetil	*(i)* Prolonged CNI use and disease relapses;*(ii)* CNI-resistant SRNS	No recommendation	*(i)*eGFR<30 mL/min/1.73 m^2; *(ii)* CNI therapy for 1-yr; *(iii)* steroid sensitive relapses
Use of rituximab	*(i)* Prolonged CNI use & disease relapses; *(ii)* CNI-resistant SRNS; *(iii)* allograft recurrence	No recommendation	*(i)* CNI-resistant SRNS; *(ii)* allograft recurrence
Prednisone alternate day	Taper over 6-9 months	Taper over 1-1.5 yr	Taper and stop by 6 months
Statins; in addition to dietary advice	LDL cholesterol >160 mg/dL; >130 mg/dL if cardiovascular risk factors	Total cholesterol >200 mg/dL,or LDL >130 mg/dL	LDL cholesterol >160 mg/dL; >130 mg/dL if cardiovascular risk factors
CNI-resistant disease	Rule out monogenic cause; consider rituximab; addition of MMF	Not discussed	Switch to MMF, rituximab; enroll in clinical trials
Renal transplantation	Evaluation of recipient, donor; managing recurrent FSGS	Not discussed	Evaluation of recipient, donor; managing recurrent FSGS

eGFR estimated glomerular filtration rate; FSGS focal segmental glomerulosclerosis; LDL low density lipoproteins; MMF mycophenolate mofetil; NS nephrotic syndrome

Table II Dosing and Monitoring of Immunosuppressive Therapy

Medication	*Dose*	*Adverse effects*	*Monitoring*
First line therapy			
Tacrolimus	0.1-0.2 mg/kg/day in 2-divided doses; maximum initial dose 4 mg/day; trough level 4-8 ng/mL*	*Both:*Acute kidney injury, nephrotoxicity,hyperkalemia, hepatotoxic *Tacrolimus*: tremors, seizures, headache; diarrhea; glucose intolerance; hypomagnesemia *Cyclosporine:* Gingival hyperplasia, hypertrichosis; hypertension; dyslipidemia	Screen for cosmetic side effects, tremors, diarrhea, hypertension Creatinine, potassium at 2-4 wk, q 3-6 months Liver function tests, uric acid, magnesium, lipids q 3-6 months Blood glucose q 3-6 months (with tacrolimus)
Cyclosporine	3-5 mg/kg/day in 2-divided doses; maximum initial dose 200 mg/day; trough level 80-120 ng/mL*		
Prednisolone onalternate days	1.5 mg/kg for 4-wks; 1 mg/kg for 4-wks; taper to 0.3-0.5 mg/kg for ~6-9 months	Weight gain, Cushingoid habitus, glucose intolerance, hypertension, raised intraocular pressure, cataract, myopathy, osteoporosis	Blood pressure, screen for cosmetic effects; eye evaluation q 12 months Blood glucose every 6-12 months
*Other agents***			
Cyclophosphamide	500-750 mg/m^2 IV; every month for 6-months	Leukopenia, hemorrhagic cystitis, vomiting, alopecia, risk of infections; gonadal toxicity, malignancies	Blood counts prior to infusion; withhold if total leukocyte count <4000/mm^3 Ondansetron,mesna prevent adverse effects
Rituximab	375 mg/m^2 everywk for 2-4 doses	Infusion reactions: Chills, fever, serum sickness, bronchospasm Neutropenia;*P. jirovecii* pneumonia; reactivation of hepatitis B, JC virus;acute lung injury; hypogammaglobulinemia	*Pre dose*: Hemogram, transaminases; hepatitis & HIV serology; immunoglobulin (IgG) level Monitor CD19 count, hemogram,IgG level
Mycophenolate mofetil	600-1200 mg/m^2/day in 2-divided doses	Leukopenia; liver dysfunction; pain abdomen, nausea, diarrhea; headache; warts; weight loss	Hemogram, liver functions q 3-6 months

*Dose titrated to blood trough level obtained 12-hr after last dose; measure 2-weeks after initiating therapy. Subsequently, if: *(i)* suspected drug toxicity, *(ii)* medications that affect levels (*Web Table* IV), or *(iii)* unsatisfactory response or relapses while on therapy

**Patients on intense immunosuppression (combination of calcineurin inhibitors and rituximab or mycophenolate mofetil) should receive prophylaxis with trimethoprim (5 mg/kg; 150 mg/m^{2}on alternate days)

Table IV Supportive Care of Children with Steroid-Resistant Nephrotic Syndrome

Complication	*Pathophysiology*	*Management*
Thromboembolism	Urine loss of coagulation regulators; hepatic production of hemostatic proteins; lack of ambulation; dehydration; thrombocytosis; platelet aggregation	Prevention: Ensure ambulation, optimal hydration; remove central venous catheters, avoid arterial punctures;use compression stockings Treatment: Heparin, low molecular weight heparin;warfarin Preventive anticoagulation: If previous thrombosis,risk factors
Hypertension	Glomerular disease; high renin, aldosterone, epinephrine, norepinephrine; reduced ANP	Target blood pressure 50-75thpercentile for age Lifestyle measures; restrict salt intake Angiotensin converting enzyme inhibitors (ACE-I), angiotensin receptor blockers
Acute kidney injury	Hypovolemia (associated hypoalbuminemia, diuretic therapy); medications (ACE-I, calcineurin inhibitors)	Supportive care: Attention to fluid and electrolytes; management of complications of acute kidney injury
Linear growth retardation	Exposure to glucocorticoids; malnutrition;adrenocortical suppression	Regular monitoring of height, height velocity; steroid minimization Limited evidence for growth hormone
Obesity	Exposure to steroids; reduced physical activity	Monitor weight, body mass index; minimize steroids; modify lifestyle
Dyslipidemia	Increased low density lipoproteins (LDL) Reduced clearance of chylomicron, very LDL	Modify lifestyle (dietary change,physical activity, weight control) ≥8-yr-oldwith LDL cholesterol >160 mg/dL, or >130 mg/dLwith risk factors*:Atorvastatin 10-20 mg daily
HPA suppression	Corticosteroid therapy	Stress dose if receiving oral steroids >2-weeks within past 1-yr
Bone health	Urinary loss of vitamin D; osteoblast suppression, osteoclast induction	Vitamin D (400-800 IU); calcium (250-750 mg)supplements
Hypothyroidism	Urinary loss of thyroidbinding globulin, transthyretin and albumin	No treatment if remission is expected; follow-up borderline levels Low free T4, TSH>10 mU/L: treat with thyroxine

HPAhypothalamo-pituitary axis
**Risk factors: Chronic kidney disease stage 3-5; blood pressure >90th centile for age; BMI > 95th centile; family history of cardiovascular disease*

Table V Monitoring of Patients with Steroid-Resistant Nephrotic Syndrome

Parameter	*Frequency*
Home urine dipstick for protein	Daily for 1-2 weeks; 2-3 times/week until remission; once-weekly thereafter
Spot urine protein/creatinine ratio*	Baseline; 2-4 weeks; then every 6-12 months
Weight, height; growth velocity; body mass index	Every 3-6 months (frequent in infantsandstage 3-5 chronic kidney disease)
Blood pressure Ambulatory blood pressure monitoring 2-D echocardiography	At each hospital visit Every 1-2 yr Annually, if hypertensive
Blood creatinine, electrolytes, albumin,eGFR	Baseline; 2-4 weeks; then every 3-6 months
Hemoglobin, glucose, calcium, phosphate, alkaline phosphatase, 25-hydroxyvitamin D; thyroid profile	Every 6-12 months with partial remission or non-response; every 12 months with complete remission; additional investigations may be required forstage 3-5chronic kidney disease
Monitoring drug toxicity	See Table II
Fasting lipid profile	Every 6-12 months
Eye examination (cataract, glaucoma)	Annually, if receiving long-term steroids
Repeat renal biopsy	Calcineurin inhibitor therapy beyond 30-36 months; recommencing therapy for second course Non-recovery from acute kidney injury
Nutritional status and advice	Every 6 months; more frequent in infants, malnourished children, stage 3-5 chronic kidney disease
Immunization	Check and complete every 12 months, as appropriate

eGFR estimated GFR(ml/min per 1.73 m^2) = $\frac{0.413 \times \text{height (cm)}}{\text{creatinine (mg/dl)}}$

**24-hr urine protein estimation may be considered instead*

ANNEXURE-VII

PARTICIPANTS INFORMATION SHEET

DEPARTMENT OF PEDIATRICS
KALAWATI SARAN CHILDREN'S HOSPITAL
DELHI - 110001

You are being invited to participate in a research study. Before you take part in this research study, we wish to explain the study to you and give you the chance to ask questions. Please read carefully the information provided here. If you agree to participate, please sign the informed consent form.

Title of study: A Study To Evaluate Trough Concentration of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome

Principal investigator: Mr Kaptan Singh Sehrawat
Supervisors: Dr. Ranjana S Patnaik, PhD, Dean, Galgotias University
Co. Supervisor: Dr Abhijeet Saha, Professor of Pediatrics, KSCH
Dr Preeti Chauhan, Professor of Biochemistry

Purpose of Study:

Nephrotic Syndrome (NS) is a common kidney disorder in children. The diagnosis and treatment of SRNS in children could be a challenging task for Nephrologist due to limited treatment options. The constant and reliable therapeutic monitoring of given drug level is utmost important to avoid toxicity effect of drug and to evaluate the safety and efficacy of treatment. To study the correlation of tacrolimus levels with clinical remission in children with SRNS and to study to side effect profile of tacrolimus in children with SRNS

Study Procedure:

You will be one of the 50 patients; we plan to recruit in this study. Your blood sample (3 ml) will be withdrawn after 12 weeks of standard treatment for your disease and regularly followed up in OPD.

Expected Duration of Subject Participation

Patient will be involved in the study for 12 weeks. 2 blood samples will be taken 12 weeks apart.

Possible benefits to subject and other people:

The results of the research may provide benefits to the society in terms of advancement of medical knowledge and/or therapeutic benefit to future patients.

Possible risks to subjects:

The only discomfort will be due to taking out blood samples. All precautions will be taken to minimize the complications due to sampling by performing it under strict aseptic precautions.

Compensation:

Not applicable

Cost to the participant:

The expenses for investigations will be borne by the hospital

Withdrawl from the Study:

You are free to not to participate, or withdraw from the study at any time. The treatment of the child will continue as before.

Confidentiality of the information obtained from you:

Information obtained about the patient during the research will be kept strictly confidential. The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity. By signing the consent form you authorise the sharing of your study related medical records to the regulatory authority and institutional ethical committee.

Contact person-

For further information / questions, you can contact us at the following address:

Principal investigator: Mr Kaptan Singh Sehrawat

Co. Supervisor: Dr Abhijeet Saha, Professor of Pediatrics Nephrology, KSCH

ANNEXURE- VII

(Hindi)

रोगी सूचना पत्र

बालरोग विभाग

लेडी हार्डिंग मेडिकल कॉलेज और

कलावती सरन बाल अस्पताल ,दिल्ली-110001

आपको एक शोध अध्ययन में भाग लेने के लिए आंमत्रित किया जा रहा है। इस अध्ययन में भाग लेने से पहले, हम आपके लिए अध्ययन की व्याख्या करना चाहते हैं और आपको सवाल पूछने का मौका देना चाहते हैं । अगर आप भाग लेने के लिए सहमत है तो सूचित सहमति पत्र पर हस्ताक्षर करें ।

अध्ययन का शीर्षक: स्टेरॉयड रेसिस्टेंट नेफ्रोटिक सिंड्रोम (एस.आर.एन.स) के मरीजों में टेक्रोलिमस ड्रग के निम्न स्तर का का मूल्यांकन करना है ।

प्रधान अन्वेषक : कप्तान सिंह सहरावत

सह-अन्वेषक : डॉ अभिजीत साहा (प्रोफेसर, बाल चिकित्सा नेफ्रोलॉजी विभाग बालरोग विभाग) डॉ प्रीती चौहान, प्रोफेसर, जीव रसायन विभाग, लेडी हार्डिंग मेडिकल कॉलेज दिल्ली

अध्ययन का उद्देश्य

स्टेरॉयड रेसिस्टेंट नेफ्रोटिक सिंड्रोम (एस.आर.एन.स) के मरीजों में टेक्रोलिमस ड्रग के निम्न स्तर का
का मूल्यांकन करना है ।

अध्ययन प्रक्रिया

स्टेरॉयड रेसिस्टेंट नेफ्रोटिक सिंड्रोम (एस.आर.एन.स) के मरीज जिन्हे मानक इलाज के लिए टेक्रोलिमस ड्रग को दिया जायेगा उनके रक्त के नमूने 12 सप्ताह के अंतराल पर लिए जाएंगे और टेक्रोलिमस ड्रग के निम्न स्तर को मापा जायेगा ।

अध्ययन का संभावित समय

रोगी 12 सप्ताह के लिए अध्ययन में शामिल किया जाएगा एवं रक्त का नमूना 12 सप्ताह के अंतराल पर जायेगा ।

विषय और अन्य लोगों के लिए संभावित लाभ

अनुसंधान के परिणाम भविष्य के रोगियों को चिकित्सा ज्ञान और/या चिकित्सीय लाभ की उन्नति के रूप में समाज को लाभ प्रदान कर सकते हैं।

विषयों के लिए संभावित जोखिम

रक्त की जांच असुविधा का एक मात्र कारण होगी। जांच निकालने की वजह से होने वाली नुकसान को कम करने के लिए सभी एसेप्टिक सावधानियां बरती जाएंगी.

नुकसान भरपाई

लागूनहीं

प्रतिभागी की लागत

जांच के लिए खर्च अस्पताल द्वारा वहन किया जाएगा

अध्ययन में भाग लेने से वापसी

इस शोध में भागीदारी विशुद्ध रूप से स्वैच्छिक है। रोगी किसी भी समय बिना कारण दिए बिना, अपने इलाज पर प्रभाव डाले बिना, अपनी मर्जी से अनुसंधान से हट सकता है।

आपसे प्राप्त जानकारी की गोपनीयता

अनुसंधान के दौरान रोगी के बारे में प्राप्त जानकारी पूरी तरह गोपनीय रखी जाएगी। इस अध्ययन की जानकारी यदि वैज्ञानिक पत्रिकाओं में प्रकाशित या वैज्ञानिक बैठकों में प्रस्तुत की जाती है तो आपकी पहचान उजागर नहीं होगी। सहमति फॉर्म पर हस्ताक्षरकर के आप नियामक प्रधिकारणों और संस्थागत नैतिक समिति को अपने अध्ययन से संबंधित चिकित्सा रिकॉर्ड साझा करने का अधिकार देते हैं ।

संपर्क व्यक्ति

अधिक जानकारी / प्रश्नों के लिएए आप हमें निम्नलिखित पते पर संपर्क कर सकते हैं
प्रधान अन्वेषक-कप्तान सिंह सहरावत
तकनीकी अधिकारी, के.एस.सी.एच.

ANNEXURE-VIII

INFORMED CONSENT

Patient identification number:

I …….father/mother of…….., a resident of …… ……., hereby declare that I give informed consent to participate in the study titled "*To evaluate trough level concentration of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome"*

The contents of the information sheet dated …….. that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask question.

The nature and purpose of the study, details of blood investigations, potential risks / benefits from study, expected duration of the study, and other relevant details of the study have been explained to me in detail in my language. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals. I give permission for these individuals to have access to my records.

I give my free full voluntary consent for being enrolled in above study

Name of the Subject Date

Signature of the Guardian

Name of the Guardian Date

Relation to the Child:

Signature of the Witness

Name of the witness Date

This is to certify that the above consent has been taken in my presence.

