Renal Biopsy in Children

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Renal biopsy is an important investigation to make the diagnosis of an underlying glomerular or tubular disease, and is commonly done by trained pediatricians. In this review, we discuss the procedure and also detail important points in interpretation of renal biopsy in children.

Keywords: *Diagnosis, Glomerular disease, Management, Nephrotic syndrome, Steroid-resistant, Tubular disease.*

Renal biopsy is an important diagnostic tool in the hands of a pediatric nephrologist. While the first biopsy was done more than 100 years ago in United States, its utility in diagnostics has increased in the last few decades [1]. Since its regular introduction in 1951 by Iverson and Brun, renal biopsy has made a revolution in the study of renal diseases [2]. Renal pathology can be better delineated with the advent of newer stains, immunofluorescence and electron microscopy. While a renal biopsy is more useful in diagnosing glomerular diseases, it often provides information on tubular conditions as well.

PRE- PROCEDURE CARE

The parents/ caregivers should be counseled and explained the procedural details and a written consent should be taken. The prerequisites for a biopsy are hemoglobin above 8 gm/dL, platelet count above 1 lakhs/ mm^3, normal INR and normal blood pressure. In the pre-biopsy checklist, it is important to take history of bleeding tendencies, allergies to povidone/iodine, ketamine, midazolam and lidocaine. Drugs like aspirin should be discontinued seven days before, warfarin 48 hr before and any other NSAIDs should also be stopped 48 hours prior to the procedure. The biopsy site should be inspected for any superficial infection. If the child is on hemodialysis, the procedure should be done after at least 24 hours of last dialysis session as heparin during the dialysis procedure may lead to excessive bleeding. For patients with prolonged BT (>8-10 minutes, *e.g.,* in SLE, azotemia), 0.3 µg/kg IV desmopressin can be administered 30 min prior, or 2-4 µg/kg DDAVP intranasal 2 hours before the procedure. Desmopressin reduces the bleeding by improving the platelet functions.

PROCEDURE

The renal biopsy is done under sedation and local anesthesia in prone position for native kidneys and in supine position for transplanted kidneys. Preferably the procedure should be done under real-time ultrasound guidance by a pediatric nephrologist/trainee in pediatric nephrology.

In conditions like abdominal distension and ascites the biopsy can be done in lateral decubitus or sitting position. A sandbag/rolled sheet or blanket is used under the abdomen to decrease the mobility of the kidney. An IV access is established and heart rate, saturation and blood pressure are monitored during the procedure. For procedural sedation the most preferred drugs are 1-2 doses of midazolam (0.1mg/kg) and ketamine 0.5-1.0 mg/ kg. Intravenous atropine 0.01 mg/kg is administered 1-2 minutes after midazolam. The left renal angle is the most preferred site for the renal biopsy. The lower pole of the kidney is located at this position. Local anesthesia is given at the site by infiltration of lignocaine injection after draping and cleaning (with spirit and povidone iodine). In the real time procedure under ultrasound guidance the automated biopsy gun should be introduced at the site in such a manner that its tip reaches the renal cortex.

Technique

The sample is usually obtained from the lower pole of the left kidney located between the erector spinae muscle and the lower border of the 12th rib. A 16 or 18 gauge biopsy gun/automated needle is used for taking the per-cutaneous renal sample. Use of an 18 gauge needle is preferred in infants and young children while thicker bore should be used in all other age groups. The yield of

glomeruli is better with 16 gauge needle [3]. The use of an 18 gauge needle resulted in a significantly smaller sample size (9 *vs* 11 and 15 glomeruli) and less diagnostic success (53% *vs* 76% and 85%), with no significant differences in complication rates [3]. The sample should be taken from the renal cortex which harbors the glomeruli. The cortical thickness in an adult kidney is about 10 mm.

