THE VALUE OF IMMUNOFLUORESCENCE IN RENAL DISEASES WITH SPECIAL REFERENCE TO PERIODIC ACID SCHIFF AND JONE'S METHANAMINE SILVER STAIN

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M.D. DEGREE

In

PATHOLOGY – BRANCH III

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DECLARATION

I hereby declare that the dissertation entitled **THE VALUE OF IMMUNOFLUORESCENCE IN RENAL DISEASES WITH SPECIAL REFERENCE TO PERIODIC ACID SCHIFF AND JONE'S METHANAMINE SILVER STAIN** is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College, Coimbatore during the period from October 2012 to July 2014 under the guidance and supervision of **Dr. D. Kavitha M.D.,** Associate Professor, Department of Pathology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai towards the partial fulfilment of the requirement for the award of M.D., Degree (Branch III) in Pathology.

I have not submitted this dissertation on any previous occasion to any University for the award of any Degree.

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Date: **Dr. C.S. AKSHATHA**

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This is to certify that dissertation entitled **THE VALUE OF IMMUNOFLUORESCENCE IN RENAL DISEASES WITH SPECIAL REFERENCE TO PERIODIC ACID SCHIFF AND JONE'S METHANAMINE SILVER STAIN** is a bonafide work done by **Dr. C.S. Akshatha,** a postgraduate student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore under the supervision and guidance of **Dr. D. Kavitha M.D.,** Associate Professor, Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore in partial fulfilment of the regulations of the Tamilnadu Dr. M.G.R. Medical University, Chennai towards the award of M.D. Degree (Branch III) in Pathology.

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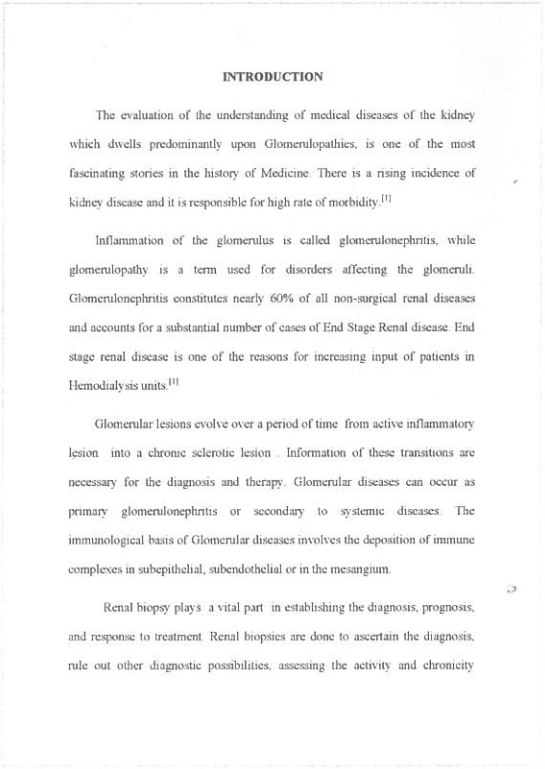


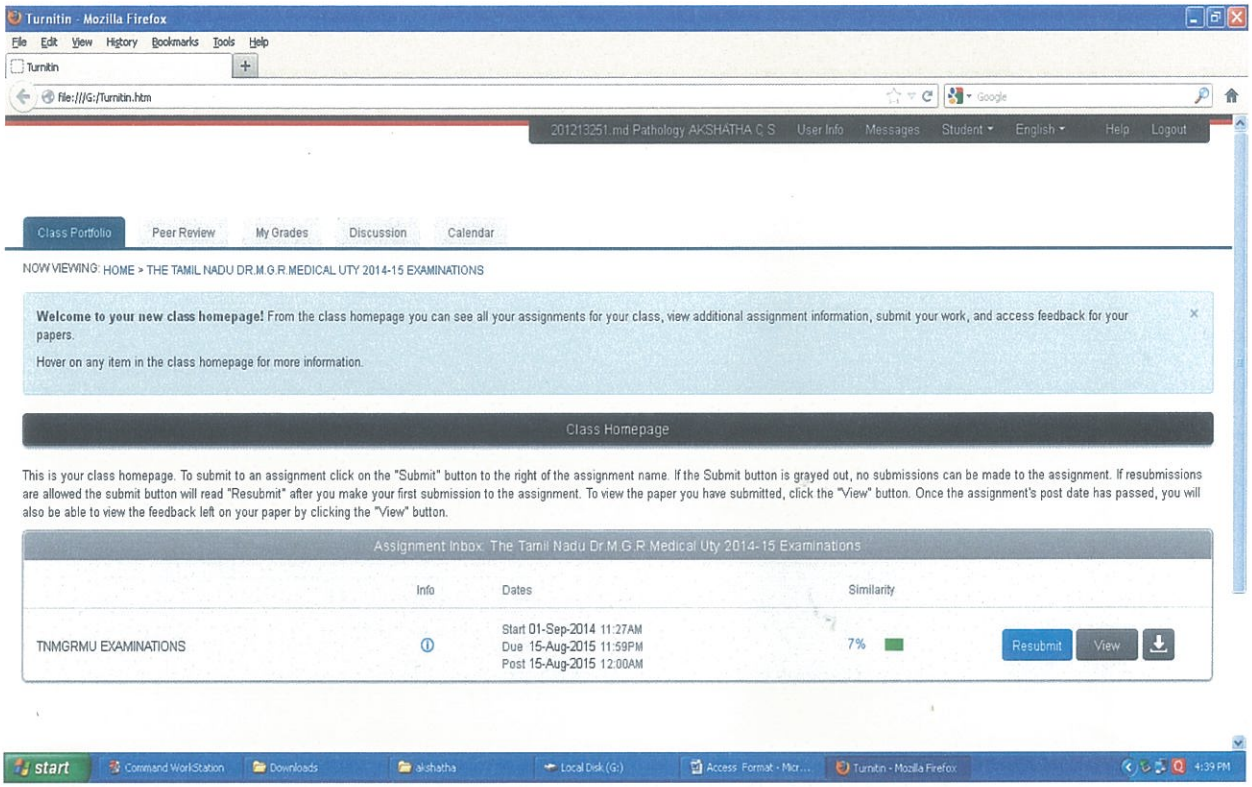
INTRODUCTION

The evaluation of the understanding of medical diseases of the kidney which dwells predominantly upon Glomerulopathies, is one of the most fascinating stories in the history of Medicine. There is a rising incidence of kidney disease and it is responsible for high rate of morbidity.[1]

Inflammation of the glomerulus is called glomerulonephritis, while glomerulopathy is a term used for disorders affecting the glomeruli. Glomerulonephritis constitutes nearly 60% of all non-surgical renal diseases and accounts for a substantial number of cases of End Stage Renal disease. End stage renal disease is one of the reasons for increasing input of patients in Hemodialysis units.[1]

Glomerular lesions evolve over a period of time from active inflammatory lesion into a chronic sclerotic lesion . Information of these transitions are necessary for the diagnosis and therapy. Glomerular diseases can occur as primary glomerulonephritis or secondary to systemic diseases. The immunological basis of Glomerular diseases involves the deposition of immune complexes in subepithelial, subendothelial or in the mesangium.

Renal biopsy plays a vital part in establishing the diagnosis, prognosis, and response to treatment. Renal biopsies are done to ascertain the diagnosis, rule out other diagnostic possibilities, assessing the activity and chronicity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ABSTRACT

Background:

The evaluation of the understanding of medical diseases of the kidney which dwells predominantly upon Glomerulopathies, is one of the most fascinating stories in the history of Medicine. There is a rising incidence of kidney disease and it is responsible for high rate of morbidity. Glomerulonephritis constitutes nearly 60% of all non-surgical renal diseases and accounts for a substantial number of cases of end stage renal disease. End stage renal disease is one of the reasons for increasing input of patients in hemodialysis units.

Aim of the study:

To analyse the histomorphology, the extent of involvement of GBM using special stains like Periodic acid sciff and Jone's methanamine silver stains and specific immunofluorescence pattern of the renal diseases to diagnose and categorize them.

Materials and methods:

The study was done for a period of 20 months between October 2012 and July 2014. A total number of 58 renal biopsies were received from the Department of Nephrology, Coimbatore Medical College and

Hospital, Coimbatore. The biopsy was subjected to light microscopic examination and immunofluorescence studies.

Results:

A total of 58 renal biopsies were analysed in Coimbatore Medical College and Hospital, Coimbatore between October 2012 and July 2014. The most common age group affected was between 31 years to 40 years. Females (51.72%) were slightly more affected than males (48.27%). Out of 58 cases, 46 (79.31%) showed primary glomerular lesions, 10 (17.24%) showed secondary glomerular lesion and 2 (3.45%) showed tubulointerstitial nephritis. Diffuse proliferative glomerulonephritis was the most common primary glomerular lesion with a total of 13 out of 58 cases (22.41%). Lupus nephritis was the most common secondary glomerular lesion with a total of 7 out of 58 cases (12.07%). Jones's methanamine silver stain along with PAS stain aided in demonstrating the extent of GBM involvement and thereby helped in typing/staging of membranous glomerulopathy and membranoproliferative glomerulonephritis. Immunofluorescence studies showed positivity in 42 patients accounting for 72.41%. The predominant pattern was granular glomerular basement membrane which was noted in 18 patients (31.03%). The diagnostic utility of IF was noted in 4 cases (6.90%)

whose diagnoses included IgA nephropathy and C1q nephropathy. The IF studies helped in modification of the final diagnosis in 1 case (1.72%) whose final diagnosis was lupus nephritis class I.

Conclusion:

Immunofluorescence studies have complemented the clinical, histomorphological findings in 53 patients both in primary and secondary glomerular diseases. However, it was even more of diagnostic importance in 5 patients including IgA nephropathy, C1q nephropathy and Lupus nephritis class I where a confident diagnosis could be rendered only because of availability of immunofluorescence studies. Hence, immunofluorescence studies when combined with histomorphologic findings by light microscopy, clinical, biochemical and serological markers can yield a better and precise diagnosis.

Key words: Renal biopsy, Immunofluorescence, Glomerulonephritis.

INTRODUCTION

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The evaluation of the understanding of medical diseases of the kidney which dwells predominantly upon glomerulopathies, is one of the most fascinating stories in the history of Medicine. There is a rising incidence of kidney disease and it is responsible for high rate of morbidity.[1]

Inflammation of the glomerulus is called glomerulonephritis, while glomerulopathy is a term used for disorders affecting the glomeruli. Glomerulonephritis constitutes nearly 60% of all non-surgical renal diseases and accounts for a substantial number of cases of end stage renal disease. End stage renal disease is one of the reasons for increasing input of patients in hemodialysis units.[1]

Glomerular lesions evolve over a period of time from active inflammatory lesion into a chronic sclerotic lesion. Information of these transitions are necessary for the diagnosis and therapy. Glomerular diseases can occur as primary glomerulonephritis or secondary to systemic diseases. The immunological basis of glomerular diseases involves the deposition of immune complexes in subepithelial, subendothelial or in the mesangium.

Renal biopsy plays a vital part in establishing the diagnosis, prognosis, and response to treatment. Renal biopsies are done to ascertain the diagnosis, rule out other diagnostic possibilities, assessing the activity and chronicity (scarring) of the lesion. Light microscopy is the standard procedure to evaluate kidney biopsies and haematoxylin and eosin stain along with special stains like Periodic-acid Schiff, Jone's methenamine silver stain are routinely employed to identify the involvement of the basement membrane. Periodic acid Schiff and Jone's methanamine silver stain defines very well glomerular basement membrane and Bowman'capsule, and they are the best stain to evaluate the glomerulus.[3] Direct immunofluorescence (DIF) on frozen tissue biopsy is the most widely applied method for the detection of immune deposits in the kidney.[2]

The introduction of a safe and reliable percutaneous biopsy method by Iver and Brun in 1951 opened the door to the modern classification of glomerular diseases. The final diagnosis of renal disease is made possible with the interpretation of renal biopsy using light microscopy, immunofluorescence studies and electron microscopy.[2,3]

Light microscopic morphology is assessed by staining the sections with standard stains like haematoxylin and eosin and other stains. When

light microscopic appearances are equivocal, immunofluorescence studies may reveal a pattern which enables the glomerular lesions to be identified.[2] Electron microscopy is expensive and may not be feasible in all situations.

The present study aims to analyse the clinical features in renal diseases, histomorphology of renal biopsy, Periodic acid Schiff and Jone's methanamine silver staining to highlight the extent of involvement of glomerular basement membrane. Also, specific immunofluorescence pattern is studied by applying the panel of immunofluorescent markers IgG, IgA, IgM, C3, C1q and fibrinogen.

Based on the above findings, the etiopathogenesis of renal diseases in the patient is analysed and better therapeutic strategies can be formulated and administered to improve the clinical outcome of the patient.

AIMS AND OBJECTIVES

AIM OF THE STUDY

To analyse the clinical features, histomorphology and use of Periodic acid Schiff and Jone's methanamine silver staining to highlight the extent of involvement of glomerular basement membrane in selective cases and to study the specific immunofluorescence pattern by applying the immunofluorescent markers.

OBJECTIVES

1. To analyse the clinical features and parameters in the spectrum of renal diseases and to correlate with the histomorphological diagnosis.
2. To correlate histomorphological diagnosis and special stains including Periodic acid Schiff and Jone's methanamine silver stains in renal diseases.
3. To correlate the clinical features and parameters, histomorphology, immunofluorescence pattern and special stains in diagnosing and categorizing renal diseases.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

EMBRYOLOGY OF KIDNEY:

The human kidney arises from two different sources namely the metanephros and ureteric bud. The excretory tubules or nephrons are derived from metanephros. The collecting part of the kidney is derived from the ureteric bud. As the ureteric bud grows cranially towards the metanephros, its growing end becomes dilated to form an ampulla. The ampulla divides repeatedly. The first three to five generations of branches fuse to form the pelvis of the kidney. The next divisions form the major calyces and further divisions form the minor calyces and the collecting ducts. The cells of metanephros form solid clumps in relation to the ampullae. Each solid clump becomes a vesicle and the vesicle becomes pear shaped and opens into the ampulla. Its distal end becomes invaginated by a tuft of capillaries which form a glomerulus. The kidneys are located in the sacral region during embryogenesis and ascends to the lumbar region in the subsequent development.[4]

GROSS ANATOMY:

The kidney is a paired bean shaped organ measuring 12 cm (length), 6 cm (width), and 2.5cm (thickness), and weighing between 120 and 175 grams.

It is covered by a thin fibrous capsule called the renal capsule which ends at the renal sinus. Cortical surface of the kidney is covered by fat called perirenal fat. Enclosing the perinephric fat is a condensed extraperitoneal fascia called as Gerota's fascia. Each kidney has 8-18 lobes.[5,7]

The cut surface of the kidney shows an outer cortex and an inner medulla. The cortex is usually 1cm to 1.2cm thick over the pyramids. The medulla consists of renal pyramids whose apices are called as papillae, each of which is connected to a calyx. Cortical tissue in between two adjacent pyramid is called as renal columns of bertini.[5]

HISTOLOGY:

One to two million nephrons can be seen in each kidney, which forms the structural and the functional element. Each nephron consists of a glomerulus, proximal and distal tubules, connecting segment, and the collecting duct.[6]

Renal corpuscle (Fig A) has a Bowman's capsule and glomerulus. The Bowman's capsule consists of visceral layer called as podocyte and the parietal layer. The space in between them is called as Bowman's space. This space continues with the lumen of the renal tubule. The glomerulus consist of an intricate branching system of capillaries arising

at the afferent arteriole and draining into the efferent arteriole. The entry and exit of the arterioles (vascular pole) lies opposite to the entrance of the renal tubule (tubular pole).[1,6]

The glomerulus consists of three types of cells namely mesangial, endothelial and epithelial cells. The outer surface of the capillaries is lined by the visceral epithelial layer or podocytes. The components of glomerular basement membrane are type IV collagen, laminin, proteoglycans predominantly heparan sulphate and several other minor components like enactin, fibronectin, glycoproteins. The endothelial cells are thin which line the inner surface of the capillaries. The capillary tufts are supported by the mesangium which comprises of two components like the mesangial cells and the extracellular mesangial matrix.[1,6]

The proximal tubule, the loop of Henle and distal tubules are lined by single layer of cuboidal cells which has microvillous luminal border. The tubules are completely surrounded by a basement membrane. Adjacent tubular basement membrane are close to each other with little intervening connective tissue stroma that contains peritubular capillaries.[1,6]

HISTOLOGY OF NORMAL GLOMERULUS.

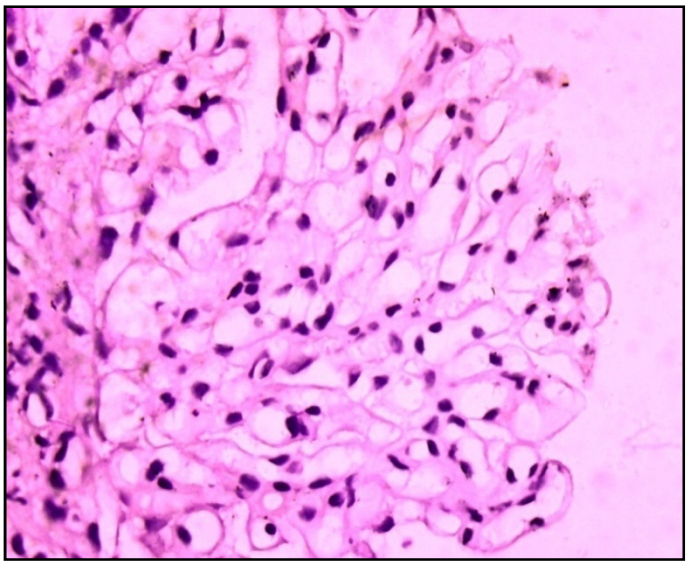


Fig A: showing normocellular glomerulus with patent capillary loops and normal thickness basement membrane.

ELECTRON MICROSCOPY OF THE GLOMERULAR FILTERING MECHANISM:

The capillary endothelium has multiple fenestrae, each being 70 to 100 nanometer in width.

The GBM in adults measures approximately about 340 nanometers to 360 nanometers and is thicker in males compared to females. It is a trilaminar structure with central thick electron dense layer called lamina densa and thinner electron lucent layers on either side which lies beneath endothelial and epithelial layers called lamina rara interna and lamina rara externa respectively.

Each podocyte has a nucleus and cytoplasmic extensions which divide and form finger-like extensions that inter-communicate with similar structures from neighbouring cells and wrap the capillaries. These are known as pedicles or foot processes. The gaps between the foot processes is called filtration slits and are bridged by slit diaphragms composed of transmembrane protein called nephrin.[1,7,8]

PATHOGENESIS OF GLOMERULAR INJURY:

The immune mechanisms underlie most types of glomerular diseases. Antibody mediated injury plays the major role and cell mediated immunity and other mechanisms also can cause glomerular injury.

Following are the types of antibody-mediated injury that have been recognized:

1. Glomerular injury caused by deposition of circulating soluble antigen-antibody complexes.
2. Injury within the glomerulus by antibodies reacting with either insoluble fixed native glomerular antigens or with planted molecules in the glomerulus which may be endogenous like DNA, nuclear proteins, immunoglobulins, immune complexes formed elsewhere in the body or exogenous like drugs, infectious agents.
3. Antibodies directed against glomerular cell components.

The antigen-antibody complexes that are formed within the glomeruli or in the circulation are trapped in the glomeruli, producing injury, causing complement activation, leucocyte recruitment and proliferation of endothelial, mesangial, and parietal epithelial cells.[1]

Interaction between immune complexes in-situ and trapped circulating complexes, local hemodynamics and structural determinants in the glomerulus all contribute to the variety of morphologic and functional changes leading to glomerulonephritis.[8]

The factors affecting the localization of antigen, antibody, or immune complexes are the molecular charge and size of the reactants, glomerular haemodynamics, mesangial function and the integrity of the charge-selective glomerular barrier.[1]

Planted antigens reacting with the antibodies tend to deposit in the mesansium and give a granular pattern of immunoglobulin deposition and antibodies that react against fixed antigens of the glomeruli, for eg; GBM, result in the linear deposition along the GBM which can be visualized with immunofluorescent microscopy.[1]

Once immune complexes are deposited in the kidney, they are degraded or phagocytosed by the leucocytes and the mesangial cells if the antigen exposure is of a short duration. If there is chronic antigen

exposure, constant cycles of immnune complexes formation, deposition and injury occurs leading to chronic glomerulonephritis like in case of hepatitis B virus infection, or systemic lupus erythematosus.[1]

Antibodies and immune complexes cause injury by complement activation, leucocyte recruitment releasing a variety of mediators and even sometimes direct podocyte injury. Podocyte injury is reflected by flattening of foot processes, cell vacuolization, shrinkage and denudement.[1,8,9]

When there is glomerular filtration rate reduction to almost half of normal, end stage kidney disease occurs inevitably. Patients have proteinuria with their kidneys showing sclerosis. Adaptive changes start occurring in the remaining glomeruli undergoing hypertrophy trying to maintain renal function. These adaptive changes become maladaptive due to disturbances in renal haemodynamics leading to further injury to the kidney and progressing to segmental or global sclerosis of the glomeruli. A vicious cycle sets in ultimately reducing the nephron mass and leading to glomerulosclerosis.[1,8,28]

NEPHROTIC SYNDROME

Many primary and secondary glomerulonephritis are associated with nephrotic syndrome and it is important to distinguish all of them into

different types because of the diverse glomerular lesions, and their different clinical outcomes, treatments and prognoses. Furthermore differentiating them helps in the development of disease-specific therapies.[8]

The nephrotic syndrome comprises of heavy proteinuria, hypoalbunemia, hyperlipidemia and edema. The kidney loses about 80 to 150 milligram of urinary protein per day in normal adult of which 60% is excreted by the glomeruli and remaining portion is secreted by the tubules (Tamm-Horsfall protein). Nephrotic range proteinuria is excretion of 3.5grams or greater protein per day per 1.73 meter square surface area. Addition of tubular protein (Tamm-Horsfall protein), alterations in the glomerular permeability and tubular resorption may result in proteinuria.[7]

Recently, altered glomerular permeability has been studied and observed that increased glomerular permeability owes to the proteinuria. The characteristic lesions seen in the glomeruli by electron microscopy are replacement of the foot process by continuous sheets of flattened cytoplasm, reduction in the number of epithelial slits with formation of occlusion junction, epithelial vacuolization and focal areas of epithelial cell detachment.[8]

The basic defect is urinary protein loss, and the hypoalbuminemia, production of edema, and hyperlipidemia stem from it. Hypoalbuminemia in the nephrotic syndrome is resulted from the combination of increased urinary loss and increased catabolism of albumin, chiefly in the kidney. The liver reacts to hypoalbuminemia by increasing albumin synthesis, but in the NS the response is inadequate . The hypoalbuminemia then leads to both hyperlipidemia and edema formation. Hyperlipidemia in NS has many different mechanisms. The principle alteration is the higher levels of low density lipoproteins (LDL), apolipoprotein B, very low density lipoproteins. Increased levels of Apoprotein B leads to both hypoalbulinemia and reduced colloid oncotic pressure. Increase in these proteins are because of decreased catabolism and not because of increased synthesis. High density lipoproteins (HDL) show slight change in their levels so that the LDL/HDL ratio is increased. In addition, there is increase in triglycerides and lipoprotein-a levels by synthesis alone.[7,8]

Underlying mechanism for edema formation is now called as underfill hypothesis which states that hypovolemia is the primary stimulus for edema formation by driving the kidney to retain sodium and water through Starling forces. However, most NS patients are either normovolemic or hypervolemic. Thus, overfill hypothesis was developed

according to which sodium retention was primary, leading to increased blood volume and increased blood pressure, changing the Starling forces thereby causing edema. Several factors result in sodium retention like reduced colloid oncotic pressure causing decrease in sodium filtration, distal tubular injury causing resistence to atrial natriuretic peptide, resulting in decreased natriuresis; chronic tubulointerstitial nephritis resulting in reduced GFR and sodium retention. Rostoker et al found that steroid therapy resolved abnormal glomerular permeability in patients with nephrotic syndrome. They proposed that a cytokine or other vascular permeability factor might be responsible for edema formation.[8]

Conditions associated with nephrotic syndrome are minimal change disease, focal segmental glomerulosclerosis, membranous glomerulonephritis, membranoproliferative glomerulonephritis, crescentic glomerulonephritis, mesangioproliferative glomerulonephritis including IgA nephropathy, congenital and familial nephrotic syndromes, amyloidosis, diabetes mellitus, SLE, HSP, drugs, intravenous drug abuse, pregnancy, obesity, sarcoidosis, bee stings and infections.[7,8]

NEPHRITIC SYNDROME

Nephritic syndrome comprises of hematuria, increased blood urea nitrogen and serum creatinine causing uremia, oliguria and mild to

moderate hypertension, proteinuria but not in the nephrotic range, mild edema manifested by facial puffiness. Urinalysis shows sediments having leucocytes, RBCs and RBC casts.[7]

Many primary and secondary glomerulonephritis manifest with nephritic syndrome. Primary glomerulonephritis presenting with nephritic syndrome are IgA nephropathy, acute postinfectious glomerulonephritis, crescentic glomerulonephritis, dense deposit disease and secondary glomerulonephritis like lupus nephritis, HSP, mixed connective tissue disease, mixed cryoglobulinemia.[7,8,9]

CLASSIFICATION OF GLOMERULAR DISEASES[1,7]

Primary glomerular diseases:

- Minimal change disease
- Focal segmental glomerulosclerosis
- Membranous nephropathy
- Membranoproliferative glomerulonephritis
- Diffuse endocapillary proliferative glomerulonephritis
- Diffuse mesangioproliferative glomerulonephritis
- Fibrillary and immunotactoid glomerulonephritis
- Diffuse crescentic glomerulonephritis

Secondary glomerular diseases :

- Lupus nephritis
- Diabetic nephropathy
- Hypertensive nephropathy
- Amyloidosis
- Good pasture syndrome
- Glomerulonephritis secondary to lymphoplasmacytic disorders
- Glomerulonephritis secondary to systemic vasculitis
- Glomerulonephritis secondary to bacterial endocarditis

Hereditary disorders :

- Alport syndrome
- Fabry disease
- Thin basement membrane disease
- Congenital nephrotic syndrome – Finnish type and Diffuse mesangial sclerosis.

