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Original Research Article

Immunologic glomerulopathies-diagnostic role of immunofluorescence study of renal biopsies

Kaushlendra K. Pandey[1]*, Anjali Tiwari[2], Asha Agarwal[3]

[1]Department of Medical Laboratory Technology, University Institute of Health Sciences, CSJM University, Kanpur, Uttar Pradesh, India
[2]Department of Laboratory Sciences, Regency Hospital, Kanpur, Uttar Pradesh, India
[3]Department of Pathology, GSVM Medical College, Kanpur, Uttar Pradesh, India

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***Correspondence:**
Dr. Kaushlendra K. Pandey,
E-mail: kaushlendra22@gmail.com

ABSTRACT

Background: The kidney is a structurally complex organ that has evolved to subserve a number of important functions: excretion of the waste products of metabolism, regulation of body water and salt, maintenance of appropriate acid balance, and secretion of a variety of hormones and autacoids. Some clinical disorders affect more than one structure. In addition, the anatomic interdependence of structures in the kidney implies that damage to one almost always secondarily affects the others. Thus, severe glomerular damage impairs the flow through the peritubular vascular system, conversely, tubular destruction, by increasing interglomerular pressure, may induce glomerular atrophy. Thus, whatever the origin, there is a tendency for all forms of chronic renal disease ultimately to destroy all four components of the kidney, culminating in chronic renal failure and what has been called end-stage contracted kidneys. The functional reserve of the kidney is large, and much damage may occur before functional impairment is evident. The circulating immune complexes play a very major role in various types of glomerular nephropathies.
Methods: The present study was conducted on renal biopsies referred to Pathology Department of G.S.V.M. Medical College, Kanpur and Regency Hospital Ltd., Kanpur. Frozen section of renal biopsy was taken for IF studies. Renal biopsy tissue was received in IF fluid containing Ammonium sulphate, N-ethyl malcimide, Magnesium sulphate.
Results: The lgG class of immunoglobulins was found to be most fatal to the G13M, the 1gM and IgA were also found to cause glomerular damage. This mechanism was seen responsible for most cases of ICGN. In the present study, fluorescent study of renal biopsy tissue was also done using Hollande's fixative and it was observed that a better diagnosis could be done when used with routine H & E and immunofluorescent studies.
Conclusions: The immunofluorescence microscopy proved to be very useful and essential, for proper diagnosis and therapy of a renal disease. IFM comes out to be a very good indicator of the deposition site and class of immunoglobulin involved in the Immune-complex deposit.

Keywords: Glomerulopathies, Immunoglobulins, Immune complexes, Kidney, Membranous glomerulonephritis

INTRODUCTION

The kidney is a structurally complex organ that has evolved to subserve a number of important functions: excretion of the waste products of metabolism, regulation of body water and salt, maintenance of appropriate acid balance, and secretion of a variety of hormones and autacoids. Diseases of the kidney are as complex as its structure, but their study is facilitated by dividing them into those that affect the four basic morphologic

components: glomeruli, tubules, interstitium, and blood vessels. This traditional approach is useful because the early manifestations of diseases that affect each of these components tend to be distinctive. Further, some components appear to be more distinctive. Further, some components appear to be more vulnerable to specific forms of renal Injury; for example, glomerular diseases are most often immunologically mediated whereas tubular and interstitial disorders are more likely to be caused by toxic or infectious agents. Nevertheless, some disorders affect more than one structure. In addition, the anatomic interdependence of structures in the kidney implies that damage to one almost always secondarily affects the others.

Thus, severe glomerular damage impairs the flow through the peritubular vascular system, conversely, tubular destruction, by increasing intraglomerular pressure, may induce glomerular atrophy. Thus, whatever the origin, there is a tendency for all forms of chronic renal disease ultimately to destroy all four components of the kidney, culminating in chronic renal failure and what has been called end-stage contracted kidneys. The functional reserve of the kidney is large, and much damage may occur before functional impairment is evident.

The objectives of the present study were

- Identification, isolation and characterization of immune complexes in various renal disorders for understanding etiopathogenesis and clinical behavior of the disease.
- To find out the correlation between Immune complex levels intensity with extent of glomerular damage.
- To study the possible role of Immune complex in relation to pathogenesis of disease.

The application of irnmunopathologic techniques adds a new dimension to the diagnosis of renal disease by providing insight into the pathogenesis and potentially the aetology of the patient’s problem. If one views the diagnosis of renal disease in terms of clinical, morphologic and immunopathologic features, then a reasonable assessment of the Individual’s illness should follow. The clinical and morphologic classifications of renal disease are generally well known and have been extensively presented and reviewed since percutaneous renal biopsy gave pathologists access to pre-end stage renal tissue, Immunopathologic classification is much newer, dating back less than 20 years and being applied with increasing frequency only in the last 10 years.