A renal sample is considered adequate for opinion if the yield of glomeruli is between 10-20. Minimum sample size for diagnosis varies greatly with the specific diagnosis. For instance, membranous glomerulonephritis (MGN) can be diagnosed even from a single glomerulus while focal segmental glomerulosclerosis (FSGS) can be missed if less than 10 glomeruli are obtained. Two to three passes with the automated gun are sufficient to yield tissue for light microscopy, immunofluoresence (IF) study and electron microscopy (EM). The sample should be removed from the biopsy needle with gentleness, taking care not to stretch or crush the tissue. Forceps should be avoided. An 18-gauge needle or a thin, wooden stick, such as a toothpick can be used. It is advisable to confirm adequacy of cortical tissue on the table itself with the help of a pathologist using the stereoscopic microscope. Once it is determined that suitable cortical tissue is obtained, about 2 mm tissue are cut off from each cortical and medullary ends of the two cores (***Fig.* 1**). One cortical tissue and one medullary tissue is placed for IF study in a vial containing Michel transport media. Antigens of interest in the renal biopsy are protected for as long as a week in this media and the sample is stable at room temperature. The cortical and medullary samples for EM are sent in 1-3% glutaraldehyde that acts as a fixative. This fixative must be refrigerated and has a short half life. Care must be taken that no cross contamination of fixative fluids occur while placing the biopsy pieces in their respective vials.

Complications

Complications of renal biopsy are few with the use of automated gun. Macroscopic hematuria following a biopsy has been reported to vary from 5-20% in different studies [4-6]. Rarely patients may develop colicky pain due to passage of clots in urine. Although clinically significant peri-nephric hematomas occur in less than 6% of the biopsies, peri-nephric hematomas have been demonstrated at 24-72 hours after biopsy in >90% of cases evaluated prospectively. Microscopic hematuria occurs in almost all patients and disappears over a 48-72 hours period. Serious complications like need for blood transfusion and development of an arteriovenous fistula are less frequent. A meta-analysis on complications following renal biopsy in children reported the need for blood transfusion in 0.9% and need for another intervention due to the procedure in 0.7% of the biopsied children [7]. Absolute contraindications of the procedure are uncontrolled bleeding diathesis, uncontrolled severe hypertension, hydronephrotic kidneys while presence of a single kidney is a relative one.

POST – BIOPSY CARE

Patient should stay in supine position for 4-6 hours and bed rest is recommended for 24 hours. The vitals should be monitored every 30 min for the first 2 hours and then hourly till 6 hours. Maintenance intravenous fluids (normal saline or N/2 saline or ringer lactate) are administered for the first 6 hours. Oral fluids are offered to the child when fully conscious and on demand. Paracetamol is used for pain relief if required. Most patients can be discharged after 24 hours of biopsy; however they should be instructed to avoid climbing of stairs, heavy work and play for one week following the procedure.

Treatment of Complications: (*i*) Gross hematuria: If coagulation is deranged it is recommended to use fresh frozen plasma or cryoprecipitate for reduction of bleeding. Also an extra dose of vitamin K should be administered. Blood transfusion may be necessary if 6 hour post biopsy hemoglobin falls by 10-15% of the baseline or the child clinically becomes pale. An urgent ultrasound abdomen should be done to visualize bleed,

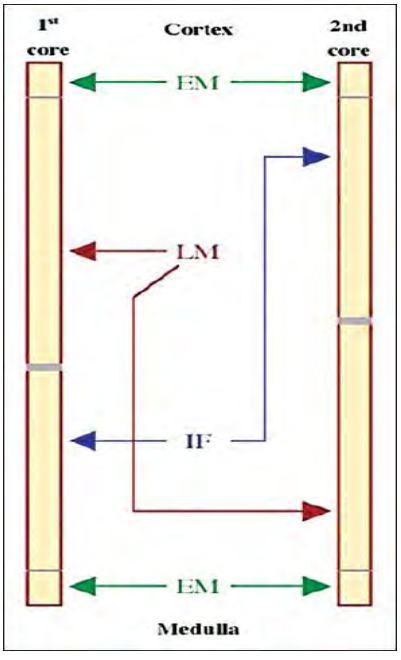

Fig. 1 *Division of kidney biopsy cores for light microscopy, immunofluorescence and electron microscopy.*