MINIMAL CHANGE DISEASE

It is the most common cause of nephrotic syndrome in children accounting to about 80% to 90%. The peak incidence in children is between 2 to 4 years. Males are more commonly affected than females. Majority of the cases are idiopathic. Minority of the cases can be associated with recent immunizations, viral infections, food allergies,

dust, bee stings, heavy metal ingestion, drug reactions to lithium, gold, interferon and ampicillin. Edema is the most common presenting symptom with some cases having microscopic hematuria.[7,9,34,35,38,39]

Light microscopy shows no or minimal glomerular changes like mesangial hypercellularity. Tubular epithelial cells may show vacuolization or hyaline droplets. Edema and focal fibrosis in the interstitium can be seen in elderly patients.[7]

Immunofluorescence studies are negative for immunoglobulin and complement. Mesangial deposits of IgM and C3 may be seen in 10% of cases.[8,9] Electron microscopy shows total effacement of foot process.[36]

Differential diagnosis includes IgM nephropathy, C1q nephropathy, congenital nephrotic syndrome and FSGS.[8]

IgM nephropathy shows no or mild mesangial proliferation on light microscopy with bright staining of IgM on immunofluorescence. Restriction of IgM nephropathy is given to only when there is bright staining of IgM on immunofluorescence.[8,9]

C1q nephropathy shows variety of lesions ranging from no or minimal mesangial proliferative to active glomerulonephritis. When there is no change seen in the glomeruli, the diagnosis of MCD should be

considered. In immunofluorescence studies, MCD shows no staining whereas C1q nephropathy demonstrates bright staining for C1q.[9,11]

FSGS poses problem in distinguishing it from MCD when the biopsy is small as FSGS affects only some glomeruli (focal). However, if there is classic segmental glomerulosclerosis there is no problem in making the diagnosis of FSGS. In case of small biopsies some features help in favoring one diagnosis or the other by considering glomerular size, presence of tubular atrophy, sampling from the deep cortex. Fogo et al found that patients with MCD and larger glomeruli had more likely chances of having FSGS on subsequent biopsies. Nyberg et al found that patients with FSGS had larger glomerular volume and diffuse mesangial sclerosis than in patients with MCD. Tubular atrophy near the glomeruli suggests the possibility of segmental lesion in the tissue near the biopsy, especially in children. If there is no segmental lesion then it is better to get the sample from juxtamedullary cortex as it is the first location of segmental lesions in FSGS.[8,36,37]

Demonstration of mutations in the nephrin gene helps in the diagnosis of congenital nephrotic syndrome.[8,9]

4% to 5% of the pediatric patients with minimal change nephrotic syndrome patients go for end stage renal disease or may die from

complications and 95% of them do well with steroid therapy according to Tarshish et al, 1997.[35]

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal segmental glomerulosclerosis can occur as primary form due to unknown cause or secondary due to familial mutations in various proteins like nephrin, podocin, integrin, laminins etc; viral infections; drugs; hereditary diseases; immunologic diseases. Primary FSGS accounts for 20% to 30% and 10% to 15% of nephrotic syndrome in adults and children respectively.[7,83,84]

Kidney involvement starts at the juxtamedullary region and spreads towards the periphery. Light microscopy shows sclerosis in one or more lobules of glomerular tufts with adhesions between the Bowman's capsule and the tuft. There is often distortion and destruction of the glomerular architecture. There may be associated glomerular hypercellularity owing to podocyte proliferation and capillary collapse. As the FSGS progresses more number of glomeruli are involved with atrophy of the tubules and interstitial fibrosis. Sclerotic part of the glomeruli is PAS positive and JMS stain shows remaining portion of glomerular basement membrane in the sclerotic areas as wrinkled lines. Immunofluorescence studies show non-specific staining for IgM and C3

in the sclerotic areas. Electron microscopy shows extensive foot process effacement which is more widespread in primary compared to secondary FSGS which shows patchy involvement.[7,8,41,47]

Morphologic variants and histologic classification of FSGS :

Columbia classification.

Tip variant: It is localized to the adjacent portion of the origin of the proximal convoluted tubule in the glomeruli. The lesion is small involving one or two lobules. Histologically, the lesion comprises of capillary loop occlusion by foam cells, endocapillary cell proliferation and sclerosis. Recent investigations show that tip variant has higher incidence of remission and 3 year renal survival rate than other variants.[7,42]

Collapsing variant: It is characterized by segmental or global collapse of glomerular tuft along with podocyte hyperplasia or hypertrophy. Immunofluorescence findings are IgM and C3 positivity in segmental portion of the glomeruli. This variant has poor prognosis with virtually no response to therapy and rapid loss of kidney function.[7,43,46,85]

Perihilar variant : This variant shows perihilar sclerosis with hyalinosis in more than 50% of involved glomeruli.[9]

Cellular variant: This variant is characterized by atleast one glomerulus showing segmental endocapillary proliferation occluding the capillary lumen.[7,9]

Not otherwise specified: This term is used when none of the above variants' features are seen and segmental solidification of the glomerular tuft is noted. This is a diagnosis of exclusion.[7,9,10,41]

Nasar Yousuf Alwahaibi et al, 2013 found that in Saudi Arabia and Sudan, FSGS and MCD are the commonest primary glomerulonephritis respectively. Reports from India showed that FSGS is the commonest primary glomerular disease.[28]

A study on Focal segmental glomerulosclerosis – morphologic diagnosis on evolution was done by David B. Thomas. This was an analytical study which included 197 patients with FSGS. According to this study, it was observed that FSGS Not otherwise specified was the most common form, followed by the perihilar type, collapsing type and tip lesion. Also patients that had collapsing and tip type of lesions manifested with higher degree of proteinuria than perihilar and FSGS NOS types.[44]

MEMBRANOUS NEPHROPATHY

Membranous nephropathy accounts for 1% to 9% in children and 20% to 30% in adults of all cases of idiopathic nephrotic syndrome. It can occur as primary (idiopathic) or secondary to infection, neoplasia or systemic lupus erythematosus, drugs and toxins, immunologic disorders.[7,8,51,82]

It is characterized by the subepithelial immune deposits with variable GBM thickening without mesangial cell proliferation or inflammatory cell infiltration. Mechanism of injury occurs by deposition of the immnune complexes that are formed in-situ by binding of the circulating antibodies to the antigen that are present in the subepithelial location of glomerulus or with the extrinsic antigens that are planted as free antigens in the subepithelial region.[7,8]

By light microscopy four stages have been described based on the structural features of the glomerular capillary wall.[9,10]

Stage 1: This is the early stage where there is no change in the glomeruli or in the thickness of the GBM.

Stage 2: The capillary walls become thickened with subepithelial deposits. Silver impregnation techniques show spike formation along the thickened basement membrane.[9]

Stage 3: The deposits are surrounded by a newly formed basement membrane. The basement membrane becomes markedly thickened with narrowing of the capillary lumina. PAS and silver stains show reduplication or moth eaten appearance of the basement membrane.[8]

Stage 4: The basement membrane show vacuolation, thickening and folding. Deposits may no longer be evident. Glomerulat tufts show segmental or global sclerosis and obliteration of the capillary lumina.[10]

Tubules may show progressive atrophy and interstitial fibrosis as the glomerular lesion progresses.

Immunofluorescence studies show generalized granular pattern of IgG and C3 along the GBM. Electron microscopy show subepithelial and sometimes intramembranous location of electron dense deposits.[7,8,9]

Differential diagnosis includes minimal change disease, focal segmental glomerulosclerosis. Based on the characteristic histomophology, immunofluorescence studies and electron microscopic findings, membranous glomerulonephritis can be differentiated from other entities. Primary membranous glomerulonephritis should be differentiated from secondary form.[8,9]

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Membranoproliferative glomerulonephritis is characterized by mesangial cell proliferation with basement membrane thickening. The main age group affected is between 5 years to 15 years. Males and females are equally affected.[7,8,55,56]

Based on the causes, MPGN can be divided into primary and secondary. Primary is idiopathic and secondary occurs due to infections, immunologic disorders, neoplasia, and systemic diseases.[8,57,81]

Based on histology and location of the immune deposits MPGN can be subdivided into three types.

MPGN type 1 most commonly occurs in children. There is global and diffuse endocapillary and mesangial cell proliferation showing lobular accentuation and expansion of mesangial matrix. There may be mononuclear cell infiltration with occasional neutrophils. There is marked thickening of GBM producing a double contour or tram- track pattern when stained with PAS and silver stains.

Immunofluorescence studies are characteristic and consistently show irregular chunky deposits in mesangium and capillary walls for IgG and C3. Sometimes IgM, C1q and C4 can also be seen in the same location. Electron microscopy shows abundant deposits in subendothelial

and mesangial areas, mesangial hypercellularity and accumulated matrix.[7,8]

MPGN type 2 is also called as Dense Deposit Disease. It is less common than type 1 and type 3 MPGN. It is characterized by the intramembranous dense deposits in the GBM which can be visualized by the electron microscopy. This is considered as pathognomonic feature of MPGN type 2 according to Appel et al, 2005.[51]

Light microscopy shows typical features of MPGN with abnormally refractile, eosinophilic ribbon-like thickening of the basement membrane. The basement membrane changes can be demonstrated with PAS, Masson trichrome and Toluidine blue and silver stains in the glomeruli, tubules and peritubular capillaries. In addition, mesangium also shows dense deposits as homogenous nodules on electron microscopy.[8,9]

Immunofluorescence studies show typical, diagnostic intense staining for C3 in the capillary walls and in the mesangium. C3 can also be detected in Bowman's capsule and tubular basement membrane. Sometimes mild staining of C1q and C4 can be noted. Electron microscopy shows hallmark finding of dense, osmiophilic deposits in the

GBM, tubular basement membrane, Bowman's capsule basal lamina and mesangial region.[7,8]

Type 3 MPGN has mixed features of type 1 MPGN and membranous nephropathy. Hence light microscopy shows lobular accentuation on glomeruli, mesangial cell proliferation, diffuse capillary wall thickening and electron microscopy shows subendothelial and subepithelial deposits and silver stains demonstrate basement membrane spike. Immunofluorescence techniques show C3 in a finely granular pattern along glomerular capillaries and mesangium.[8,9]

Appel et al, 2005 in their study concluded that in MPGN type 2, half of the patients develop end stage renal disease. The pathophysiology behind this type is high activation of the alternative pathway.[52]

PD Walker et al, 2007 studied 81 cases of dense deposit disease and categorized them based on the histomorphological appearance into five different subtypes. The patterns observed were membranoproliferative (endocapillary proliferation with lobular accentuation in 17 cases); mesangioproliferative (30 cases); crescentic (12 cases); acute proliferative (8 cases) and unclassified (2 cases). They concluded that typical membranoproliferative pattern was seen in only 17 cases and majority of the cases showed other patterns. Hence dense deposit disease

should be considered as a different entity and not a subtype (type 2) of membranoproliferative glomerulonephritis.[53]

DIFFUSE ENDOCAPILLARY PROLIFERATIVE GLOMERULONEPHRITIS.

Diffuse endocapillary proliferative glomerulonephritis is characterized by lesions having mesangial and endocapillary cell proliferation. It is synonymous with acute post-streptococcal glomerulonephritis but this term is seldom used now since this may also occur after the infections by other organisms like staphylococci, meningococci, pneumococci, enterococci, Klebsiella, Salmonella, Brucella, Mycobacteria and various ricketssial and viral infections. It is a disease of childhood affecting children between 5 and 15 years, but can affect any age group. Clinically, patients present with nephritic syndrome after 1 to 4 weeks of nephrogenetic strain of beta hemolytic streptococcal infection in the skin or pharynx.[8,9,58,59,60,61]

Light microscopy shows diffuse enlargement of the glomerular tufts by endocapillary,mesangial and epithelial proliferation with leucocytic infiltration (neutrophils and monocytes). Three patterns of immunofluorescence can be noted which are garland pattern (dense, discrete deposits of IgG and C3 seen as many humps on the subepithelial

side of the basement membrane); starry sky pattern (diffuse, irregular deposits of fine and granular type seen in both capillary loops and mesangium) and mesangial pattern (granular deposition of predominantly C3 and small amounts of IgG in the mesangium). Electron microscopy show subepithelial dome shaped deposits called humps.[8,9,10] Garland pattern is considered to have worse prognosis compared to starry sky and mesangial pattern.[10]

Differential diagnosis: Non-streptococcal origin of acute post-infectious glomerulonephritis should be considered as a differential diagnosis for post streptococcal glomerulonephritis. Clinical history of pharyngitis and skin infections (impetigo) and laboratory evidence of the organism help in differentiating these two entities. Also, electron microscopy shows more of subendothelial and intramembranous deposits in nonstreptococcal origin of acute post-infectious glomerulonephritis.

Membranoproliferative glomerulonephritis can be differentiated from DPGN by subendothelial deposits in electron microscopy.

Lupus nephritis class IV- diffuse proliferative type shows endocapillary proliferation with leucocytic infiltration. This can be differentiated from DPGN by clinical history, typical immunofluorescence and electron microscopy findings.[8]

DIFFUSE MESANGIOPROLIFERATIVE GLOMERULONEPHRITIS.

Diffuse proliferation of mesangial cells and matrix expansion without involvement of capillary walls or lumina occurs in a variety of renal conditions including Ig A nephropathy, systemic lupus nephritis, Henoch Schonlein purpura, and resolving phase of post-infectious glomerulonephritis.[8,9]

IgA NEPHROPATHY

IgA nephropathy can occur at any age but most common age group affected is the second and third decades. Most common symptom is macroscopic hematuria. IgA nephropathy results from either deposition of immune complexes that are circulating because of binding of IgA to specific antigen planted in the mesangium or binding of IgA (abnormally glycosylated IgA1) to the mesangium in the absence of antigen. The circulating autoantigen-antibody complexes go through glomerular capillary fenestrae and stimulate the mesangial cells. These complexes play an important role in the pathogenesis of Ig A nephropathy.[63,64]

Several biomarkers have been established recently in order to study the disease severity and progression. Among the biomarkers, elevated levels of galactose deficient IgA1, glycan-specific IgG and IgA1 auto-

antibodies have been assessed and found that elevated levels of galactose deficient IgA1 associated with worse prognosis and patient outcome. Serum levels of IgG and IgA auto-antibodies are strongly associated with IgA nephropathy progression.[64,65]

Light microscopy demonstrates a variety of histologic patterns ranging from normal to a diffuse crescentic glomerulonephritis.[8,62]

Based on the light microscopy, IgA nephropathy can be classified into five stages (lee et al):[8]

Stage I: Normal glomeruli or may show mesangial thickening with or without hypercellularity. Tubular and interstitial changes are absent.

Stage II: Mesangial hypercellularity in less than 50% of glomeruli. Sclerosis and crescents are noted rarely. Tubular and interstitial changes are absent.

Stage III: Diffuse along with focal and segmental mesangial proliferation variability is noted. Adhesions and crescents are infrequently seen. Rarely tubular atrophy and focal interstitial edema and inflammatory infiltration may be seen.

Stage IV: Striking diffuse mesangial proliferation and sclerosis is seen with crescents in 45% of glomeruli. Tubulointerstitial system shows atrophy, inflammatory infiltration and occasional foam cells.

Stage V: Similar glomerular, tubular and interstitial changes as Stage IV but more severe. Crescents are seen in more than 45% of glomeruli.

Stage IV and V indicate worse prognosis.

IgA nephropathy is diagnosed by immunofluorescence findings which show dominant diffuse mesangial and capillary loop deposits of IgA. Frequently C3 shows similar pattern as that IgA. Uncommonly, IgM and IgG can also show reactivity.[8,9]

Differential diagnosis: Henoch Schonlein purpura has the similar histomorphological features as that of IgA nephropathy but can be distinguished from IgA nephropathy by the presence of extrarenal manifestations.[8]

When IgG staining is as intense as IgA, lupus nephritis should be considered for differential diagnosis. If C1q staining is positive then the diagnosis of lupus nephritis is made because C1q staining is rarely seen in IgA nephropathy.[8,9]

In IgA- dominant post-infectious glomerulonephritis, dominant C3 deposits in immunofluorescence studies, hump-like deposits on electron microscopy, polymorphs in histomorphology favours post-infectious glomerulonephritis.[8,9]

C1Q NEPHROPATHY

C1q nephropathy patients present with nephrotic syndrome and it usually affects adolescents and young adults. Males are more commonly affected than females. It has relatively poor prognosis with 5 year survival rate being 78%.[9]

Light microscopy shows spectrum of lesions ranging from minimal glomerular changes, mesangium expansion with mesangial hypercellularity, focal or diffuse proliferative glomerulonephritis. Segmental glomerulosclerosis may or may not be seen.

Immunofluorescence studies show predominantly C1q accompanied by C3 staining which is usually not as intense as C1q. IgA may be seen in about 60% of cases. Electron microscopy show mesangial immune complex deposits and foot process effacement.[9,10]

Differential diagnosis includes lupus nephritis. Patients with Lupus nephritis have typical clinical history of systemic lupus nephritis, and immunofluorescence studies show 'full house' pattern. Electron

microscopy shows characteristic reticular aggregates in lupus nephritis which is absent in C1q nephropathy.[9,10]

FIBRILLARY AND IMMUNOTACTOID GLOMERULONEPHRITIS.

These two types of glomerulonephritis are very rare and occur in older individuals. Light microscopic picture of both are very similar, showing mesangial hypercellularity, thickening of glomerular capillary walls, deposition of amorphous PAS-positive material. This deposit can be differentiated from amyloid by using with Congo red and thioflavin T stains.[7]

Immunofluorescence studies in both show IgG and C3 distribution corresponding with the microtubules and fibrils.These two conditions can be differentiated only by electron microscopy studies. In both these conditions deposits can be found in subepithelial, subendothelial, GBM and mesangium. But immunotactoid glomerulonephritis show parallel arrangement of microtubular structures measuring 30 to 50 nm in width whereas fibrillary glomerulonephritis show deposition of nonbranching, randomly arrayed fibrils measuring approximately 20 nm in diameter.[7,8]

DIFFUSE CRESCENTIC GLOMERULONEPHRITIS.

Diffuse crescentic glomerulonephritis is termed as rapidly progressive glomerulonephritis or extracapillary proliferative glomerulonephritis. It is a severe type of glomerulonephritis with brisk and progressive loss of renal function accompanied by hematuria, proteinuria, RBC casts in urine and severe oliguria.[1,7]

Histopathologically, the characteristic finding is crescent formation in 50% or more glomeruli. Crescent formation occurs due to disruption in the glomerular capillaries which allows leucocytes, plasma proteins, fibrin into the Bowman's space where they induce epithelial cell proliferation, macrophage maturation forming cellular crescents. Cellular crescents should be atleast two cell layer thick. As the disease progresses, cellular crescents become fibrous which are now called as fibrocellular crescents. With further disease progression, cellularity is almost totally reduced forming a fibrous crescent.[7,8,9]

There are three types of crescentic glomerulonephritis based on underlying pathologic mechanisms

1. Anti-GBM glomerulonephritis
2. Immune complex crescentic glomerulonephritis
3. Pauci-immune crescentic glomerulonephritis

ANTI-GLOMERULAR BASEMENT MEMBRANE DISEASE

This accounts for 15% cases of cresentic glomerulonephritis. It is an auto-immune disease occurring as kidney limited or as pulmonary kidney syndrome (Goodpasture syndrome). This results from antibodies against carboxyl terminus of the NC1 domain of the alpha 3 chain of type IV collagen.[7,8]

The light microscopy shows typical necrotizing glomerulonephritis with crescent formation. Glomerular capillaries are often disrupted with neutrophilic infiltration and fibrin deposition. Severe involvement of glomeruli show intense periglomerular inflammation and Bowman's capsule disruption which can be better visualized with silver stains.

Immunofluorescence microscopy shows continuous linear staining in the glomerular capillaries for C3 and IgG. Electron microscopy show no immune complex deposition which is an important negative finding but disruption of GBM and fibrin deposition can be visualized.[8,9]

IMMUNE COMPLEX CRESCENTIC GLOMERULONEPHRITIS.

Immune complex crescentic glomerulonephritis accounts for 25% of all crescentic glomerulonephritis commonly occuring in children. It can be idiopathic or due to a complication of post-infectious

glomerulonephritis, types I and II MPGN, cryoglobulinemic glomerulonephritis, SLE, IgA nephropathy and HSP.

Light microscopy show variable degree of glomerular capillary wall thickening and endocapillary cell proliferation along with some amount of necrosis. Immunofluorescence study show IgG granular deposition in capillary walls.[7,8,9,10]

PAUCI-IMMUNE CRESCENTIC GLOMERULONEPHRITIS.

It is the most common type of crescentic glomerulonephritis occurring in elderly patients and 80–90% of them are ANCA-positive. Serological markers for pauci-immune crescentic glomerulonephritis are anti-neutrophil cytoplasmic autoantibodies (either proteinase-3 or myeloperoxidase).

Light microscopic picture is similar to anti-GBM disease. Immunofluorescence studies show small irregular focal staining for C3. Crescents show fibrinogen positivity. Electron microscopy demonstrates disruption of GBM and fibrin deposition.[7,8,9]

LUPUS NEPHRITIS

Systemic lupus erythematosus (SLE) is a chronic inflammatory auto-immune disease involving various systems the etiology of which is

unknown. Prevalence of SLE in India ranges from 14 to 64 per 100,000 population. Incidence of SLE in women is higher than in males with female to male ratio being 12:1. The common age group affected ranges from 15 to 45 years. Renal manifestation in SLE is called as lupus nephritis.[7,78]

Most of the patients have no or slight renal symptoms. Usual finding is the abnormal urinary sediment or altered renal functional parameters. The clinical presentation does not always predict the underlying histological class of renal involvement.[72,73,74]

All the four compartments i.e, glomeruli, tubules, interstitium, and blood vessels may be affected in lupus nephritis.

International Society of Nephrology (ISN) / Renal Pathology Society (RPS) classification of lupus nephritis[7,8,9]

It is the recent and widely accepted classification of lupus nephritis.

Class I : Minimal mesangial lupus nephritis show no changes in glomeruli in light microscopy. Immunofluorescence studies show only mesangial deposits.

Class II : Mesangial proliferative lupus nephritis is defined by any degree of mesangial cell proliferation and mesangial expansion.

Immunofluorescence shows mesangial immune complex deposits. Electron microscopy demonstrates rare subepithelial and subendothelial deposits.[7,8]

Class III : Focal lupus nephritis consists of the global or focal and segmental, endocapillary or extracapillary cell proliferation affecting less than half of the glomeruli. Active lesions are characterized by endocapillary proliferation and chronic lesions by healed,sclerosed changes. Some degree of necrosis, crescent formation and sclerosis can be seen. In necrotizing lesion, there can be hematoxylin bodies which are round masses to minute fragmented nuclei that show a lilac tinge on hematoxylin and eosin stained sections. These bodies are considered as pathognomonic of lupus nephritis but it can be seen only in 1-2% of cases. Active lesions show interstitial edema, inflammation whereas chronic stage shows atrophy of the tubules and fibrosis of the interstitium. Immunofluorescent microscopy reveals diffuse and global capillary wall and mesangial deposits for IgG, IgA, IgM, C3, and C1q. This is called as a 'full house' pattern. Fibrinogen is identifiable in crescents and necrotizing lesions. Electron microscopy shows mesangial, subepithelial and subendothelial deposits.[8,9,75]

III A : Active lesions

III A/C : Active and chronic lesions

III C : Chronic lesions

Class IV : Diffuse lupus nephritis involves more than half of the glomeruli showing diffuse global or segmental endocapillary or extracapillary cell proliferation. There may be active and chronic lesions. The characteristic immune deposition in subendothelial location produces marked thickening of the capillary walls to form 'wire loop' lesions. Capillary lumina may be occluded by heavy deposition of the immune complexes called as 'hyaline thrombi'. The activity and chronicity of the tubulointerstitial part corresponds to the activity and chronicity of the glomerular lesions. Immunofluorescence shows a coarse granular pattern in the mesangium and in the capillary walls for IgG, IgA, IgM, C3, and C1q. Fibrin positivity is seen in crescentic necrotizing lesions. Electron microscopy shows mesangial and subendothelial deposits.[8,9,10,76]

IV A: Active lesions

IV A/C: Active and chronic lesions

IV C: Chronic lesions

Jindal et al, who studied fatal complications of SLE in 25 cases observed that 96% of the cases showed kidney involvement. Of them, commonest lesion noted was diffuse proliferative glomerulonephritis comprising 60%. Rapidly progressive glomerulonephritis was seen in 7 cases. All of them belonging to Class IV.[72]

Tateno et al observed that massive lumpy deposits in the glomeruli were associated with severe diffuse proliferative class of Lupus nephritis which had a poor prognosis.[73]

Class V: Membranous lupus nephritis shows the thickening of capillary basement membrane by the deposition of the immune complexes in subepithelial location. Glomeruli show mesangial cell proliferation and mesangial expansion. Silver stains show spike and dome pattern in the thickened capillary basement membrane. Immunofluorescence studies show IgG, IgA, IgM, C3 and C1q positivity in the capillary walls and IgG, IgM, C3 positivity in mesangium. Electron microscopy show subepithelial and mesangial deposits.[7,8]

Class VI : Advanced sclerosing lupus nephritis is characterized by global sclerosis affecting more than 90% of glomeruli. Tubulointerstitium shows chronic changes like atrophy, inflammation, fibrosis and vessels show atherosclerotic changes. Some of the glomeruli

may have residual mesangial cellularity. Immunofluorescence and electron microscopy demonstrate granular immune deposits in the capillary wall and mesangium.[8,9]

Klemperer et al., 1941 did a study on autopsy material and Muchreke et al.,1957 on percutaneousrenal biopsies and both these studies showed that kidney involvement in SLE is a frequent finding and a serious manifestation. Death by kidney involvement and need for kidney replacement therapy was seen in 15- 20% cases.