Signature of the Investigator

Name of the Investigator Date

ANNEXURE-VIII (Hindi)

सहमति पत्र

मैं_____का/की माता/पिता,_____
_____का रिहायशी निम्नलिखित अध्ययन हेतु अपनी सहमति देता/देती हूँ।

□ीर्षक : स्टेरॉयड रेसिस्टेंट नेफ्रोटिक सिंड्रोम के मरीजों में (स.एन.आर.एस) टेक्रोलिमस ड्रग के निम्न स्तर काका मूल्यांकन करना

हमें रोगी सूचनापत्र प्रदान कर दिया गया है। रोगी सूचना पत्र दिनांक _____
_____को हमनें ध्यानपूर्वक पढ़ लिया/हमें हमारी भाषा में समझा दिया गया है। हमें इसमें प्रश्न पूछने की अनुमति एवं अवसर प्रदान किये गए है। मुझे इस अध्ययन के सभांवित **उद्देश्य, प्रकृति, इसमें होने वाले सभी जांचे,** लाभ/नुकसान के बारे में समझा दिया गया हैं। मुझे बता दिया गया है कि अध्ययन में मेरा भाग लेने पूर्ण रूप से स्वैच्छिक हैं और मै किसी भी समय बगैर कोई कारण दिये इससे निकलने को स्वतंत्र हूँ। इससे मेरे कलावती सरन बाल चिकित्सालय में होने वाले इलाज प्रभावित नहीं होगा। मुझे समझा दिया गया है कि एकत्रित जानकारी एवं इलाज से संबंधित दस्तावेज नैतिक रूप से जिम्मेदार व्यक्ति देख सकता है। मै इसकी अनुमति देता हूँ।

मैं इस अध्ययन में भाग लेने के लिए पूर्ण सहमति प्रदान करता हूँ।

रोगी का नाम : _____ दिनांक :_____

अभिभावक के हस्ताक्षर :_____दिनांक :_____

अभिभावक का नाम : _____

बच्चे से संबंध : _____

गवाह के हस्ताक्षर : _____दिनांक :_____
_____

गवाह का नाम :_____

अन्वेषक के हस्ताक्षर : _____दिनांक :_____

अन्वेषक का नाम : _____

ANNEXURE-IX

ASSENT FORM

Patient identification number:

I…… , a resident of ……..., hereby declare that I give informed consent to participate in the study titled "To evaluate trough level concentration of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome"

The contents of the information sheet dated …….. that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask question.

The nature and purpose of the study, details of blood investigations, potential risks / benefits from study, expected duration of the study, and other relevant details of the study have been explained to me in detail in my language. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals. I give permission for these individuals to have access to my records.

I give my free full voluntary consent for being enrolled in above study.

Signature of Participant Date
Name of the Participant

We are witness that the child has signed the acceptance letter with his full consent.

Signature of the Witness

Name of the witness Date

Signature of the Investigator

Name of the Investigator Date

ANNEXURE-IX-Hindi

स्वीकृति पत्र

मैं_____ का

रिहायशी निम्नलिखित अध्ययन हेतु अपनी सहमति देता/देती हूँ।

शिर्षिक : .....

हमें रोगी सूचना पत्र प्रदान कर दिया गया है। रोगी सूचना पत्र दिनांक_____को हमनें ध्यान पूर्वक पढ़ लिया/हमें हमारी भाषा में समझा दिया गया है। हमें इसमें प्रश्न पूछने की अनुमति एवं अवसर प्रदान किये गए है। मुझे इस अध्ययन के सभांवित उद्देश्य, प्रकृति, इसमें होने वाले सभी जांचें, लाभ/नुकसान के बारे में समझा दिया गया हैं। मुझे बता दिया गया है कि अध्ययन में मेरा भाग लेना पूर्ण रूप से स्वैच्छिक हैं और मै किसी भी समय बगैर कोई कारण दिये इससे निकलने को स्वतंत्र हूँ। इससे मेरे कलावती सरन बाल चिकित्सालय में होने वाला इलाज प्रभावित नहीं होगा। मुझे समझा दिया गया है कि एकत्रित जानकारी एवं इलाज से संबंधित दस्तावेज नैतिक रूप से जिम्मेदार व्यक्ति देख सकता है। मै इसकी अनुमति देता हूँ।

मैं इस अध्ययन में भाग लेने के लिए पूर्ण सहमति प्रदान करता हूँ।

प्रतिभागी के हस्ताक्षर : _____ दिनांक _____

प्रतिभागी का नाम : _____

हम गवाह है कि बच्चे ने अपने पूर्ण सहमति के साथ स्वीकृति पत्र पर हस्ताक्षर किया है।

गवाह के हस्ताक्षर : _____ दिनांक _____

गवाह का नाम : _____

अन्वेषक के हस्ताक्षर : _____ दिनांक _____

अन्वेषक का नाम : **कप्तान सिंह सहरावत**

ANNEXURE-X

A Study to Evaluate Trough Concentration of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome

CASE PERFORMA

Date of contact:	Name:
C.R. No.	Age/Sex:
Fathers Name:	Address:
Ph. No.	

HISTORY

A. FEATURES AT ONSET- NEPHROTIC SYNDROME

Month/year: Age at onset:

Hematuria: Gross Microscopic Nil

Hypertension: Oliguria:

Fever: Rash: Joint Pain:

Family history of Idiopathic Nephrotic Syndrome

Family history of CVD

B. TREATMENT HISTORY OF FIRST EPISODE

- Steroid treatment at onset
  Drug:
  Daily dose: Duration:
  Alternate day dose: Duration:
- Any Other Medication

C. COURSE DURING TREATMENT OF FIRST EPISODE

Remission: Yes/ No

Spot UP:UC:

Serum Protein: Seruma Albumin:

T.Chol:

D. RELAPSES (IF ANY)

Relapses No	**Treatment**	**Response to Treatment**

E. SRNS Details

- Date on which SRNS diagnosed
- Age on diagnosis of SRNS
- Renal Biopsy
- Date on which Tacroliums Started:
- Fill Counts- Yes/No
- Compliance: Poor/Good
- Immuno Suppression Details (Duration)
- Initial/ Late Resistance

F. EXAMINATION

General Physical Examination

Weight: Height:

BMI:

PR: RR: BP:

Edema: Ascites

BP Centiles	SBP	DBP
50th		
90th		
95th		
95+12		

BP (Father) : BP (Mother):

Systemic Examination

- Respiratory:
- CVS:
- Per Abdomen:
- CNS:

INVESTIGATIONS

	At diagnosis of SRNS	At 3 months
Haemogram		
HB		
TLC		
DLC		
Platelets		
Kidney Function Test		
Urea		
Creat		
Uric acid		
Serum Electrolytes		
Na		
K		
Cal/po4		
ALP		
TP/ALB		
Lipid Profile		
Total Chl.		
HDL		
LDL		
TG		
Serum Mg^{++}		
Urine		
UP/UC		
Urinary Mg^{++}		
Urinary Creatinine		

TREATMENT

(a) Specific Treatment

Indication: Initial Resistance/ Late Resistance

Drug:

Dose:

(b) Lipid Lowering Treatment

Diet:

Physical Exercise:

Pharmacological Agent:

(c) Any Other Medication

ANNEXURE-XI

DEPARTMENT OF BIOCHEMISTRY
DRUG ASSAY & ADVANCED BIOCHEMISTRY LAB
KALAWATI SARAN CHILDREN'S HOSPITAL, NEW DELHI

Name:			
Ward/OPD		Age/Sex	
UHID/C.R. No.		Sample Id	
Date of Reporting			

Investigation	Result	Unit
TACROLIMUS (FK506), Whole blood (Immunoassay)		ng/mL

*Reportable Range-1 to 30ng/ml

*Therapeutic range is based on whole blood specimen drawn 12hrs post dose or prior to next dose (trough).

*The optimal therapeutic range for tacrolimus in whole blood has not been stabilised with this assay.

LIMITATIONS:

1. Immunoassays are non-specific and cross react with metabolites. Because of this immunoassay may overestimate the concentration of tacrolimus.
2. The test findings should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
3. Differences in sensitivity to immunosuppressive and nephrotoxic effects of tacrolimus, co administration of other immunosuppressant's, type of transplant, time post-transplant and a number of other factors contribute to different requirements for optimal blood levels of tacrolimus

COMMENT: Tacrolimus is a macrolide antibiotic with potent immunosuppressive functions used to prevent transplant rejection. Its half-life is 12 to 18 hrs, which suggest 2.5 day should elapse to assess effect of dose adjustment on tacrolimus level. Large intra-patient and inter-patient variability has been noted. So careful and frequent monitoring of tacrolimus is recommended, to minimize Nephrotoxic effect with adequate immunosuppression.

Performed By:	Verified by:	
K.S Sehrawat Technical Officer	Dr. Saroj Choudhary Assistant Professor	Dr. Smita Tripathi Professor & Lab I/C

ANNEXURE-XII

कलावती सरन बाल चिकित्सालय

KALAWATI SARAN CHILDREN'S HOSPITAL, NEW DELHI

बंगला साहिब मार्ग, नई दिल्ली–110001 / BANGLA SAHIB MARG, NEW DELHI-110001

24 घंटे आपातकालीन प्रयोगशाला विभाग / DEPARTMENT OF 24 HOURS EMERGENCY LAB SERVICES

CLINICAL BIOCHEMISTRY REPORT

Name	Age/Sex
C.R. No.	Consultant
Ward/OPD	Unit/Bed No.
Date/Time	Lab Reference No.
Diagnosis/History :-	Signature of the Doctor

- *Please note that incomplete test requisition form will not be accepted.*
- *Samples of patient registered in KSCH will only be accepted in Lab.*
- *Samples of all admitted patients and OPD patients of KSCH will be received from wards/Units and reports of same will also be dispatched in respective section by the lab auxiliary staff.*
- *Duplicate Reports of admitted patients, required if any, will have to be collected by the doctors of respective units/wards.*

✓ Please tick marks the required investigation in the box. Please don't write anything on back side of requisition form. Lab Report is to be printed there.

		Reported Value	Units	Normal Range
	Glucose (Fasting)		Mg/dl	60-100
	Glucose (PP)		Mg/dl	65-140
	Glucose (R)		Mg/dl	60-140
	Sodium		MEq/l	130-149
	Potassium		MEq/l	3.5-5.0
	Chloride		MEq/l	98-108
	Urea		Mg/dl	10-40
	Creatinine		Mg/dl	0.5-1.0
	Uric Acid		Mg/dl	3.0-6.5
	Serum Bilirubin-Total		Mg/dl	0.2-1.0
	Serum Bilirubin-Conjugated		Mg/dl	0-0.2
	Serum Bilirubin-Unconjugated		Mg/dl	Up to 1.0
	SGOT (AST)		IU/L	10-40
	SGPT (ALT)		IU/L	10-45
	Alkaline Phosphatase (ALP)		IU/L	Up to 350
	Total Protein		gm/dl	6.6-8.0
	Albumin		gm/dl	3.5-5.0
	Globulin		gm/dl	1.8-2.5
	Serum Calcium		Mg/dl	9-11
	- Ionized Calcium		Mg/dl	4.0-5.5
	- Unionised Calcium		Mg/dl	4.5-5.5
	Inorganic Phosphorus		Mg/dl	2.5-5.0
	Total Cholesterol		Mg/dl	150-250
	HDL		Mg/dl	40-60
	LDL		Mg/dl	130-190
	Triglycerides		Mg/dl	50-150
	CPK Total		IU/L	20-195
	CPK-MB		IU/L	0-24
	CSF Protein		Mg/dl	15-40
	CSF Sugar		Mg/dl	40-70
	Other Body Fluids, (Please Mention the type of body fluid)		Mg/dl	

Signature of the Doctor

ANNEXURE-XIII

(Master Sheet)

S.No.	Gender	Age at onset of SRNS (Months)	Hematuria (Gross)	Hematuria (Microscopic)	Hypertension	Oliguria	Fever	Rash	Joint Pain	Family history of Idiopathic NS	Family of history CVD	Steroid treatment at onset drugname	Daily dose of steroid in (mg)	Duration in Weeks
1	1	72	1	1	0	0	1	0	0	0	0	1	2	6
2	1	148	0	0	0	0	0	0	0	0	0	1	2	6
3	0	166	0	1	0	0	0	0	1	0	0	1	2	6
4	1	19	0	0	0	0	0	1	0	0	0	1	2	6
5	0	84	1	1	0	0	0	0	0	0	0	1	2	6
6	0	36	0	0	0	0	0	0	0	0	0	1	2	6
7	1	117	0	0	0	0	0	0	0	0	0	1	2	4
8	0	51	0	0	0	0	0	0	0	0	0	1	2	5
9	1	60	0	0	0	0	0	0	0	0	0	1	2	6
10	1	36	0	0	0	0	0	0	0	0	0	1	2	6
11	0	114	0	0	0	0	0	0	0	0	0	1	2	4
12	1	36	1	1	0	0	0	0	0	0	0	1	2	5
13	0	50	0	0	0	0	1	0	0	0	0	1	2	6
14	0	128	0	0	0	0	0	0	0	0	0	1	2	6
15	0	24	1	1	0	0	0	0	0	0	0	1	2	6
16	1	44	0	0	0	1	0	0	0	0	0	1	2	6
17	0	39	0	0	0	1	0	0	0	0	0	1	2	5
18	0	68	0	0	0	0	0	0	0	0	0	1	2	3
19	1	37	0	0	0	0	1	1	0	0	0	1	2	6
20	0	18	0	0	0	0	0	0	0	0	0	1	2	4
21	0	36	0	0	0	0	0	0	0	0	0	1	2	6
22	1	36	0	0	0	0	0	0	0	0	0	1	2	5
23	0	84	0	0	0	0	0	0	0	0	0	1	2	6
24	0	103	0	0	0	0	0	0	0	0	0	1	2	3
25	1	96	0	0	0	0	0	0	0	0	0	1	2	6
26	0	144	1	1	0	0	0	0	1	0	0	1	2	6
27	0	128	0	0	0	0	0	0	0	0	0	1	2	6
28	1	12	0	0	0	0	0	0	0	0	0	1	2	4
29	1	29	1	0	0	0	0	0	0	0	0	1	2	6
30	1	49	0	0	0	0	0	0	0	0	0	1	2	6
31	0	72	0	0	0	0	0	0	0	0	0	1	2	6
32	0	36	0	0	0	0	0	0	0	0	0	1	2	6
33	0	168	0	0	0	0	0	0	0	0	0	1	2	6
34	0	96	0	0	0	0	1	0	0	0	0	1	2	6
35	0	62	0	0	0	0	0	0	0	0	0	1	2	6
36	1	24	0	0	0	0	0	0	0	0	0	1	2	6
37	0	84	0	1	0	0	1	0	0	0	0	1	2	6
38	0	32	0	0	0	0	0	0	0	0	0	1	2	6
39	0	84	0	0	0	0	0	0	0	0	0	1	2	6
40	0	84	0	0	0	0	0	0	0	0	0	1	2	6
41	1	96	0	0	0	0	0	0	0	0	0	1	2	6
42	0	16	0	0	0	0	0	0	0	0	0	1	2	6
43	0	84	0	0	0	0	0	0	0	0	0	1	2	6
44	0	38	0	0	0	0	0	0	1	0	0	1	2	6
45	0	120	0	0	0	0	0	0	0	0	0	1	2	6
46	0	108	0	0	0	0	0	0	0	0	0	1	2	6
47	1	111	0	0	0	0	0	0	0	0	0	1	2	6
48	1	24	0	0	0	0	0	0	0	0	0	1	2	6
49	0	18	0	0	0	0	0	0	0	0	0	1	2	6
50	1	24	0	0	0	0	0	0	0	0	0	1	2	6
51	0	88	0	0	1	0	0	0	0	0	0	1	2	6
52	1	31	0	0	1	1	0	0	0	0	0	1	2	5
53	1	74	0	0	1	0	0	0	0	0	0	1	2	4
54	0	43	1	1	1	1	1	0	0	0	0	1	2	6
55	0	36	1	1	1	0	0	0	0	0	0	1	2	6
56	0	72	1	0	1	0	0	0	0	0	0	1	2	6
57	0	132	0	0	1	0	1	0	0	0	0	1	2	6
58	1	32	0	0	1	0	0	0	0	0	0	1	2	6
59	0	48	0	0	1	0	0	0	0	0	0	1	2	6
60	0	24	0	0	1	0	0	0	0	0	0	1	2	6