Box I Common Indications for Renal Biopsy in Children

Glomerular causes

Steroid resistant nephrotic syndrome

Congenital nephrotic syndrome

Atypical nephrotic syndrome

Rapidly progressive glomerulonephritis

Non resolving post-infectious glomerulonephritis.

Recurrent gross hematuria

HBSAg/anti HCV positivity with proteinuria/ hematuria

Tubulo-interstitial nephritis

Acute kidney injury >4 wks without cause

hematoma in such a situation. Rarely radiographic transcatheter embolization or surgical intervention may be required for continuous bleeding. (*ii*) Sedation related complications: Brief hypoxia, transient airway complications, vomiting or minor aspiration, laryngospasm are complications of sedation and might need repositioning of the child with suctioning of the airways, oxygen administration and rarely ventilation.

INDICATIONS

The primary indication for renal biopsy in a child is steroid resistant nephrotic syndrome (SRNS) [8-9]. A recent retrospective review showed that 36.1% of the pediatric biopsies were for SRNS, 22.1% for steroid sensitive disease and 12% for acute kidney injury (AKI) [10]. Glomerular diseases (62.6%) predominated in the national Turkish registry review of all pediatric biopsies between 1991 and 2010 [11]. The most likely biopsy findings in patients with nephrotic syndrome are minimal change disease (MCD), FSGS and mesangioproliferative glomerulonephritis (Mes PGN) [12]. While MCD predominates in younger children, FSGS is more common in older children and adolescents [13]. Biopsy findings of membranoproliferative glomerulonephritis (MPGN) and MGN occur in less than 5% of patients with steroid resistant disease in children. Primary glomerular diseases accounted for almost 85% of all biopsies in older children in a recent study [13]. The indications for renal biopsy are listed in ***Box* I**.

All children with steroid sensitive or resistant nephrotic syndrome require a biopsy prior to starting calcineurin inhibitors (cyclosporine and tacrolimus) which are potentially nephrotoxic [9]. Besides children with nephrotic syndrome on calcineurin inhibitors for more then 2-3 years are often re-biopsied to look for features of nephrotoxicity before further continuation of these agents. Other indications of biopsy are in patients with rapidly progressive renal failure where a suspicion of crescentic glomerulonephritis is kept. In patients with acute nephritic syndrome, renal biopsy is needed if the kidney functions are worsening or the investigations are not suggestive of a post streptococcal glomerulonephritis. Renal biopsy may be done in patients with AKI to identify the underlying cause where recovery is delayed beyond one month to differentiate acute tubular necrosis from other causes of AKI. Other conditions like acute and chronic interstitial nephritis can be identified on kidney biopsy. While kidney biopsy is not required for the diagnosis of chronic kidney disease (CKD) it may be done where the kidneys appear normal in size and corticomedullary differentiation on sonography and the cause of CKD is not explicable. In post transplant patients, the biopsy of the grafted kidney provides information on acute and chronic rejection. Biopsy of kidneys with structural anomalies should be done carefully under ultrasound guidance. In this article we would be discussing the biopsy interpretation of some common glomerular conditions occurring in native kidneys.

INTERPRETATION

Light Microscopy

For light microscopic examination of renal biopsy specimen, stains used include hematoxylin-eosin stain (HE stain), periodic acid Schiff (PAS) stain, Masson trichrome and silver stains. Identification of cortex or medulla, number of glomeruli, and cells infiltrating the interstitium of the kidney like neutrophils and lymphocytes are best identified on the HE stain. For glomerular structure, PAS stain is better as it delineates mesangial cells and matrix. PAS and silver stains effectively stain the basement membrane while Masson's trichrome and silver stains are used for identification of fibrosis.