Kanjanabuch et al., has shown in a study that lupus nephritis is the most common secondary form of glomerulonephritis followed by post-infectious glomerulonephritis in developing countries.

Rohi wani et al,2012., in a study on lupus nephritis showed that majority of the patients were females accounting 91.4% and the most common class of lupus nephritis was class IV accounting for 54. 29%.

Banff et al, in a study of 147 patients with lupus nephritis found mesangial pattern in class II and in few cases of class I and III, the peripheral pattern (lumpy and granular) was most common in class IV and VI whereas membranous pattern was noted in class V.

Gladman et al, in a study of 148 biopsies, found that both active and chronic lesions are seen most commonly in Class III and IV of lupus

nephritis. Also, class I and class II LN showed active lesions in 17% and 8% respectively which was due to interstitial inflammation. In class V there were 18% of cases having both active and chronic lesions.[77]

McLaughlin found that 5 year survival rate was 86% in class I and 30% to 79% in class IV. In the follow up study of cases of lupus nephritis, rapid increase in the serum creatinine levels is considered as a predictive marker for the progression of irreversible renal failure. The main causes of death in SLE patients are irreversible renal failure, neurological manifestations and infections.

DIABETIC NEPHROPATHY

Diabetic nephropathy is considered as the leading cause for end-stage renal disease. It is characterized by deposition of extracellular matrix in the glomerular and tubulointerstitial compartments along with the thickening and hyalinization of intrarenal vessels.[66,67,71]

The following lesions are seen in the diabetic nephropathy:

Diffuse glomerulosclerosis is the most common lesion and is characterized by expansion of the eosinophilic, PAS-positive material in the mesangial matrix with segmental or diffuse capillary basement membrane thickening. There can be mild mesangial cell proliferation.[7,8]

Nodular glomerulosclerosis (Kimmelstiel Wilson lesion) is first described in 1936 by Kimmelstiel and Wilson. The lesion is characterized by accumulation of the round homogenous eosinophilic extracellular material in the mesangium and in capillary basement membrane. This material can be demonstrated by PAS, Masson trichrome, Mallory stains. Both nodular and diffuse glomerulosclerosis can be seen in the same glomeruli.[7]

Insudative lesions, also called as hyalinosis are the electron dense, finely granular material containing lipid droplets seen in various regions of the glomeruli. When they are in the periphery of the loop in subendothelial region it is called fibrin cap and when they are within the basement membrane of the Bowman's capsule it is called capsular drops.[7,8]

Armani–Ebstein lesion is defined by vacuolization of the proximal tubular epithelial cells because of glycogen accumulation.

Immunofluorescence studies are negative for immune deposits but show diffuse linear reaction for IgG along the glomerular capillary, tubular, and Bowman capsular basement membranes due to non-immunological trapping of proteins in the altered basement membrane.[8]

Pathological classification of diabetic nephropathy (Thijs W. Cohen Tervaert et al, 2010)[70]

Class I: Light microscopy show mild or nonspecific changes. Electron microscopy shows glomerular basement thickening (>395 nanometer in females, >430 nanometer in males of 9 years of age or older). Biopsy should not meet any of the criteria for class II, III or IV.

Class II a: Mild mesangial expansion in >25% of the observed mesangium. Biopsy does not meet the criteria for class III or IV.

Class II b: Severe mesangial expansion in >25% of the observed mesangium. Biopsy does not meet the criteria for class III or IV.

Class III: Nodular sclerosis (Kimmelstiel- Wilson lesion). Alteast one Kimmelstiel-Wilson lesion should be seen and biopsy does not meet the criteria for class IV.

Class IV: Advanced diabetic glomerulosclerosis (Global sclerosis in >50% of glomeruli).

Differential diagnosis: Hyalinosis lesion seen in diabetic nephropathy can also be seen in FSGS where usually the changes are focal and in DN it is more diffuse. The immunofluorescence and electron microscopy studies aid in distinguishing between these two lesions.[8]

Immune-complex mediated glomerulonephritis may be confused with DN. The presence of linear staining for IgG and albumin in immunofluorescence studies, absence of immune complex deposits in electron microscopy, thickening of glomerular basement membrane helps in making the diagnosis of diabetic nephropathy.[7,9]

Hypertensive nephropathy or age related changes can be confused with diabetic nephropathy since both have thickening of glomerular basement membrane. Presence of hyaline arteriosclerosis is usually seen in DN which aids in making the correct diagnosis.[8]

Nodular glomerulosclerosis in diabetes can be confused with amyloidosis, light chain deposition disease, membranoproliferative glomerulonephritis and immunotactoid glomerulonephritis. Amyloid is acellular and stains positive with Congo red stain and gives an apple green birefringence when visualized under polarized light unlike the nodules of diabetic nephropathy which stain negative for Congo red and positive for PAS stain. Furthermore, electron microscopy demonstrates amyloid as the classic rigid nonbranching fibrils of 8 to 10 nanometer in diameter.[7,8,9]

Light chain deposits are granular in appearance and immunofluorescence studies show either kappa or lamba staining in the

tubular basement membrane and sometimes in mesangial nodules and in GBM thus helping in distinguishing light chain deposition disease from diabetic nephropathy.[8,9]

Immunotactoid glomerulonephritis can be differentiated from diabetic nephropathy by electron microscopy. The characteristic microtubules of 30 to 50 nm in width is seen in immunotactoid glomerulonephritis.[8]

Membranoproliferative glomerulonephritis show diffuse involvement with similar degree of changes in all the glomeruli whereas DN show focal changes in glomeruli. Mesangial cellularity is more pronounced in MPGN but in DN it is mild. With respect to nodules, diffuse and global involvement is seen in MPGN whereas it is focal and segmental in diabetic nephropathy. Methanamine silver stains demonstrates double contour of basement membrane, immunofluorescence studies show granular peripheral deposits and electron microscopy shows subendothelial and mesangial deposits in type I and intramembranous deposits in type II MPGN which further rules out the diagnosis of DN.[8,9,10]

Diabetic nephropathy is the leading cause for end stage renal disease. A recent study from India shows that 31.3% of renal failure is

caused by diabetic nephropathy. Out of 114 cases of diabetes, 86 patients (75.43%) had diabetic nephropathy and remaining 28 had non-diabetic renal disease. Currently, not all the patients with a history of diabetes undergo renal biopsy. Biopsy is indicated only when there are atypical features noted in diabetic patients like absence of diabetic retinopathy, rapid onset of nephrotic syndrome or proteinuria, low or rapid decrease in glomerular filtration rate, presence of active urinary sediment and presence of signs and symptoms of other systemic diseases. They concluded that most of the patients with diabetes with kidney dysfunction have diabetic nephropathy. Most common class of diabetic nephropathy was class IV followed by class III, class II and class I. Renal biopsy helps in staging of renal lesions in patients having diabetes with kidney dysfunction (M. Sahay et al, 2014).[69]

HYPERTENSIVE NEPHROPATHY

Hypertension is the elevation in systolic blood pressure more than 140mm of Hg and diastolic blood pressure more than 90mm of Hg. When the gradient of the progressive rise in the blood pressure is slow it is called as benign phase of hypertension and when it is very steep it is called as malignant hypertension with blood pressure greater than 200/120 mm of Hg.[1]

Benign hypertensive changes in the kidney

Light microscopy shows wrinkling, thickening of the GBM with collapse, shrinkage of tuft; loss of tuft cellularity and obliteration of capillary lumina.[7,8]Tubules show atropy with thickening of basement membrane. Homogenous eosinophilic tubular casts may be seen. Arteries and arterioles show thickening of the walls with hyalinization.[8]

Malignant hypertensive changes in the kidney

Light microscopy show diffuse sclerosis in more than 50% of the glomeruli. Few glomeruli show fibrinoid necrosis with increase in cellularity by polymorphs and proliferation of the parietal cells. Few glomeruli show cellular crescents. Tubules show marked atrophy with hyaline casts and RBC casts in them. Arteries show thickening of the wall with onion skin appearance. Arterioles show typical fibrinoid necrosis.[8]

AMYLOIDOSIS

It is a group of disorders characterized by the extracellular deposition of amorphous, eosinophilic, nonbranching, linear, fibrillary protein. Amyloid is best demonstrated with Congo red stain and display a pathognomonic apple green birefringence when viewed under polarized light.[1,7,8]

Amyloidosis involving kidney can be of the following subtypes:

AL amyloidosis which is related with plasma cell dyscrasias.

AA amyloidosis which is associated with chronic inflammatory conditions.

Dialysis related amyloidosis which occurs in patients undergoing long term dialysis and is caused by deposition of fibrillar beta-2 microglobulin.

Light microscopic changes include amyloid deposition in the mesangium initially which then involves the capillary walls. Tubular deposition of amyloid causes atrophy of the tubules and interstitial fibrosis. A variety of stains are used to demonstrate amyloid like Congo red, Thioflavin T and S, crystal violet stains. The most reliable method is demonstration of an apple-green color by polarized light in Congo red stained sections.

Immunofluorescence studies show that amyloid is auto-fluorescent under ultraviolet light. Non-specific staining of deposits for IgM and C3 are sometimes noted.[7,8]

MYELOMA CAST NEPHROPATHY

Myeloma cast nephropathy is the most common kidney disorder in the multiple myeloma patients. It is seen in approximately half of the patients who have kidney disease in multiple myeloma. Other renal manifestations that are encountered are amyloid (light chain or heavy chain deposition), acute tubular damage or necrosis, inflammatory tubulointerstitial nephritis, heavy chain deposition. Infiltration of the plasma cells into the kidney is a rare finding.

Cast nephropathy typically presents with acute renal failure with nephrotic range proteinuria composed predominantly of Bence Jones protein.

The glomeruli and vessels may appear normal by light microscopy. The distal and proximal tubules show casts which appear dense, eosinophilic, irregular, angulated, fracture planes. The casts are composed of Bence Jones, Tamm-Horsfall or light chain proteins. The tubules are frequently surrounded by giant cells and inflammatory cells giving a granuloma-like inflammatory reaction. There is associated chronic inflammatory cell infiltration, tubular atrophy, denudation and necrosis.

Immunofluorescence studies show either kappa or lambda positivity in the casts. Electron microscopy show casts having crystalline structures of varying sizes and shapes.[7,8,9]

GLOMERULAR LESIONS ASSOCIATED WITH VASCULITIS

A variety of primary and secondary vasculitis are known to have renal involvement. Polyarteritis nodosa, Henoch-Schonlein purpura, Wegener's granulomatosis and microscopic polyangiitis are some of the common lesions affecting the kidney.[1]

POLYARTERITIS NODOSA

The kidneys are affected in 80-90% of the cases. The acute phase shows fibrinoid necrosis of the vessel wall. Initially, the inflammation and necrosis is seen on the inner wall which spreads transmurally leading to the perivascular involvement. In the chronic phase there is extensive destruction of the wall with replacement by fibrosis. Immunofluorescence studies and electron microscopy show no immune complex deposition.[1,7,8]

HENOCH-SCHONLEIN PURPURA

The light microscopic changes in HSP are variable. Glomerular changes may range from apparently normal looking to diffuse proliferative and crescentic glomerulonephritis. Immunofluorescence studies show predominant mesangial deposition and weak staining in the capillary walls for IgA.[7,8]

MICROSCOPIC POLYANGIITIS

Light microscopy shows effacement of the glomerular architecture by extensive sclerosis along with destruction of Bowman's capsule, periglomerular fibrosis and chronic inflammation. Immunofluorescence studies show intense fibrin staining in the interlobular arterial wall.[8]

WEGENER'S GRANULOMATOSIS

The most common lesion in Wegener's granulomatosis is focal necrotizing glomerulonephritis with crescents. Interstitium shows inflammatory infiltrates.[7,8]

HEREDITARY GLOMERULAR DISEASES

ALPORT SYNDROME

It is an inherited disorder of the basement membrane (type IV collagen). 90% of the patients have X-linked dominant inheritance with remaining cases being autosomal recessive or autosomal dominant.[7,8]

Light microscopy findings are nonspecific with glomeruli showing mild mesangial cell proliferation and mild thickening of the capillary basement membrane. Interstitium show foamy cells. As the disease progresses the glomeruli undergo global or segmental sclerosis.

Immunofluorescence studies show scattered deposits of IgM and C3. Electron microscopy shows transformation of the lamina densa into multiple interwoven lamellae that enclose electron-lucent areas containing round granules of variable density.[8]

THIN BASEMENT MEMBRANE DISEASE

This is a hereditary renal disease characterized by the uniform thinning of the basement membrane. Light microscopy show erythrocytes in the Bowman's space and renal tubules but otherwise appear normal.

The diagnosis is made by the characteristic ultrastructural demonstration of uniform thinning of the GBM (lamina densa). The

width is reduced to one-third (approximately 200 nm), and on occasion ruptures of the GBM may be seen. Immunofluorescence studies are usually negative for immunoglobulin and complement.[7,8,9]

FABRY DISEASE

It is an uncommon inherited X-linked disease. It is caused by a deficiency of a-galactosidase A enzyme found in lysosomes resulting in accumulation of neutral glycosphingolipid globotriaosylceramide in the tissues. Kidney involvement manifests in the second decade with hematuria and proteinuria.[7]

On light microscopy, vacuolization of the visceral and parietal epithelial cells, endothelial cells, mesangial cells, tubular epithelial cells are noted. These vacuoles are PAS negative. As the disease progresses, glomeruli undergoes segmental or global sclerosis with interstitial scarring and arteriolar hyalinosis.

On electron microscopy, characteristic ‘zebra bodies’ are seen in the affected cells. Zebra bodies are laminated inclusions which are either round with a concentric myelin-like structure or ovoid with parallel layers seen in the cytoplasm.[7,8]

CONGENITAL NEPHROTIC SYNDROME

The patients present with clinical symptoms at birth or within few months of age. Two major types of congenital nephrotic syndrome are congenital nephrotic syndrome of the Finnish type and diffuse mesangial sclerosis.[7]

CONGENITAL NEPHROTIC SYNDROME OF THE FINNISH TYPE

It is a rare disease with autosomal recessive mode of inheritence having mutations of the *NPHS1* gene located on chromosome 19q13.

The most characteristic feature in light microscopy is proximal and distal tubular ectasia with flattening of the tubular epithelial cells. There may be increased number of immature glomeruli.[8]

Immunofluorescence studies may show mesangial and capillary staining for IgM and C3.[7,8]

DIFFUSE MESANGIAL SCLEROSIS

It is characterized by the early onset of severe proteinuria. Patients will rapidly progress to end-stage renal failure within 3 years of age.

Light microscopy show diffuse mesangial sclerosis but do not show increased cellularity. Occasionally crescents may be seen.

Immunofluorescence studies show IgM and C3 deposits outlining the sclerosed glomeruli and IgM, C3 and C1q deposits in the mesangium of the slightest affected glomeruli.[7,8]

TUBULOINTERSTITIAL DISEASES

ACUTE TUBULAR NECROSIS

The two features of ATN are acute renal failure and epithelial cell injury. The two types of ATN are ischaemic and toxic ATN.

ISCHAEMIC ATN:

Ischaemic ATN is caused by decreased perfusion of the kidneys and is the most common type. Hypoperfusion of the kidneys results from various causes like burns, shock following surgeries, septic shock after pancreatitis, dehydration because of vomiting, diarrhea or increased sweating.

The light microscopy changes depend upon the extent of severity of renal failure and evolution of the disease. Glomeruli are usually spared. Tubular epithelial cells show swelling, vacuolation, loss of brush border, and denudation. Tubules may commonly show epithelial, hyaline or granular casts which are PAS-positive. Interstitium shows edema and

mononuclear cell infiltration. Later stages may show evidence of tubular epithelial cell regeneration.[7,8,9]

TOXIC ATN:

Toxic acute tubular necrosis can be caused by wide variety of substances like chemotherapeutic agents, organic solvents, heavy metals, antibiotics and radiographic contrast agents. Some of the endogenous components like hemoglobin and myoglobins also causes ATN.[7,68]

Light microscopy shows extensive necrosis of the tubular epithelial cells with pigmented casts. Immunofluorescence studies show non-specific linear staining for IgG and C3 in the tubular basement membrane in some drug-induced ATN. Electron microscopy shows evidence of interstitial edema, inflammatory cell infiltration and tubulitis. Immune complex deposits are not seen.[7,8]

RENAL BIOPSY

The first renal biopsy was performed a century ago in 1901 for the treatment for Bright disease as a part of renal decapsulation procedure. Open renal surgical procedure was done by Gwyn in 1923 and percutaneous renal biopsy was performed only in 1951 by Iverson and Brun. A few years later in 1954, a descriptive procedure of patients lying in prone position for renal biopsy was published by Kark and Muehrcke. Presently, new ultrasound guided renal biopsy is the gold standard procedure with minimal complications.[2,3,12,17]

Indications for renal biopsy :

Isolated hematuria, mild, moderate and severe proteinuria, acute and chronic renal failure, glomerulopathies, renal manifestations of systemic diseases. Renal biopsy also plays an important role in renal transplantation.[3,12]

Contraindications for renal biopsy:

There are absolute and relative contraindications for renal biopsy. Some of the absolute contraindications are uncooperative patients, pregnancy, uncontrolled bleeding diathesis and anatomic malformations. Relative contraindications are renal abscesses, hydronephrosis, pyelonephritis, severe anemia, marked obesity, uremia, uncontrolled

hypertension or hypotension, large renal tumors, arterial aneurysms and cysts.[3,14]

Procedure :

Ultrasound guided percutaneous renal biopsy is the gold standard method to obtain renal biopsy. It is usually carried out by the nephrologist or radiologist according to the local practice.[12,18]

Adequacy of the tissue sample :

1. Biopsy must include 8 to 10 glomeruli.
2. Juxtamedullary glomeruli (preferential involvement in FSGS).
3. In case of focal lesions, a minimum of 25 glomeruli should be in the biopsy tissue to look for the evidence of renal injury.
4. In case of diffuse lesions even one glomeruli is considered sufficient to make a diagnosis.[3,12,13,14]

Complications of the procedure :

Minor : Gross hematuria, silent hematuria

Major : Hematoma

Catastrophic : Loss of functional mass, death.[3]

Clinical information necessary for the pathologist:

Pathologists will need to know the detailed clinical history, past history of diabetes mellitus, hypertension, laboratory investigations like

urine analysis particularly hematuria, proteinuria, pyuria, serum creatinine, blood urea nitrogen values, total protein, cholesterol, C3 and C4 levels, antinuclear antibodies, anti-neutrophil cytoplasmic antibodies, current treatment if any.[14]

Handling of the specimen :

Two core biopsy specimens, each divided into three portions for light microscopy, immunofluoroscence studies and electron microscopy. Biopsy for immunofluorescence studies is sent in Michel transport medium. For light microscopy 10% neutral buffered formalin can be used as transport medium as well as fixative. For electron microscopy, ice cold 1% to 3% glutaraldehyde can be used as fixative.[2,12,14]

Light microscopy :

2 to 3 micron thickness serial sections are taken for light microscopy. Number of stains can be used for light microscopy. Routine haematoxylin and eosin stain is considered best to visualize the cell morphology. Periodic acid Schiff stain can be used to highlight the basement membrane and connective tissue matrix. Methanamine silver stains are mainly used to see the basement membrane and are better than PAS in highlighting the basement membrane abnormalities. Use of haematoxylin and eosin in place of neutral green in methanamine silver

stain has an added advantage of examining the relations between matrix and glomerular cells. Other stains like Congo red can be used in suspicious cases of amyloidosis (heavy proteinuria, systemic amyloidosis), elastin stain can be used in cases of vascular renal diseases.[12,14]

Immunofluorescent microscopy:

Tissue is snap-frozen for immunofluorescence and serial sections of 2 to 3 micron thickness is cut and placed on the air-dried slides. A panel of antibodies for the immunoglobulins (IgG,IgA,IgM), complements (C3, C4, C1q), fibrin, kappa and lambda light chains are added to the sections. The fluorescence is visualized under the fluorescent microscope. While reporting the positive staining the pathologist should note the intensity and pattern or localization (linear or granular deposits along basement membrane, mesangial) of the fluorescence.[14]

There are few advantages and disadvantages of this method. This method is comparatively easy and quick. Disadvantages would be a separate core tissue must be taken at the time of biopsy for this study. A cryostat for sectioning and fluorescent microscope to visualize the slides are a must for this method. An aqueous medium which is the mounting medium is not permanent. Exposure to light causes bleaching. To prevent

from bleaching, slides can be stored in dark in the refrigerator. Photographs of the relevant slides can be taken for future references.[12,14]

Electron microscopy:

Tissue for electron microscopy is transformed into plastic and ultra-thin sections are taken and stained with heavy metal stains like lead citrate and uranyl acetate. One or two glomeruli, vascular structures and tubulointerstitial areas are examined under low, medium and high magnification. Photomicrographs are taken of the pathological abnormalities.[12,13]

Reporting of the renal biopsy: The final report should include the following information:

- Number of glomeruli and the arteries (adequacy of the tissue).
- Histomorphological changes in each compartment of the kidney tissue in a systematic order (glomeruli, tubules, interstitium, vessels).
- Immunofluorescence study results.
- Electron microscopy results.

The final diagnosis is given after carefully correlating all the above findings.[13,16]

MATERIALS AND METHODS

MATERIALS AND METHODS

SOURCE OF DATA

The present study "The value of immunofluorescence in renal diseases with special reference to Periodic acid Schiff and Jone's methanamine silver stain" was conducted in the Department of Pathology, Coimbatore Medical College, Coimbatore from October 2012 to July 2014. A total of 58 cases, two renal biopsies for each case, one in formalin and other in Phosphate buffer solution were received. The study was performed based on the following proforma.

INCLUSION CRITERIA

Renal biopsy specimens of the patients of all age groups and both sexes with altered renal function suggestive of kidney disease from the Department of Nephrology, Coimbatore Medical College and Hospital, Coimbatore were included in this study.

EXCLUSION CRITERIA

1. Specimen not received in phosphate buffer solution for immunofluorescence studies.

2. Specimens that are very tiny for processing and considered inadequate with no glomeruli in subsequent serial sections for light microscopy.
3. Specimen without required clinical and histopathological details.
4. Clinically suspected cases of diabetic nephropathy.
5. Patients that are considered unfit for biopsy (coagulation abnormalities, poor cardiac function).

Indications for biopsy:

1. All nephrotic syndrome and nephritic syndrome patients who are willing for renal biopsy.
2. Patients with acute renal failure not recovering within 4 weeks of duration.
3. All patients with systemic lupus erythematosus who are willing for biopsy.

METHODS OF COLLECTION OF SAMPLE

Before the procedure a pre-renal anaesthetic assessment including prothrombin time, bleeding time, complete blood count were checked and xylocaine needle test dose was given.

After obtaining informed consent, under local anaesthesia and aseptic precautions, two cores of percutaneous ultrasound guided biopsy

specimens of kidney were taken from the patients with altered renal functions. One core was sent in 10% neutral buffered formalin for routine light microscopic examination and other was sent in phospate buffer solution (pH 7.4) for immunofluorescence studies. The procedure was performed with an informed consent by the clinician as a routine procedure for diagnosis and treatment.

LIGHT MICROSCOPY

The renal tissue obtained in 10% neutral buffered formalin is kept for fixation for 12 hours to 24 hours and it is then processed and embedded in paraffin. The sections of 3µ to 4µ thickness were cut and stained using haematoxylin and eosin.

Special stains like Periodic Acid Sciff and Jone's methanamine silver stain were performed to look for the abnormalities in the glomerular basement membrane.

HAEMATOXYLIN AND EOSIN STAIN

Materials required:

A. Ehrlich's haematoxylin

B. Xylol

C. Absolute isopropyl alcohol I and II

D. 90% isopropyl alcohol

E. 1% eosin (1gram eosin + 100 ml of distilled water)

F. 1% acid alcohol (99 ml of isopropyl alcohol + 1 ml concentrated hydrochloric acid)

Procedure :

1. Paraffin sections of thickness 3µ to 4µ were taken on egg albumin coated slides.
2. Air dry the slides and dewax them (62□c to 64□c).
3. Transfer the sections immediately to xylene for 30 minutes.
4. Sections are then transferred to absolute alcohol I and II and 90% alcohol for 15 minutes.
5. Bring the sections to water.
6. Clean the slides around the sections.
7. Transfer the sections to Ehrlich's haematoxylin for 15 to 20 minutes.
8. Drain the slides and wash them in tap water.
9. Dip the slides 2 to 3 times in 1% acid alcohol.
10. Wash the slides in tap water.
11. Keep the slides in washing tray (for blueing) for 10 to 15 minutes.
12. Slides are dipped 3 to 4 times in 1% eosin.