S.No.	Remission status after steroid treatment	Spot UP:UC	Total Protein in gm/dl	Albumin	Cholestrol	Status of Steroid Resistant	Age at diagnosis of SRNS	Renal Biopsy	Fill Count	Compliance	Immuno Suppression Duration (Weeks)	Weight in Kg	Weight after SRNS Treatment	Height in cm	Height after treatment SRNS
1	1	0.18	6.8	3.5	140	1.0	87	0.0	1	1	12	17.8	17.0	123	124.0
2	1	0.14	5.9	3.6	188	1.0	156	0.0	1	1	12	40.8	40.5	152	152.0
3	0	16.00	7.3	4.2	285	0.0	168	0.0	1	1	12	35.2	35.0	152	154.0
4	1	0.14	5.2	2.5	227	1.0	29	0.0	1	1	12	9.5	9.0	82	82.0
5	1	0.08	3.9	1.3	283	1.0	96	0.0	1	1	12	27.0	26.5	131	132.0
6	1	0.19	6.3	3.0	141	1.0	98	0.0	1	1	12	16.0	16.0	95	95.0
7	0	1.80	5.4	1.6	231	0.0	120	2.0	1	1	12	34.0	34.0	138	138.0
8	0	4.60	4.7	1.3	186	0.0	53	0.0	1	1	12	13.6	15.0	98	98.0
9	0	2.81	6.3	2.8	186	0.0	63	0.0	1	1	12	16.3	16.0	102	103.0
10	1	0.14	6.8	3.4	166	1.0	49	0.0	1	1	12	13.4	15.0	104	104.0
11	0	13.70	5.0	1.8	193	0.0	117	0.0	1	1	12	20.1	20.0	129	130.0
12	0	2.71	5.8	2.3	192	0.0	38	0.0	1	0	12	14.0	14.0	101	100.0
13	1	0.05	6.1	2.2	162	1.0	60	0.0	1	1	12	16.0	16.0	95	95.0
14	1	0.10	6.3	3.0	163	1.0	165	0.0	1	1	12	43.2	42.0	156	156.0
15	0	2.37	5.7	1.9	253	0.0	26	0.0	1	1	12	13.0	13.0	98	98.0
16	1	0.13	6.6	3.4	124	1.0	72	0.0	1	1	12	16.0	15.0	112	112.0
17	0	2.41	6.3	1.9	156	0.0	42	1.0	1	1	12	20.1	20.0	110	110.0
18	0	2.91	6.1	1.8	256	0.0	72	1.0	1	0	12	24.0	24.0	123	123.0
19	1	0.13	6.7	3.4	166	1.0	58	0.0	1	1	12	16.5	16.0	98	100.0
20	0	17.11	5.9	1.3	192	0.0	20	0.0	1	1	12	9.7	9.0	76	76.0
21	1	0.14	6.7	3.1	166	1.0	42	1.0	1	1	12	20.0	20.0	107	107.0
22	0	11.73	5.5	1.6	166	0.0	39	0.0	1	1	12	14.0	14.0	96	96.0
23	1	0.16	6.7	3.3	122	1.0	88	1.0	1	1	12	27.2	27.0	118	118.0
24	0	11.50	5.1	2.1	283	0.0	105	0.0	1	1	12	31.0	30.0	131	131.0
25	0	11.31	5.8	1.4	141	0.0	98	0.0	1	1	12	63.0	61.0	155	155.0
26	1	0.17	6.5	3.2	166	1.0	192	1.0	1	1	12	32.0	32.0	156	156.0
27	0	6.11	5.6	2.1	183	0.0	132	0.0	1	1	12	19.0	19.0	141	141.0
28	0	3.39	5.6	1.6	286	0.0	15	1.0	1	1	12	10.3	10.0	94	94.0
29	1	0.16	6.3	3.2	112	1.0	36	0.0	1	1	12	16.4	16.0	101	101.0
30	0	2.29	5.5	2.4	213	0.0	53	2.0	1	1	12	18.3	18.0	98	98.0
31	0	4.11	5.1	1.4	287	0.0	76	1.0	1	1	17	22.0	22.0	108	109.0
32	1	0.14	5.8	1.7	267	1.0	42	1.0	1	1	15	13.0	13.0	106	106.0
33	1	0.13	5.1	1.7	219	1.0	192	2.0	1	1	12	48.6	47.0	156	156.0
34	1	0.04	5.9	2.8	180	1.0	108	0.0	1	1	12	27.3	27.0	128	128.0
35	1	0.19	5.5	2.7	189	1.0	72	0.0	1	1	12	19.5	20.0	109	109.0
36	1	0.12	5.6	2.0	176	1.0	39	0.0	1	1	12	15.8	16.0	94	94.0
37	0	7.31	4.8	1.7	189	0.0	90	0.0	1	1	16	26.4	25.0	116	116.0
38	1	0.11	5.8	2.6	166	1.0	38	0.0	1	1	12	15.7	16.0	107	108.0
39	1	0.01	4.9	1.5	244	1.0	180	1.0	1	1	12	43.6	43.0	158	158.0
40	1	0.11	5.8	3.0	226	1.0	144	1.0	1	1	12	42.3	43.0	152	152.0
41	1	0.16	6.6	3.0	176	1.0	156	2.0	1	1	12	30.0	30.0	146	146.0
42	1	0.17	6.4	3.1	174	1.0	24	1.0	1	1	15	15.0	15.0	104	105.0
43	1	0.16	5.9	2.9	214	1.0	101	2.0	1	1	12	22.5	22.0	125	125.0
44	1	0.11	6.7	3.2	164	1.0	18	1.0	1	1	12	10.6	10.0	95	95.0
45	1	0.13	5.9	1.8	261	1.0	125	2.0	1	1	12	27.6	27.0	140	140.0
46	1	0.14	5.9	2.8	206	1.0	114	0.0	1	1	12	24.0	24.0	132	133.0
47	1	0.17	6.4	3.4	146	1.0	114	1.0	1	1	12	44.9	43.0	154	154.0
48	1	0.13	6.0	3.1	186	1.0	57	2.0	1	1	12	15.7	15.0	105	105.0
49	1	0.19	5.7	1.6	233	1.0	26	1.0	1	1	12	14.0	14.0	102	102.0
50	1	0.14	6.4	2.8	166	1.0	32	2.0	1	1	12	15.5	15.0	100	101.0
51	0	2.35	3.9	1.3	436	0.0	90	0.0	1	1	12	29.6	30.0	130	129.5
52	0	11.63	6.5	3.6	331	0.0	29	0.0	1	1	12	8.1	9.0	92	93.0
53	0	6.30	3.6	0.9	209	0.0	72	0.0	1	1	12	15.0	15.0	102	102.0
54	1	0.12	6.7	3.1	157	1.0	48	0.0	1	1	12	14.7	15.0	102	103.0
55	1	0.17	6.3	2.8	122	1.0	46	0.0	1	1	12	17.8	18.0	99	99.0
56	1	0.17	6.8	3.1	139	1.0	77	0.0	1	1	12	22.0	22.0	114	114.0
57	1	0.18	6.0	1.9	192	1.0	137	1.0	1	1	16	27.5	27.0	140	141.0
58	0	6.37	5.5	1.9	314	0.0	34	1.0	1	1	12	21.0	20.0	105	105.0
59	1	0.13	5.8	1.8	142	1.0	56	2.0	1	1	12	19.8	20.0	115	116.0
60	1	0.17	6.1	3.0	128	1.0	37	1.0	1	1	12	22.0	22.0	121	122.0

S.No.	BMI at diagnosis SRNS	BMI after 12 Weeks treatment of SRNS	PR	RR	BP-Systolic	BP-Diastolic	Edema	Ascites	BP Centiles 50th SBP	BP Centiles 50th DBP	BP Centiles 90th SBP	BP Centiles 90th DBP	BP Centiles 95th SBP	BP Centiles 95th DBP	BP Centiles 95th SBP
1	11.8	11.1	95	22	96	52	0	0	95	56	108	70	111	73	123
2	17.7	17.5	86	22	116	74	0	0	105	63	118	75	122	79	134
3	15.2	14.8	94	20	120	80	0	0	104	60	118	74	122	77	134
4	14.1	13.4	86	24	88	62	1	0	86	43	100	57	103	61	115
5	15.7	15.2	90	26	98	74	1	0	97	58	109	72	113	75	125
6	17.7	17.7	80	20	102	52	1	0	89	47	101	59	106	62	118
7	17.9	17.9	88	26	120	70	1	0	99	60	111	73	115	76	127
8	14.2	15.6	110	34	100	62	0	0	90	48	102	60	107	63	119
9	15.7	15.1	82	24	86	64	0	0	89	51	103	64	107	68	119
10	12.4	13.9	80	21	80	50	1	0	103	64	118	73	121	75	133
11	12.1	11.8	90	16	93	60	1	0	97	58	108	71	112	74	124
12	13.7	14.0	82	24	100	60	1	0	99	48	103	61	106	65	118
13	17.7	17.7	82	20	80	60	1	0	88	48	100	61	105	63	117
14	17.8	17.3	72	22	108	60	0	0	106	61	119	74	123	78	135
15	13.5	13.5	86	21	92	64	1	0	90	48	102	60	107	63	119
16	12.8	12.0	76	26	102	66	1	1	93	54	105	67	109	70	121
17	16.6	16.5	82	22	100	60	0	0	92	54	104	66	108	69	120
18	16.0	15.9	82	21	106	70	1	1	96	57	109	68	112	72	124
19	17.2	16.0	86	22	84	60	0	0	89	50	103	62	107	66	109
20	16.8	15.6	98	26	80	55	1	0	84	40	97	52	101	54	113
21	17.5	17.5	82	24	90	68	0	0	91	53	103	65	108	68	120
22	15.2	15.2	84	19	92	64	1	0	90	49	104	62	107	66	119
23	19.7	19.4	85	22	96	68	1	0	94	56	106	68	110	71	122
24	18.1	17.5	88	23	98	74	1	0	98	59	110	71	113	74	125
25	26.2	25.4	76	26	102	70	1	1	106	63	119	76	124	79	136
26	13.1	13.1	90	18	110	70	0	0	108	61	122	75	127	78	139
27	9.6	9.6	86	21	112	74	1	0	100	62	112	75	116	78	128
28	11.7	11.3	86	28	90	62	0	0	84	48	103	61	106	65	118
29	16.1	15.7	84	24	90	62	0	0	90	51	104	63	108	67	120
30	19.1	18.7	135	28	120	80	0	0	88	50	102	62	106	67	118
31	18.9	18.5	92	22	100	66	0	0	91	54	103	66	108	69	120
32	11.6	11.6	92	26	76	40	0	0	92	50	105	62	108	66	120
33	20.0	19.3	92	17	120	74	0	0	108	61	122	75	127	78	139
34	16.7	16.5	86	20	100	50	0	0	97	58	109	71	113	74	125
35	16.4	16.8	84	22	94	50	0	0	92	53	104	66	108	69	120
36	17.9	18.1	90	20	98	62	0	0	89	48	103	61	106	65	118
37	19.6	18.6	86	24	102	60	0	0	94	56	106	68	110	71	122
38	13.7	13.7	96	26	90	52	1	0	92	52	104	64	108	67	120
39	17.5	17.2	83	18	116	70	0	0	109	62	123	75	128	78	140
40	18.3	18.6	74	20	110	64	1	0	105	59	119	74	124	77	136
41	14.1	14.1	76	20	90	60	0	0	104	76	117	76	122	79	134
42	13.9	13.6	88	24	76	38	0	0	91	51	103	63	107	66	119
43	14.4	14.1	84	22	74	50	0	0	96	58	108	70	112	73	124
44	11.7	11.1	94	26	66	40	1	0	89	46	102	59	106	61	118
45	14.1	13.8	78	20	108	70	0	0	100	62	112	75	115	78	127
46	13.8	13.6	76	22	100	64	0	0	98	60	109	73	113	76	125
47	18.9	18.1	76	20	90	50	0	1	105	60	118	74	122	78	134
48	14.2	13.6	88	26	88	50	0	0	90	52	104	65	108	69	120
49	13.5	13.5	84	24	90	60	1	0	92	46	106	58	109	61	121
50	15.5	14.7	88	26	82	60	0	0	90	51	104	63	108	67	120
51	17.7	17.9	86	20	118	88	0	0	97	58	109	71	113	74	125
52	9.6	10.4	82	22	110	80	1	1	89	48	103	60	106	64	118
53	14.4	14.4	76	18	84	50	0	0	89	51	102	64	107	68	119
54	14.1	14.1	94	24	100	80	1	0	91	49	103	61	107	64	119
55	18.2	18.4	88	26	110	70	0	0	90	48	102	60	107	63	119
56	16.9	16.9	120	26	100	70	1	0	92	55	106	68	109	72	121
57	14.0	13.6	76	18	120	80	0	0	101	61	113	75	115	78	127
58	19.0	18.1	78	26	100	70	1	0	90	52	103	65	108	69	120
59	15.0	14.9	82	22	84	56	0	0	94	55	106	67	110	70	122
60	15.0	14.8	86	26	122	86	1	0	95	57	107	69	111	72	123

S.No.	BP Centiles95th DBP	Hb	TLC	DLC-N	DLC-L	DLC-M	DLC-E	Platelets	Urea	Creatinine	Uric Acid	Na	K	Calcium	Phosphorus
1	85	10.6	9820	46	40	12	2	1.96	36.0	0.32	3.4	138	4.1	8.6	3.1
2	91	12.5	12850	44	51	5	0	4.14	31.0	0.40	4.6	138	3.8	8.5	4.3
3	89	13.2	8200	55	39	6	0	2.10	55.6	0.48	3.6	142	4.8	9.7	4.6
4	73	9.5	10600	46	52	2	0	3.24	27.0	0.30	3.6	136	4.4	8.6	3.3
5	87	14.2	6740	45	51	4	0	3.17	25.0	0.34	4.1	136	4.3	9.2	4.3
6	74	10.2	7300	60	38	2	0	2.81	38.0	0.70	4.8	142	3.4	8.7	4.6
7	88	12.7	9720	42	55	3	0	3.81	18.1	0.35	3.8	145	3.8	8.9	4.8
8	75	12.1	7800	56	40	2	2	3.12	26.0	0.30	3.8	138	4.1	8.0	4.3
9	80	9.5	7430	56	41	3	0	3.66	21.0	0.37	3.5	146	4.5	8.0	3.6
10	87	8.7	11370	46	49	4	1	2.73	27.0	0.27	2.9	139	4.3	9.0	3.7
11	86	9.3	6820	45	53	3	0	2.72	50.0	0.62	4.8	139	4.5	8.0	4.3
12	77	11.3	16100	43	48	7	2	8.50	11.5	0.13	3.8	146	3.6	9.6	4.7
13	75	10.7	9760	51	43	6	0	2.31	32.0	0.40	3.1	142	3.2	8.2	3.3
14	90	13.1	6940	53	41	6	0	2.71	16.0	0.27	4.5	143	4.2	9.6	4.7
15	75	11.2	7940	41	54	5	0	4.12	19.0	0.31	4.4	138	3.9	8.7	4.7
16	82	9.3	6400	55	41	3	1	3.74	29.6	0.41	3.8	139	4.5	9.3	4.0
17	81	11.4	9430	46	50	3	1	3.12	22.0	0.40	5.3	144	4.0	8.7	4.2
18	84	14.7	10400	61	32	7	0	2.83	32.0	0.46	4.8	138	3.9	8.7	4.3
19	78	10.3	9650	41	55	4	0	3.11	27.0	0.38	2.8	140	3.8	8.3	3.1
20	66	6.0	7700	43	55	2	0	6.18	34.0	0.40	4.1	137	4.4	8.1	3.2
21	80	11.3	6700	57	40	3	0	1.83	32.0	0.23	3.1	145	5.2	8.5	4.2
22	78	8.4	9100	43	52	4	1	1.94	32.0	0.39	2.9	142	3.7	8.9	3.5
23	83	11.4	10250	51	41	8	0	2.11	26.0	0.34	4.6	139	4.5	8.7	4.1
24	86	10.3	7780	44	52	4	0	3.27	28.0	0.32	3.7	137	3.8	8.7	3.8
25	91	12.9	7960	56	32	10	2	4.11	27.0	0.41	3.1	139	4.3	8.6	3.6
26	90	13.3	9840	61	35	4	0	3.60	26.0	0.27	3.7	142	4.5	9.3	4.1
27	90	10.3	9100	41	56	3	0	3.83	28.0	0.29	3.1	138	3.7	9.4	4.1
28	77	11.4	8450	46	48	5	1	4.63	28.0	0.31	3.1	138	3.9	8.7	3.8
29	79	13.1	10500	25	59	16	0	3.19	27.2	0.35	4.7	139	4.1	8.5	3.5
30	79	9.4	10780	56	41	3	0	3.68	29.0	0.41	3.6	144	4.6	9.4	4.7
31	81	10.3	11400	68	30	2	0	3.98	39.0	0.52	4.7	146	4.0	8.3	4.2
32	78	11.4	9100	57	40	3	0	3.12	34.0	0.37	4.3	139	4.5	9.1	4.0
33	90	12.8	6910	56	38	6	0	3.16	30.0	0.45	5.8	141	3.9	8.8	4.3
34	86	12.2	11400	56	41	3	0	2.98	36.0	0.57	6.3	136	3.4	8.7	4.9
35	81	10.9	8400	53	48	5	0	2.81	26.0	0.33	4.8	135	4.9	9.4	4.6
36	77	10.3	8700	43	52	5	0	3.13	29.0	0.32	4.2	143	4.6	8.7	4.4
37	83	9.8	10400	56	30	4	0	4.61	39.0	0.81	4.9	143	5.5	8.4	4.2
38	79	9.8	9410	48	39	11	2	3.94	36.0	0.48	4.9	147	4.6	8.1	4.9
39	90	10.4	11400	56	34	10	0	4.73	94.0	0.54	9.6	140	4.8	7.0	6.1
40	89	12.8	7740	57	36	7	0	2.43	18.0	0.41	4.7	142	4.4	9.1	3.8
41	91	11.9	10600	71	25	4	0	3.91	36.0	0.78	4.7	138	5.1	9.2	3.9
42	78	12.8	8900	44	53	3	0	3.47	26.0	0.41	3.7	141	4.5	9.0	4.1
43	85	11.7	13800	71	25	4	0	3.19	21.0	0.34	3.1	141	4.3	8.4	4.5
44	73	9.4	7910	56	40	4	0	3.16	26.0	0.33	3.2	141	4.1	8.8	4.5
45	90	10.7	11400	38	57	5	0	2.93	32.0	0.42	4.1	139	4.3	9.1	3.5
46	88	11.3	8100	50	41	7	2	3.17	29.0	0.24	3.8	142	4.2	9.3	3.6
47	90	12.2	7300	54	33	10	3	2.97	19.0	0.32	3.6	139	4.1	8.8	4.1
48	81	8.7	9240	44	51	4	1	3.19	33.0	0.44	4.5	138	4.3	9.5	3.5
49	73	9.5	10350	59	26	12	3	3.82	31.0	0.41	4.0	140	3.9	8.7	4.2
50	79	11.3	6800	51	36	3	0	3.55	17.0	0.29	3.9	137	4.4	8.5	4.1
51	86	11.7	12900	56	41	3	0	9.10	146.0	0.55	5.3	135	4.6	8.4	4.2
52	76	8.4	9950	42	46	9	3	3.37	54.0	0.56	4.2	135	5.3	8.2	3.8
53	80	7.9	14500	51	44	3	2	6.43	26.0	0.20	7.0	143	4.4	8.1	4.9
54	76	9.6	9100	58	37	5	0	2.13	31.0	0.43	2.6	142	3.9	8.3	3.7
55	75	8.9	9870	46	51	3	0	4.31	32.3	0.23	4.6	138	4.7	7.8	3.4
56	84	12.5	14700	57	37	6	0	8.31	29.0	0.34	4.2	141	3.9	8.7	4.2
57	90	9.4	9740	40	50	7	3	4.43	27.0	0.71	4.9	142	5.7	9.0	3.6
58	81	11.3	9400	55	41	4	0	3.47	38.0	0.67	5.0	139	4.9	4.8	5.2
59	82	11.1	14400	33	57	8	2	4.84	36.0	0.67	4.8	146	4.6	9.1	3.7
60	84	12.2	9300	50	44	5	1	2.63	22.0	0.45	4.4	142	4.1	8.9	4.5