There are two main types of immunologically induced glomerulonephiritis:

- Deposition in the glomeruli of circulating antigen-antibody complexes, and.
- Deposition of antibody directed against the glomerular basement membrane.

In the first type of glomerulonephritis, the antigen does not usually originate from the kidney, and antibody is not directed against any of the glomerular constituents. The Immune complexes localize in the kidney because of tl1ir physical properties and because they are extracted from the circulation by a phagocytic system of the glomeruli-the mesangium. Both the endothelial cells and smooth muscle cells can also be phagocytic. A single injection of foreign protein can induce glomerulonephritis (acute serum sickness), as can recurrent injections of a similar protein over a period of weeks or months (chronic serum sickness). Other studies have induced glomerular damage by the injection of antigen-antibody complexes which have been prepared invitro (passive serum sickness).[1]

In the second type of immunologically- induced glomerulonephritis, antibodies from the circulation react with the glomerular basement membrane to cause nephrotoxic serum nephritis or antiglomerular basement membrane disease (anti GBM disease).[2]

The division of tissue was modified from transplant biopsies so as to expedite the diagnosis when rejection was suspected. Approximately half of the cortex was submitted for frozen sections while the remainder was processed for light microscopy and small (1mm) portions were saved for immunofluorescence microscopy. Frozen sections were taken immediately for light microscopy stains (H&E, PAS) and immunofluorescence microscopy. These procedures could be completed within 1-2 hours, so that in many patient's rejections could be determined.[3]

METHODS

The present study was conducted on renal biopsies referred to Pathology Department of G.S.V.M. Medical College, Kanpur and Regency Hospital Ltd., Kanpur, India. The patients were mainly selected from indoor and outdoor departments of L.L.R. and Associated Hospitals and Regency Hospital Pvt. Ltd., Kanpur, India.

Tissue processing for light microscopy

The tissue was fixed in 10% formalin for overnight. After fixation, the tissue was dehydrated and embedded in paraffin wax because it permitted the use of H&E for routine light microscopy and allows further use of special stains. Normally, seven slides were prepared with 2 mm thick sections. The first, fourth and seventh slides were stained with H&E, the second and fifth with PAS and third and sixth with PAMS. Additional stains, like Congo red, Methyl violet, Crystal violet and Thioflavin-T, were used to demonstrate amyloid.[4]

Immunofluorescence (IF)

Direct technique: Renal biopsy tissue was received in IF fluid containing Ammonium sulphate, N-ethyl malcimide, Magnesium sulphate. Frozen section of renal biopsy was taken for IF studies.[5]

Staining procedure

Sections were washed in 0.1 M phosphate buffered saline (PBS) with 3 changes over a period of 30 minutes.

i. Excess PBS was drained off and wipe around section with filter paper. Section was covered with diluted conjugate and allowed to reach for 30 minutes at room temperature.
ii. Conjugate was drained off and washed with 3 changes of PBS over a period of 30 minute.
iii. Excess PBS was drained off and the area round the section dried with filter paper. The sections are mounted using a PBS solution.
iv. The preparation was seen under fluorescent microscope as soon as possible. Indirect technique[6]

- The substrate tissue section was air dried according to the antibody to be detected in patient's serum.
- Washed in PBS-for 5 minutes.
- The serum was applied on section and incubated at room temperature for 30 min.
- Washed with PBS at least three times for 15 min.
- Reacted for 30 min. with anti-immunoglobulin (anti-IgG) conjugated with fluorescence at room temperature.
- Washed with PBS using two changes for 10 min.
- Mounted with buffered glycerine.

Antigen retrieval

The enzymatic digestion involved dewaxing, rehydrating and rinsing the specimen in running water.[7] The specimen was the equilibrated with pepsin (0.05% v/v in 2N HC1) and trypsin (0.05% v/v in PBS with 0.1% Calcium chloride) (Table 1). The enzymatic activity was stopped by placing specimen in cold buffer at 40C.[8] The antibody used was lgG of Binding Site Limited. U. K. (Batch no. PFOG4, lgG antihuman), Antihuman 1gm (PFOI2 and antihuman IgA (PFOIO) from binding site Ltd. U. K. (Table 2).