The number of glomeruli, size, presence of any sclerosis, focal or diffuse changes, and presence of any crescents or mesangial cell proliferation can be checked on light microscopy. Lesions involving <50% of glomeruli are called focal while more than that are called diffuse. If only a part of the glomerulus is involved it is termed segmental while involvement of the whole glomerulus is defined as global. Basement membrane thickening or splitting is seen in conditions like MGN and MPGN and is identified on PAS or silver stain. Vessel wall thickening, medial sclerosis or fibrinoid necrosis in case of vasculitis is better seen with PAS stain [14-16]. Stains like von Kossa for calcification and Congo red for identification of amyloidosis are used infrequently in specialized situations [14]. Chronic tubulointerstitial

damage can be identified on light microscopy as tubular atrophy and interstitial fibrosis. In acute interstitial nephritis, interstitial edema, infiltration by neutrophils, lymphocytes and plasma cells can be seen while in chronic interstitial nephritis; fibrosis instead of edema is a prominent feature [2].

Immunofluorescence

IF study is done with labeled antisera and antibodies. Antisera or monoclonal antibodies against immunoglobulins (IgA, IgG and IgM), components of the classical or alternative complement pathway (C1q, C3c and C4d), protein light chains (kappa and lambda), albumin and fibrinogen are used for identification of different immunofluoresence patterns. The pattern of staining can be linear or granular; linear staining occurs in anti-GBM disease while granular in immune complex mediated injury. The location of deposits can be mesangial or in the peripheral capillary walls (PCW).

In conditions like MPGN and MGN, the immunoglogulin (IgG) deposits are primarily subendothelial and subepithelial respectively. Mesangial deposits of IgA are primarily seen in IgA nephropathy. Similarly granular C3 deposits in the PCW are consistent with a diagnosis of post infective glomerulonephritis (PIGN) while deposits of all immunoglobulins and complements (full house staining) are a hallmark of lupus nephritis.

Using immunohistochemistry procedures, antibodies against viruses like cytomegalovirus and polyoma virus can identify these in the biopsy specimens. Antibodies against hepatitis B and C antigens can be detected on renal tissue and nature of amyloid whether primary or secondary identified by AA Amyloid stain. Additional immunohistochemical study with antibodies, such as collagen IV alpha chains can be performed for identification of Alport's syndrome. In post transplant renal biopsies immunostaining for complement factor C4d can be done to identify humoral rejection.

Electron Microscopy

It is not necessary, but helpful to do EM for all renal biopsies. The conditions in which electron microscopy will help confirm the light microscopy diagnosis are in the identification of podocyte structure alteration (effacement of foot processes) in MCD, changes of glomerular basement membrane especially thinning, thickening or splicing and the site of immune deposits (subendothelial or subepithelial). EM is essential for diagnosis of basement membrane abnormalities like thinning in thin basement membrane disease and irregular thickening with basket weave pattern in Alport's syndrome. EM is also essential in sub defining the nature of deposits in immune complex deposition diseases like immunotactoid glomerulonephritis (GN) and fibrillary GN. Metabolic disease like Fabry disease also require EM for diagnosis. ***Box* II** gives in a nutshell what to look for in a renal biopsy specimen.

BIOPSY PICTURE IN GLOMERULAR DISORDERS

Some salient biopsy characteristics of renal disorders are given in ***Table* I**; the biopsy picture in different conditions is discussed briefly below.

MCD: The glomeruli in MCD look almost unremarkable. There is no significant increase in mesangial matrix and cellularity and no thickening of basement membrane is identified. Tubules may show hyaline droplets representative of resorbed proteins following the heavy proteinuria. Immunofluorescence studies are generally negative for all immunoglobulins (***Fig.* 2*a, b***). MCD is part of set of diseases called as podocytopathies characterized by abnormalities in the podocytes or visceral epithelial cells lining the glomerular capillary loops. There is simplification of foot processes of podocytes seen as diffuse effacement on electron microscopy (***Fig.* 2*c***); which is the hallmark of the disease.