S.No.	ALP	Total Protein	Albumin	Total Cholesterol	HDL	LDL	TG	ANA	UP:UC after Steroid	Drug Tacrolimus	Daily Dose of Tac.	Dose mg/Kg/day	Hb after Treatment	TLC after Treatment	TLC-N after Treatment
1	122	7.4	3.1	126	36	83	166	0	2.9	1	1.50	0.084	12.1	9980	46
2	88	5.9	3.6	188	100	75	130	0	2.3	1	4.00	0.098	12.1	9780	50
3	66	7.3	4.2	285	123	129	101	0	6.0	1	3.00	0.085	12.2	7900	50
4	163	6.9	3.6	170	56	120	137	0	4.3	1	1.00	0.106	8.2	7300	55
5	132	3.9	1.3	233	51	143	281	0	4.5	1	3.00	0.111	12.6	6960	38
6	266	5.5	3.3	188	46	111	146	0	3.4	1	1.50	0.094	9.6	9300	53
7	112	4.8	2.1	231	58	142	320	1	17.3	1	3.00	0.088	13.2	8610	50
8	119	6.2	2.6	172	76	92	188	0	2.2	1	1.50	0.110	11.8	10200	52
9	122	6.0	2.4	188	56	111	281	0	3.1	1	1.50	0.092	11.4	7160	52
10	127	5.4	2.8	267	56	178	243	0	9.8	1	1.50	0.112	12.7	6780	45
11	83	5.6	2.1	175	60	98	204	0	12.3	1	2.50	0.124	11.7	9400	50
12	144	3.6	0.9	189	38	116	283	0	2.2	1	1.50	0.107	11.0	6120	48
13	112	5.0	2.6	189	36	128	112	0	3.1	1	2.00	0.125	12.6	7140	58
14	148	7.8	3.1	241	66	122	311	0	3.1	1	4.00	0.093	12.8	8760	50
15	111	6.1	3.1	336	61	189	381	0	3.7	1	2.00	0.154	12.7	9640	47
16	143	5.3	1.8	193	55	114	128	0	9.7	1	1.50	0.094	13.4	7443	47
17	122	6.1	1.3	189	47	112	146	0	4.8	1	2.00	0.100	12.9	7430	50
18	92	5.8	1.8	196	52	126	231	0	11.4	1	2.50	0.104	13.0	7300	54
19	87	6.9	2.8	190	39	133	207	0	4.6	1	2.00	0.121	12.6	7630	48
20	112	4.2	1.4	286	57	116	201	0	11.3	1	1.00	0.103	7.7	16300	45
21	92	6.6	3.3	204	63	124	196	0	3.7	1	2.50	0.125	12.6	11200	51
22	112	7.0	3.1	166	31	112	263	0	7.3	1	2.50	0.179	12.9	10350	52
23	98	5.8	3.1	189	56	106	313	0	7.1	1	2.50	0.092	13.4	7043	54
24	112	4.9	1.8	183	32	122	282	0	16.1	1	3.50	0.113	12.8	8130	66
25	98	6.3	3.1	168	49	112	129	0	3.6	1	3.50	0.056	13.6	8600	51
26	157	6.7	2.9	141	39	86	156	0	4.0	1	3.50	0.109	13.8	7220	55
27	109	6.1	3.0	181	50	116	192	0	4.1	1	2.50	0.132	13.2	10600	53
28	119	6.9	2.8	126	48	68	162	0	11.3	1	2.00	0.194	12.0	9860	42
29	125	6.1	2.8	166	56	93	213	0	17.1	1	1.50	0.091	13.6	7310	40
30	129	6.0	2.8	124	38	70	166	0	2.2	1	2.00	0.109	10.4	7910	51
31	144	5.4	1.9	98	39	184	290	0	8.2	1	2.00	0.091	12.7	7300	47
32	112	6.3	2.8	212	44	116	184	0	4.1	1	1.50	0.115	13.3	8310	50
33	83	6.9	4.0	186	48	111	126	0	3.4	1	3.00	0.062	13.9	10400	69
34	140	6.0	1.8	254	48	176	194	0	3.8	1	3.00	0.110	12.7	7410	50
35	110	6.4	3.5	174	48	112	169	0	6.1	1	2.00	0.103	11.8	9810	46
36	121	6.0	3.1	226	51	116	212	0	9.1	1	1.00	0.063	11.6	9400	55
37	144	5.5	1.9	244	49	155	247	0	9.1	1	2.50	0.095	11.9	5810	52
38	126	4.8	2.3	224	48	161	216	0	3.5	1	1.50	0.096	11.7	7420	54
39	407	3.9	1.7	401	68	272	242	0	3.9	1	4.00	0.092	10.8	9500	47
40	109	6.0	2.8	259	49	188	156	0	4.7	1	4.00	0.095	13.1	8710	52
41	114	6.7	2.8	219	36	166	178	0	4.1	1	4.00	0.133	12.8	8300	65
42	114	6.3	1.9	236	42	171	193	0	17.3	1	2.00	0.133	13.1	7800	41
43	119	6.0	2.7	281	62	192	196	0	3.9	1	2.00	0.089	12.8	8410	65
44	124	4.6	1.4	244	46	170	144	0	2.9	1	1.50	0.142	12.3	9100	50
45	148	5.7	1.8	256	54	161	139	0	4.1	1	3.00	0.109	12.2	7900	50
46	89	5.5	1.5	193	38	143	104	0	2.6	1	2.50	0.104	12.3	14100	41
47	104	6.1	2.7	271	51	180	121	0	5.6	1	5.00	0.111	11.1	8400	33
48	136	6.3	2.9	284	63	178	117	0	7.8	1	2.00	0.127	9.5	9100	59
49	92	5.4	2.2	382	75	244	223	0	17.3	1	1.00	0.071	12.2	9300	50
50	129	6.2	3.1	284	46	196	244	0	8.1	1	2.00	0.129	12.4	7640	50
51	79	4.7	2.2	438	44	331	198	0	2.4	1	2.50	0.084	9.5	8600	52
52	171	3.4	1.3	455	71	288	453	0	11.7	1	0.75	0.093	11.4	7840	50
53	58	4.6	2.1	245	48	162	248	0	3.5	1	2.00	0.133	9.6	7800	48
54	123	5.4	1.9	311	43	272	183	0	17.3	1	1.50	0.102	12.6	7700	55
55	110	5.5	1.5	198	44	120	226	0	3.8	1	3.00	0.169	11.8	8450	42
56	122	5.3	1.7	221	47	170	330	0	6.3	1	3.50	0.159	12.0	9350	61
57	144	6.9	3.1	184	32	114	192	0	3.8	1	2.50	0.091	11.8	7330	48
58	124	5.6	3.1	311	58	201	267	0	5.4	1	2.00	0.095	10.9	8310	50
59	144	6.5	2.5	332	66	221	186	0	3.8	1	2.00	0.101	11.3	8500	51
60	139	5.2	1.3	233	41	168	172	0	4.3	1	2.50	0.114	8.2	7300	55

S.No.	TLC-L after Treatment	TLC M after Treatment	TLC-E after Treatment	Platelets after Treatment	Urea after Treatment	Creatinine after Treatment	Uric acid after Treatment	Na after Treatment	K after Treatment	Calcium after Treatment	Phosphorus after Treatment	ALP after Treatment	Total Protein after Treatment	Serum Albumin after Treatment	Total Chol after Treatment
1	40	12	2	2.6	24.0	0.3	3.1	141	3.6	9.5	3.4	142	7.2	4.1	138
2	38	10	2	2.5	18.8	0.4	3.6	135	4.1	9.6	3.5	95	6.5	4.0	112
3	46	4	0	2.7	56.0	0.5	4.0	140	4.0	9.0	4.1	82	6.4	3.5	183
4	41	4	0	2.8	36.0	0.3	3.1	141	3.8	9.0	3.6	187	5.8	2.7	156
5	30	11	20	1.8	23.9	0.5	7.3	140	4.5	10.1	4.6	194	6.5	4.0	106
6	44	3	0	2.4	27.0	0.5	5.3	143	4.4	9.3	3.7	182	6.2	2.8	219
7	43	7	0	2.6	37.0	0.6	4.3	149	4.4	9.5	4.6	88	6.8	2.9	211
8	43	5	0	2.6	36.0	0.4	4.0	139	4.0	9.1	4.6	204	6.7	3.4	136
9	45	3	0	2.9	18.0	0.2	4.3	140	4.2	9.6	4.2	159	5.5	3.6	203
10	51	3	1	3.7	22.0	0.4	3.4	142	4.0	8.7	3.9	89	6.8	3.6	126
11	41	6	3	3.1	19.0	0.3	4.3	140	3.9	9.6	4.0	116	6.6	4.4	122
12	41	9	2	2.9	16.3	0.4	4.2	136	4.6	9.4	4.2	181	6.0	2.4	189
13	35	6	1	2.6	25.0	0.3	3.4	138	3.6	9.0	3.9	76	6.2	3.8	159
14	48	2	0	2.2	19.0	0.3	4.0	141	4.4	9.2	4.2	194	7.3	3.0	156
15	51	2	0	3.1	14.0	0.2	3.4	142	4.4	9.6	4.3	126	7.4	4.0	112
16	50	2	1	2.8	25.5	0.3	4.5	142	3.8	11.2	4.6	185	6.0	3.9	135
17	43	5	2	2.1	18.0	0.3	3.9	140	3.9	9.4	3.9	166	7.6	3.6	147
18	41	5	0	2.0	26.0	0.5	4.2	144	4.6	9.1	4.0	122	6.1	2.8	171
19	41	9	2	3.6	18.0	0.2	3.1	141	4.0	9.6	4.0	118	7.5	4.1	163
20	48	6	1	3.2	26.9	0.3	3.7	141	4.9	8.7	3.6	126	6.4	3.1	166
21	43	5	1	2.6	19.0	0.3	4.0	139	4.3	9.2	4.0	106	7.0	3.7	167
22	43	5	0	2.4	18.0	0.2	3.8	140	3.3	9.3	4.1	89	7.4	3.6	126
23	28	13	5	2.5	18.0	0.2	4.1	143	4.2	9.6	4.4	112	6.6	3.5	143
24	31	3	0	3.8	18.0	0.2	3.1	141	4.1	9.6	4.1	136	7.1	3.8	114
25	43	6	0	3.2	18.0	0.3	3.4	141	3.8	9.3	4.2	88	7.7	3.5	122
26	41	3	1	1.9	17.0	0.3	4.1	140	4.1	9.7	4.7	119	7.7	3.9	91
27	41	6	0	2.2	26.0	0.3	2.8	142	3.9	9.6	4.2	79	6.4	3.5	162
28	51	7	0	2.7	17.0	0.3	4.3	141	4.1	9.6	3.4	88	7.3	3.6	146
29	54	6	0	2.9	19.0	0.3	4.8	142	4.2	9.4	4.0	116	7.8	3.4	122
30	46	3	0	2.0	12.0	0.2	4.1	140	4.0	9.8	4.1	154	7.5	3.7	177
31	46	5	2	2.0	26.0	0.4	3.9	144	3.7	9.4	3.8	116	7.4	4.5	122
32	43	7	0	2.7	11.0	0.1	4.7	142	4.1	9.0	3.8	124	7.6	3.9	114
33	25	6	0	2.3	17.0	0.3	5.1	143	4.2	9.4	4.5	98	6.3	4.1	126
34	43	7	0	1.9	13.0	0.3	4.9	141	4.0	9.3	4.1	118	6.8	4.1	168
35	48	5	1	1.7	19.0	0.2	3.9	144	4.2	9.1	4.1	126	6.2	4.0	174
36	41	4	0	2.2	13.0	0.2	4.1	145	4.0	9.3	4.1	144	6.0	4.1	187
37	38	10	0	2.6	13.0	0.3	4.3	139	4.1	9.7	4.0	111	7.8	4.7	161
38	40	6	0	2.1	14.0	0.3	4.0	139	4.2	9.3	4.9	133	6.3	3.8	182
39	30	20	3	1.9	16.0	0.2	4.3	140	3.9	9.2	4.8	141	6.9	4.4	176
40	40	6	2	2.1	24.0	0.4	4.4	140	4.0	9.5	4.0	88	7.6	3.2	156
41	30	5	0	1.9	16.0	0.3	4.4	141	4.5	9.6	4.0	126	7.7	3.8	113
42	56	3	0	2.1	12.0	0.2	4.3	139	3.8	9.6	4.6	127	7.8	3.9	136
43	30	3	2	1.9	14.0	0.3	3.8	144	4.2	9.3	4.2	144	7.8	3.8	166
44	38	10	2	2.3	17.1	8.3	3.6	136	4.3	9.5	3.5	96	6.6	3.1	124
45	46	4	0	2.7	56.0	0.5	4.0	140	4.0	9.0	4.1	82	6.4	3.5	183
46	55	4	0	2.9	29.0	0.3	3.7	141	4.0	9.3	4.2	122	6.4	3.5	144
47	57	8	2	3.1	19.0	0.4	4.8	143	4.2	9.1	3.7	144	6.5	3.4	114
48	26	12	3	2.9	24.0	0.4	4.0	140	3.9	8.7	4.2	92	5.4	3.5	156
49	44	5	1	2.6	17.0	0.5	4.4	142	4.1	8.9	4.5	139	5.2	3.1	176
50	43	7	0	1.9	16.0	0.3	4.4	140	4.2	9.2	4.1	118	7.1	3.5	168
51	41	7	0	3.6	37.0	0.4	6.0	139	4.0	9.0	4.7	88	5.6	3.4	236
52	46	4	0	2.8	38.0	0.4	4.1	141	4.3	9.2	4.3	126	4.9	2.6	234
53	45	5	2	3.6	33.0	0.5	4.6	140	4.1	8.6	4.6	78	6.2	3.1	165
54	42	3	0	2.0	22.0	0.3	3.2	140	3.7	9.3	2.8	116	6.7	3.2	183
55	53	5	0	3.1	18.0	0.3	4.3	137	4.0	8.9	3.6	126	7.4	3.4	132
56	33	6	0	3.1	19.0	0.3	4.5	139	4.1	9.3	4.5	162	6.9	3.6	129
57	43	7	2	2.1	13.0	0.3	4.1	139	4.0	8.7	4.3	156	7.8	3.9	114
58	36	11	3	2.7	26.0	0.5	4.3	143	4.0	9.4	3.8	109	7.0	3.3	192
59	36	3	0	2.7	17.0	0.3	3.9	137	4.4	8.5	4.1	129	6.2	3.6	182
60	41	4	0	2.8	36.0	0.3	3.1	141	3.8	9.0	3.6	187	4.8	1.5	257