The autoclaving or pressure cooking method Involved the specimen to be brought to full pressure as quickly as possible and pressure to be released at the end of heating time (1-2mm.). The sections were washed in buffer and not allowed to dry.[9] The microwave irradiation also involved the temperature to be taken suddenly from maximum to minimum within five minutes.[10]

RESULTS

In the present study, fluorescent study of renal biopsy tissue was also done using Hollande's fixative and it was observed that a better diagnosis could be done when used with routine H & E and immunofluorescent studies. The method was found effective in identifying IC and was helpful in detecting the site where the IC deposits were situated but like EM it was not able to detect that which class of immunoglobulin is involved in IC deposits. Thus, it is recommended to be used with routine H & E and not as an alternative to the IF method. Where particular class of immunoglobulin involved in IC deposit can be identified. The CIC were found to be actively responsible for the glomerular assault in various forms of GN.

Table 1: Results of immunofluorescence microscopy findings.

Types of disease	Immunofluorescence microscopy findings
APGN n=6	Granular deposits of IgG and IgM along capillary walls
MCD n=10	NO immuno deposits in 4 cases. Only one case shows focal IgG deposit in mesangium.
MGN n=24	Subepithelial granular deposits of IgG following contour of GBM
MPGN n=8	Granular lumpy deposition of IgG along capillary wall and mesangium in all
MSGN n=6	Immune deposits of IgG, IgM in mesangium
FSGS n=8	IgM and IgA deposits in solid area and capillary wall
Amylidosis n=8	No deposits seen
SLE n=4	Messangial IgG and IgA deposits
Diabetic n=4	No deposit seen
	n = number of cases

Type 2: Type of immunoglobulins identified in immune complex deposits in various glomerulonephrities by IEF.

Type of disease	Type of immunoglobulin			No. of cases found positive
	IgG	IgM	IgA	
APGN n=6	4	2	0	6 (100%)
MCD n=10	2	0	0	2 (20%)
MGN n=24	18	0	0	18 (75%)
MPGN n=8	8	0	0	8 (100%)
MSGN n=6	2	4	0	6 (100%)
FSGS n=8	0	4	2	6 (75%)
Amylidosis n=8	0	0	0	0
SLE n=4	2	0	2	4 (100%)
Diabetic n=4	0	0	0	0

The high CD values of CIC in SLE. FSGS, MGN and MPGN and APGN correlate well with their high prevalence responsible for the glomerular injury in various GN. There were adequate amounts of IC deposits identified in the glomeruli under FM Hollande's fixed sections. which later were confirmed to be IgG type by IFM.

Thus, lgG class was found to be the most significant in causing glomerular injury followed by 1GM and IGA. It is clearly evident that IC play a very important role in the etiopathogenesis of various GN (whether fixed IC or

CIC) and their estimation and recognition is very Important for the diagnosis, management and prognosis of various renal disorders.

DISCUSSION

Glomerulonephritis constitutes nearly 60% of all non-surgical renal disease and accounts for a substantial number of cases of end-stage renal disease. Glomerulonephritis has unique place in the evolution of understanding the immune-mediated disorders.

The immune complexes play a very important role in various types of glomcrular injury. The fixed as well as circulating immune complexes (CIC) are equally important in their involvement leading to renal damage.[11] Keeping in view the role of IC in renal damage the present study was performed to isolate and characterize the immune complexes (IC) in immunologic glomerulopathies.

The immune complexes comprise of the antigen (usually not related to glomerular antigens), antibody (immunoglobulin) arid complement. These are also called as “immune deposits" and are identified by Immunofluorescence as irregular granular deposits along the capillary wall and in the mesangium.[12] Immune deposits are localized in the subepithelial, subendothelial or mesarigial regions, depending upon the molecular size of the IC and subtype of the glomerulonephritis.

The antigen may be derived from the products of viruses, bacteria or other parasites. Antibody-induced damage may be from antibodies against intrinsic glomerular antigens or from circulating antigens planted in the glomerulus and injury resulting from, deposition of CIC entrapped or fixed in glomerulus. EM though a good technique for identification of these complexes in tissues, however is unable to characteristic the type of immune complex i.e. whether lgG, 1gM or so on but also shows some diagnostic.

The lgG class of immunoglobulins was found to be most fatal to the G13M, the 1gM and IgA were also found to cause glomerular damage. Formation of glomerular deposits of IC in situ occurred as a result of combination of antibodies with autologous non- basement membrane antigens or non-glomerular antigens planted on glomeruli. This mechanism was seen responsible for most cases of ICGN.

CONCLUSION

The immunofluorescence microscopy proved to be very useful and essential, for proper diagnosis and therapy of a renal disease. IFM comes out to be a very good indicator of the deposition site and class of immunoglobulin involved in the Immune-complex deposit. Identification and characterization of IC will help in evaluating and understanding their role in etiopathogenesis of various renal disorders. It not only gives an insight into the basic disease mechanism, but also enables to understand the disease progression and varied final outcomes in different renal disorders ultimately guiding the management and suggesting prognostic outcome.

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