FSGS: The classical lesion in FSGS is a focal solidification of the glomerular tuft by an acellular extracellular matrix that is positive on PAS and silver stains (***Fig.* 2*d,e***). The segmental sclerosis is often accompanied by attachment to the Bowman's capsule called as "synechie" formation. These lesions are identified in only a portion of the glomeruli and do not

Box II Features to Look for in Renal Biopsy with Different types of Processing

Light microscopy

- Glomerular proliferation or sclerotic changes can be identified best
- Basement membrane thickening can be identified
- Tubulointerstial damage like tubular atrophy and fibrosis can be seen
- Blood vessels may show medial sclerosis

Immunoflorescence

- Helps in identifying immune deposits like C3, IgG, IgM, IgA, fibrin etc.

Electron microscopy

- Most useful in identifying the structural defects of podocytes like effacement in MCD, identifying the exact location of immune deposits (subepithelial or subendothelial), basement membrane thickening or thinning (in Alport disease and thin basement membrane disease)

involve the entire glomerular tuft; hence the term focal and segmental. FSGS can further be pathologically classified as glomerular tip, perihilar, cellular, collapsing and mixed variants according to Columbia classification. On IF study, segmental glomerular staining for IgM and C3 is identified which represents a non specific entrapment in the area of sclerosis. Staining for immunoglobulins is generally negative (***Fig.* 2f**). EM shows effacement and obliteration of podocyte foot processes, mesangial sclerosis (***Fig.* 2 g**).

MGN: This is a disease caused by immune complex deposition in the sub-epithelial zone i.e. over the basement membranes of the capillary loops. The capillary basement membranes show spike formation due to deposition of type IV collagen around this material in an

Table I Salient Features on Renal Biopsy in Different Conditions

Condition	*Light microscopy*	*Immunofluorescence*	*Electron microscopy*
Minimal change disease	Glomeruli look unremarkable, no significant increase in mesangial matrix & cellularity, normal looking BM, blood vessels look normal, tubules may show hyaline droplets	No or minimal immune deposits	Diffuse effacement of podocyte foot processes
Focal segemental glomerulosclerosis (FSGS)	Focal solidification of glomerular tuft by acellular extracellular matrix positive on PAS & silver stains; changes in few glomeruli; tubular atrophy & interstitial fibrosis may be seen	No to minimal immune deposits (C3 & IgM)	Epithelial cell detachment from glomerular BM, extensive foot process obliteration, mesangial sclerosis &collapsed glomerular loops
Mesangioproliferative glomerulonephritis *(MesPGN)*	Diffuse or focal increase in mesangial cells & matrix, BM normal, blood vessels look normal, tubules may show atrophy	No to minimal deposits of IgA, IgM, IgG along mesangial capillary walls	Mesangial deposits of immune complexes
Membranous nephropathy	Glomeruli are enlarged with mild increase in mesangial matrix & cellularity,thickened capillary walls with prominent spike formation; best seen on PAS & silver stains.	The immune complexes deposited in the peripheral capillary walls are granular deposits, positive for IgG & C3; IgA& IgM may also be seen	Granular electron dense deposits in subepithelial zone on outer aspect of the glomerular BM
Membranoproliferative glomerulonephritis (MPGN)	Lobular accentuation of enlarged glomeruli, mesangial hypercellularity & splitting of BM	Primarily mesangial & subendothelial C3 deposits; C1q, C4 deposits may occur	Subendothelial & mesangial electron dense deposits, increased mesangial matrix, podocyte foot process fusion
IgA nephropathy	Mesangial hypercellularity, endocapillary proliferation, segmental sclerosis & varying degree of tubular atrophy & interstitial fibrosis	Granular deposits of IgA in the mesangial areas	Mesangial, subendothelial & subepithelial immune deposits
Crescentic glomerulonephritis	Presence of crescents (extracapillary proliferation of cells resulting in collapse of glomerular capillary loop) in >50% glomeruli; there may be cellular, fibrocellular or fibrosed crescents	Immune deposits are absent in paucimmune condition while there may be C3,IgG, IgA, IgM deposits in immune mediated conditions	Rupture of GBM and Bowman's capsule, focal effacement of podocyte foot processes
Alport syndome	May show variable thickening of the GBM	Absence of α3, α4 and α5 chains of type IV collagen from BM of glomeruli characteristic	Thinning of the membrane that later change to basket weaving & lamellation of GBM