S.No.	HDL after Treatment	LDL after Treatment	TG after Treatment	UPUC after treatment	Level of Tacrolimus	Treatment outcome
1	5	8	121	0.1	6.8	1
2	58	44	174	0.2	5.4	1
3	46	119	90	0.5	10.9	1
4	45	100	88	12.8	3.1	3
5	61	31	138	0.1	13.0	1
6	49	133	174	2.7	5.7	3
7	41	122	162	3.0	4.8	3
8	56	77	173	0.1	10.5	1
9	46	123	126	0.1	8.3	1
10	46	73	122	0.2	5.7	1
11	41	62	117	0.1	4.2	1
12	46	90	136	4.1	3.0	3
13	41	85	95	0.2	4.3	1
14	43	113	183	0.1	8.5	1
15	43	61	143	0.2	5.5	1
16	58	68	66	0.1	5.8	1
17	38	93	137	0.0	4.5	1
18	46	109	178	4.2	3.5	3
19	46	112	167	0.2	8.8	1
20	42	96	122	0.0	5.2	1
21	52	112	126	0.2	6.9	1
22	31	76	176	0.2	7.5	1
23	48	79	86	0.2	6.1	1
24	39	68	88	0.2	4.6	1
25	43	7	126	0.1	10.8	1
26	40	46	88	0.1	4.3	1
27	38	83	116	0.2	6.6	1
28	41	111	91	0.2	4.8	1
29	47	64	156	0.1	7.6	1
30	52	96	156	0.2	4.4	1
31	40	88	93	0.2	5.4	1
32	29	67	109	0.1	6.7	1
33	42	94	118	0.2	7.3	1
34	43	112	126	0.1	5.6	1
35	38	102	126	0.0	5.0	1
36	48	114	166	0.1	8.7	1
37	38	94	188	0.0	7.1	1
38	40	121	176	0.1	7.4	1
39	51	113	124	0.1	10.2	1
40	43	103	112	1.4	5.7	2
41	38	76	103	0.1	10.4	1
42	39	93	124	0.2	5.0	1
43	44	112	96	0.1	8.2	1
44	52	44	174	1.8	4.3	2
45	46	119	90	0.0	6.9	1
46	38	81	130	0.1	4.7	1
47	36	89	156	0.1	11.7	1
48	44	167	167	0.1	7.0	1
49	41	111	89	1.6	5.8	2
50	43	110	125	0.1	6.5	1
51	38	168	152	0.1	8.9	1
52	43	177	126	3.7	5.4	3
53	58	82	126	0.2	7.2	1
54	36	126	148	0.1	4.9	1
55	42	67	188	0.2	5.9	1
56	43	72	188	0.1	8.2	1
57	38	76	81	0.0	8.6	1
58	52	156	167	1.6	7.5	2
59	46	133	112	0.1	5.3	1
60	45	134	126	6.9	3.8	3

ANNEXURE-XIV

Certificates

GALGOTIAS UNIVERSITY

Plot No. 2, Yamuna Expy, Opposite, Buddha International Circuit, Sector 17A, Greater Noida, Uttar Pradesh 203201

Desire

International e-Conference on Forensic Science & Criminology

Bridging the Gap in Criminal Justice System Conference Series: Forensis Agora

This is to certify that Prof./Dr./Mr./Ms./Mrs. K S Sehrawat of Galgotias University has presented a poster entitled Quality Assurance in Therapeutic Drug Monitoring of Calcineurin Inhibitors in Children using Particle Enhanced Turbidimetric Immunoassay in the conference held on **15th - 16th May, 2021** through online mode.

Dr. Arvind Kr. Jain
Dean School of Basic & Applied Sicences
Galgotias University

Dr. Rajeev Kumar
Program Chair
Division of Forensic Science
Galgotias University

Mrs. Vinny Sharma
Assistant Professor
Organizing Secretary

Id: 4233639

GALGOTIAS UNIVERSITY

CERTIFICATE

of PARTICIPATION

This is to certify that

KAPTAN SINGH SEHRAWAT

has successfully participated in ***Virtual Artificial Intelligence Summit-Solutions to COVID-19*** organized by Center of Excellence in AI & ML, Galgotias University on ***May 9th, 2020***

Mrs. Sofia K. Pillai
ORGANIZING CHAIR

GALGOTIAS UNIVERSITY

RESEARCH PAPER WRITING WORKSHOP SERIES

School of Law, Galgotias University, Uttar Pradesh, 201310

1st Lecture, 10 October, 2020

On

"Research Paper Writing"

CERTIFICATE OF PARTICIPATION

This certificate is awarded to

Mr. Kaptan Singh Sehrawat

for attending the lecture on "Research Paper Writing" under RESEARCH PAPER WRITING WORKSHOP SERIES orgainized by Think Tank Committee, School of Law, Galgotias University on 10th Oct, 2020.

Prof. (Dr.) Azim Bahadur Pathan	Dr. Namita Singh Malik	Dr. Prashna Samaddar	Prof. (Dr.) P.P. Mitra
Professor & Dean	Co-Coordinator, Think Tank Committee	Co-Coordinator, Think Tank Committee	Co-Coordinator, Think Tank Committee
School of Law, Galgotias University	School of Law, Galgotias University	School of Law, Galgotias University	School of Law, Galgotias University

GALGOTIAS UNIVERSITY

CERTIFICATE OF PARTICIPATION

This is to certify that

KAPTAN SINGH SEHRAWAT

has successfully participated in two day *International Virtual Summit* under **Theme: Global Impacts and Trends of the Pandemic** organised by International Research & Collaboration Cell, Galgotias University on

MAY 01 & 02' 2020

DR. NAGA SWETHA PASUPULETI

Summit Organizer & Head, International Research and Collaboration Cell

MIMLS
MALAYSIAN INSTITUTE OF MEDICAL LABORATORY SCIENCES
INSTITUT SAINS MAKMAL PERUBATAN MALAYSIA

In collaboration with
Oxford Immunotec
Biomed Global

IT'S TIME END TB

CERTIFICATE OF PARTICIPATION

This is to verify that

KAPTAN SINGH SEHRAWAT

has actively participated in the

WEBINAR ON TUBERCULOSIS
Advances in Tuberculosis Detection - How to Accurately Diagnose TB Infection

on

10th April 2021

END TB

Khew Jye Kang
President, MIMLS

myCPD
APPROVED 4 CPD POINTS
Category A3 (Workshops/Course etc)

GALGOTIAS UNIVERSITY

ICTACADEMY

Certificate of Participation

One Week Virtual Workshop on Learning Practical Research Skills and Techniques

This certificate is awarded to

Kaptan Singh Sehrawat

for participating in 'Learning Practical Research Skills and Techniques' on Date (10th Aug 2020 to 14th Aug 2020), Organized by University Center of Research and Development(UCRD) Galgotias University, India.

Prof.(Dr.) Preeti Bajaj
Vice Chancellor, Galgotias University

Dr Anbuthambi Rhojarajan
President, ICT Academy

Prof.(Dr.) Meenakshi Sharma
DORD, Galgotias University

Prof.(Dr.) D. Balamurugan
UCRE, Galgotias University

AHPCON 2022

4th National Allied Health Professionals' Conference of Joint Forum of Medical Technologists of India (JFMTI)

"Allied Health Sciences : Transforming Healthcare"

21st - 22nd May, 2022 | Indira Gandhi Medical College & Hospital, SHIMLA (H.P.)

CERTIFICATE OF PARTICIPATION

Presented to

Dr. / Mr. / Ms. Kaptan Singh Sehrawat

for participating as SPEAKER in the conference held at Indira Gandhi Medical College & Hospital Shimla, Himachal Pradesh on 21st - 22nd May, 2022

G S Wadhawan
Organising Chairperson

Dr. Pankaj Kaul
Co-Organising Chairperson

Kaptan Singh Sehrawat
Organising Secretary

GALGOTIAS UNIVERSITY

NATIONAL EDUCATION POLICY 2020

A GATEWAY TO ACADEMIC EXCELLENCE

17th-18th February 2021

Certificate of Participation

This is to certify that
Kaptan Singh Sehrawat
has successfully participated
in Day-2 of
Two Days Seminar on National Education Policy 2020 :A Gateway to Academic Excellence,
conducted by Galgotias University on 17th - 18th Feb 2021.

Registrar

Vice Chancellor

WEBINAR ON

IMMUNOHISTOCHEMISTRY – PRINCIPLES & PRACTICE

National Instituite of Mental Health and Neurosciences

(NIMHANS), Bangalore

27th November 2021

Certificate

This is to certify that Dr/Mr/Ms K.S. Sehrawat ..... participated as a Delegate and contributed to the success of the programme.

Dr. Anita Mahadevan
Organising Chairperson

Mr. Kaptan Singh Sehrawat
President, ICMLS

Dr. Nandeesh BN
Organising Secretary

ESTRO
European SocieTy for Radiotherapy & Oncology

Association of Radiation Therapy Technologists of India

ARTTICON 2019

13th-15th December | AIIMS, New Delhi

First World Congress, 23rd National Conference & 1st ARTTI-ESTRO Train the Trainer Course

Theme: Focusing to improve the standards of delivery and verification by acting and promoting Quality patient care, Education and Research in the Science of Radiotherapy Technology

CERTIFICATE

This is to certify that

Kaptan Singh Sehrawat

has participated as Faculty

in "ARTTICON 2019" organized by All India Institute of Medical Sciences, New Delhi, India from 13th to 15th December 2019

ARTTI has accredited ARTTICON 2019 for 18 CME credit hours after Scientific Evaluation

Ajeet Singh
(Organising Secretary & President ARTTI)

Mary Coffey
(ESTRO)

Gurvinder S Wadhawan
(Chairman Scientific Committee)

Indian Confederation of Medical Laboratory Sciences

(National Registered Voice of Indian Medical Laboratory Professionals' Associations)

Affiliated with International Federation of Biomedical Laboratory Sciences (IFBLS) Hamilton, Canada

Member-Asia Association of Medical Laboratory Scientists, Seol, South Korea

Registration No. 1256/2016 www.icmls.com, president.icmls@gmail.com

This certificate of appreciation is presented to

Mr. Kaptan Singh Sehrawat

for presenting scientific talk on the topic

COVID-19: Sharing work experiences & Challenges of Medical Laboratory Corona Warriors. Future perspective and plannings on dated 25th May, 2020

during webinar series-1 titled COVID-19: Updates for Medical Laboratory Professionals organised from 11th May 2020 to 25th May 2020

Kaptan Singh Sehrawat
President,
ICMLS

Dr Surendra Kr. Sharma
Org. Secretary,
ICMLS-Webinars

Dr Chandra Prakash Pandey
Co. Org. Secretary,
ICMLS-Webinars

Dr. Pankaj Kaul
General Secretary,
ICMLS

Under the Aegis of
Rajiv Gandhi Cancer Institute
and Research Centre

FRONTIERS IN URO-ONCOLOGY

RGCON 2022

CERTIFICATE

OF APPRECIATION

THIS CERTIFICATE IS PROUDLY PRESENTED TO

KAPTAN SINGH SEHRAWAT

has participated as a **Delegate** in

"20th Annual Conference RGCON 2022 - Frontiers In Uro-Oncology"

organized by Rajiv Gandhi Cancer Institute & Research Centre,

New Delhi, India from 15th - 17th April 2022.

Dr. Sudhir Rawal
Medical Director
Chief Urogenital Oncology
Rajiv Gandhi Cancer Institute & Research Centre, Delhi

Dr. Vineet Talwar
Director - Medical Oncology
Rajiv Gandhi Cancer Institute, Delhi

Dr. Munish Gairola
Director - Radiation Oncology
Rajiv Gandhi Cancer Institute, Delhi

Dr. Amitabh Singh
Consultant,
Dept. of Genito Uro-Oncology
Rajiv Gandhi Cancer Institute, Delhi

Dr. Ashish Khanna
Consultant,
Dept. of Genito Uro-Oncology
Rajiv Gandhi Cancer Institute, Delhi

Delhi Medical Council has accredited RGCON 2022 for 21 credit hours vide letter no. 3419/DMC/CME/16C/2/2022/ dated 13th April, 2022

GALGOTIAS UNIVERSITY

Certificate of Participation

Kaptan Singh Sehrawat

PhD Scholar-Healthcare & Clinical Research, Galgotias University

Has attended 5 Days Global E-FDP on "Unleashing Research Potential: Global Trends and Practices" Organised by Office of International Relations, Galgotias University, Greater Noida, Uttar Pradesh held from March 7-11, 2022.

Prof. (Dr.) B. Balamurugan
Director, International Collaboration

Prof. (Dr.) Anamika Pandey
School of Business

IFBLS International Federation of Biomedical Laboratory Science

35th WORLD CONGRESS OF IFBLS

& 2022 The 60th Conference of the Korean Association of Medical Technologists

KAMT KOREAN ASSOCIATION OF MEDICAL TECHNOLOGISTS

Certificate of Presentation

This is to certify that

Kaptan Singh SEHRAWAT
(India)

has presented a clinical laboratory chemistry symposium presentation entitled

Trough level of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome

at the 35th World Congress of Biomedical Laboratory Science &
2022 the 60th Conference of the Korean Association of Medical Technologists
held from October 5 to 9, 2022 in Suwon, Korea.

October 9, 2022

Alan WAINWRIGHT
President, International Federation of Biomedical Laboratory Science

In Ho JANG
President, Korean Association of Medical Technologists
President, 35th World Congress of IFBLS

ANNEXURE-XV

GALGOTIAS UNIVERSITY

Date: 07 September 20 22

Certificate of Plagiarism

This is to certify that Plagiarism check of Ph .D. Thesis of Mr./Ms. Kaptan Singh Sehrawat, Registration No. 17SCRH301012, in Department of Clinical Research , School of Basic and Applied Sciences, titled "A Study To Evaluate Trough Concentration of Tacrolimus In Children With Steroid Resistant Nephrotic Syndrome" has been done through iThenticate and found 10% similarity index .

Thanking you,

(Dr. Debal C. Kar)
University Librarian

ANNEXURE-XVI
(Publication -1)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 3323–3331
Received 15 April 2021; Accepted 05 May 2021

Measurement of Tacrolimus: A Review of Laboratory Methods

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ABSTRACT
Tacrolimus is an immunosuppressive agent prescribed in various medical conditions like organ transplantation, malignancies, autoimmune diseases, and for the treatment of Nephrotic Syndrome. It has a narrow therapeutic index and even at a low trough level (4-6 ng/mL) has been found to be linked with nephrotoxicity. Therapeutic drug monitoring of immunosuppressive drugs is required because of their varied metabolism, absorption, and drug interactions. The pharmacokinetics data of Tacrolimus is also very limited for the treatment of steroid resistant nephrotic syndrome in children. There are various laboratory detection methods which have an important role in treatment outcomes. Aim of this study is to review various laboratory methods in terms of functional feasibility, cost-effectiveness, and turnaround time (TAT) for therapeutic drug monitoring of tacrolimus. Published data of various laboratory detection methods have been evaluated in this study and found that LC-MS and Immunoassay are two important techniques that are applied for the therapeutic monitoring of Tacrolimus. The LC-MS is a gold standard method that requires a high degree of technical competence and extensive training to perform therapeutic drug testing. Turbidimetric immunoassays can also be an alternative to LC-MS in resource-constraint laboratory facilities.
Keywords: Tacrolimus; Steroid Resistant; Nephrotic Syndrome; LC-MS; PETIA; Pediatrics; QMS Tacrolimus Immunoassay

Introduction: Tacrolimus is an important antibiotic of fungal origin, *Streptomyces tsukubaensis* with a potent immunosuppressive function. It is used in multiple clinical conditions including organ transplantation, autoimmune diseases, malignancies, and for treatment of Nephrotic Syndrome [1] It has a very narrow therapeutic index and even at a low trough level (4-6 ng/mL) has been linked to nephrotoxicity [2]. It is a calcineurin inhibitor that inhibits the production of IL-2 and discourages the proliferation of T cells [3, 4]. It is metabolized in the liver, mainly via CYP3A [5]. Common interactions of tacrolimus are with grapefruit, antimicrobials, and anti-fungal which increase the levels. It has high inter-individual and intra-individual pharmacokinetic variability. The pharmacokinetics data of tacrolimus and its correlation with therapeutic efficacy is very limited in the Indian population particularly in children which requires regular therapeutic

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monitoring of the drug. It is also important for clinical laboratories to evaluate laboratory monitoring methods which have a crucial role in treatment outcomes. The purpose of this review is to discuss various laboratory methods in terms of their advantages and disadvantages, functional feasibility, cost-effectiveness, and required turnaround time (TAT) for therapeutic drug monitoring of tacrolimus.