13. Wash the slides in several changes of water till the water becomes colourless.
14. Air dry and clear the sections using xylol
15. Sections are mounted with DPX mountant.

Results :

Nuclei – Blue. Cytoplasm – shades of pink.

PERIODIC ACID-SCHIFF STAIN

Materials required :

A. Periodic acid
B. Basic fuschin
C. 1 Normal Hydrochloric acid
D. Sodium metabisulphite
E. Activated charcoal
F. Distilled water
G. Haematoxylin

0.5% periodic acid preparation :

Periodic acid - 0.5 gram

Distilled water - 100 millilitre

Schiff reagent preparation:

Dissolve 1 gram of Basic fuchsin in 200 ml of boiling water. Cool to 50˚C, add 20 millilitre of 1 Normal Hydrochloric acid . Cool further and add 1 gm of anhydrous sodium bisulphate. Keep it in the dark for 48 hours. Then add 2 gms of activated charcoal until the solution becomes straw yellow colour. Filter the solution. Keep it in brown bottle at 4˚C.

Procedure:

1. Deparaffinize the sections and bring it to water .
2. Oxidize with 0.5% Periodic acid for 5 minutes.
3. Wash with tap water for 5 minutes.
4. Use Schiff reagent on the sections for 15 minutes.
5. Wash with water for 10 minutes.
6. Transfer the sections to Haematoxylin for 15 minutes.
7. Wash with water (till blueing) for 10 minutes.
8. Differentiate with 1% Acid alcohol.
9. Wash in running tap water for 5 minutes.
10. Dry, clear the sections with xylene and mount with DPX mountant.

Results:

Nuclei – blue.

Basement membrane and PAS positive material – magenta pink

JONE'S METHANAMINE SILVER STAIN

Reagents required:

1. 1% aqueous periodic acid
2. Hexamine silver solution.
3. 5% borax.
4. 0.1% aqueous gold chloride
5. 5% aqueous sodium thiosulphate
6. 0.2% light green in 0.2% acetic acid.

Stock hexamine silver solution:

Mix 5 ml of 5% aqueous silver nitrate and 100ml of 3% aqueous hexamine (synonym: methanamine or hexamethylenetetranium). A white precipitate forms that dissolves on shaking. The solution is kept for the limited time (1 to 2 months) if stored in a dark container at 4˚c.

Working Hexamine Silver solution:

Dilute 2ml of a 5% aqueous sodium borate solution with 25ml of distilled water. Mix and then add 25ml of the stock Hexamine Silver solution.

Procedure :

1. Bring sections to distilled water.
2. Treat with 1% periodic acid solution for 10 minutes.
3. Wash well in several changes of distilled water.
4. Place in working hexamine silver solution in 56˚c for 20 minutes and examine subsequently at frequent intervals until the basement membranes are blackened.
5. Wash well in two changes of distilled water each for 5 minutes.
6. Tone in 0.1% aqueous yellow gold chloride for 2 to 5 minutes.
7. Wash in water and treat with 5% aqueous sodium thiosulphate for 5 minutes.
8. Wash in water, counterstain in 0.2% light green in 0.2% acetic acid for 1 minute.
9. Dehydrate, clear and mount in DPX mountant.

Results:

Basement membranes (Basal lamina) - Black.

Background - Green.

IMMUNOFLUORESCENCE

Preparation of phosphate buffer saline (pH 7.4)

Disodium hydrogen phosphate - 8.5gram

Potassium dihydrogen phosphate - 1.5 gram

Sodium chloride - 8.0 gram

Distilled water - 1000 milliliter

Preparation of Buffered Glycerol mounting medium:

Glycerol – 9 ml

Phosphate Buffer Saline – 1 ml

Procedure:

1. The biopsy was received in phosphate buffer saline (pH 7.4).
2. Wash the tissue in distilled water to remove blood clot if any.
3. Take sections in Leica CM 1510 S cryostat machine at -24 degree Celsius temperature using Tissue tek embedding medium at 3 to 4 micron thickness.

4. Dry the sections at room temperature in dark for 2 hours atleast. Keep the sections in the chill tray of refrigerator.
5. Take the slides from the chill tray and keep them in dark room for 30 minutes to 1 hour to bring them to room temperature.
6. Fan dry the slides at room temperature for 10 minutes.
7. Choose the slide with glomeruli by observing under phase contrast microscopy. Slides with even one glomerulus were selected and subjected for immunofluorescence studies.
8. Mark the slides with diamond marker.
9. Wash the sections with PBS for 10 minutes.
10. Drain off the excess PBS and wipe around the sections using tissue paper.
11. Cover the sections with diluted Fluorescent isothiocyanate conjugated antibodies IgG, IgA, IgM, C3c, C1q and Fibrinogen (1:20 dilution with PBS) for two hours in room temperature in dark environment.
12. Wash the sections with PBS for 10 minutes each (3 changes).
13. Drain off the excess PBS and wipe around the sections using tissue paper.
14. Mount the slides with one to two drops of buffered glycerol mounting medium and cover slip was applied.

15. Examine under the fluorescent microscope in a dark environment.

16. Pictures were taken using the camera and preserved in the computer for future reference.

17. The slides were stored in the refrigerator for a week.

Result: Green fluorescence - Positive.

Background – black.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

The present study is a prospective study of renal biopsies received over a period of 20 months. The total number of biopsies received are 58 from the Department of Nephrology, Coimbatore Medical College and Hospital, Coimbatore.

AGE DISTRIBUTION OF CASES

The patients were divided into six groups depending on their age at presentation.

GROUP 1 : 1-10 YEARS

GROUP 2 : 11-20 YEARS

GROUP 3 : 21-30 YEARS

GROUP 4 : 31-40 YEARS

GROUP 5 : 41-50 YEARS

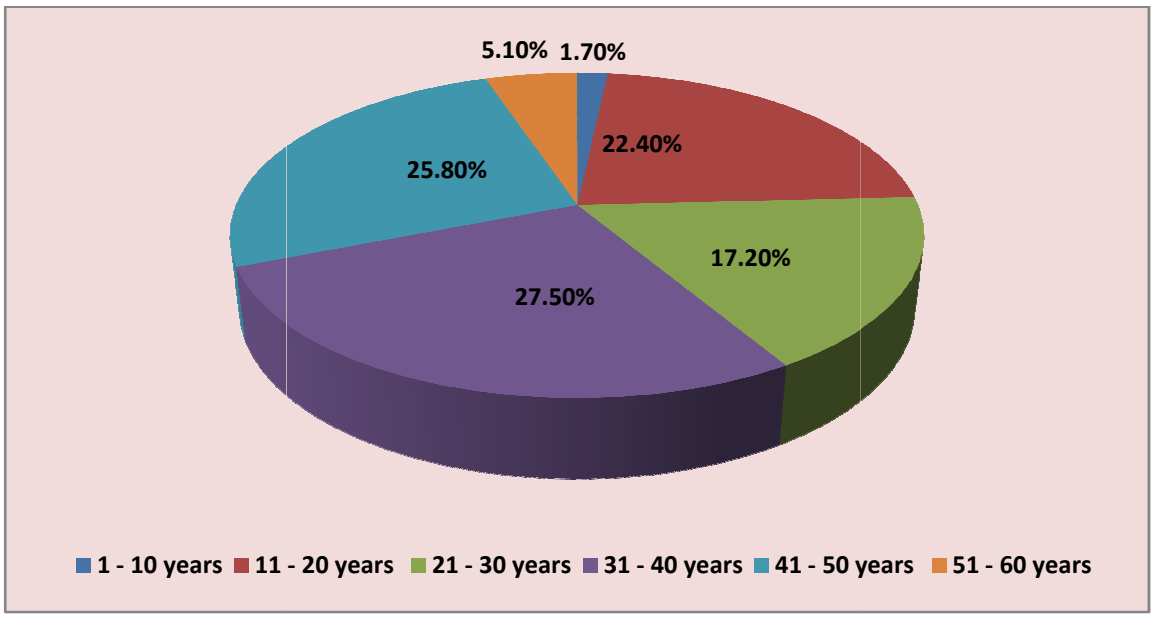
GROUP 6 : 51-60 YEARS

TABLE 1: AGE DISTRIBUTION OF THE CASES

Age	Frequency	Percent
1 – 10 years	1	1.7
11 – 20 years	13	22.4
21 – 30 years	10	17.2
31 – 40 years	16	27.5
41 – 50 years	15	25.8
51 – 60 years	3	5.1
Total	58	100.0

N	Mean (Years)	Median (years)	Standard deviation	Minimum (years)	Maximum (years)
58	33.03	35.00	12.54	9	57

CHART 1: AGE DISTRIBUTION OF THE CASES



The highest number of patients were in the age group 31years to 40 years (16) which constituted 27.5% of patients followed by the age group 41 years to 50 years (15) which constituted 25.8% of the patients. The mean age was 33.03 years and median was 35 years. The youngest patient was 9 years and the oldest patient was 57 years.

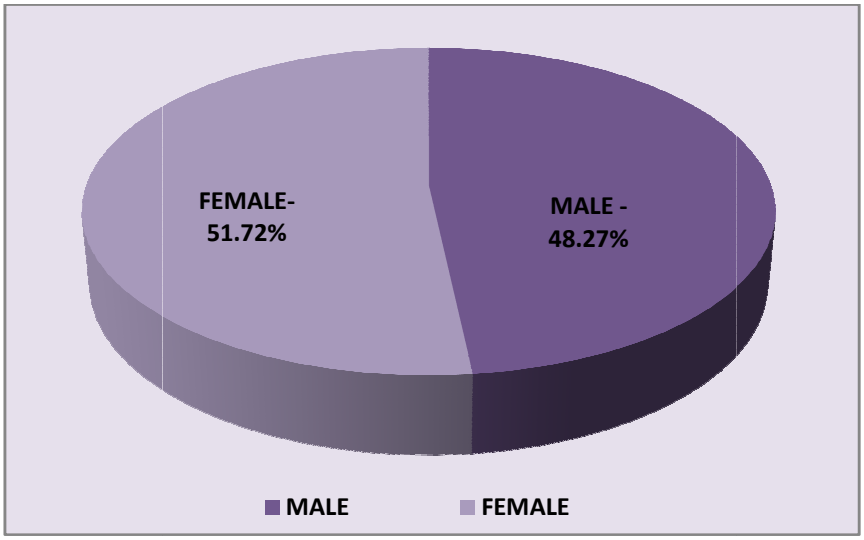
The most common lesion in the age groups 3 (between 21years to 30 years) and 4 (31years to 40 years) was diffuse proliferative glomerulonephritis. The most common lesion in the groups 1 and 2 (between 1 year to 20 years) was focal segmental glomerulosclerosis and in the age group 5 (41 years to 50 years) was membranous glomerulonephritis. Patients belonging to group 6 (between 51 years to

60 years) had the diagnoses of diffuse proliferative glomerulonephritis, membranoproliferative glomerulonephritis and mesangioproliferative glomerulonephritis.

TABLE 2: GENDER DISTRIBUTION OF THE CASES

Gender	Frequency	Percent
Male	28	48.27
Female	30	51.72
Total	58	100.0

CHART 2: GENDER DISTRIBUTION OF THE CASES



To the above table Z test has been applied and it was noted that there was equidistribution of patients among males and females. Of the 58 cases, 28 patients were males constituting 48.27% and 30 patients were females constituting 51.27%. The male to female ratio was found to be 0.933:1. The most common glomerular lesion noted in males was diffuse

proliferative glomerulonephritis (10 out of 28 cases) and in females was Lupus nephritis (7 out of 30 cases).

TABLE 3: AGE AND GENDER DISTRIBUTION OF THE CASES.

AGE	GENDER		TOTAL
	MALE	**FEMALE**	
1 – 10 YEARS	1	0	1
	3.5%	0%	1.7%
11 – 20 YEARS	9	4	13
	69.23%	30.77%	22.4%
21 – 30 YEARS	5	5	10
	17.85%	16.66%	17.2%
31 – 40 YEARS	6	10	16
	37.5%	62.5%	27.5%
41 – 50 YEARS	5	10	15
	17.85%	33.33%	25.8%
51 – 60 YEARS	2	1	3
	17.14%	3.33%	5.1%
TOTAL	28	30	58
	100.0%	100.0%	100.0%

CHART 3: AGE AND GENDER DISTRIBUTION OF THE CASES

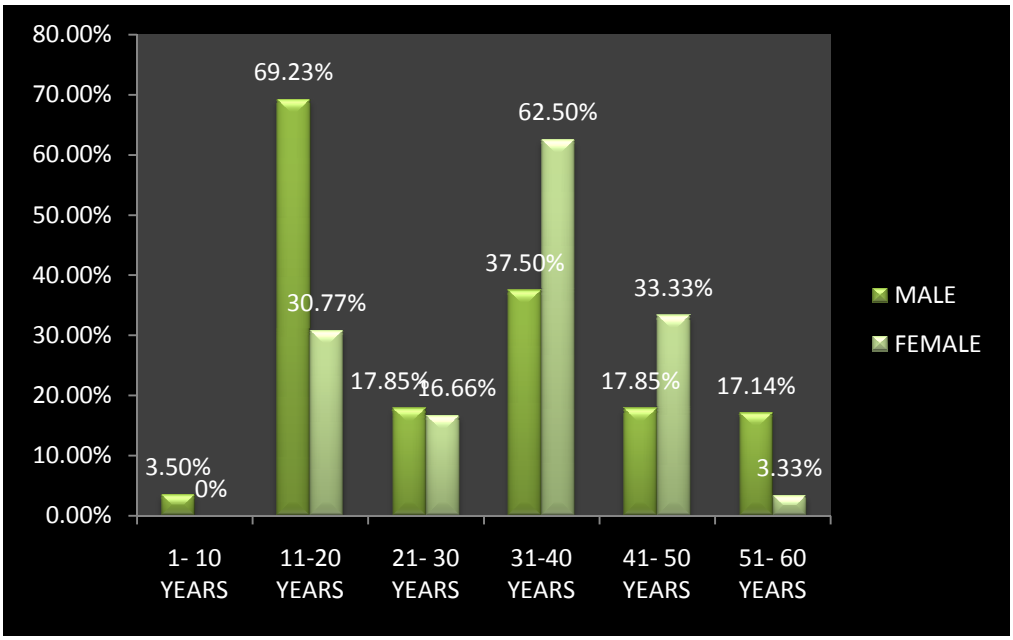
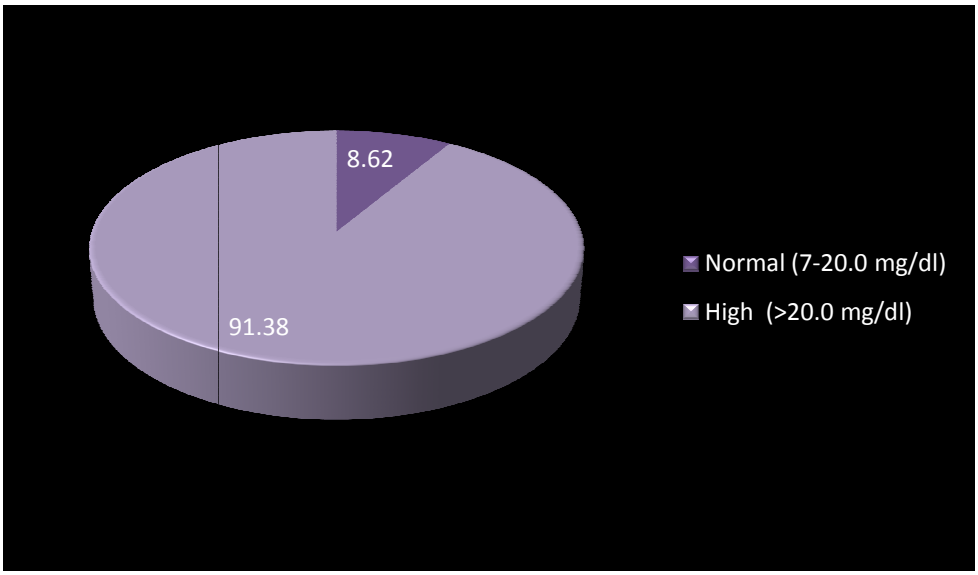


TABLE 4: DISTRIBUTION OF CASES BASED ON BLOOD UREA NITROGEN VALUE (mg/dl)

BUN (mg/dl)	Frequency	Percent
Normal (7-20.0)	5	8.62
High (>20.0)	53	91.38
total	58	100.0

CHART 4: DISTRIBUTION OF CASES BASED ON BLOOD UREA NITROGEN VALUE (mg/dl)

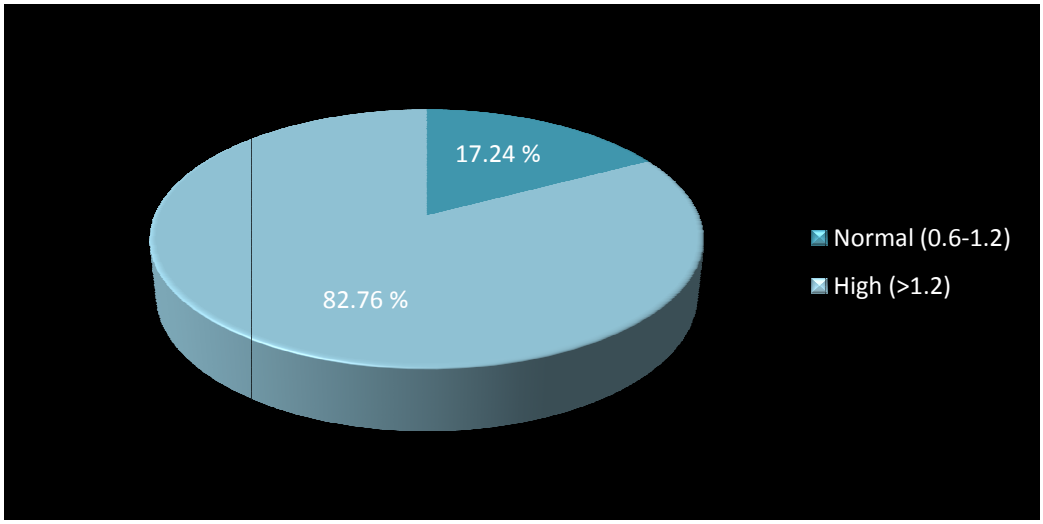


Of the 58 patients, 53 patients (91.38%) had high blood urea nitrogen value (> 20.0 mg/dl). Blood urea nitrogen value was normal in 5 patients (8.62%) whose diagnoses included focal segmental glomerulosclerosis (1patient), minimal change disease (1patient), membranoproliferative glomerulonephritis (1patient), IgA nephropathy(1patient) and acute tubular necrosis(1patient).

TABLE 5: DISTRIBUTION OF CASES BASED ON SERUM CREATININE LEVEL (mg/dl)

Serum creatinine (mg/dl)	Frequency	Percent
Normal (0.6-1.2)	10	17.24
High (>1.2)	48	82.76
total	58	100.0

CHART 5: DISTRIBUTION OF CASES BASED ON SERUM CREATININE LEVEL (mg/dl)

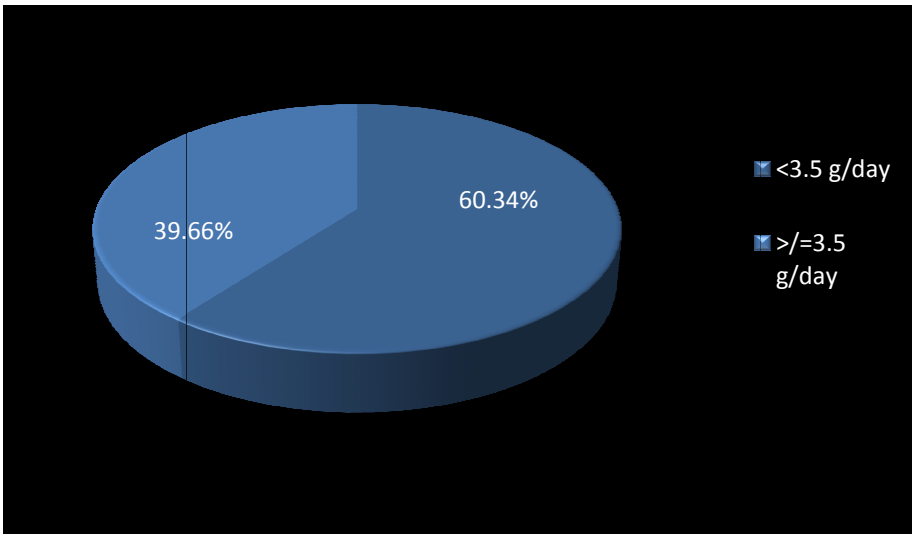


Of the 58 patients, 48 patients (82.76%) had high serum creatinine value and 10 patients (17.24%) had normal serum creatinine value whose diagnoses included diffuse proliferative glomerulonephritis (4 patients), focal segmental glomerulosclerosis(2 patients), IgA nephropathy (2 patients), mesangioproliferative glomerulonephritis (1 patient), and membranoproliferative glomerulonephritis (1patient).

TABLE 6: DISTRIBUTION OF CASES BASED ON 24 HOURS URINE PROTEIN LEVELS (g/day)

Urine protein (g/day)	Frequency	Percent
<3.5	35	60.34
>/=3.5	23	39.66
Total	58	100.0

CHART 6: DISTRIBUTION OF CASES BASED ON 24 HOURS URINE PROTEIN LEVELS (g/day)



Of the 58 patients, 23 patients (39.66%) had a 24 hour urine protein excretion of more than 3.5 grams/day (nephrotic range proteinuria) whose diagnoses included diffuse proliferative glomerulonephritis (6 patients), Focal segmental glomerulosclerosis (4 patients), membranoproliferative glomerulonephritis (4 patients), membranous nephropathy (3 patients), lupus nephritis (3 patients), minimal change disease (2 patients) and chronic glomerulonephritis (1 patient).

TABLE 7: DISTRIBUTION OF RENAL DISEASES.

Diagnosis	Frequency	Percent
Primary glomerulonephritis	46	79.31
Secondary glomerulonephritis	10	17.24
Tubulointerstitial nephritis	2	3.45
Total	58	100.0

CHART 7: DISTRIBUTION OF RENAL DISEASES.

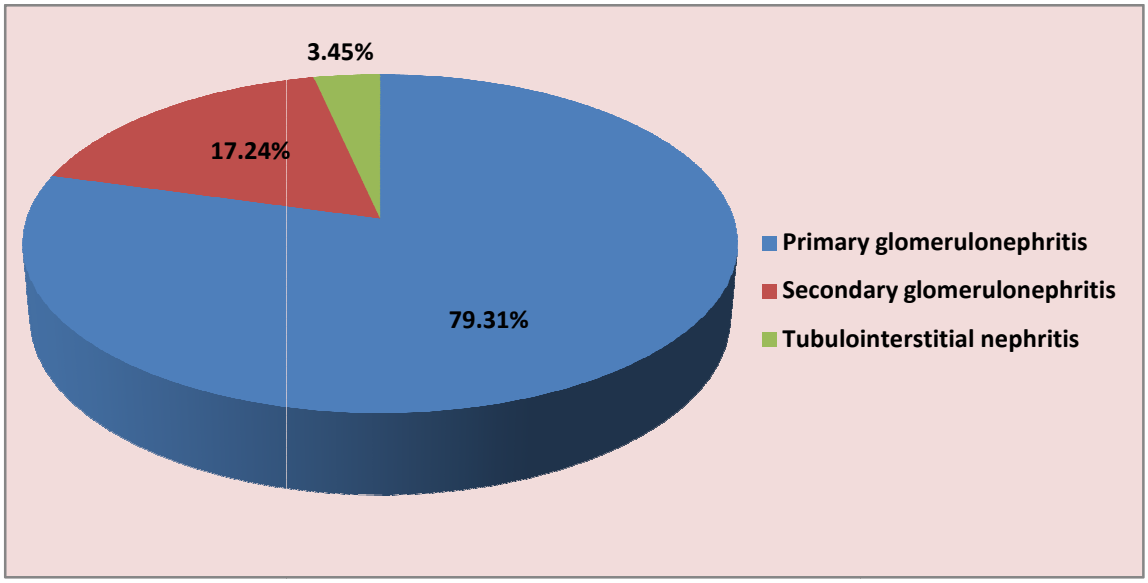


TABLE 8: DISTRIBUTION OF CASES BASED ON DIAGNOSIS

Final diagnosis	Frequency	Percent
A.Primary glomerulonephritis	**(N=46)**	**79.31**
Diffuse proliferative glomerulonephritis	13	28.26
Membranoproliferative glomerulonephritis	8	17.39
Focal segmental glomerulosclerosis	7	15.22
Membranous nephropathy	5	10.87
Minimal change disease	5	10.87
Ig A Nephropathy	2	4.35
C1q Nephropathy	2	4.35
Mesangioproliferative glomerulonephritis	2	4.35
Chronic glomerulonephritis	1	2.17
Sclerosing glomerulonephritis	1	2.17
B.Secondary glomerulonephritis	**(N=10)**	**17.24**
Lupus nephritis	7	70
Hypertension glomerulopathy	2	20
Myeloma cast nephropathy	1	10
C.Tubular interstitial disease	**(N=2)**	**3.45**
Acute tubular necrosis	2	100.0
Total	**(N=58)**	**100.0**

Of the 58 patients, diffuse proliferative glomerulonephritis constituted highest number of cases accounting for 28.26% (13 cases) followed by membranoproliferative glomerulonephritis accounting for 17.39% (8cases) overall and also among primary glomerulonephritis. Out of 10 secondary glomerulonephritis cases, Lupus nephritis was the most common lesion noted constituting 70% (7 cases) and all of them were females.