BM: basement membrane; GBM: glomerular basement membrane.

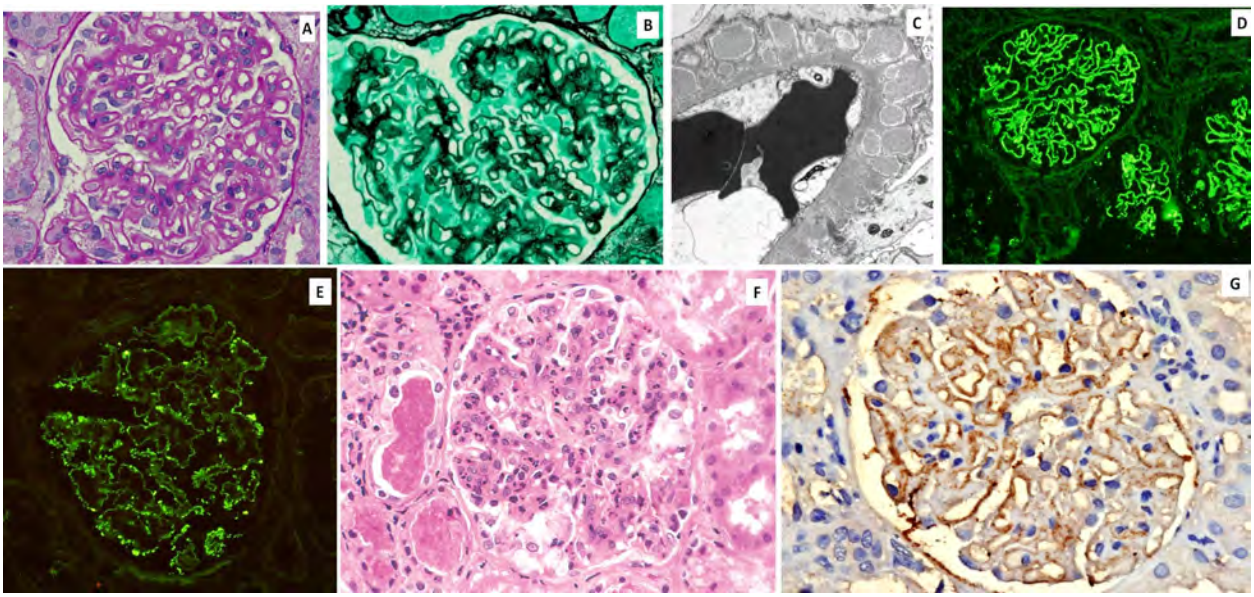

Fig. 2 *Minimal Change Disease: A:PAS stained section of a glomerulus in Minimal Change Disease showing only mild increase in mesangial matrix (x400x); B: IgG stained cryosection showing lack of immune deposits and protein reabsorption granules in parietal epithelial cells (x200x); C: Electron micrograph showing complete effacement of foot processes of podocytes (x4300x). Focal and Segmental Glomerulosclerosis: D: HE stained glomerulus in focal and segmental glomerulosclerosis showing segmental sclerosis of glomerular tuft and foam cell infiltration [arrow] (x400x); E: JSM Stained section showing segmental sclerosis in glomerulus (x400x); F: Electron micrograph showing complete effacement of foot processes of podocytes (x4300x); G: C3 stained cryosection showing segmental deposits in glomerular tuft (x200x).*