Significance of Therapeutic Drug Monitoring of Tacrolimus: A Trough level of tacrolimus is essential in patients to prevent rejection of kidney, heart, or liver transplants [6, 7]. It is useful for determining adequate therapeutic concentration and also to avoid the toxicity of a drug. The management of organ transplant rejection and graft vs. host disease (GVHD) remains a challenging task for the clinician. Tacrolimus toxicity is mostly seen in children when plasma levels exceed 15 to 20 ng/mL which may include life-threatening complications like hyperkalemia, insulin-dependent diabetes mellitus, reversible left ventricular hypertrophy, cardiomyopathy, and encephalopathy[7]. Therefore, proper compliance with immunosuppressive therapy is of utmost importance for long-term survival. Therapeutic drug monitoring of immunosuppressive drugs is essential because of diverse metabolism, absorption, and drug interactions. Timely monitoring of drug levels is also important to improve efficacy and reduce the toxicity of individual drug dosage.

Management of steroid-resistant nephrotic syndrome (SRNS) is always challenging for clinicians. There is a dearth of pharmacokinetic data of tacrolimus regarding nephrotic syndrome in children. Gut edema, heavy proteinuria, hypercholesterolemia, hypoalbuminemia, and hypertriglyceridemia are distinctive features of nephrotic syndrome thus the pharmacokinetic data of organ transplant studies may not be appropriate to children with nephrotic syndrome. Hypoalbuminemia may lead to reduced protein binding, whereas gut edema can lead to uneven absorption of the drug which may also have an altered volume of drug distribution or clearance. Further, renal transplant data also indicates a narrow therapeutic index of a drug, significant inter-individual inconsistency in tacrolimus trough concentration (C0), and inter-dose area under the curve (AUC0–12 h). However, the Tacrolimus has gained clinical acceptance in the management of steroid-resistant nephrotic syndrome (SRNS) in children nevertheless therapeutic range is still extrapolated from pediatric renal transplant recipients due to the limited availability of pharmacokinetic data in SRNS patients.

Laboratory Methods: Various laboratory methods are being applied in laboratories for therapeutic drug monitoring like Liquid Chromatography-Mass Spectrometry (LC-MS/MS), Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), Particle Enhanced Turbidimetric Immunoassay (PETIA), and Dried-blood-spot analysis etc.

Particle Enhanced Turbidimetric Immunoassay (PETIA): The QMS Tacrolimus Immunoassay is a homogeneous particle-enhanced turbidimetric immunoassay. The assay is

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based on competition between the drug in the sample and the drug-coated onto a microparticle for antibody binding sites of the tacrolimus antibody reagent. The tacrolimus-coated microparticle reagent is rapidly agglutinated in the presence of the anti-tacrolimus antibody reagent and the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically at 700 nm. When a sample containing tacrolimus is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained with the maximum rate of agglutination at the lowest tacrolimus concentration and the lowest agglutination rate at the highest tacrolimus concentration. [8]

Liquid Chromatography-Mass Spectrometry (LC-MS): Tacrolimus (TAC) has a narrow therapeutic index and a high inter-individual and intra-individual pharmacokinetic variability, which necessitates therapeutic drug monitoring to individualize dosage. Mass spectrometers operate by converting the analyte molecules to a charged (ionized) state, with subsequent analysis of the ions and any fragment ions that are produced during the process, based on their mass to charge ratio (*m/z*). [10] Liquid chromatography-mass spectrometry (LC-MS) is now a commonly used technique with the development of electrospray ionization (ESI) providing a simple and robust interface [9]. LC-MS is mostly preferred in labs due to its high specificity and reduced run time. This technique uses molecular fragmentation for the separation of particles. Single quadrupoles, triple quadrupoles, and quadrupole ion-trap instruments are the most used mass analyzers in routine laboratories, in the fields of forensic toxicology and therapeutic drug monitoring [11]. Lower drug concentrations can also be quantified. It further helped in the quantification of lower drug concentrations in the blood samples. The advantages of HPLC-mass spectrometry are high sensitivity, specificity, small sample requirements, minimal sample preparation, rapid throughput, and simultaneous measurement [12]. The application of immunoassay methods may lead to an over-estimation of blood trough levels and under-dosage of the drug. Overestimation of the concentration using immunoassay methods occurs because of the cross-reaction with metabolites. The occurrence of such overestimation indicates the general need for more precise methods for drug monitoring. The high selectivity of LC/MS/MS methods prevents an overestimation of the concentration of immunosuppressive agents in patient samples [13]. The disadvantage of this method is that it required high upfront costs and full validation for use. However, to perform this LC-MS testing; a high degree of technical ability and extensive training is required.

High-Pressure Liquid Chromatography (HPLC): It is the sensitive and specific method used for measuring the tacrolimus. The stationary phase used is usually a C18 column and the mobile phase is methanol with formic acid. The principle of separation is adsorption. When a mixture of components is introduced into an HPLC column, with high pressure they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards adsorbent generally travels slowly. The component which has less affinity will travel faster finally the components get separated. They were mostly combined with techniques like ELISA

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and mass spectrometry for the analysis of tacrolimus. The disadvantages of HPLC-MS were the high cost of equipment and the availability of suitably skilled scientific staff. The advantages of HPLC-mass spectrometry were high sensitivity, specificity, small sample requirements, minimal sample preparation, rapid throughput, and simultaneous measurement [14].

Gas Chromatography-Mass Spectrometry (GC-MS): This is a method that uses very high temperature for causing sample vaporization. Vaporized fractions are then passed through the electric field where they get separated based on their molecular weight. The pattern of separation is unique for each drug, therefore establishes a fingerprint for identification [15]. GC MS has limited use as it is preferable only for volatile substances. GC MS is not the preferred method these days.

Radio Immuno Assay (RIA): It generally, uses radioactivity for the detection of the presence of the analyte. In RIA the sample is incubated with an antibody and a radiolabeled drug (mostly used radio labeled substance I125). The amount of radioactivity measured is compared to the radioactivity present in the known standards which are included in each run and results are quantitated. Mostly used of determination of drugs of abuse. The advantages of RIA were high specificity and sensitivity. The side effects mostly included radiation hazards, the use of radio labeled reagents, the requirement of specially trained persons. Labs also require a special license to handle radioactive material. Further a special arrangement for storage, and waste disposal of radioactive materials is also a big challenge [16].

Particle Enhanced Turbidimetric-Inhibition Immunoassay (PETINIA): It is also an immune turbidimetric method. It mostly uses the creation of light scattering particles to measure drug levels. The free drug in the sample competes for the antibody fragment, thereby decreasing the rate of particle aggregation. The rate of aggregation is inversely proportional to the concentration of a drug in the sample. It's a developing technique and not much literature is available.

Cloned Enzyme Donor Immunoassay (CEDIA) : Competitive homogenous enzyme immunoassay. It uses a genetically formulated enzyme βgalactosidase. This assay has two component fragments of an enzyme: enzyme acceptor and an enzyme donor which are generally inactive, but in solution, they become activated and reassemble. As a single subunit, they can react with the substrate. Drug bound to the enzyme donor competes with the drug or with metabolite in the sample for antibody binding site. If the drug bound to the enzyme donor binds to the antibody, it is prevented from reassembling with the enzyme acceptor and activating the enzyme. If the drug is present the unbound enzyme donor reassembles with the enzyme acceptor and reacts with the substrate to produce a change of absorbance. Linearity generally ranges from 0-30 ng/ml. It is well correlated with the gold standards LCMS ($r = 0.964$) and MEIA ($r = 0.874$) [17]. The reportable range varies from 2-30 ng/ml. Moreover follows 2-point calibration throughout standard curve assay.

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Chemiluminescent Microparticle Enzyme Immunoassay (CMIA): Before the initiation of the procedure, a manual pretreatment step is performed in which the whole blood sample is extracted with a precipitation reagent and centrifuged. The supernatant is transferred into a Transplant Pretreatment Tube, which is placed onto the System. Sample, assay diluent, and anti-tacrolimus coated paramagnetic microparticles are combined to create a reaction mixture. Tacrolimus present in the sample binds to the anti-tacrolimus coated microparticles. After a delay, tacrolimus acridinium-labeled conjugate is added to the reaction mixture. The tacrolimus on the acridinium-labeled conjugate competes for the available binding sites on the microparticles. Following incubation, the microparticles are washed and pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). An indirect relationship exists between the amount of tacrolimus in the sample and the RLUs detected by the system optics. A study by Marubashi, *et al* had compared the method with MEIA and found that the correlation between the two methods was highly significant ($r = 0.941$). They found that CMIA was much superior to MEIA in detecting the low levels [18].

Dried Blood Spot (DBS): In this method sampling using a finger prick is an emerging alternative to venous sampling. Important advantages of DBS sampling include the less amount of blood is needed, the possibility of home-based sampling after which the sample can be sent to the laboratory, easier sampling at desirable sampling times (eg, trough concentrations), and the quick results being available to the patient before the next outpatient visit[19]. This process of TDM with DBS sampling has recently been demonstrated to be cost-effective, with lifelong concentration monitoring [20] There has been an increase in the development of DBS assays for TDM for a wide range of drug therapies, including immunosuppressants [21, 22]. The challenge is to increase the applicability of DBS sampling as adequate clinical validation is required. Second, the acceptability of DBS sampling needs to be evaluated to assess the possible bottlenecks for implementation at an early stage. Only then can widespread home-based sampling for TDM can be implemented.

Discussion: The adjustment of dose for tacrolimus based on daily therapeutic drug monitoring (TDM) is important to prevent rejection and its severe adverse reactions like central neurotoxicity and nephrotoxicity. The factors like hematocrit, plasma proteins as well as drug concentration are known to affect the distribution of tacrolimus between whole blood and plasma [23, 24]. Tacrolimus is known to be metabolized by CYP3A into at least 8 metabolites through demethylation and hydroxylation [25]. Therefore frequent monitoring of the drug is recommended. LC-MS/MS is used as the primary chromatographic detection method across the world due to its high specificity and sensitivity [26]. Detection of tacrolimus by immunoassay is another preferred method nowadays. These methods mostly include the use of anti-tacrolimus antibodies conjugated with specific antigens. Antibodies used in immunoassay are well known to cause cross-reactivity with a variety of metabolites [27]. Advances in immunoassay measurement involve automated specimen pretreatment, enhanced reagent stabilities to lower potential matrix effect, and new anti-Tac antibodies that provide more sensitivity and affinity to the target drug.

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Immunoassay continues to be used in many laboratories across the country because of its ease of use and lower costs associated with services. Many laboratories find this option appealing because it does not involve a high level of technical skill from staff; the equipment can be leased; and the manufacturer often provides training, support, and maintenance for these systems. A study held in Japan had mentioned that the Microparticle enzyme immunoassay was the most widely used method for more than 20 years. After that, another new technique was introduced as chemiluminescent enzyme immunoassay (CLIA and an affinity column-mediated immunoassay had been introduced and used in Japan. These 2 immunoassay methods were based on anti-tacrolimus antibodies, which had different properties in the cross-reaction with tacrolimus metabolites. Tacrolimus concentrations were measured in the peripheral blood of 102 patients using MEIA, CLIA, ACMIA, and LC-MS/MS. Additional blood samples of 54 patients, who also underwent liver transplantation at Kyoto University Hospital, were analyzed using the newly developed FIA-MS/MS and LC-MS/MS. CLIA had shown the highest accuracy among all the 3 assays [28]

Another study by Marubashi et al also mentioned that CLIA had a highly significant association with the results of the gold standard LC-MS (r=0.964)[18]. With the added influence of pharmacokinetic and pharmacodynamic factors affecting drug therapy, continuous monitoring of drug concentration is recommended. Since the physicians need to maintain the Tacrolimus levels between 3–7 ng/ml, methodologies capable of quantifying up to 1ng/ml need to be developed. Techniques like LC-MS, CLIA, ACMIA, and newer techniques Like PETIA and CEDIA have made rapid assays possible.

Although there has been literature suggesting LC-MS as the gold standard assay due to its high specificity it has a major disadvantage of a high-ended and costly setup, moreover requires extremely trained professionals. Ultimately labs now prefer immunoassays as they are more cost-effective and do not require specialized training. Although the major disadvantage the immunoassays exhibit is cross-reactivity with different metabolites, with advances in technology newer methods like PETIA, CEDIA, and Dried blood spot analysis have been implemented. Studies have exhibited that these techniques significantly correlated with the gold standard techniques and they were found to be cost-effective. CEDIA correlated well with LC-MS (r= 0.924) and could detect TAC concentration between1-30ng/ml. It further followed 2 point calibration throughout standard curve assay. On the other hand, PETIA is emerging as the most cost-effective procedure nowadays. Correlation studies also established a highly significant association between test specimens with PETIA to that of LC-MS (r = 0.972) and the Abbott Architect assay (r=0.937). PETIA can detect as low as 1ng/ml with linearity of 0-30ng/ml. It also follows 6 point calibration which makes this technique highly sensitive. Dried spot analysis using finger prick is usually a less invasive, painless method for clinical analysis [20] but this technique needs proper validation for implementation.

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Conclusion: LC-MS and Immunoassay are two important techniques that are used for the therapeutic monitoring of Tacrolimus. The LC-MS is a gold standard method which requires high costs and full validation for use. A high degree of technical ability and extensive training is required to perform this type of testing. Turbidimetric immunoassays can be considered a better alternative to LC-MS in terms of cost and turnaround time (TAT), especially in resource-constraint laboratory facilities.

References:

1. Thölking G, Gerth HU, Schuette-Nuetgen K, Reuter S. Influence of tacrolimus metabolism rate on renal function after solid organ transplantation. World J Transplant. 2017 Feb 24; 7(1):26-33.

2. Starzl T, Todo S, Fung J, et al. FK506 for human liver, kidney and pancreas transplantation. Lancet1989; 2:1000–4.

3. McCauly J, Fung J, Jain A, et al. The effects of FK506 on renal function after liver transplantation. Transplant Proc 1990; 22:17–20.

4. Tamura K, Kobayashi M, Hashimoto K, et al. A highly sensitive method to assay FK-506 levels inplasma. Transplant Proc 1987;19: 23–9.

5. Elens L, Capron A, van Schaik RH, De Meyer M, De Pauw L, Eddour DC, Latinne D, Wallemacq P, Mourad M, Haufroid V. Impact of CYP3A4*22 allele on tacrolimus pharmacokinetics in early period after renal transplantation: toward updated genotype-based dosage guidelines. Ther Drug Monit. 2013 Oct; 35(5): 608-16.

6. Port FK, Dykstra DM, Merion RM, Wolfe RA. Trends and results for organ donation and transplantation in the United States, 2004. Am J Transplant. 2005;5(4 Pt 2):843–849. 6. Gounden V, Soldin SJ. Tacrolimus measurement: building a better immunoassay. Clin Chem. 2014;60(4):575–576.

7. Scott LJ, McKeage K, Keam SJ, et al. FK506: a further update of its use in the management of organ transplantation. Drugs. 2003;63:1247-1297

8. Christians U, Pokaiyavanichkul T, Chan L, Tacrolimus, Applied Pharmacokinetics and Pharmacodynamics: Principals of Therapeutic Drug Monitoring. 4th Edition. Lippincott Williams & Wilkins, Philadelphia, PA 2005: 529-562.