CHART 8: DISTRIBUTION OF CASES BASED ON DIAGNOSIS (PRIMARY GLOMERULONEPHRITIS) N=46

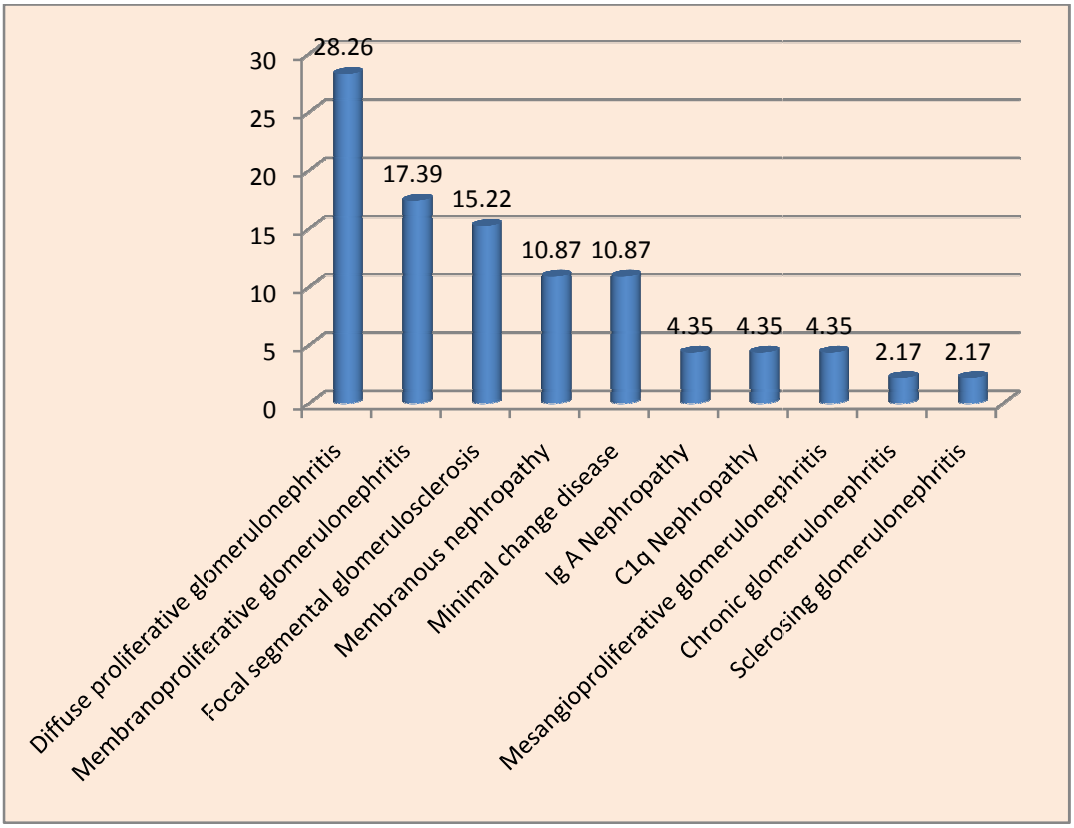
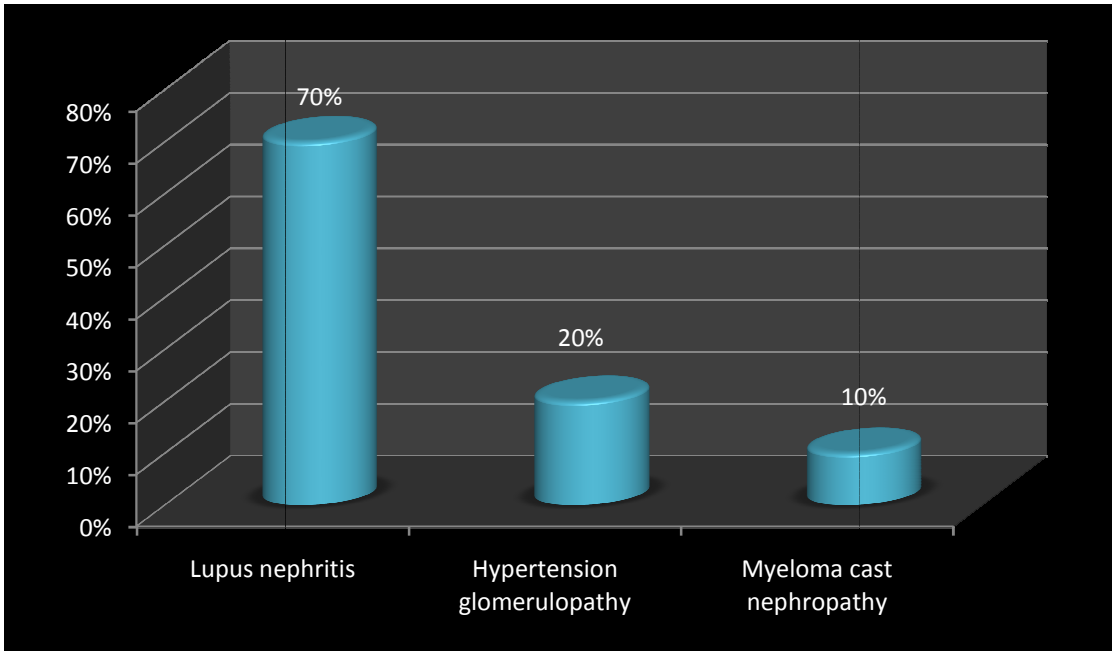


CHART 9: DISTRIBUTION OF CASES BASED ON DIAGNOSIS (SECONDARY GLOMERULONEPHRITIS) N=10



Out of 10 secondary glomerulonephritis cases, Lupus nephritis was the most common lesion noted constituting 70% (7 cases) and all of them were females.

TABLE 9: SPECIAL STAINS FINDINGS

Diagnosis	Stains performed	Findings
Focal segmental glomerulosclerosis (n=7)	A. PAS	PAS-positive sclerosed part of glomeruli (n=7)
	B. JMS	Wrinkled lines of GBM in sclerosed part of glomeruli (n=7)
Membranous nephropathy (n=5)	A. PAS	Thickened GBM (n=5)
	B. JMS	Spike formation in GBM (n=2) Thickened GBM (n=2) Moth eaten appearance of GBM (n=1)
Membraproliferative glomerulonephritis (n=8)	A. PAS	Thickened GBM (n=8)
	B. JMS	Double contour of GBM (n=3) Thickening of GBM (n=5)
Myeloma cast nephropathy (n=1)	A. PAS	Weak Positive in tubular casts (n=1)
	B. JMS	Negative in tubular casts (n=1)
	C. Congo red	Negative in tubular casts (n=1)
	D. Masson Trichrome stain	Casts appear green color (n=1)

Jones's methanamine silver stains helped in typing/staging of membranous glomerulopathy and membrano proliferative glomerulonephritis.

In 5 patients of MGN, spike formation was noted in GBM in 2 patients which is seen in stage II MGN, thickening of GBM was noted in 2 patients which is seen in stage IV MGN and moth eaten appearance of GBM was seen in 1 patient which is noted in stage III MGN.

Out of 8 patients of MPGN, double contour of GBM was noted in 3 patients which is seen type I MPGN and thickening of GBM was noted in 5 patients which is seen in type II MPGN. However, confirmatory typing/staging should be done when special stains findings are combined with electron microscopy findings of location of immune complex deposits.

In a patient of myeloma cast nephropathy, tubular casts stained negative with Congo red which was used to differentiate it from amyloid deposits. Other stains like PAS, JMS and Masson trichrome were performed which stained weakly positive, negative and green color respectively.

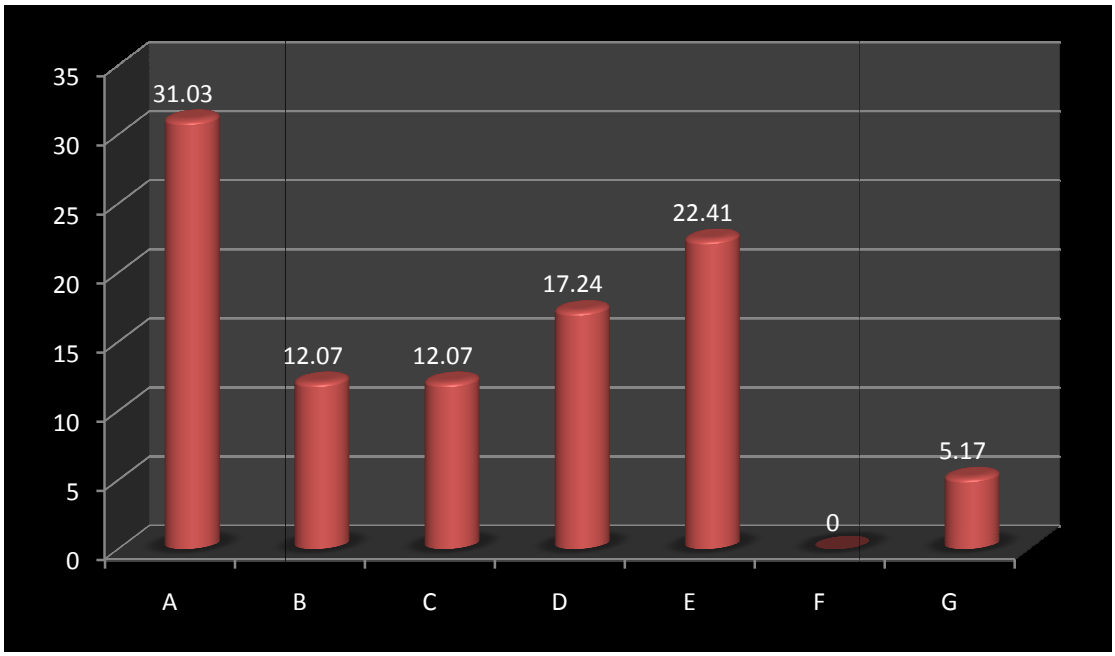
TABLE 10: IMMUNOFLUORESCENCE FINDINGS

Immunofluorescence	Frequency	Percent
Uniform granular staining of GBM	18	31.03
Uniform granular staining of GBM and mesangial staining	7	12.07
Mesangial staining only	7	12.07
Non-specific staining	10	17.24
Negative	13	22.41
Linear staining of glomerular basement membrane	0	0
No core	3	5.17
Total	58	100.0

Immunofluorescence studies showed positivity in 42 patients accounting for 72.41%. The predominant pattern was granular staining in glomerular basement membrane which was noted in 18 patients (31.03%).

CHART 10: IMMUNOFLUORESCENCE FINDINGS

(In Percentage)



A = Uniform granular staining of glomerular basement membrane.

B = Uniform granular staining of glomerular basement membrane and mesangial staining.

C = Mesangial staining only.

D = Non-specific staining.

E= Negative.

F = Linear staining of glomerular basement membrane.

G = No core.

Granular GBM positivity was noted in 18 patients whose diagnoses included diffuse proliferative glomerulonephritis (5 patients), membranous nephropathy (5 patients), membranoproliferative glomerulonephritis (4 patients) and Lupus nephritis (4 patients).

Non-specific staining in IF was noted in 10 patients whose diagnoses included focal segmental glomerulosclerosis (6 out of 7 cases), Acute tubular necrosis (1 out of 2 patients), sclerosing glomerulonephritis(1 patient), hypertensive glomerulopathy (1 out of 2 patients) and myeloma cast nephropathy (1 patient).

Negative staining was noted in 13 patients whose diagnoses included minimal change disease (all 5 patients), diffuse proliferative glomerulonephritis (4 out of 13 cases), focal segmental glomerulosclerosis (1 patient), hypertensive nephropathy (1 patient), acute tubular necrosis (1 patient) and chronic glomerulonephritis (1 patient).

No core was obtained for IF in two cases whose diagnoses included mesangioproliferative glomerulonephritis and diffuse proliferative glomerulonephritis.

Out of 58 patients subjected for light microscopy and immunofluorescence studies, the immunofluorescence findings were of

diagnostic utility in 4 patients. The final diagnosis was modified based on immunofluorescence findings in 1 patient.

In two patients of mesangioproliferative glomerulonephritis, one of them showed intense mesangial staining of IgA and weak mesangial staining for C3 and the other patient showed intense mesangial staining of C1q. In one patient with light microscopic diagnosis of focal proliferative glomerulonephritis the IF finding was intense mesangial staining of IgA. In another patient with light microscopic diagnosis of focal segmental glomerulosclerosis, the IF findings showed intense mesangial staining of C1q. In these patients the diagnosis was given as IgA nephropathy and C1q nephropathy accordingly. Hence, the diagnostic utility of IF was noted in 4 cases (6.90%).

In a case of minimal change disease, the diagnosis was modified to Lupus nephritis – class I after performing the immunofluorescence studies which showed C3 mesangial staining. Hence the IF studies helped in modification of the final diagnosis in 1 case (1.72%).

EQUIPMENTS

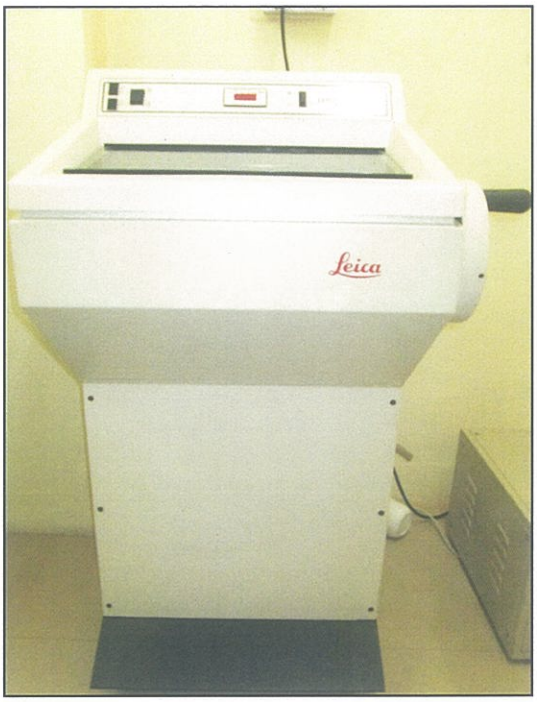


Fig 1.1 : Cryostat Leica CM 1510

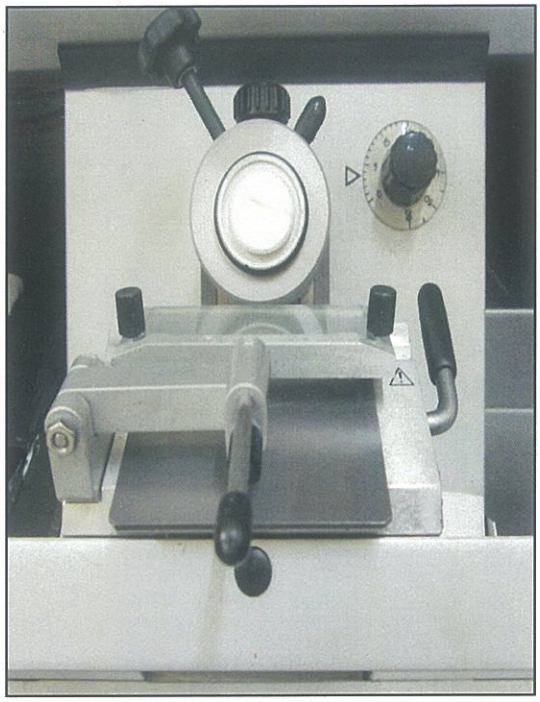


Fig 1.2 : Cryostat Leica CM 1510 with a block in the chuck

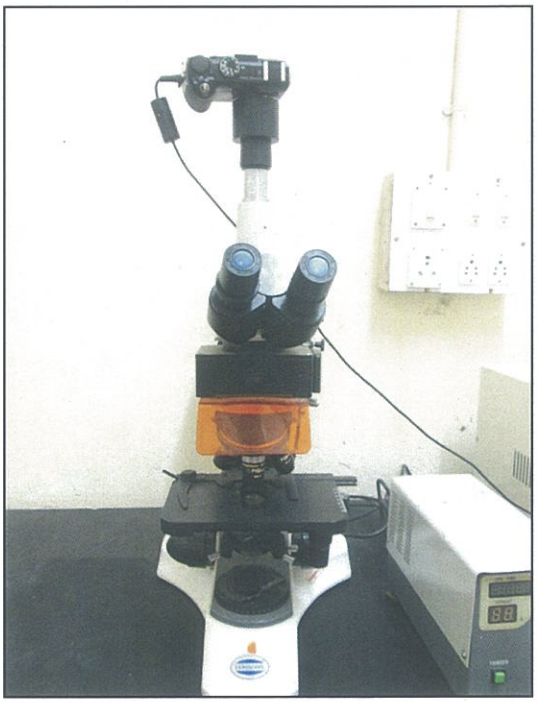


Fig 1.3 : Immunofluorescent microscope

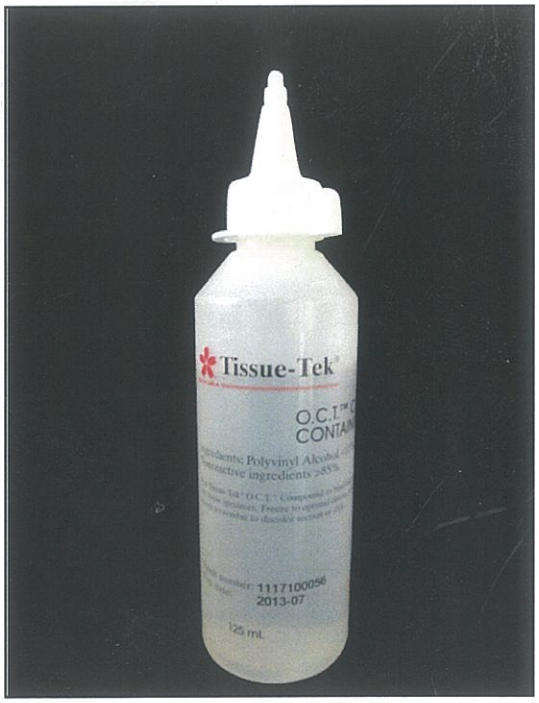


Fig 1.4 :Tissue Tek -embedding Medium

MINIMAL CHANGE DISEASE

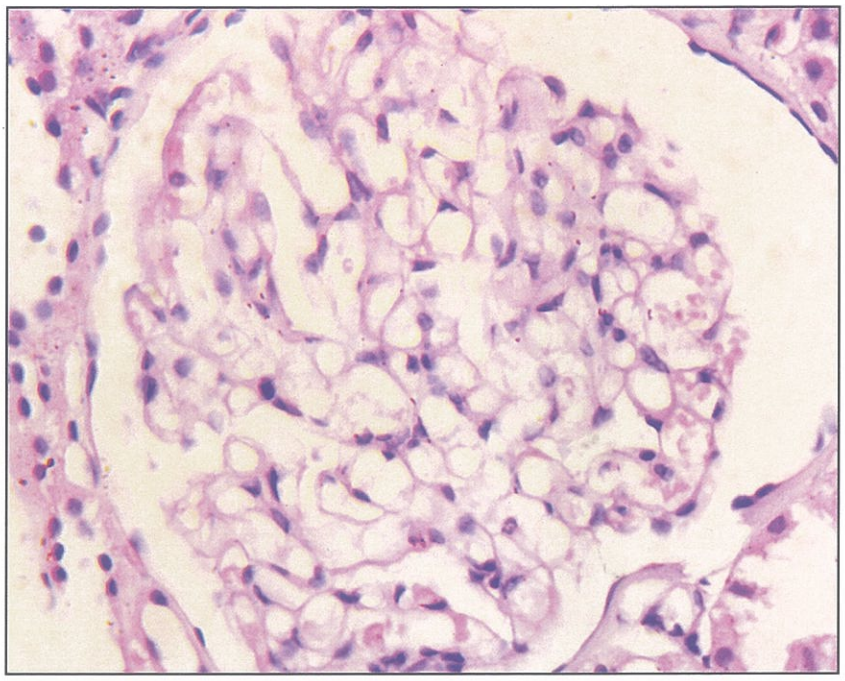


Fig : 2.1 Showing Normal Glomerulus H & E (x 40)

HYPERTENSIVE NEPHROPATHY

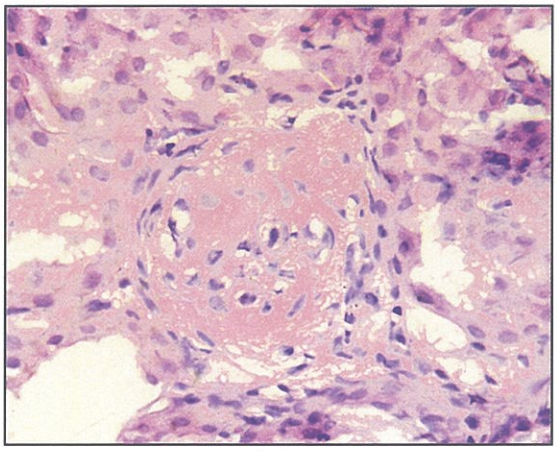


Fig : 3.1 Showing Sclerosed Glomerulus H & E (x 40)

ACUTE TUBULAR NECROSIS

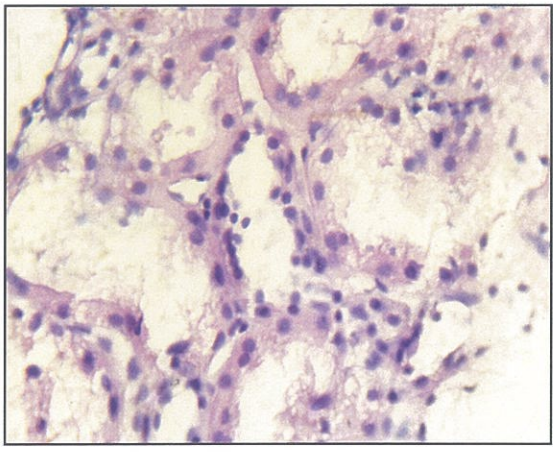


Fig 4.1 : Showing tubular epithelial cell necrosis with denudement H & E (x 40)

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

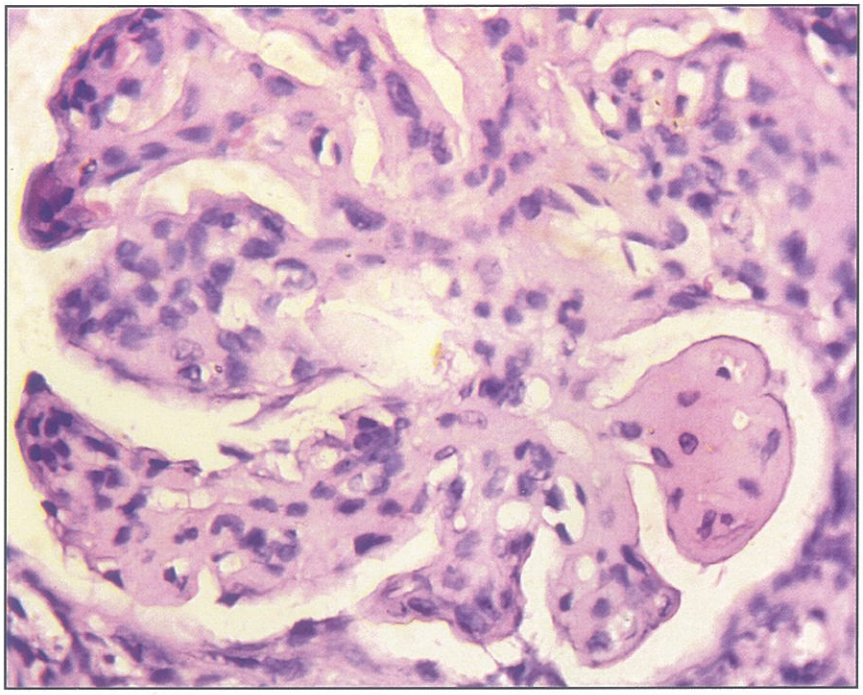


Fig 5.1 : Glomerulus showing focal and segmental sclerosis H & E (x 40)

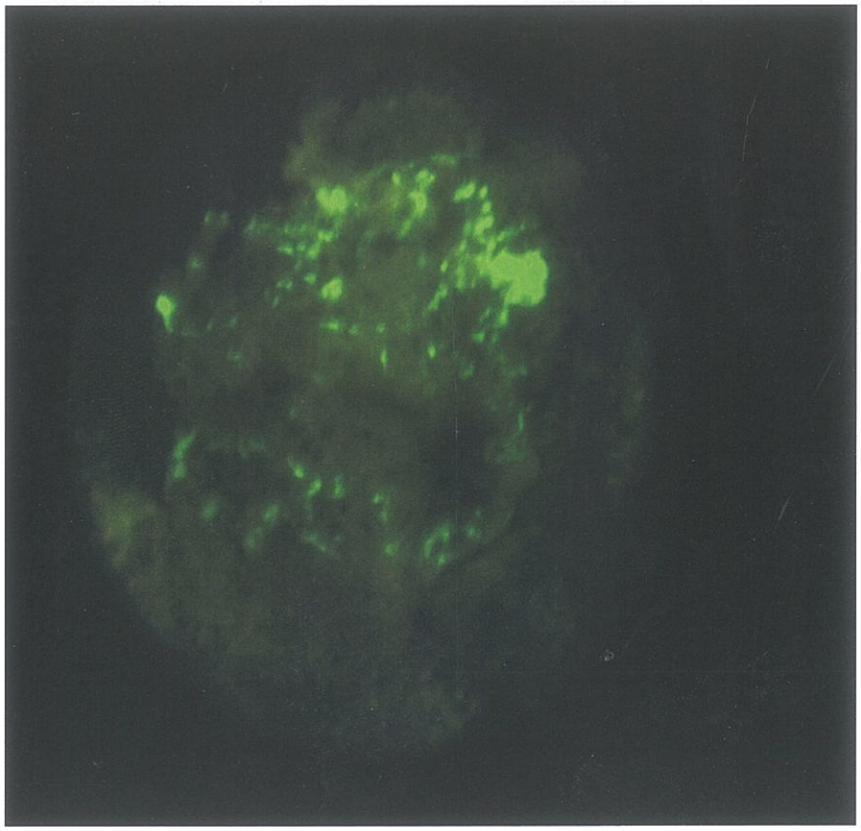


Fig 5.2 : Immunofluorescence of focal IgM Deposits (x 40)