attempt to wall them off and decrease their inflammatory reaction (***Web Fig.* 1*a, b***). The glomeruli are enlarged with mild increase in mesangial matrix and cellularity. Activity in the form of endocapillary proliferation or crescent formation are not a feature of primary MGN but usually representative of a membranous nephropathy secondary to a systemic cause like SLE, other auto immune or infectious diseases. On IF, the immune complexes deposited in the peripheral capillary walls are classically identified as granular deposits, positive for IgG and C3 (***Web Fig.* 1*d***). Other immunoglobulins like IgA and IgM are often seen. Further a diagnosis of primary MGN may be confirmed by demonstration of anti-PLA_2R antibodies in the podocytes by immuno-fluorescence staining (***Web Fig.* 1*e***). On EM, granular electron dense deposits are identified in the sub-epithelial zone on the outer aspect of the glomerular basement membrane (***Web Fig.* 1*d*).**

MPGN: This term indicates thickening of the basement membrane accompanied by mesangial proliferation. The kidney biopsy in MPGN shows a classical lobular accentuation of glomeruli, mesangial hypercellularity and splitting of basement membranes on silver stains. Secondary MPGN pattern of injury is seen in cases of long standing infectious pathology, auto-immune conditions, dysproteinemias, transplant glomerulopathy and various other miscellaneous conditions. Primary MPGN is caused by abnormalities of the alternate complement pathway and is known as C3 glomerulopathy. It is primarily diagnosed on IF by strong deposition of C3 in the kidney biopsy in the absence of immunoglobulin deposition. The diagnosis can only be confirmed on EM, based on which, it is further divided into dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). Dense, band-like osmophilic deposits in the GBM on EM is the classical feature of DDD. C3GN is characterized by sub-endothelial, mesangial and sub-epithelial C3 deposits on EM.

IgA nephropathy: IgA nephropathy is one of the commonest forms of primary glomerulonephritis the world over. It is characterized by granular deposits of IgA in the mesangial areas identified on IF. On light microscopy, these biopsies present a diverse histological presentation ranging from no detectable histological finding to diffuse proliferative and crescentic glomerulonephritis. The grade of histological changes determines the clinical prognosis. The histological changes in the form of mesangial hypercellularity, endocapillary proliferation, segmental sclerosis, tubular atrophy and interstitial fibrosis have been graded by the Oxford classification into 4 grades each (0-4). A sum total of grades in all the four compartments represents the activity of the disease and determines the clinical prognosis [17].

Lupus nephritis: The renal biopsy in lupus nephritis shows a wide variety of changes which commensurate with the disease activity and have a bearing on the prognosis of the patient. The renal biopsy changes in lupus have been classified into 6 groups by the ISN /RPS classification system into Class I (minimal lupus nephritis), Class II (mesangial lupus nephritis), Class III (focal lupus nephritis), Class IV (diffuse lupus nephritis), Class V (membranous lupus nephritis) and Class VI (advanced sclerosing glomerulonephritis). Some modifications have been added to the classification [18].The diagnosis is confirmed on IF by presence of a full house pattern in the form of immunoglobulins IgG, IgA and IgM along with complements C3 and C1q; deposits of immunoglobulins are also indentified in the walls of tubules and blood vessels.

Tubulointerstitial changes: The tubules should be examined for features of acute tubular necrosis as seen in AKI. The interstitium shows edema and a mixed inflammatory cell infiltrate. Other findings are interstitial fibrosis, tubular atrophy, arteriolar sclerosis, and occasionally, patchy mononuclear cell infiltration. The degree of chronic parenchymal damage in the tubulo-interstitial compartment is an important prognostic indicator in all glomerular diseases and is assessed on PAS and MT stains. Blood vessels changes secondary to hypertension are often seen in glomerular diseases.