9. Ho CS, Lam CWK, Chan MHM, Cheung RCK, Law LK, Lit LCW, et al. Electrospray ionisation mass spectrometry: principles and clinical applications. Clin Biochem Rev. 2003;24:3–12.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 3323–3331
Received 15 April 2021; Accepted 05 May 2021

10. Gallardo E, Barroso M, Queiroz JA. LC-MS: a powerful tool in workplace drug testing. Drug Test Anal. 2009 Mar;1(3):109-15

11. Jansen R, Lachatre G, Marquet P. LC-MS/MS systematic toxicological analysis: comparison of MS/MS spectra obtained with different instruments and settings. Clin Biochem. 2005;38:362–72

12. Yang Z, Wang S. Recent development in application of high performance liquid chromatography-tandem mass spectrometry in therapeutic drug monitoring of immunosuppressants. J Immunol Methods. 2008;336:98–103

13. Agrawal YP, Cid M, Westgard S, Parker TS, Jaikaran R, Levine DM. Transplant patient classification and tacrolimus assays: more evidence of the need for assay standardization. Ther Drug Monit. 2014;36(6):706–709.

14. Taylor PJ. Therapeutic drug monitoring of immunosuppressant drugs by high-performance liquid chromatography-mass spectrometry. Ther Drug Monit. 2004 Apr;26(2):215-9.

15. Kapur BM. Drug-testing methods and clinical interpretations of test results. Bull Narc. 1993;45(2):115-54.

16. Mould G.P., Aherne G.W., Morris B.A. *et al.* Radioimmunoassay of drugs and its clinical application. *European Journal of Drug Metabolism and Pharmacokinetics* 2, 171–184 (1977)

17. Liang SL, Breaud A, Dunn W, Clarke W. Comparison of the CEDIA and MEIA assays for measurement of tacrolimus in organ transplant recipients. Clin Chim Acta. 2008 Oct;396(1-2):1-6.

18. Marubashi S, Nagano H, Kobayashi S, Eguchi H, Takeda Y, Tanemura M, Umeshita K, Monden M, Doki Y, Mori M. Evaluation of a new immunoassay for therapeutic drug monitoring of tacrolimus in adult liver transplant recipients. J Clin Pharmacol. 2010 Jun;50(6):705-9.

19. Demirev P.A. Dried blood spots: Analysis and applications. Anal. Chem. 2013;85:779–789.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 3323–3331
Received 15 April 2021; Accepted 05 May 2021

20. Zakaria R., Allen K.J., Koplin J.J., Roche P., Greaves R.F. Advantages and Challenges of Dried Blood Spot Analysis by Mass Spectrometry Across the Total Testing Process. EJIFCC. 2016;27:288–317.

21. Hinchliffe E, Adaway JE, Keevil BG. Simultaneous measurement of cyclosporin A and tacrolimus from dried blood spots by ultra high performance liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2012; 883–884: 102–107.

22. Koop DR, Bleyle LA, Munar M, Cherala G, Al-Uzri A. Analysis of tacrolimus and creatinine from a single dried blood spot using liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2013; 926: 54–61

23. Jusko WJ, Thompson AW, Fung J, et al. Consensus document; therapeutic monitoring of tacrolimus(FK-506). The Drug Monit 1995;17:606-614

24. Physicians' Desk Reference, 58th ed. Thomson PDR at Montvale, NJ. 2004; PROGRAF®: 1323-1327

25. Christians U, Pokaiyavanichkul T, Chan L, Tacrolimus, Applied Pharmacokinetics and Pharmacodynamics: Principals of Therapeutic Drug Monitoring. 4th Edition. Lippincott Williams & Wilkins, Philadelphia, PA 2005: 529-562.

26. Polledri E, Mercadante R, Ferraris Fusarini C, Maiavacca R, Fustinoni S. Immunosuppressive drugs in whole blood: validation of a commercially available liquid chromatography/tandem mass spectrometry kit and comparison with immunochemical assays. Rapid Commun Mass Spectrom. 2017 Jul 15;31(13):1111-1120.

27. Bazin C, Guinedor A, Barau C, et al. Evaluation of the ARCHITECT tacrolimus assay in kidney, liver, and heart transplant recipients. J Pharm Biomed Anal. 2010;53(4):997–1002.

28. Hashi S, Masuda S, Kikuchi M, Uesugi M, Yano I, Omura T, Yonezawa A, Fujimoto Y, Ogawa K, Kaido T, Uemoto S, Matsubara K. Assessment of four methodologies (microparticle enzyme immunoassay, chemiluminescent enzyme immunoassay, affinity column-mediated immunoassay, and flow injection assay-tandem mass spectrometry) for measuring tacrolimus blood concentration in Japanese liver transplant recipients. Transplant Proc. 2014 Apr; 46(3):758-60.

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Clinical Profile and Therapeutic Level of Tacrolimus in Children withSteroid Resistant Nephrotic Syndrome-A Single Centre Retrospective Study

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Abstract:

Background: Nephrotic Syndrome is a chronic kidney disease in children.Majority of the children usually respond to standard steroids treatment and achieve complete remission of proteinuria but 10-15% of these patients do not respond to steroid. This condition is categorized as steroid resistant nephrotic syndrome (SRNS) and treated with Calcineurin inhibitors. The Tacrolimus is one of the important Calcineurin inhibitor prescribed for thetreatment of SRNS. The aim of this study is tostudy Clinical profile and therapeutic level of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome.
Method: A retrospective study of follow up patients referred to Drug Assay Lab was done. Therapeutic drug monitoring of tacrolimus was done for the SRNS patients from January, 2021 to April, 2021.The clinical profile 55 children with steroid resistant nephrotic syndrome were assessed. Data was evaluated on the basis of laboratory report of tacrolimus and hospital patient's card of these patients.

Results: 55 patients were studied. Mean age at onset of Nephrotic syndrome was 5.45±3.79. Gender distribution ration was 2.43:1. Kidney Biopsy results showed thatmost common histological diagnosis was minimal change disease (47.27%) followed by Focal Segmental Glomerulosclerosis (34.54%).Themean trough concentration of tacrolimus at follow up visit after one year was found 6.41 ng/mL and 61.81% achieved a remission and 30.90% patients showed partial remission.

Conclusion:61.81% patients achieved a complete remission and 30.90% patients showed partial remission.Clinical profile and trough level of tacrolimus of children showed a better treatment outcome with tacrolimus therapy.

Keyword: Steroid Resistant Nephrotic Syndrome (SRNS); Tacrolimus; Nephrotic Syndrome, Calcineurin inhibitors.

Introduction: Nephrotic Syndrome is a common glomerular disorder in children characterised by combination of massive proteinuria, hyperlipidemia, hypoalbuminemia and edema. The prevalence of childhood nephrotic syndrome (NS) varies in different population from 12–16/ 100000 children [1]. It affects all ages and ethnic background. The reported incidence of nephrotic syndrome is 2-3/100,000 children in western countries, slightly higher in 2- 7/100,000 in south Asian origin [2].The majority of patients of Nephrotic syndrome are idiopathic while a small proportion of cases may be secondary or congenital [3]. The common causes of nephrotic syndrome in children consist of minimal change disease (MCD), Focal segmental glomerulosclerosis (FSGS), IgA nephropathy and membranous nephropathy. The patients of nephrotic syndrome are treated with multiple immunosuppressive therapies which include calcineurin inhibitors (cyclosporine and tacrolimus), cyclophosphamide, Mycophenolatemofetil and Rituximab [4, 5].

Steroid Resistant Nephrotic Syndrome (SRNS): Most of the children with Nephrotic

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Syndrome initially respond to steroids and achieve remission of proteinuria following 4-6 weeks of treatment with prednisolone, however 10-15% patients do not achieve complete remission and classified as steroid resistant nephrotic syndrome (SRNS) [6]. Recent studies have indicated significant increase in the number of steroid resistant Nephrotic syndrome particularly in south east Asia.Management of Steroid Resistant Nephrotic Syndrome requires combination of immunosuppressants, angiotensin converting enzyme inhibitor, low dose steroids and statin etc. Failure of immunosuppressive medications leads to a high risk of developing end stage renal disease (ESRD). The management of SRNS is a challenging task for nephrologists due to various side effects related to immunosupression such as infections, nephrotoxicity, cytopenia neurotoxicity and malignancies. Regular therapeutic monitoring of calcineurin inhibitor drug are important and the choice of immunosuppressant primarily depends upon their efficacy and safety of drugs. Tacrolimus and cyclosporine are important drugs primarily prescribed for the treatment of SRNS. The aim of this study is to evaluate clinical profile and therapeutic level of Tacrolimus in children receiving treatment of with Steroid Resistant Nephrotic Syndrome.

Material and Methods: A retrospective study of all patients referred to Drug Assay Lab at Kalawati Saran Children's Hospital (KSCH) for therapeutic drug monitoring of Calcineurin Inhibitor was done. Chart review of 55 children in the age group of 1–18 years of age, attended Nephrology Clinic from January 2021 to March, 2021 was performed. The study was approved by the Institutional Ethics Committee of LHMC & Associated Hospitals vide letter no LHMC/IEC/2021/01. Trough concentration of tacrolimuswas evaluated in children with steroid resistant nephrotic syndrome receiving treatment from Kalawati Saran Children's Hospital, New Delhi.KSCH is a tertiary care children hospital which provides a comprehensive care and facility for diagnosis, treatment and management of pediatric patients. Our hospital runs special clinic for patients with nephrotic syndrome every Tuesday and Saturday. Regular follow-up and laboratory monitoring of all patients is done. The patients are examined by specialist medical practitioners. Hospital has a dedicated Drug Assay Laboratory provide facility for therapeutic drug monitoring for various important drugs. Therapeutic drug monitoring is also available for Calcineurin inhibitors like tacrolimus and cyclosporine. Tacrolimus level are done on Indiko™(Thermo Fisher) fully automated clinical chemistry analyzer using particle enhanced turbidimetric immunoassay (PETIA) Technology. Clinical Data of duly registered patients of Nephrotic Syndrome like clinical history, treatment, laboratory reports of patients was evaluated. All analyses were carried out using statistical software, SPSS, after data collection.

Inclusion Criteria:

- Children in the age group of 1–18 years were included in the study. Nephrotic Syndrome was diagnosed as per the ISPN guidelines (nephrotic range proteinuria 40 mg/$m^{2/}$ hr or >1000 mg/m^2 /day; spot Up/Uc>2 mg/mg; 3-4+ by dipstick; with spot hypoalbuminemia (albumin <3.0 g/dL; and edema.

Exclusion criteria:

- Children with secondary causes of nephrotic syndrome were excluded from the study.

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• Children whose records were not complete were excluded from the study

Box I Definitions Related to Nephrotic Syndrome [7]

Nephrotic syndrome	Nephrotic range proteinuria (40 mg/m2/hr or >1000 mg/m2/day; spot Up/Uc>2 mg/mg; 3-4+ by dipstick); hypoalbuminemia (albumin <3.0g/dL); and edema.
Steroid sensitivenephrotic syndrome	Complete remission within 6-weeks' treatment with prednisolone at a dose of 60 mg/m2/ day (2 mg/kg/day; maximum 60 mg/day)
Initial steroid-resistance	Failure to achieve complete remission after 6-weeks initial therapy with prednisolone (as defined above)
Late (secondary) steroid-resistance*	Initially steroid-sensitive; steroid resistance in a subsequent relapse
Complete remission	Urine protein nil-trace by dipstick for 3 consecutive days, Up/Uc<0.2, or 24-hr protein <100 mg/m2/day
Partial remission	Urine protein 1+/2+ (dipstick), Up/Uc between 0.2-2, or 24-hr urine protein 100-1000 mg/m2/day; serum albumin ≥3.0 g/dL; and absence of edema
Non-response	Urine protein 3+/4+ (dipstick), Up/Uc>2, or 24-hr urine protein >1000 mg/m2/day; albumin <3.0 g/dlL or edema
Relapse	Urine albumin 3+/4+ for 3 consecutive days, Up/Uc>2, or 24-hr protein >1000 mg/m2/day, in a patient previously in partial or complete remission
Monogenic disease	Pathogenic or likely pathogenic variation, defined by American College of Medical Genetics and Genomics, in a gene associated with nephrotic syndrome
CNI-resistant disease	Non-response to cyclosporine or tacrolimus, given in adequate doses and titrated to blood levels, for 6-months
Allograft recurrence of nephrotic syndrome	Persistent proteinuria (Up/Uc>1) if previouslyanuric;or increase of Up/Uc>1 if proteinuria at time of transplant (in absence of other apparent causes)

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CNI Calcineurin inhibitor; Up/Uc urine protein to creatinine ratio (mg/mg)
Patients with steroid toxicity may receive daily prednisolone for 4 weeks, followed by alternate-day therapy for 2 weeks. All above definitions are as per ISPN guidelines

Results:55 patients were studied. Mean age at onset of Nephrotic syndrome was 5.45±3.79 and mean age of all patients undertaking treatment in different age group was 7.65±4.42. Gender distribution ration was 2.43:1. Kidney Biopsy results showed that most common histological diagnosis was minimal change disease (47.27%) followed by Focal Segmental Glomerulosclerosis (34.54%). mean trough concentration of tacrolimus at follow up visit after one year was found 6.41 ng/mL. After one year, 61.81% showed a remission and 30.90% patients showed partial remission.

Table 1: Demographic Profile of Patients with Steroid Resistant Nephrotic Syndrome

Variable	Number	Percentage	Mean±SD
Age in years (SRNS) Patients			
1–3	5	9.09 %	7.65±4.44
3–6	22	40 %	
6–9	11	20 %	
9–12	6	10.9 %	
12–15	5	9.09 %	
15-18	6	10.9 %	
Sex			
Male	39		70.90%
Female	16		29.09%
Rural/Rural			
Rural	14		25.45%
Urban	41		74.54%

Majority of children receiving treatment of SRNS were in age group of 3-6 years (40%). Male children were higher (71%) and female children were 29% and most of the children were from urban area.

Table 2: Age at onset of Nephrotic Syndrome (in Years)

Age in years	Number	Percentage	Mean±SD
1–3	16	29.00	5.57±3.63
3–6	21	38.18	
6–9	08	14.54	
9-12	06	10.90	

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12-15	03	05.45	
15-18	01	01.81	

Table 3: Duration of SRNS Treatment

Duration in Years	Number	Percentage	Mean±SD
1-2	32	58.18	2.2±1.00
2-3	11	20.00	
3-4	07	12.72	
4-5	05	9.00	

Table 4: Treatment Outcomes in SRNS Patients

Variable	Number	Percentage
Full Remission	34	61.81
Partial Remission	17	30.90
No Remission	05	09.00

Table 5: Histological findings on renal biopsy-Histological diagnosis

Variable	Number	Percentage
Minimal Change Disease (MCD)	26	47.27%
Focal Segmental Glomerulosclerosis (FSGS)	19	34.54%
Biopsy not done/Report not available	10	18.18%

Most of the patients were in treatment duration of 1-2 years and 5 patients (9%) were taking treatment from 4-5 years due to multiple relapse. After one year, around 62% achieved complete remission, 31% showed partial remission and around 9% indicated no remission. Histopathology report indicated Minimal Change Disease (MCD) in 47%, Focal Segmental Glomerulosclerosis (FSGS) in 34% and biopsy were not done in 18% cases.

Table 6: Complication in SRNS Patients

Diseases/Condition	Number	Percentage
Urinary Tract Infection	8	14
Hypertension	2	3.6
Pneumonia	3	5.45
Tuberculosis	2	3.6

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Hypothyroidism	1	1.81
COVID-19 infection	1	1.81
MRKH with Cholelithiasis	1	1.81
No major diseases/complication	**37**	**67**

Table 6: Trough level of Tacrolimus after one year of Tacrolimus Therapy

level of Tacrolimus	Number	Percentage	Mean±SD
0- 4 ng/ mL	15	27.27	6.41±4.31
4- 8 ng/ mL	27	49.09	
8- 12 ng/ mL	09	16.36	
12-16 ng/mL	02	3.63	
16-20 ng/mL	01	1.81	
20-30 ng/mL	01	1.81	

Table 6: Laboratory Parameter in Patients

Laboratory Parameters	Total Number	Mean Reported Value
Urea	42	29
Creatinine	40	0.4
UP:CR	35	1.53

Discussion: In our study, most common histological diagnosis was minimal change disease (47.27%) followed by Focal Segmental Glomerulosclerosis (34.54%) which is in contrast to a study by Bhutani et al. which reported FSGS as a common histopathological finding as 50-60% in children with SRNS [8]

Tacrolimus has gained acceptance for treatment of SRNS yet it was found that pharmacokinetics date and correlation is very limited regarding therapeutic efficacy. There is serious dearth of target concentration for children with SRNS and treatment of SRNS is done on the basis of therapeutic concentration applied in organ transplantation.