MEMBRANOUS GLOMERULONEPHRITIS

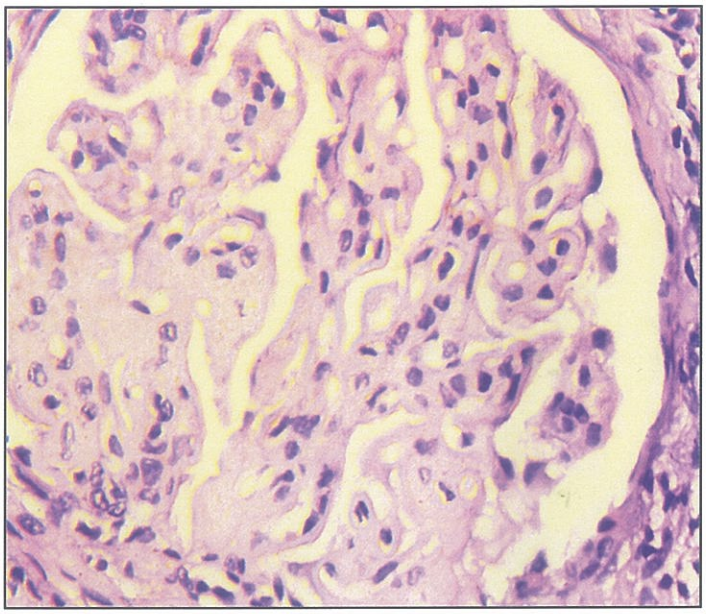


Fig 6.1 : Showing Uniform thickening of
Glomerular basement membrane H & E (x 40)

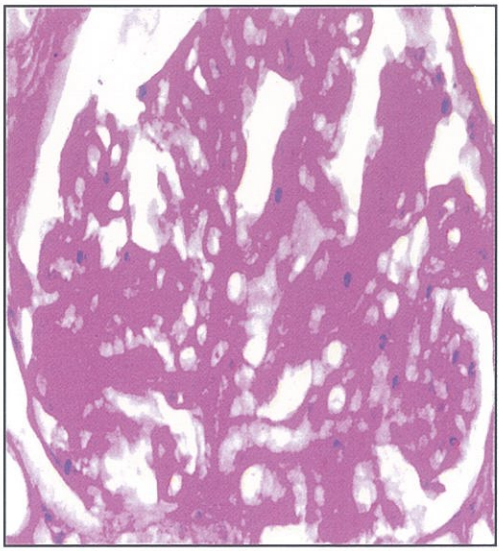


Fig 6.2 : Showing Uniform thickening of
Glomerular basement membrane - PAS (x 40)

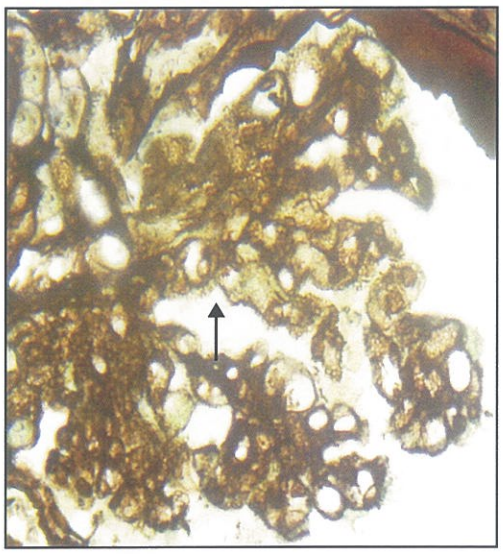


Fig 6.3 : Arrow Showing Spikes in
Glomerular basement membrane - JMS (x 40)

MEMBRANOUS GLOMERULONEPHRITIS

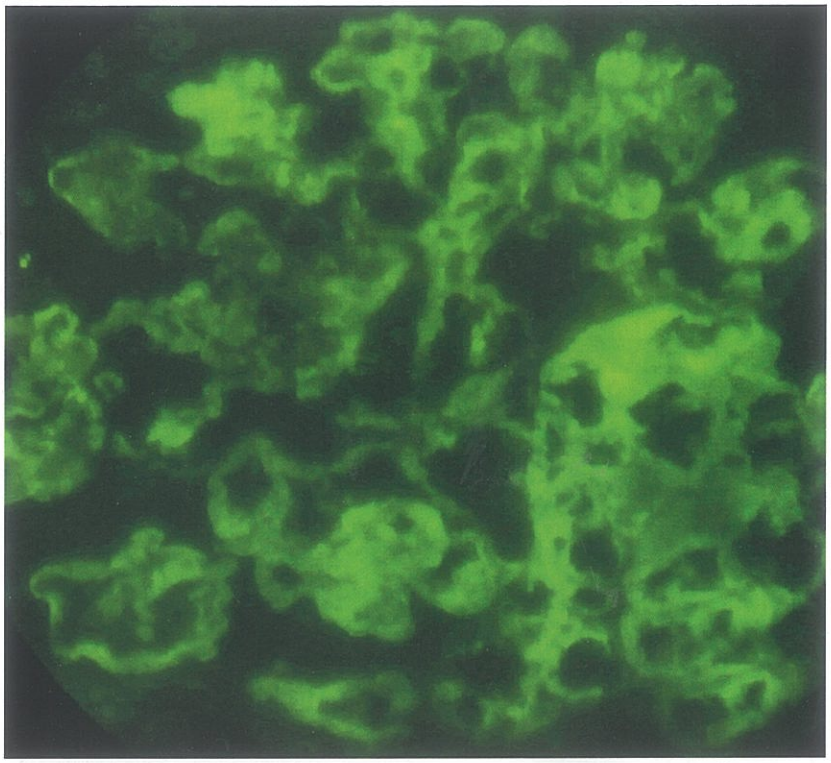


Fig 6.4 : showing uniform granular deposits of IgG along Glomerular basement membrane (x 40)

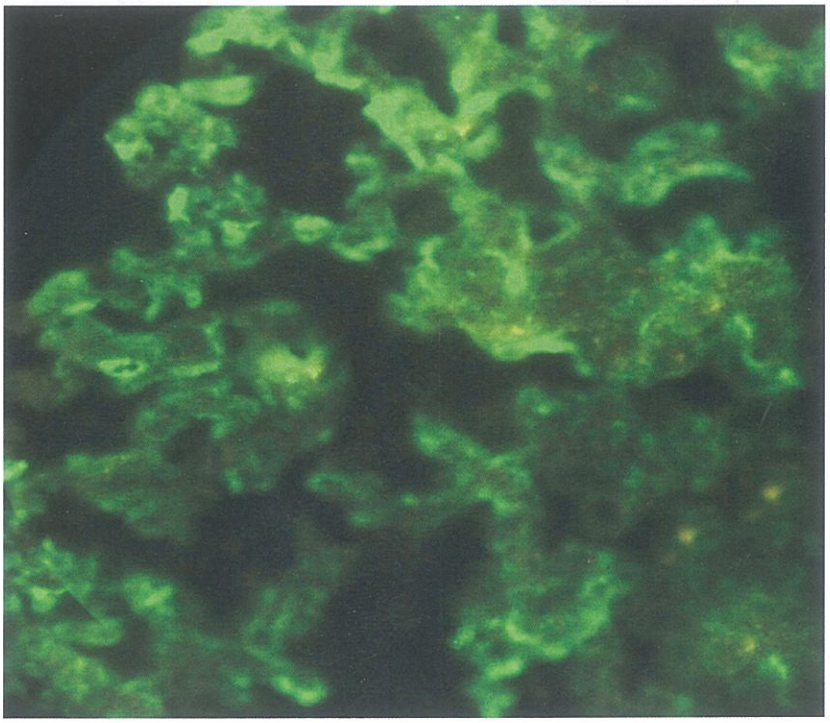


Fig 6.5 : showing uniform granular deposits of C3 along Glomerular basement membrane (x 40)

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

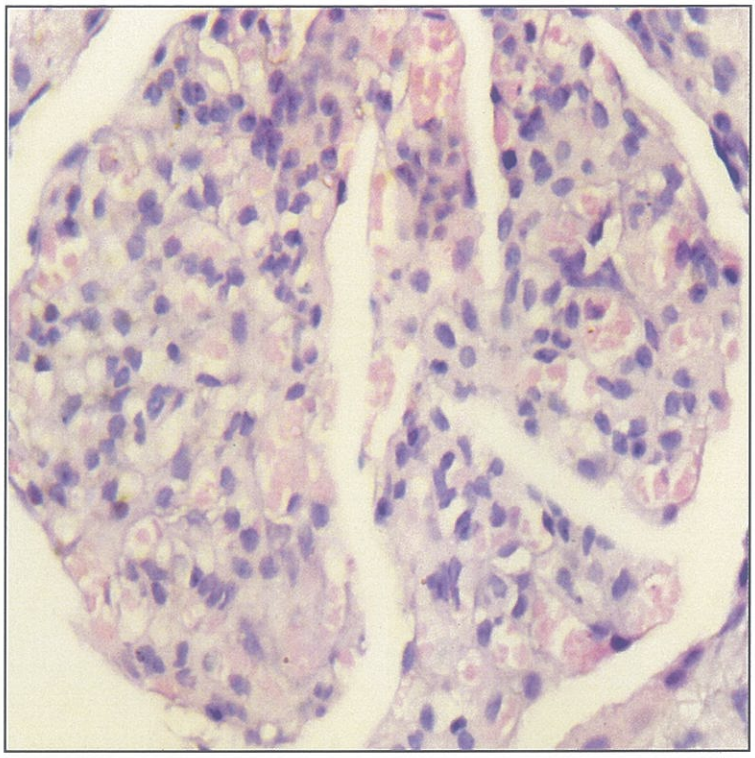


Fig 7.1 : Glomerulus showing mesangial and Endocapillary Proliferation H & E (x 40)

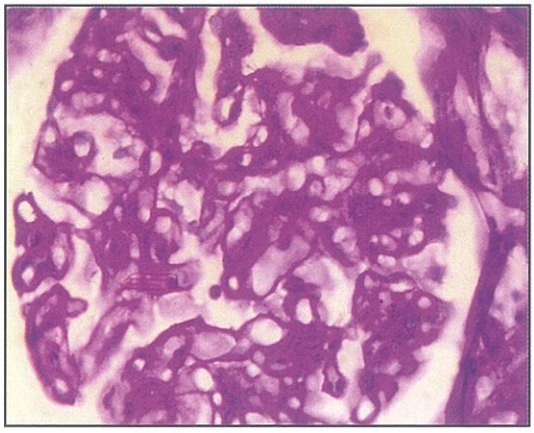


Fig 7.2 : Glomerulus showing Thickened GBM - PAS (x 40)

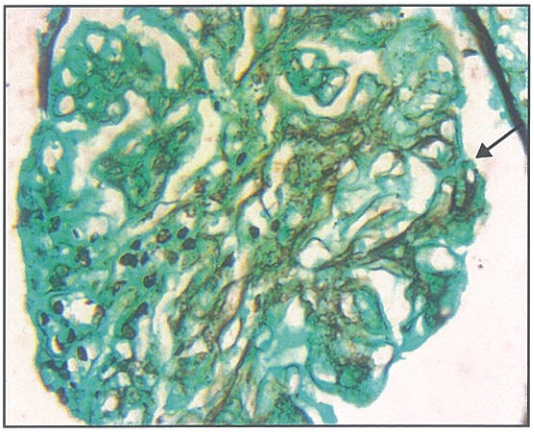


Fig 7.3 : Arrow Showing Double Contour of GBM - JMS (x 40)

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

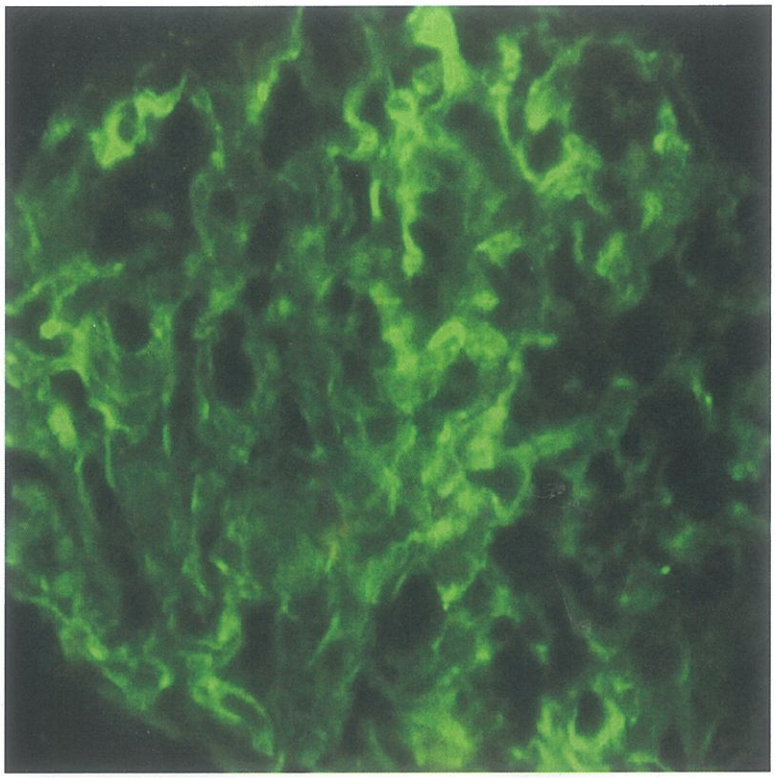


Fig 7.4 : Immunofluorescence of IgG deposits along Glomerular basement membrane & mesangium (x 40)

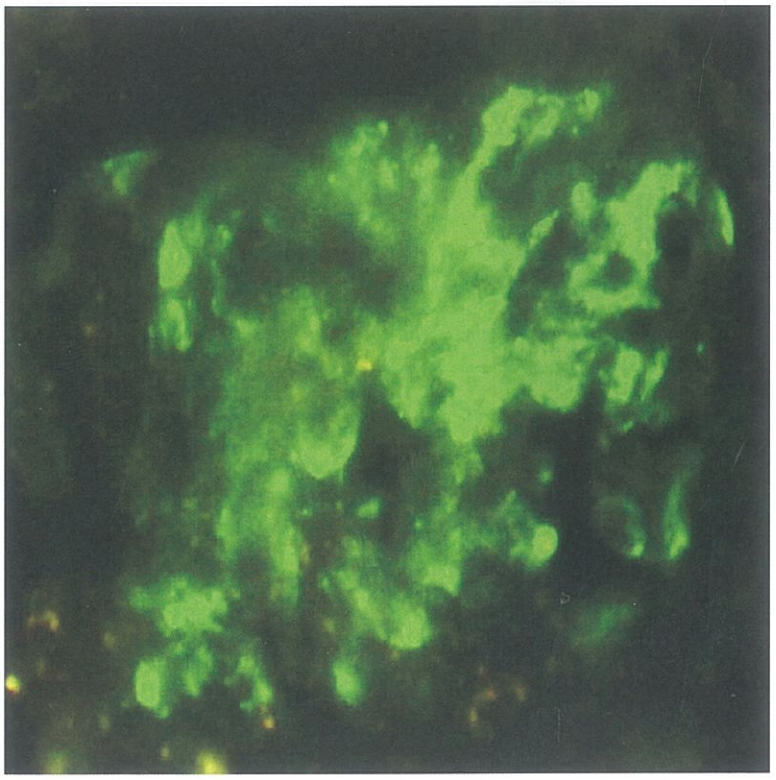


Fig 7.5 : Immunofluorescence of C3 deposits along Glomerular basement membrane & mesangium (x40)

DIFFUSE PROLIFERATIVE GLOMERULONEPHRITIS

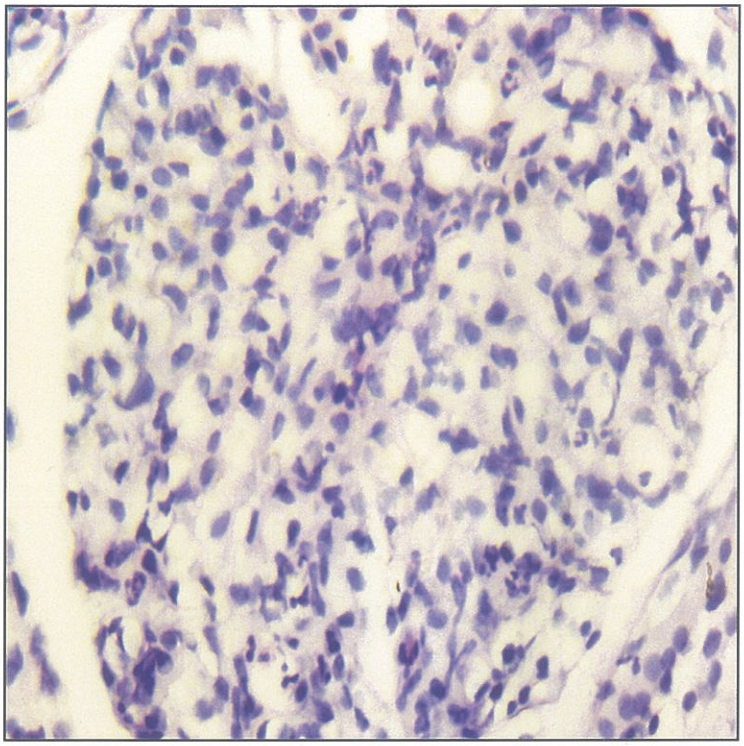


Fig 8.1 : Glomerulus showing Endocapillary Proliferation and Leucocytic infiltration H & E (x 40)

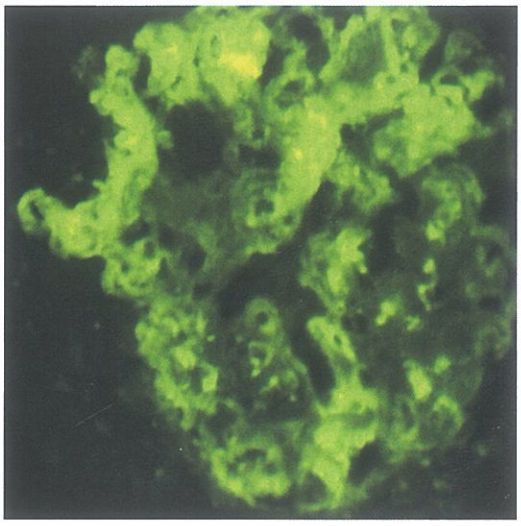


Fig 8.2 : Immunofluorescence of IgG deposits along Glomerular basement membrane (Garland pattern x 40)

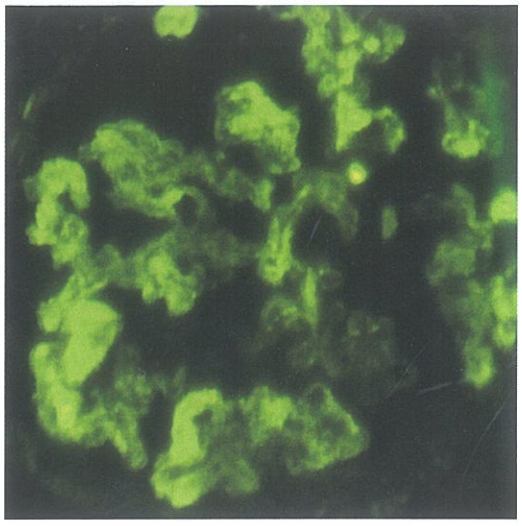


Fig 8.3 : Immunofluorescence of C3 deposits along Glomerular basement membrane (Garland pattern x 40)

IgA NEPHROPATHY

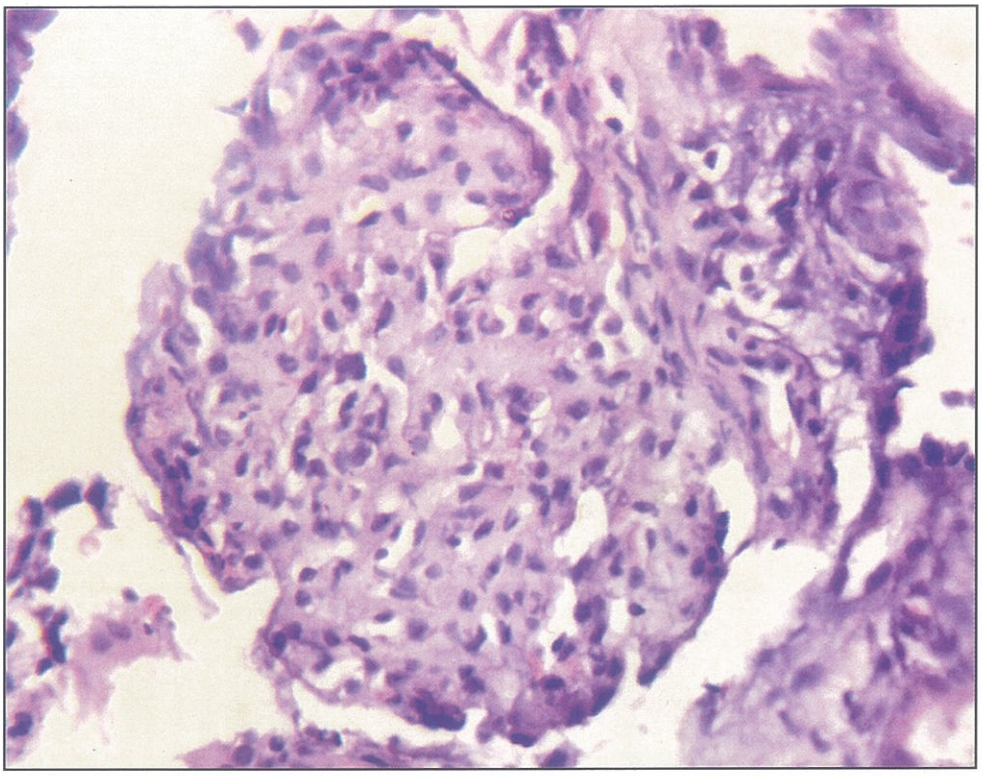


Fig 9.1 : Glomerulus showing mesangial Proliferation H & E (x 40)

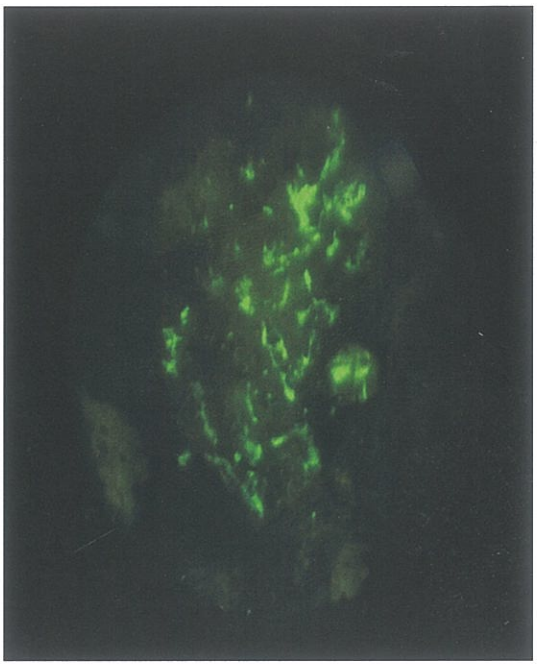


Fig 9.2 : Immunofluorescence of IgA deposits in mesangium (x 40)

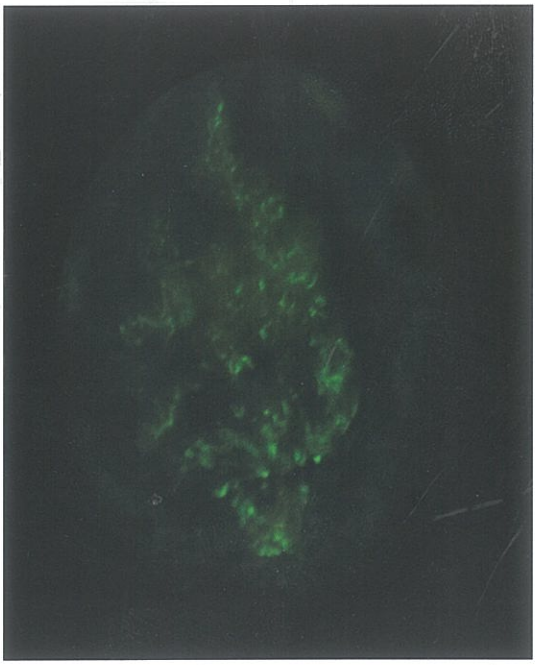


Fig 9.3 : Immunofluorescence of C3 deposits in mesangium (x 40)