To conclude, interpretation of renal biopsy in children involves procuring an adequate sample for examination and processing it for light, immunofluorescence and electron microscopy. While renal biopsy is more useful for identifying glomerular diseases, it also provides sufficient information about tubulo-interstitial changes. The biopsy changes should be carefully interpreted along with the clinical findings for making a confirmatory diagnosis.

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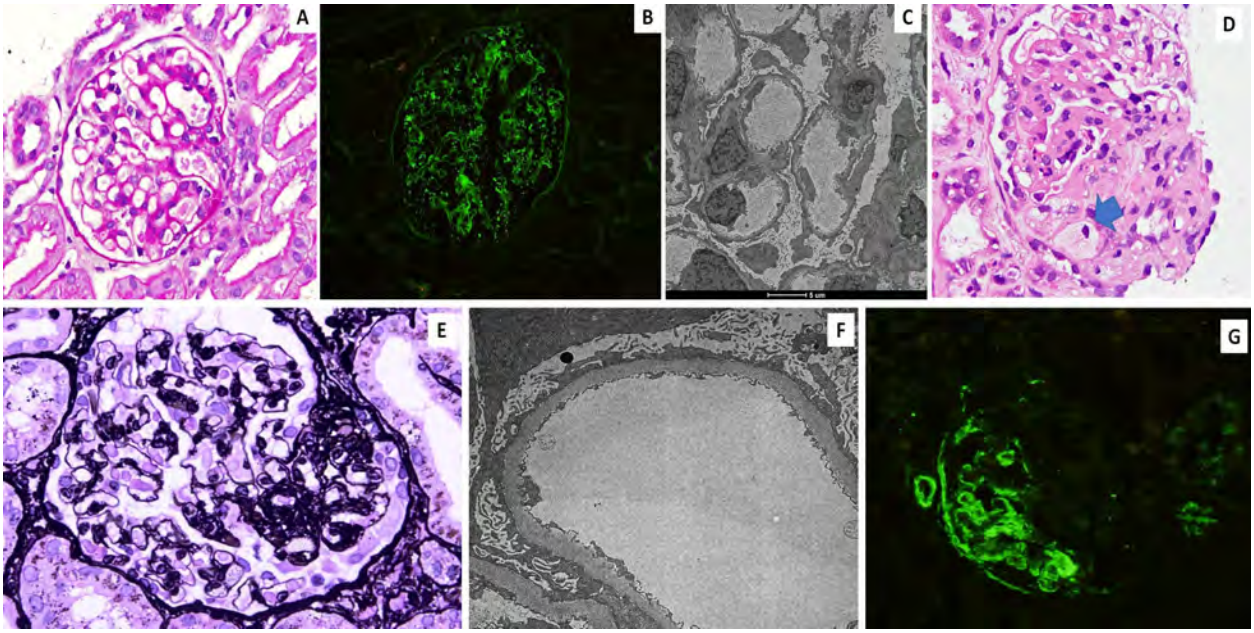

Web Fig. 1 *Membranous Glomerulonephritis: PAS stained section of a glomerulus in membranous glomerulonephritis [MGN] showing thickened basement membranes with prominent spikes on silver methenamine stain [B] (x400x); C: Electron micrograph showing electron dense depositis in the sub epithelial location of the basement membranes (x5300x); D: IgG stained cryosection showing granular immune deposits in the basement membrane in case of MGN (x200x); E : Deposits on subepithelial surface of basement membrane positive for PLA2R (x400x). Diffuse Proliferative Glomerulonephrtis (DPGN): F: HE stained glomerulus in DPGN showing endocapllary proliferation and infiltration by polymorphs [arrow] (x400x); G: JSM Stained section showing segmental sclerosis in glomerulus (x400x)*