In this study, mean trough concentration of tacrolimus was found 6.41 ng/mL.The desirable C_0 of tacrolimus in pediatric transplant immediately after transplant are 10-12 ng/mL and 5-10 ng/mL subsequently to prevent rejection of organ.[9] On the basis of transplant data, suitable target concentration of tacrolimus in SRNS is considered from 5ng/mL to 10 ng/mL in most of the studies[10].In our study we found a significant correlation of trough concentration of tacrolimus with other studies.The average value of blood urea reported in 42 patients was 29 mg/dL and average creatinine level was found 0.4 mg/dl. The average UP:CR was found 1.53 in

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35 patients.The male children were higher in number as compare to female. The mean age group of children at the onset of treatment was5.45±3.79 and maximum children were in age group of 3-6 years and maximum patients were belonging to urban background. In our study the 3-6 years age group indicated maximum number.

Conclusion: Clinical profile of children showed a better treatment outcome with tacrolimus. The similarity was found with other studies in age of children at onset of nephrotic syndrome. Mean Trough concentration of tacrolimus and clinical profile of children was similar with typical steroid resistant nephrotic syndrome in children. However, long-term follow-up with more number of patients is required to substantiate the findings.

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Conflict of Interest: None

Ethical Approval: Study was approved by the Institutional Ethics Committee of LHMC & Associated Hospitals vide letter no LHMC/IEC/2021/01

REFERENCES-

1. Noone DG, Iijima K, Parekh R. Idiopathic nephrotic syndrome in children. Lancet. 2018 Jul 7; 392 (10141):61-74.
2. Eddy AA, Symons JM. Nephrotic syndrome in childhood. Lancet 2003;362:629-39.
3. Niaudet P. Steroid sensitive nephrotic syndrome in children. In: Avner ED, Harmon WE, Neasden P, editors. Paediatric Nephrology. 1st ed. Philadelphia: Lippincott Williams and Wilkins; 2004. p. 543-56.
4. Niaudet P, Boyer O. Paediatric Nephrology. 6th ed. Berlin, Heidelberg:springer-verlag.2009:667-668.
5. Chua A, Yorgin P. Evidence based Nephrology. 1st ed. Chichester, west Sussex, UK wiley-blackwell.2009:787-789.
6. Tullus K, Webb H, Bagga A. Management of steroid-resistant nephrotic syndrome in children and adolescents. Lancet Child AdolescHealt.2018; 2:880-90.
7 Indian Pediatrics, JANUARY 04, 2021 [E-PUB AHEAD OF PRINT]
8. Butani L, Ramsamooj R. Experience with tacrolimus in children with steroid-resistant nephrotic syndrome. PediatrNephrol. 2009 Aug; 24(8):1517-23.
9. Lancia P, Jacqz-Aigrain E, Zhao W. Choosing the right dose of tacrolimus. Arch Dis Child. 2015 Apr; 100(4):406-13.
10. Lombel RM, Gipson DS, Hodson EM; Kidney Disease: Improving Global Outcomes. Treatment of steroid-sensitive nephrotic syndrome: new guidelines from KDIGO. PediatrNephrol. 2013 Mar; 28(3):415-26

Abbreviations:
NS- Nephrotic syndrome,
SDNS- steroid dependent nephrotic syndrome,
SRNS- steroid resistant nephrotic syndrome,
CR- complete remission,
PR- partial remission,
NR- no remission,
MCD- Minimal change disease,
FSGS- Focal segmental glomerulosclerosis,

(Publication -3)

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Trough Level of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome

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The objective of the study was to evaluate trough concentration of Tacrolimus in children with steroid resistant nephrotic syndrome (SRNS) in a tertiary care children's Hospital A cross-sectional study was conducted among children with SRNS receiving Tacrolimus treatment in a tertiary care children's Hospital. The trough level of Tacrolimus was measured in 60 children after 12 weeks of treatment using particle enhanced turbidimetric immunoassay (PETIA). The study was approved by the Institutional Ethics Committee. The laboratory results were evaluated with the outcomes of SRNS treatment. The results presented that 81% (49/60) of patients had complete remission while 7% (4/60) were in partial remission and 12% (7/60) were in non-remission status after minimum 12 weeks of initial treatment with Tacrolimus. The mean trough level of tacrolimus of (N=60 patients) after a minimum of 12 weeks of Tacrolimus treatment was found 6.6±2.2 ng/mL. The mean C0 of Tacrolimus was higher in the remission group than in the non-remission group (7.0 ng/mL, versus 4.18 ng/mL, *P*=0.004). The mean drug dose of Tacrolimus in patients with remission (N=49) was 0.11 mg/kg/daily and the mean dose of tacrolimus in partial remission and non-remission patients was found 0.10 mg/kg/daily. There was a significant correlation of three important laboratory parameters C0 of Tacrolimus (*P*=0.004) UP: UC ratio (*P*=0.000), and serum albumin level (*P*=0.000) in remission, partial remission, and non-remission groups. 63% patients (N=38/60) were late resistant to steroid treatment while 38% (N=22/60) were initial resistant. The mean age at onset of diseases was 67.08 months. The male children were higher at 63% (N=38/60) while female children were 37% (N=22/60). The major side effect of Tacrolimus were heartburn/acidity in 28.3% (N=17/60), loss of appetite, 23.3% (N=14/60), Nausea 13.3% (N=9/60). Our findings corroborate that maintaining a trough level of Tacrolimus 6.6±2.2 ng/mL during treatment may play an important role in treatment outcomes and the clinical efficacy of Tacrolimus in children with SRNS is related to its trough levels in children. The PETIA may be an alternative to LCMS. However, a pharmacokinetic study with a large sample size is required to substantiate our findings.

Keywords: Nephrotic syndrome, Particle enhanced turbidimetric immunoassay, Steroid resistant nephrotic syndrome, Tacrolimus

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ANNEXURE-XVI
(Author's CV)

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(India)

Academic & Professional Qualification: (Highest Degree)-
BSc MLT, MSc-TQM (Specialization in Clinical Research),
MBA in Hospital & Healthcare Administration,
WHO Fellowship, Pursuing- PhD in Clinical Research.

Matric	Board of School Education Haryana
10+2 (Vocational MLT)	Board of School Education Haryana
Diploma in Medical Laboratory Technology (DMLT)	State Govt. Public Health Department, Karnal (HR)
BA	Delhi University
BSc MLT	Mahatma Gandhi University
MSc-TQM -(Specialization in Clinical Research)	Kuvempu University, Karnataka
MBA- (Hospital & Healthcare Administration)	Vinayaka Mission University, Selam, Tamilnadu
WHO Fellowship in Cytology	ICPO, ICMR (Govt. of India)
WHO Fellowship in Virology	National Institute of Virology (ICMR), Pune
PhD in Clinical Research (Pursuing)	Galgotias University, Greater Noida
Professional Profile:	
Designation	Technical Officer-(Medical Laboratory Services)
Department/Ministry	Department of Clinical Biochemistry, Kalawati Saran Children's Hospital, New Delhi
Work Experience	More than 20 years' work experience in all major areas of Laboratory Sciences
Office Address	Room No. 229, Kalawati Saran Children's Hospital, Bangla Sahib Marg, New Delhi-110001

Awards, Certification and Professional Trainings)

Learning Practical Research Skills & Techniques	Galgotias University	2020
CME		
Workshop on Stem Cells and Clinical Application	Dept. of Research and Biochemistry Sir Ganga Ram Hospital & GRIPMER, New Delhi	7th Nov, 2019
Training on Operation & User maintenance of the Instrument ERBA XL 640 Bio-Chemistry Analyser	Transasia Ltd.	7th July, 2018
SBMLS- Young Scientist Award	Society of Biomedical Laboratory Scientist & Department of Biotechnology, Mewar University	2015
Sardar Vallabh Bhai Patel Memorial-Unity of Nation Award-2014	Mission Protect India	2014
Training Course on Laboratory Quality Management System and Internal Audit as per ISO-15189.	Indian Institute of Quality Management, Government of India (IIQM) Jaipur	2007
Workshop on Reducing Pre-analytical Errors.	Department of Genetics, Dr. Lal Path Labs Pvt. Ltd Delhi	2007
Training Course on Prevention and Control of HIV/AIDS	Lady Hardinge Medical College and DSACS, New Delhi	2002
Training Course on Laboratory Diagnosis of Tuberculosis	New Delhi Tuberculosis Centre	1998
Scientific Conferences and Workshop attended:		
RGCON 2019	Rajiv Gandhi Cancer Institute & Research Centre, New Delhi	8-10 Feb, 2019
CME on Quality Control (NMLPW-2019)	AIIMS, New Delhi	17-18 July, 2019
32nd Annual Conference & International Symposium of Indian Society for Atherosclerosis Research	Department of Biochemistry, LHMC, New Delhi	8-10 Nov, 2019
CME on Computerisation and E-Hospital Module	LHMC, New Delhi	15th Nov, 2019
36th All India Medical Laboratory Technologists' Association' National Conference	Osmania Medical College and Hospital Hyderabad	2010
Quality Council of India-Quality conclave	Lee Meridien Hotel, New Delhi	2009
28th World Congress of Biomedical Laboratory Sciences	The Hotel Ashok, New Delhi	2008
Delhi State AIMLTA's Scientific Conference	UCMS and GTB Hospital, New Delhi	2009
Delhi State AIMLTA's Scientific Conference	UCMS and GTB Hospital, New Delhi	2006

31st All India Conference & Scientific Seminar-AIMLTA	AIIMS, New Delhi	2004
Training /Conference/Seminar organized/facilitated:		
Facilitated Short Term Training Course for BSc MLT Final Year students from RIPANS, Aizawl (Mizoram)	LHMC New Delhi	6 – 8 June, 2019
Organised ICMLS-2019	NIMHANS, Bengaluru	2019
MLTA - CME on Clinical Application of Flow Cytometry in Acute Leukaemia	LHMC – New Delhi	21st July, 2018
CME on Role of Hb HPLC in Diagnosis of Hemoglobinopathies	LHMC – New Delhi	21st July, 2018
Organised AHPCON-2018	PGIMER, Chandigarh	2018
Organised ICMLS-2017	LHMC, New Delhi	2017
Organised CME on Historical Perspective Medical Lab Services in India:100 Years of LHMC Laboratories	Convocation Hall, LHMC	2016
2nd National Allied Health Professional's Conference-AHPCON-2016	AIIMS, New Delhi	2016
1st National Allied Health Professionals Conference (AHPCON-2014)	PGIMER, Dr. RML Hospital, New Delhi	2014
Pre-analytical errors in Biochemistry	Lady Hardinge Medical College & associated SSK and KSCH, New Delhi	2012
Pre-analytical errors in Hematology	Lady Hardinge Medical College & associated SSK and KSCH, New Delhi	2011
Scientific Seminar on importance of Total Quality Management in Clinical Laboratories	Lady Hardinge Medical College & associated SSK and KSCH, New Delhi	2010
Facilitated Training Course for Laboratory Technician under Revised National Tuberculosis Control Programme (RNTCP) of DGHS, Ministry of Health Government of India	LRS Institute of Tuberculosis and allied Diseases, Mahrauli, Ministry of Health & FW, Government of India Delhi	4-10-2000 to 17-10-2000
Facilitated Training Course for Laboratory Technician under Revised National Tuberculosis Control Programme (RNTCP) of DGHS, Ministry of Health Government of India	LRS Institute of Tuberculosis and allied Diseases, Mahrauli, Ministry of Health & FW, Government of India Delhi	29-05-2000 to 16-06-2002
Research Paper Presented/Professional Lecture/Talk Delivered- (International/National/State Scientific Conferences)		
Trough level of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome	35th World Congress of International Federation of Biomedical Laboratory Science, Seoul South Korea	5-9th Oct, 2022

Clinical Profile and Therapeutic Level of Tacrolimus in Children with Steroid Resistant Nephrotic Syndrome -A Single Centre Study	International Conference on Forensic Science & Criminalistics, organised by Galgotias University, Noida	15-16 May, 2021
Presented Professional Talk on Important Professional Issues of Indian Medical laboratory Professionals	6th Congress of Asia Association of Medical Laboratory Scientists (AAMLS-2019) organised by ACMTT	29-31 May, 2019
Presented an invited Talk on Recent Policy Development for AHPs in India: Emerging Issues and Future Possibilities for Radiotherapy Professionals.	ARTTICON - 2019	13-15 Dec, 2019
Presented an organisational Talk on behalf of ICMLS	AAMLS – 2017 Organised by Korean Association of Medical Technologist – Busan, South Korea	22-24 Sept, 2017
Presented a scientific poster during on accreditation of clinical Lab during 32nd World Congress of Biomedical Laboratory Sciences	32nd World Congress of Biomedical Laboratory Sciences Held in Kobe, Japan from 31st October to 4th Sept,2016	2016
Presented an Academic Lecture on the need of revamping curriculum of Medical Laboratory Science Courses	National Science Colloquium & 1st Scholar Science Meet (SCSSM-20215) organised by Mewar Institute of Management	10th October, 2015
Participated as an Expert in National Curricula Redesign Taskforce Meeting of Medical laboratory Sciences	Organised by Ministry of Health & Family Welfare, Government of India	13th October, 2014
Presented Scientific Paper on Lab Medicine: SWOT analysis during 31st World Congress of Biomedical Laboratory Sciences.	31st World Congress of Biomedical Laboratory Scientists held at Taipei October,2014, Taipei, Taiwan	2014
Presented scientific paper on Evaluating and preventing of pre-analytical errors in Blood Gases measurement in a tertiary care children's' Hospital	Asian Congress of Medical Laboratory Scientists (AAMLS) 2nd October to 4th October 2013 at Singapore	2013
Presented Scientific Paper on Identification of Pre-analytical errors in emergency Laboratory of a tertiary care Children's Hospital, New Delhi during 30th World Congress of Biomedical Laboratory Sciences.	30th World Congress of Biomedical Laboratory Scientists- 18-22 August,2012 Berlin, Germany	2012

Participated as a invited Faculty and delivered scientific lecture on Role of Medical Laboratory Services (POCT) in Emergency Medicine	7th Indo-US Emergency Summit-INDUS-EM 2011 AIIMS, New Delhi	2011
Participated as a invited Speaker and delivered lecture on emerging professional issues in Allied health services during 3rd Biennial State Conference of All Tripura Pathological and Radiological Services association	3rd Biennial State Conference of ATPRCA Agartala Medical College and GP Pant Hospital, Agartala, (Tripura)	2011
Participated as a invited speaker in Chandigarh Chapter Scientific Conference on World Biomedical Science Day	Government Medical College and Hospital, Sector 32 Chandigarh	2010
Delivered Scientific Lecture on recent trend on quality assurance in Medical Laboratories during Delhi State Conference of AIMLTA	Delhi University	2010
Participated as a Guest faculty and Delivered lecture on necessity Accreditation for Biomedical Laboratory Services and education in India	PGI Medical Technology Association Annual Conference, PGIMER, Chandigarh	2009
Participated as a Guest faculty Delivered lecture on necessity of central regulatory mechanism for Biomedical Laboratory Services and education in India	All Assam Medical Tech National Biennial Conference-2009 Jorhat, Assam	2009
Role of Rapid Malaria Testing in emergency Laboratory of a Children Hospital	Tata Memorial Hospital, Mumbai	2007
Lecture delivered on ISO-15189 Standard for Clinical laboratories: an Overview	UCMS, New Delhi	2007
Delivered lecture on Professional regulatory issues of Biomedical Laboratory Sciences	PGIMER, Chandigarh	2007
Delivered lecture on Professional regulatory issues of Biomedical Laboratory Sciences	UCMS and GTB Hospital, Delhi	2006
Presented Scientific Paper on Seroprevalance of Toxoplasma Gondii in Children's in a tertiary care Children's hospital	Bangalore Medical College & Hospital, Bangalore, Karnataka	2006
Countries Visited: Germany, France, Switzerland, Italy, Austria, Singapore, Malaysia, Thailand, Taiwan, Hongkong, Japan, South Korea		
Official Member- National Expert Task Force Committee, Ministry of Health & FW National Curricula Review Committee (MLS), Ministry of Health & FW, Govt. of India		
Extra-Curricular/Organizational Activities: 1. Organised National Debate on NCHRH		

2. Organised National discussion on Paramedical Bill

Organizational position holding Presently:
Founder & Present General Secretary, Joint Forum of Medical Technologists of India. (JFMTI)
President, Indian Confederation of Medical Laboratory Science (ICMLS)
General Secretary, Medical Laboratory Technologists Association (MLTA), LHMC & Associated Hospitals, New Delhi

Personal Profile:

Name	Kaptan Singh Sehrawat
Date of Birth	23.11.1978
Father's Name	Sh. Balbir Singh Sehrawat
Nationality	Indian
Marital Status	Married
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Languages Known	Hindi, English