C1q NEPHROPATHY

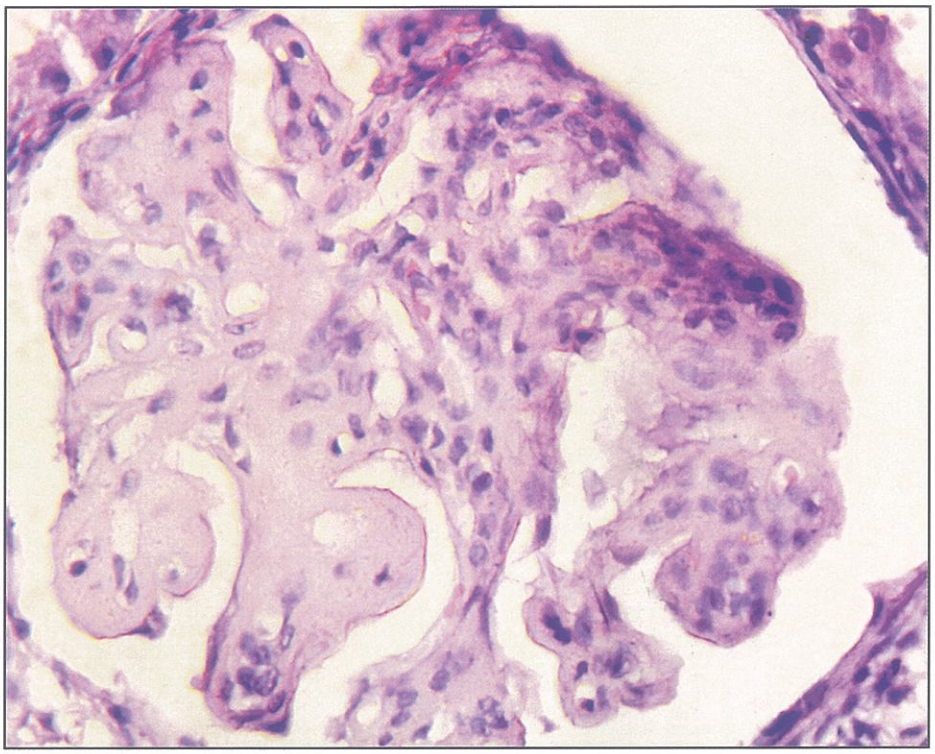


Fig 10.1 : Glomerulus showing focal and segmental sclerosis H & E (x 40)

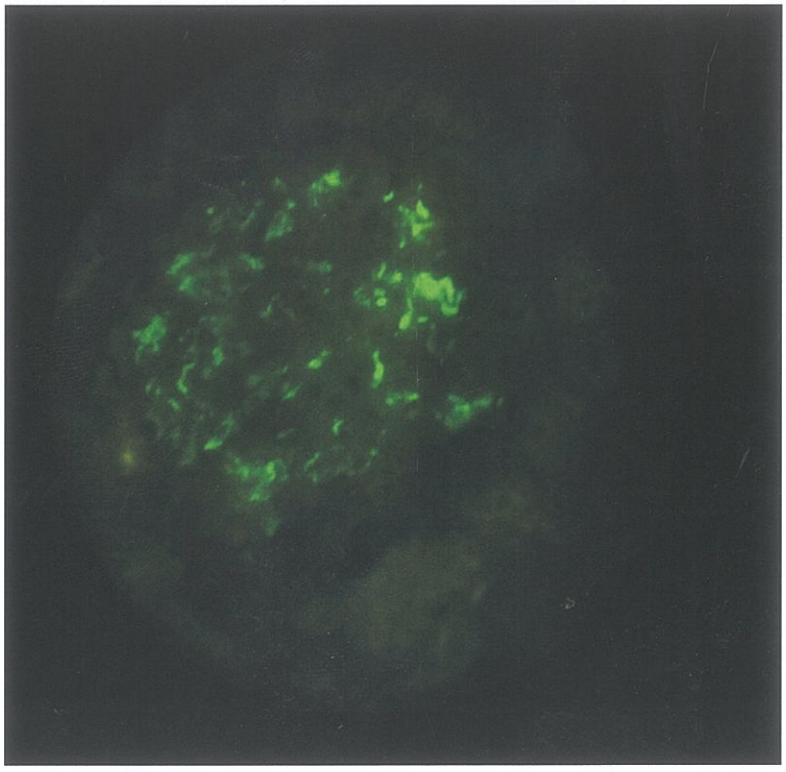


Fig 10.2 : Immunofluorescence of C1q deposits in mesangium (x 40)

LUPUS NEPHRITIS

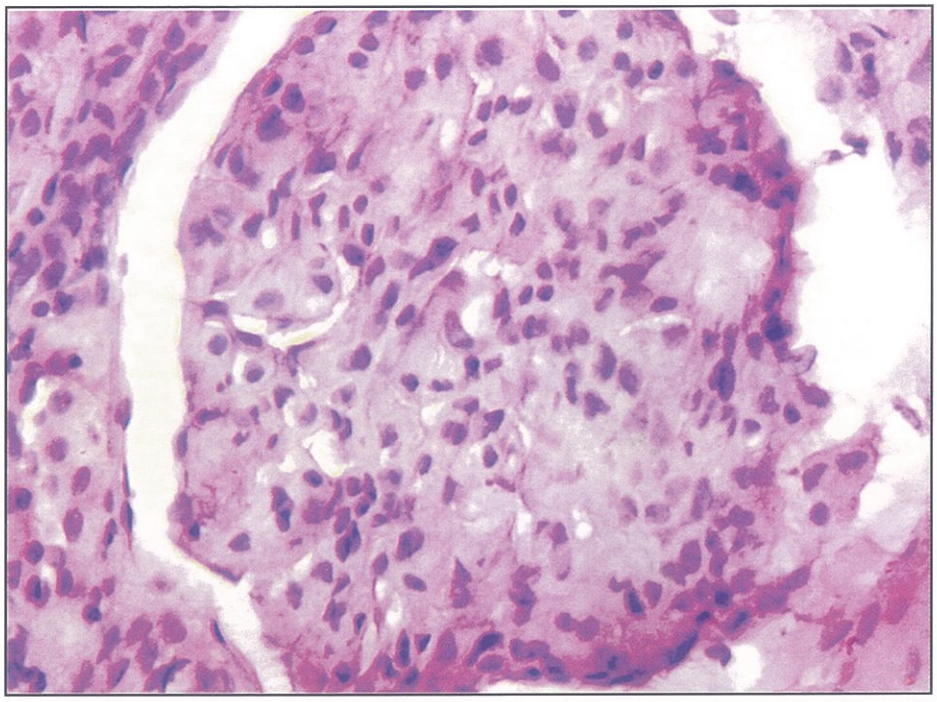


Fig 11.1 : Glomerulus showing diffuse Endocapillary proliferation H & E (x 40)

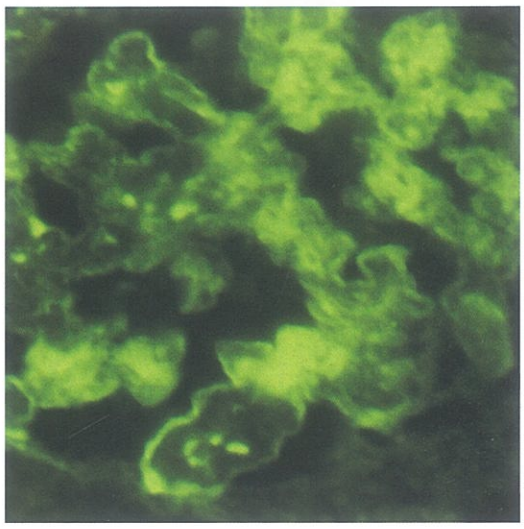


Fig 11.2 : Immunofluorescence showing full house pattern - IgG deposits along Glomerular basement membrane (x 40)

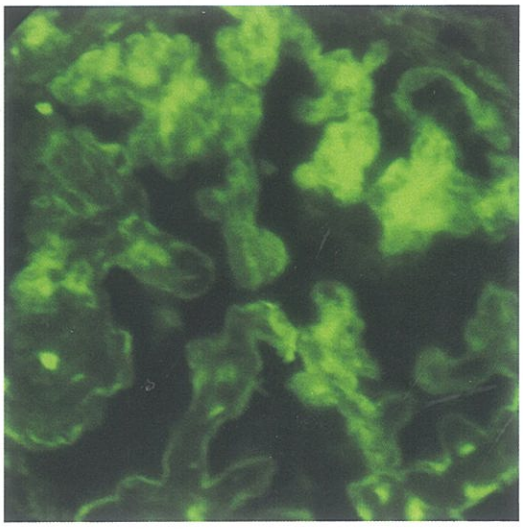


Fig 11.3 : Immunofluorescence showing full house pattern - IgA deposits along Glomerular basement membrane (x 40)

LUPUS NEPHRITIS

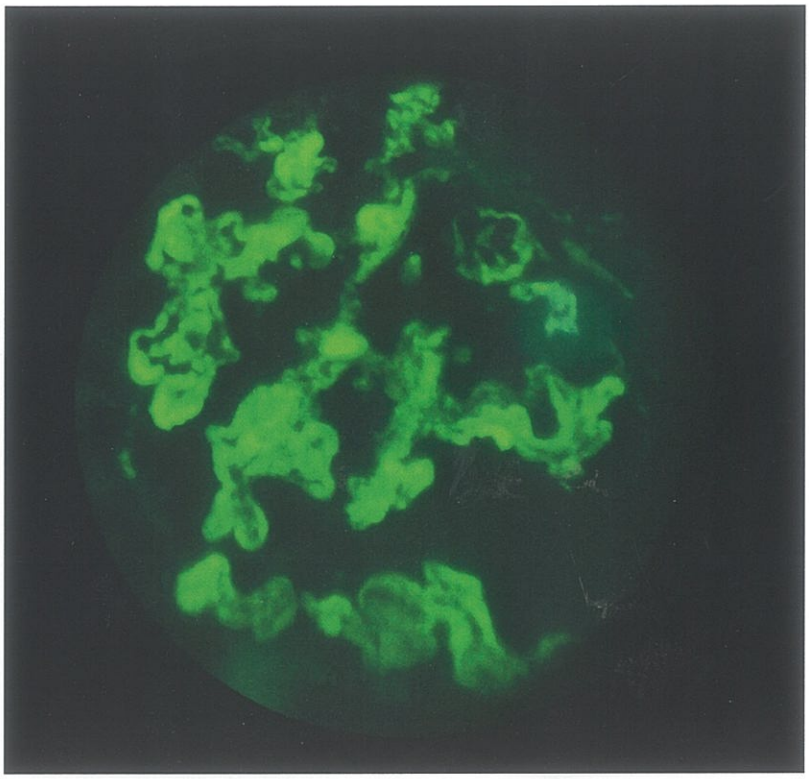


Fig 11.4 : Immunofluorescence showing full house pattern - IgM deposits along Glomerular basement membrane (x 40)

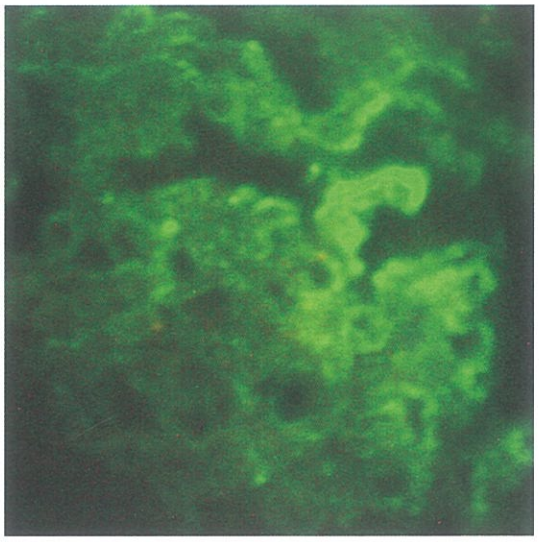


Fig 11.5 : Immunofluorescence showing full house pattern - C3 deposits along Glomerular basement membrane (x 40)

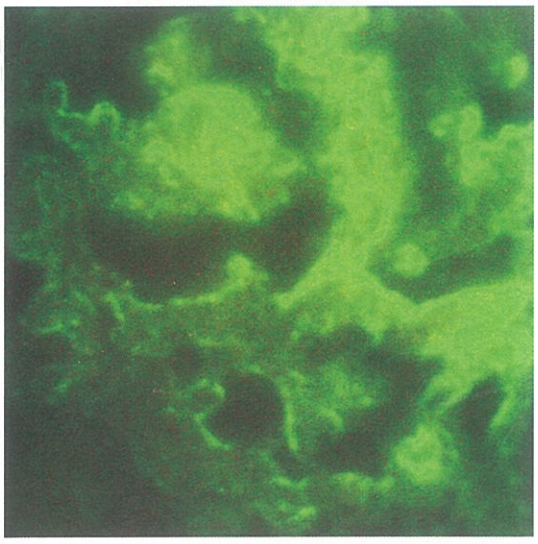


Fig 11.6 : Immunofluorescence showing full house pattern - C1q deposits along Glomerular basement membrane (x 40)

MYELOMA CAST NEPHROPATHY

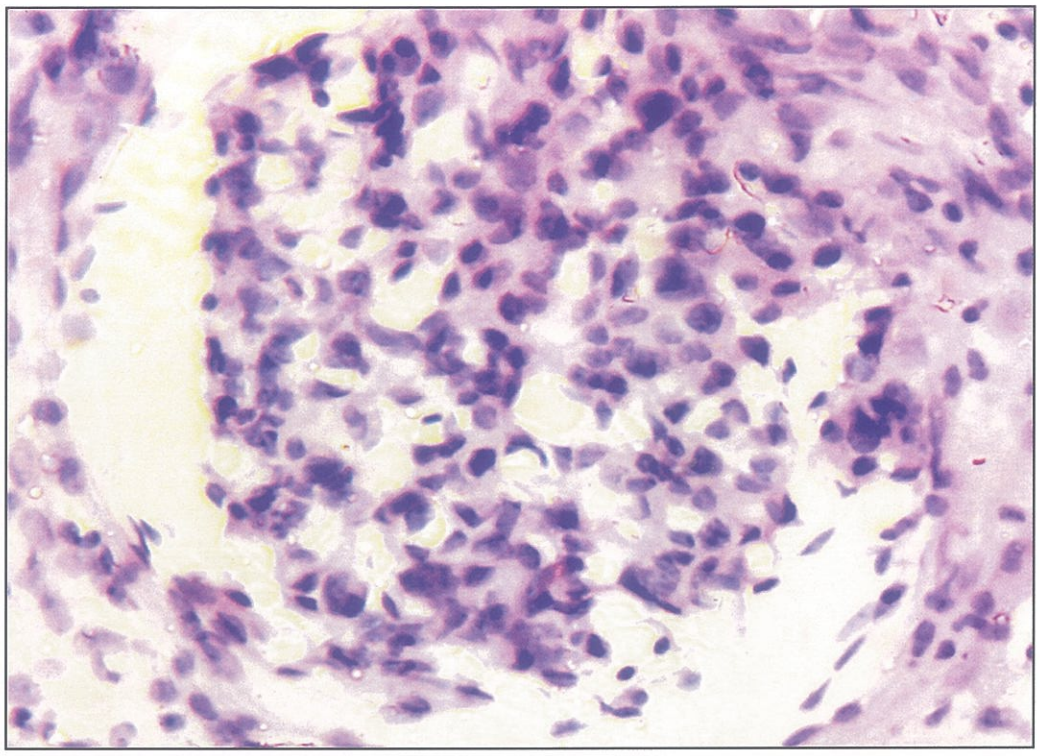


Fig 12.1 : Glomerulus showing Plasma Cell Infiltration H & E (x 40)

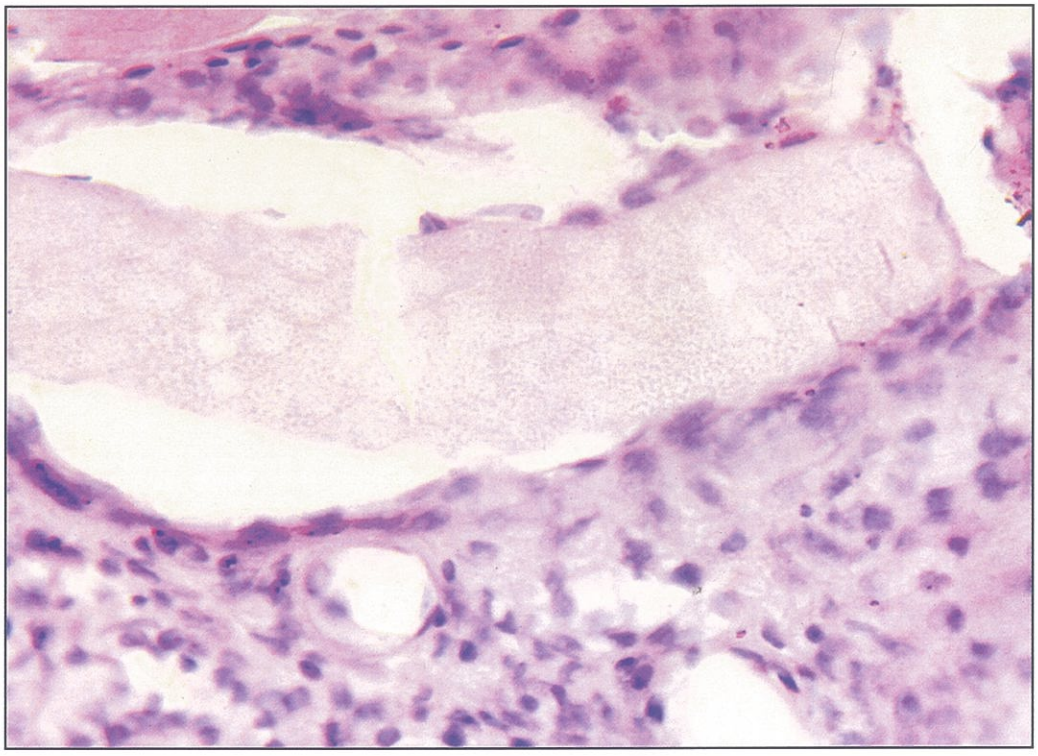


Fig 12.2 : showing Fractured Cast in the tubule H & E (x 40)

MYELOMA CAST NEPHROPATHY

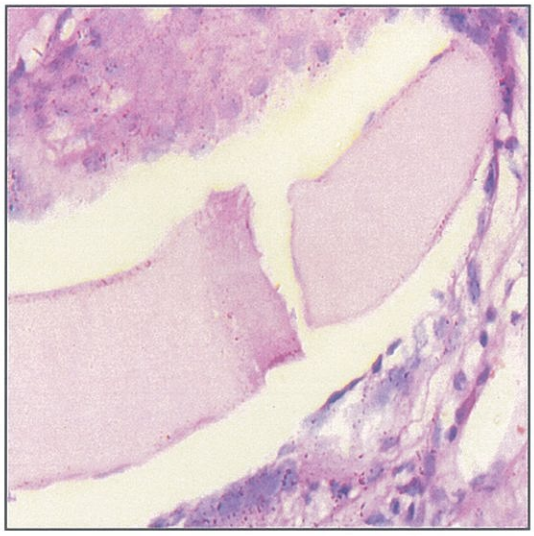


Fig 12.3 : Showing Weak PAS Positivity in Fractured Cast (x 40)

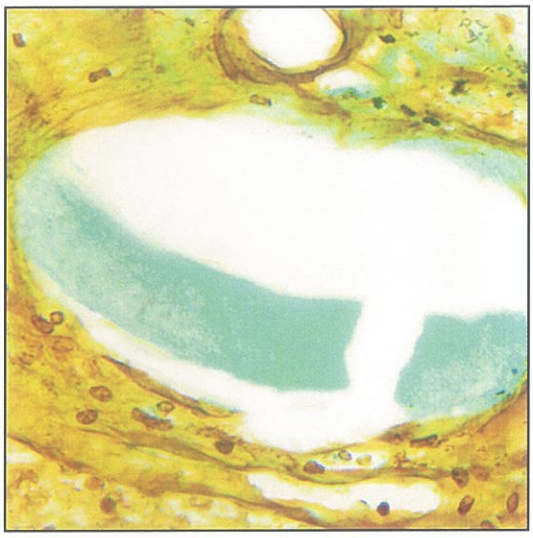


Fig 12.4 : Fractured Cast Showing Negative Staining with JMS (x 40)

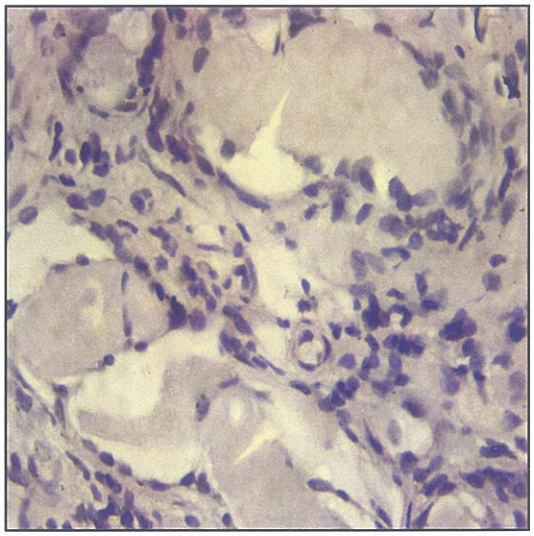


Fig 12.5 : Fractured Cast Staining Negative with congo red (x 40)

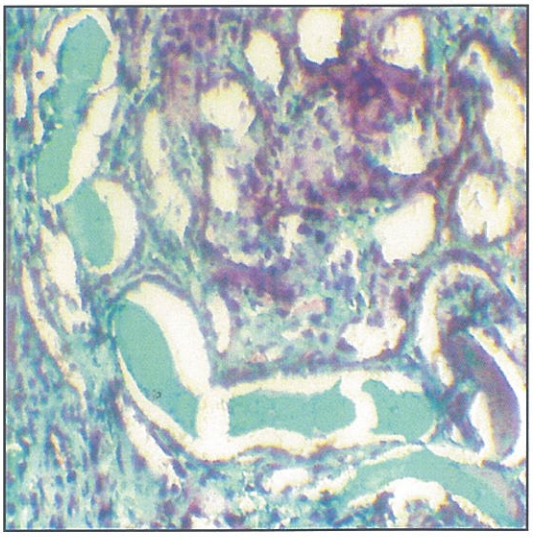


Fig 12.6 : showing Fractured Cast Staining Green Colour with Masson Trichrome stain (x 40)

DISCUSSION

DISCUSSION

In the present study 58 renal biopsies were subjected to light microscopy and immunofluorescence studies to arrive at the final diagnosis. Special stains were done for all cases. The duration of the study was from October 2012 to June 2014.

In the present study clinical, histomorphological features and immunofluorescence findings of various renal diseases were studied. The results obtained with the present study was compared with the other studies and discussed as follows.

The parameters compared with the other studies are age distribution, gender distribution, frequency and most common primary glomerulonephritis, frequency and most common secondary glomerulonephritis, comparision of frequency of tubulointerstitial nephritis.

TABLE11: COMPARISION OF AGE DISTRIBUTION WITH OTHER STUDIES

Authors	Year	Age range (years)
A.R.Reshi et al[27]	2008	1 - 72
Niang Adbu et al[45]	2003	5 – 60
Ivan Rychlı´k et al[22]	2000	1 – 85
Pierre Simon et al[19]	2002	10 - 80
Present study	2014	9 - 57

In the present study the age of the patients ranged from 9 to 57 years which is in league with the study done by Pierre Simon et al.

TABLE 12: COMPARISION OF GENDER DISTRIBUTION WITH OTHER STUDIES

Authors	Year	Male (%)	Female (%)
A.R.Reshi et al[27]	2008	66.2	33.8
Riyad Said et al[54]	2000	53.4	46.6
Ivan Rychlı´k et al[22]	2000	48.7	51.3
Ikechi Okpechi et al[26]	2009	45.20	54.80
Present study	2014	48.27	51.72

In the present study, female predominance was noted with male to female ratio being 0.933:1. This is in league with the study done by Ikechi Okpechi et al and Ivan Rychlı´k et al.

TABLE13: COMPARISION OF FREQUENCY AND MOST COMMON PRIMARY GLOMERULONEPHRITIS WITH OTHER STUDIES

Authors	Year	Primary lesions (%)	Diagnosis
A.R.Reshi et al[27]	2008	91.73	MCD
Riyad Said et al[54]	2000	72.2	MPGN
Nasar Yousuf Alwahaibi et al[29]	2010	69.1	FSGS
Niang Adbu et al[45]	2003	69.5	FSGS
Lt Col GU Deshpande et al[50]	2000	61.5	DPGN
Ivan Rychlı´k et al[22]	2000	59.8	IgA Nephropathy
Present study	2014	79.31	DPGN

In the present study primary glomerular lesions constituted 79.31% of the renal diseases which was in concordance with the other studies and diffuse proliferative glomerulonephritis was the most common primary glomerular lesion noted which is in league with the study done by Lt Col GU Deshpande et al. The present study did not correlate with the other studies and it can be attributed to two reasons. Firstly, clinically suspected cases of membranous glomerulonephritis and minimal change disease presenting with nephrotic syndrome were not willing for the renal biopsy. Hence renal biopsy could not be attempted in such cases. Secondly, most of the patients were from low socioeconomic status with

high incidence of infections and poor public awareness regarding health care.[40] Hence, infective etiologic diagnosis was the most common lesion noted in the present study.

TABLE14: COMPARISION OF FREQUENCY AND MOST COMMON SECONDARY GLOMERULONEPHRITIS WITH OTHER STUDIES.

Authors	Year	Secondary lesions (%)	Diagnosis
A.R.Reshi et al[27]	2008	8.28	Diabetic nephropathy
Ahmed Al Arrayed et al[32]	2004	33.6	Lupus nephritis
Nasar Yousuf Alwahaibi et al[29]	2010	30.9	Lupus nephritis
Niang Adbu et al[45]	2003	23.5	Lupus nephritis
Ivan Rychlı´k et al[22]	2000	25.4	Lupus nephritis
Present study	2014	17.24	Lupus nephritis

In the present study, secondary glomerular lesions constituted 17.24% of the renal diseases which correlated with Niang Adbu et al and Ivan Rychlı´k et al and the most common secondary glomerular lesion

was Lupus nephritis which is in concordance with the studies done by Ahmed Al Arrayed et al, Niang Abdu et al, Nasar Yousuf Alwahaibi et al and Ivan Rychlı´k et al. All the patients of lupus nephritis were females (7 patients).

TABLE 15: COMPARISION OF FREQUENCY OF TUBULOINTERSTITIAL NEPHRITIS

Authors	Year	TIN (%)
Ivan Rychlı´k et al [22]	2000	4.4
Patricia Malafronte et al [23]	2005	2.3
Ikechi Okpechi et al [26]	2007	5.6
Lei-shi li et al [20]	2002	3.43
Present study	2014	3.45

In the present study, tubulointerstitial nephritis cases constituted 3.45% which is in concordance with all the above mentioned studies.

SUMMARY AND CONCLUSION

SUMMARY

- The present study is a cross sectional descriptive study.
- The aim of this study is to determine the utility of direct immunofluorescence studies in renal diseases and also to study their various clinical presentations and histomorphological findings.
- During the twenty months period of study from October 2012 to June 2014, 58 biopsies were received from the Department of Nephrology, Coimbatore Medical College and Hospital, Coimbatore.
- The youngest patient was 9 years and the oldest patient was 57 years.
- The most common age group affected was 31 years to 40 years and the mean age at presentation was 33.03 years. The most common diagnosis in this age group was diffuse proliferative glomerulonephritis.
- Females were slightly more affected than males and the male to female ratio was found to be 0.933:1. The most common glomerular lesion noted in males was diffuse proliferative glomerulonephritis (10 out of 28 cases) and in females was Lupus nephritis (7 out of 30 cases).

- 23 patients (39.66%) out of 58 presented with nephrotic range proteinuria whose diagnoses included diffuse proliferative glomerulonephritis (6 patients), Focal segmental glomerulosclerosis (4 patients), membranoproliferative glomerulonephritis (4 patients), membranous nephropathy (3 patients), lupus nephritis (3 patients), minimal change disease (2 patients) and chronic glomerulonephritis (1 patient).
- 53 patients (91.38%) had high blood urea nitrogen value more than 20.0 mg/dl.
- 48 patients (82.76%) had high serum creatinine value more than 1.2mg/dl.
- Out of 58 biopsy specimens, 46 (79.31%) showed primary glomerular lesions, 10 (17.24%) showed secondary glomerular lesion and 2 (3.45%) showed tubulointerstitial nephritis.
- Diffuse proliferative glomerulonephritis was the most common primary glomerular lesion with a total of 13 out of 58 cases (22.41%).
- Lupus nephritis was the most common secondary glomerular lesion with a total of 7 out of 58 cases (12.07%).
- Jones's methanamine silver stain along with PAS stain helped in typing/staging of membranous glomerulopathy and

membranoproliferative glomerulonephritis. Various changes in GBM like spike formation, thickening and moth eaten appearance of GBM was noted which is seen in MGN stage II, IV and III respectively. Double contour and thickening of GBM was noted which is seen in type I and II MPGN respectively. However, confirmation of typing/staging could be done only when special stain findings are collaborated with electron microscopy findings which show location of immune complex deposits.

- In Myeloma cast nephropathy, tubular casts stained negative with Congo red which was used to differentiate it from amyloid deposition. Other stains like PAS, JMS and Masson trichrome were performed which stained weakly positive, negative and green color respectively. Congo red stain should be performed in such patients to rule out amyloid deposition in myeloma cast nephropathy because it will help the clinician to look for amyloid deposition in other organs in such patients.
- Immunofluorescence studies showed positivity in 42 patients accounting for 72.41%. The predominant pattern was granular glomerular basement membrane which was noted in 18 patients (31.03%). Immunofluorescence studies were negative in 13 cases (22.41%) and there was no core in 3 cases (5.17%).

- Granular GBM positivity was noted in 18 patients whose diagnoses included diffuse proliferative glomerulonephritis (5 patients), membranous nephropathy (5 patients), membranoproliferative glomerulonephritis (4 patients) and Lupus nephritis (4 patients).
- Mesangial staining only was noted in 7 patients whose diagnoses included IgA nephropathy (2 patients), C1q nephropathy (2 patients), Class I Lupus nephritis (1 patient), diffuse proliferative glomerulonephritis (2 patients).
- Uniform granular staining of glomerular basement membrane and mesangial staining was noted in 7 patients whose diagnoses included membranoproliferative glomerulonephritis (3 patients), diffuse proliferative glomerulonephritis (2 patients) and lupus nephritis (2 patients).
- Non-specific staining in IF was noted in 10 patients whose diagnoses included focal segmental glomerulosclerosis (6 out of 7 cases), acute tubular necrosis (1 out of 2 patients), sclerosing glomerulonephritis (1 patient), hypertensive glomerulopathy (1 out of 2 patients) and myeloma cast nephropathy (1 patient).
- Negative staining was noted in 13 patients whose diagnoses were minimal change disease (all 5 patients), diffuse proliferative glomerulonephritis (4 out of 13 patients), focal segmental

glomerulosclerosis (1 patient), hypertensive nephropathy (1 patient), acute tubular necrosis (1 patient) and chronic glomerulonephritis (1 patient).

- Negative staining was seen in 4 patients of DPGN which was subsequently attributed to contamination of phosphate buffer saline with formalin. This technical error prevented the positive staining in these cases.
- No tissue core for IF in three cases whose diagnoses included mesangioproliferative glomerulonephritis (2 patients) and diffuse proliferative glomerulonephritis (1 patient).
- Among two patients of mesangioproliferative glomerulonephritis, one of them showed intense mesangial staining of IgA and weak mesangial staining for C3 and the other patient showed intense mesangial staining of C1q. In one patient with light microscopic diagnosis of focal proliferative glomerulonephritis the IF finding was intense mesangial staining of IgA. In another patient with light microscopic diagnosis of focal segmental glomerulosclerosis, the IF findings showed intense mesangial staining of C1q. In these patients the diagnosis was given as IgA nephropathy and C1q nephropathy accordingly. Hence, the diagnostic utility of IF was noted in 4 cases (6.90%).

- In a case of minimal change disease, the diagnosis was modified to Lupus nephritis – class I after performing the immunofluorescence studies where the IF finding of mesangial C3 staining. Hence the IF studies helped in modification of the final diagnosis in 1 case (1.72%).

CONCLUSION

The total number of 58 renal biopsies were subjected to light microscopy and immunofluorescence studies. Final diagnosis was arrived after carefully correlating with the clinical history, biochemical and serological parameters, histomorphology using various stains like haematoxylin and eosin, periodic acid Schiff and Jone's methanamine silver stains, immunofluorescence study findings using a panel of markers (IgG, IgA, IgM, C3, C1q and Fibrinogen) in the biopsy tissue.

The cases were grouped into primary and secondary glomerular diseases and tubulointerstitial nephritis. Out of total 58 cases, primary glomerulonephritis constituted 46 cases (79.31%), 10 cases (17.24%) were secondary glomerulonephritis and 2 cases (3.45%) were tubulointerstitial nephritis.

Among the primary glomerulonephritis, diffuse proliferative glomerulonephritis was the most common lesion constituting 28.26% and the most common secondary glomerular lesion was Lupus nephritis constituting 70%. Since the exclusion criteria includes diabetic patients, the renal biopsy is attempted only when there are atypical features noted in diabetic patients like absence of diabetic retinopathy, rapid onset of nephrotic syndrome or proteinuria, low or rapid decrease in glomerular

filtration rate, presence of active urinary sediment and presence of signs and symptoms of other systemic diseases. So, the picture in the present study is different from other studies.

Immunofluorescence studies helped in the modification of the diagnosis in one case (Lupus nephritis class I)[79] after mesangial deposits for C3 was noted in immunofluorescence studies. Intense mesangial staining of IgA and C1q helped in making the diagnosis of IgA nephropathy and C1q nephropathy respectively. This shows the diagnostic value of IF studies and also helps the clinicians to plan the appropriate treatment modalities which differ from one diagnosis to another.[33]

Use of special stains like Periodic acid Schiff and Jone's methanamine silver stains helped to identify the extent of glomerular basement membrane involvement and in typing/staging of MGN and MPGN that complemented the histomorphological findings. However, special stains in parallel with electron microscopy findings of location of immune complex deposits should be done for the confirmation of staging of MGN and typing of MPGN. This is helpful for the clinicians to plan a better therapeutic strategy in the nephrology patients.

In conclusion, the epidemiology of renal diseases differ from developed countries to developing countries. Developing country like ours has shown that the incidence of post infectious glomerulonephritis is still high compared to other glomerular lesions like membranous nephropathy and focal segmental glomerulosclerosis which is more common in developed countries.[15,21,24,25] This can be attributed to the low socioeconomic status, prevalence of infections, lack of awareness regarding health care.[80]

Immunofluorescence studies have complemented the clinical, histomorphological findings in 58 patients including primary, secondary glomerular and tubulointerstitial diseases. However, it was even more of diagnostic importance in 5 patients including IgA nephropathy, C1q nephropathy and Lupus nephritis class I where a confident diagnosis could be rendered only because of availability of immunofluorescence studies. Hence, immunofluorescence studies when combined with histomorphologic findings by light microscopy, clinical, biochemical and serological markers can yield a better and precise diagnosis which can help in improved management of nephrology patients.

Further study:

Additional markers like kappa and lambda etc, could be applied in cases of myeloma kidney diseases to give a better diagnosis which includes predominant type of light chain deposition.

Electron microscopy facility, when made available would complement the histomorphology and immunofluorescence findings.

Confirmation of IF findings with immunohistochemistry markers which could be stored for a longer period, might prove as another milestone in diagnosing renal diseases.[30]

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MASTER CHART

Sl.No	Age/Sex	IP/OP no	Symptoms	Bld Urea	S. Cr	24 HR U.P	Pr. Diag	HPE. No	HPE Diag	IF no	IF findings	Final diagnosis
1	33/F	33990	PE, O, HTN	37	2.1	1.1	MGN	1598/12	MCD	4/12	C3-MES	CLASS I LN
2	45/F	61860	FP,PE,O,F,HTN	42	1.7	5	NS	2761/12	MGN	17/12	IgG,C3-Gr GBM	MGN
3	20/F	64554	FP,PE,A,F	60	2.1	1.6	NS	2799/12	MGN	22/12	IgG, C3- Gr. GBM	MGN
4	39/F	66389	FP,HTN	51	1.9	3	NS	2889/12	MPGN	24/12	IgG,C3-MES+Gr GBM	MPGN I
5	9/M	66513	FP,PE,O,A	33	1	4	NS	2958/12	DPGN	25/12	IgG,C3-MES+Gr GBM	PIGN
6	35/F	66258	FP,PE,O	140	2.8	2.5	LN	3113/12	LN - IV	33/12	IgG,C3-MES+Gr GBM	LN - IV
7	26/M	27885	FP,O,H	40	1.6	3.1	?AGN	3153/12	DPGN	35/12	NEG	DPGN
8	23/M	27912	PE,HTN	85	4.3	5	NS	3169/12	HTN N	36/12	NEG	HTN N
9	38/M	27912	PE,O	55	2.4	5	CRF	3184/12	DPGN	37/12	NEG	DPGN
10	22/M	73707	Fe,H,O	29	0.9	1.7	?IgA N	3230/12	DPGN	38/12	IgG,C3-MES+Gr GBM	PIGN
11	55/M	76878	O.PE	75	7.1	3.2	CKD/MM	3353/12	DPGN	42/12	NEG	DPGN
12	46/F	922	O,PE,FP,H	49	1.3	4.2	NS	73/13	MPGN	3/13	IgG- Gr GBM	MPGN I
13	45/M	76162	PE,FP	164	12	2.7	ARF	123/13	MCD	4/13	NEG	MCD
14	48/F	2266	FP,PE	78	4.8	5	RF	137/13	MGN	6/13	IgG,C3- Gr GBM	MGN
15	25/M	2043	FP,PE,HTN	57	3.5	2.2	ARF	162/13	HTN N	7/13	Fi-GBM	HTN N

16	44/F	2782	O	135	2.2	6.1	?SLE?GN	198/13	LN	8/13	IgG, IgA, IgM, C3, C1q- Gr GBMC3-MES	LN
17	19/M	28181	FP	35	2.2	5.2	NS	233/13	FSGS	12/13	NEG	FSGS
18	32/F	28332	Green cowdung poison	70	4	2.1	ARF	415/13	ATN	13/13	NEG	ATN
19	15/M	28365	Fe,H,Ab PAIN	34	1.2	0.76	?AGN	416/13	MCD	14/13	NEG	MCD
20	38/M	2846	PE,HTN	282	12.3	1	CRF	538/12	MPGN	17/13	IgG- Gr GBM, C3-MES	MPGN
21	25/F	9934	PE,A	39	0.9	0.75	NS	544/13	MPGN	18/13	IgG, C3- Gr GBM + MES	MPGN
22	35/F	28543	PE,FP,HTN	80	1.8	3.7	LN	730/13	MCD	21/13	NEG	MCD
23	29/F	186053	PE,FP,A,HTN	33	0.9	0.2	NS	754/13	DPGN	22/13	IgG,C3-Gr GBM	PIGN
24	29/F	11731	PE,O,SR	25	1.8	3.3	?LN	755/13	LN	23/13	IgG,IgA,IgM,C3,C1q-Gr GBM	LN
25	37/F	14284	PE,FP,O	72	2	5	LN	803/13	LN	24/13	IgG,IgA,IgM,C3,C1q-Gr GBM	LN
26	50/F	19351	PE,O	20	1.9	3.7	NS	892/13	ATN	26/13	IgG + in tubules Non-specific	ATN
27	18/M	23998	PE,O,FP,HTN	42	2.2	5	NS	1139/13	MPGN	28/13	C3- Gr GBM	MPGN
28	19/M	24861	O,PE,FP	97	4.3	5	NS	1236/13	DPGN	30/13	IgG-Gr GBM	PIGN
29	16/M	26943	H,Fe,Arthralgia	20	0.8	1	?IgA N	1301/13	MES Pr. GN	32/13	IgA, C3- MES	IgA N
30	35/F	28510	FP, Ab pain	115	6.9	5	LN	1371/13	LN-IV	34/13	IgG,IgA,IgM,C3, C1q-Gr GBM	LN - IV
31	15/M	28737	O,PE,FP,Ab Pain	80	4.1	5	?HSP	1483/13	DPGN	36/13	IgG,C3,IgA- Gr GBM, IgA- Intense + in MES	PIGN- IgA Dominant

32	18/M	31566	FP,O,H	29	1.1	5	NS	1538/13	FSGS	37/13	IgM,C3- Nonspecific staining in sclerosed glomeruli	FSGS
33	45/M	37220	O,H,FP,Fe	68	7.7	3	?PSGN	1894/13	DPGN	41/13	No tissue	DPGN
34	14/F	42954	O,FP,PE	20	0.7	2	NS	2060/13	FSGS	44/13	IgM-MES, C3-Gr GBM	FSGS
35	31/F	47307	PE,FP	27	0.8	1.6	NS	2364/13	DPGN	49/13	C3-MES	PIGN
36	42/M	52107	FP,PE,A,HTN	29	2.1	5	NS	2558/13	MGN	54/13	IgG, C3- Gr GBM	MGN
37	20/M	54131	O,PE,FP,Ab Pain	34	1.6	5	NS	2645/13	MPGN	57/13	C3- MES + Gr GBM	MPGN
38	35/F	58129	O,FP,PE	30	1.4	5	NS	2907/13	MPGN	61/13	C3-Gr GBM	MPGN
39	39/M	64016	F,FP,PE	54	3.9	6.5	?MGN	3285/13	DPGN	63/13	IgG,C3-Gr GBM	PIGN
40	50/M	61075	Anuria,O,FP,PE, A	57	4.9	5	CKD/MM	3296/13	CGN	64/13	NEG	CGN
41	16/F	64297	PE,FP	125	5.7	5	?RPGN, ?PSGN	3309/13	FSGS	65/13	C3- Non-specific staining	FSGS
42	50/F	67958	O,PE,HTN	48	2.7	5	?MGN	3366/13	FSGS	66/13	C3- Non-specific staining	FSGS
43	21/F	67958	O,PE,FP	20	1.6	3	?FSGS	3430/13	MCD	70/13	NEG	MCD
44	57/M	69390	PE,FP	20	1.8	3	?MGN	3432/13	MPGN	71/13	IgG- Gr GBM, C3-MES	MPGN
45	32/M	69651	O,PE,FP	32	4.8	3	NS, FSGS	3488/13	MCD	72/13	NEG	MCD
46	26/F	72184	PE	37	2	2	?FSGS	3587/13	FSGS	74/13	IgM, C3- Non-specific staining of sclerosed glomeruli	FSGS

47	45/F	68012	HTN, Ab Pain	59	6.4	3	Myeloma Kidney	3614/13	Myeloma cast nephropathy	75/13	IgA,IgM,C3- CastS and MES	Myeloma cast nephropathy
48	35/F	74185	O,PE,FP,Ab P,Fe	31	1.7	2	NS	3944/13	DPGN	80/13	C3-MES	PIGN
49	47/F	4642	O,PE,FP,HTN	157	6.9	2	CKD/MM	404/14	MES Pr. GN	81/13	No tissue	MES Pr. GN
50	40/M	11306	PE,FP	65	3.5	2	RPGN	674/14	FSGS	6/14	IgG-WeaK GrGBM, C1q- Intense MES	C1q N
51	18/F	11952	H	26	0.9	2.2	?HSP	705/14	FOCAL PR. GN	7/14	IgA,C3- MES	IgA N
52	50/F	11000	O,PE,HTN	54	1.8	2.4	NS	704/14	LN - IV	8/14	IgG,IgM,C3,C1q - Gr GBM, Fi - thrombi	LN
53	45/M	21340	O,PE,FP	54	1.6	3.1	NS	1463/14	MGN	11/14	IgG-Gr GBM	MGN
54	22/M	25150	PE, Joint pain	47	3.5	3	RPGN	1527/14	Sclerosing GN	13/14	IgM,C3- Nonspecific staining in sclerosed glomeruli	Sclerosing GN
55	35/M	21500	PE,O,FP	40	2.9	3	NS	1624/14	DPGN	14/14	NEG	DPGN
56	53/F	28967	PE	25	0.7	3	?MGN	1681/14	MES Pr. GN	16/14	No tissue	MES Pr. GN
57	45/F	28986	PE	44	1.7	3	NS	1682/14	MES Pr. GN	15/14	IgG,C1q- MES	C1q N
58	20/M	38959	O,PE,FP	40	2.3	2.2	?MCD/ ?FSGS	2241/14	FSGS	23/14	IgM, C3- Non-specific staining of sclerosed glomeruli	FSGS

KEY TO MASTER CHART

24 HR U.P	–	24 hours urinary protein (g/day)
A	–	Ascites
ABD pain	–	Abdominal Pain
AGN	–	Acute glomerulonephritis
ARF	–	Acute Renal Failure
ATN	–	Acute tubular necrosis
Bld Urea	–	Blood urea (mg/dl)
C1q	–	Complement 1q
C1q N	–	Complement 1q Nephropathy
C3c	–	Complement 3
CRF	–	Chronic Renal Failure
DPGN	–	Diffuse proliferative glomerulonephritis
F	–	Female
Fe	–	Fever
Fi	–	Fibrinogen
FP	–	Facial puffiness
FSGS	–	Focal segmental glomerulosclerosis
GN	–	glomerulonephritis
Gr GBM	–	granular Glomerular Basement Membrane
H	–	Hematuria

HPE Diag	–	Histopathology Diagnosis
HPE No.	–	histopathology number
HSP	–	Henoch Schonlein Purpura
HTN	–	Hypertension
IF findings	–	immunofluorescence findings
IF No	–	immunofluorescence number
Ig A N	–	Ig A Nephropathy
LN	–	Lupus Nephritis
M	–	Male
MCD	–	Minimal change disease
MES	–	Mesangial
MGN	–	Membranous glomerulonephritis
MM	–	Multiple myeloma
MPGN	–	Membranoproliferative glomerulonephritis
NS	–	Nephrotic Syndrome
O	–	Oliguria
PE	–	Pedal edema
PIGN	–	Post Infectious glomerulonephritis
Pr	–	Proliferative
Pr Diag	–	Provisional diagnosis

RF	–	Renal failure
RPGN	–	Rapidly progressive glomerulonephritis
S. Cr	–	Serum creatinine(mg/dl)
S. no	–	Serial number
SLE	–	Systemic Lupus Erythematosus
SR	–	Skin Rash

ANNEXURE - I

ANNEXURE – I:

PROFORMA

S. NO : NAME :

OP/IP NO: AGE AND SEX :

DATE : DATE OF BIOPSY :

Presenting complaint :

Anuria			Facial puffiness		
Oliguria			Fever		
Hematuria			Abdominal pain		
Dysuria			Skin rash		
Pedal edema			Joint pain		
Sore throat			Abdominal mass		

Treatment history if any:

Past history : Diabetes mellitus -

Hypertension -

Kidney disease -

Other medical diseases if any –

Family history : Diabetes mellitus -

Hypertension -

Kidney disease -

Other medical diseases if any –

Personal history :

INVESTIGATIONS :

Urine analysis – 24 hour urine protein –

Urine albumin -

RBCs -

Pus cells -

Deposits -

Casts –

Renal function tests – serum creatinine -

Blood urea –

Liver function tests – total protein –

Serum albumin –

Serum globulin –

Albumin: globulin –

Total cholesterol level –

Chest X-ray –

Abdominal ultrasound scan –

C3 levels-

C4 levels-

ANA -

ANCA –

ASO titre –

Hbs Ab –

Hepatitis C virus-

Histopathology report:

Immunofluorescence report:

Special stain report:

Final diagnosis:

ANNEXURE - II

ANNEXURE - 2: LIST OF ABBREVIATIONS

C3c	–	Complement 3
C1q	–	Complement 1q
DIF	–	Direct Immunofluorescence
DNA	–	Deoxy Ribonucleic Acid
DPGN	–	Diffuse proliferative glomerulonephritis
FSGS	–	Focal segmental glomerulosclerosis
GBM	–	Glomerular Basement Membrane
GFR	–	Glomerular Filtration Rate
HSP	–	Henoch Schonlein Purpura
Ig	–	Immunoglobulin
JMS	–	Jone's Methanamine Silver stain
LN	–	Lupus Nephritis
MCD	–	Minimal change disease
MGN	–	Membranous glomerulonephritis
MPGN	–	Membranoproliferative glomerulonephritis
NS	–	Nephrotic Syndrome
PAS	–	Periodic Acid Schiff
PBS	–	Phosphate Buffer Saline
PSGN	–	Post Streptococcal glomerulonephritis
RBC	–	Red Blood Cell

RPGN	–	Rapidly progressive glomerulonephritis
SLE	–	Systemic Lupus Erythematosus
WHO	–	World Health Organization

ANNEXURE – III

ANNEXURE - 3: GLOSSARY OF TERMS USED TO DESCRIBE HISTOLOGIC LESIONS IN GLOMERULI

Focal	Involving less than 50% of glomeruli
Diffuse	Involving 50% or more of glomeruli
Segmental	Involving part of a glomerular tuft
Global	Involving all of a glomerular tuft
Mesangial hypercellularity	4 or more nuclei in a peripheral mesangial segment
Endocapillary hypercellularity	Increased cellularity internal to the GBM composed of leucocytes, endothelial cells and/or mesangial cells.
Extracapillary hypercellularity	Increased cellularity in Bowman's space, i.e. less than one layer of parietal or visceral epithelial cells, or monocytes / macrophages.
Crescent	Extracapillary hypercellularity other than the epithelial hyperplasia of a collapsing variant of FSGS
Fibrinoid necrosis	Lytic destruction of cells and matrix with deposition of acidophilic fibrin-rich material
Sclerosis	Increased extracellular collagenous matrix that is expanding the mesangium, obliterating capillary lumens or forming adhesions to Bowman's capsule
Hyaline	Glassy acidophilic extracellular material
Membranoproliferative	Combined capillary wall thickening and mesangial or endocapillary hypercellularity

Lobular(hypersegmented)	Consolidated expansion of segments that are demarcated by intervening urinary space
Mesangiolysis	Detachment of the paramesangial GBM from the mesangial matrix or lysis of mesangial matrix.
Spikes	Projections of glomerular basement membrane intervening between subepithelial deposits (seen in membranous nephropathy)
Subepithelial	Between podocyte and glomerular basement membrane
Subendothelial	Between endothelial cell and glomerular basement membrane
Wire loop	Thick, rigid appearance of the capillary loop because of massive subendothelial deposition
Activity	Treatment-reversible lesions like cellular crescents, proliferation, necrosis, cellular infiltrate.
Chronicity	Irreversible lesions with treatment like fibrous crescents, tubular atrophy, interstitial fibrosis, sclerosis.
Tram-track	Double contour of glomerular basement membrane due to deposits and/ or circumferential interposition