

APPLICATION OF IMMUNOPEROXIDASE (PAP) TECHNIQUE FOR DEMONSTRATION OF DEPOSITED IMMUNOGLOBULINS IN RENAL BIOPSIES

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Abstract

The presence of different immunoglobulins (IgG, IgM, and IgA) was studied in 50 routinely fixed paraffin embedded renal biopsies. IgG deposits was seen independently in 26 (52%) cases, in combination with IgA in 14 (28%) cases, with IgM in 1 (2%) case and with both IgA/IgM in 1 (2%) case. IgA alone was seen in 1 (2%) case only. IgM was seen only in 2 (4%) cases. In one case it was seen in combination with IgG and in the other case with both IgG & IgM. The PAP staining technique was found to be extremely useful, It has many advantages over the established Immunofluorescence technique including lower cost and less cumbersome methodology. It was found that PAP stain along with methenamine silver stain was extremely useful in histopathological classification.(JPMA: 38 : 66 , 1988).

INTRODUCTION

Pathogenesis of renal diseases involve many mechanisms of injury among which immunological mechanisms are of paramount importance because they underlie most of the renal diseases^{1,13}. The knowledge of involved mechanisms of injury is important to forestall the course of a disease and to embark on a proper treatment modality^{7,12}. Many glomerular injuries are believed to involve local deposition of Immunoglobulins mainly IgG, IgM and IgA², the demonstration of Immunoglobulins in the renal glomeruli is of utmost importance for reaching a conclusion about involved immune mechanisms of injury. Till recently, the only reliable method of detecting deposited immunoglobulins was Immunofluorescent microscopic technique⁸ Nevertheless, this technique is cumbersome 'and costly compared to newly developed technique of Immunoperoxidase. This technique has revolutionised the issue in being highly specific, sensitive and requires the use of an ordinary light microscope giving the morphological details at the same time⁹. This study was designed to evaluate the application of Peroxidase Anti Peroxidase (PAP) staining technique in formalin fixed, paraffin embedded renal biopsies for demonstrating the presence of immunoglobulins: IgG, IgA and IgM.

MATERIALS AND METHODS

The material for study consisted of renal biopsies, originally fixed in 10% formalin of fifty selected cases of Nephrotic Syndrome, examined histologically in the Department of Pathology from January 1981 to March 1986. Detailed clinical data was collected from the department of Nephro-urology, Jinnah Postgraduate Medical Centre. Each case was studied with the help of H&E, methenamine silver stain and PAP stain. The PAP stain was done with "Histoset Immunoperoxidase staining kits" of Ortho Diagnostics Systems for Immunoglobulins. The PAP stain was used to confirm and further clarify the diagnosis made with the help of H & E and methenamine silver stain. It demonstrated the type, site (subendothelial, subepithelial or mesangial) and the patterns (linear, granular, lumpy or linear

with interrupted granular) of immunoglobulins deposits. The intensity of staining was recorded as 1+ to 4+. Sections were considered to be positive, when the glomeruli showed reddish brown deposits in the mesangium or para-mesangial areas against a yellow blue counter staining background, while its negative control showed no staining. Palatine tonsils of juvenile/adolescent group were used as known positive controls. The cases were classified in various morphological entities according to the classification by Heptinstall (1983).

OBSERVATIONS AND RESULTS

The cases which comprised this study were from age 1 year to 65 years. The distribution of 50 cases according to age, sex and histological diagnosis is shown in Table 1.

TABLE - I
Distribution of 50 Cases according to Histological Diagnosis, Age and Sex.

Histopathological Dx	MCD		AGN		MPGN		MGN		FGN		CGN		LN		Total	Percentage
	M	F	M	F	M	F	M	F	M	F	M	F	M	F		
Age Group (years)																
0-7	2	-	-	-	-	1	-	-	-	-	-	-	-	-	3	6
8-15	-	1	-	1	3	1	-	-	-	-	2	1	-	-	9	18
16-25	3	1	-	-	3	3	-	1	1	-	-	-	-	1	13	26
26-35	-	-	-	-	4	1	-	-	2	-	2	2	1	-	12	24
36-65	2	-	-	-	4	2	3	-	-	1	1	-	-	-	13	26
Total	7	2	-	1	14	8	3	1	3	1	5	3	1	1	50	100

KEY: MCD = Minimal Change Disease, AGN = Acute Glomerulonephritis
 MPGN = Membrano Proliferative Glomerulonephritis, MGN = Membranous Glomerulonephritis
 FGN = Focal Glomerulonephritis, CGN = Chronic Glomerulonephritis
 LN = Lupus Nephritis
 M,F = Male, Female

Some form of deposited immunoglobulins was seen in 43 (86%) out of 50 cases in the glomeruli. These cases showed deposition of IgG, IgA or IgM or a combination of them (Table II).

TABLE - II
Distribution of Immunoglobulins (Igs) deposits in Glomeruli in different Histopathologically diagnosed Groups.

Immunoglobulins	No. of Cases	IgG	IgA	IgM	Combination Deposits			
					IgG/IgA	IgG/IgM	IgA/IgM	IgG/IgA/IgM
Histopathological Dx		+	+	+	+	+	+	+
MCD	9	7	-	-	-	-	-	-
AGN	1	-	-	-	1	-	-	-
MPGN	22	11	-	-	5	1	-	1
MGN	4	3	1	-	-	-	-	-
FGN	4	-	-	-	3	-	-	-
CGN	8	7	-	-	1	-	-	-
LN	2	1	-	-	1	-	-	-
TOTAL	50	29	1	-	11	1	-	1

NOTE: The cases with the doubtful Immunoglobulins (Igs) deposits are excluded.

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Out of 7(14%) cases which showed no Igs, 4 were of Membranoproliferative glomerulonephritis 2 of

Minimal change disease and 1 of Focal glomerulonephritis. No immunoglobulin deposition was seen in tubular basement membrane. One of the problems noted was non specific staining. It was seen in 8(16%) cases at the edges and in the tissue cracks in 2(4%) cases. However, the major problems was the staining of serum proteins within the glomeruli, blood vessels and in tubules seen in 15 (30%) cases. This required very careful interpretation specially in the glomeruli. Even then 8(16%) cases were labelled as doubtful having doubtful deposits of anyone of the Immunoglobulins.

DISCUSSION

The PAP staining technique was found more useful, less cumbersome and cheaper compared to the Immunofluorescence technique, the standard method so far. PAP method has many advantages over the Immunofluorescence technique, e.g.:

- * more sensitive than immunofluorescence technique¹⁰.
- * Easily applied in our laboratory set up in Pakistani not requiring any special equipment or training of the staff.
- * Important diagnostic stain for retrospective studies and obviates the need for a fresh specimen and frozen sections with its affiliated formalities.
- * Can be done on already stained sections with H&E¹⁴. Thus even if the tissue is not available or if the glomeruli are subsequently not seen in the serial sections, it can be of immense help.
- * Stained sections studied with ordinary light microscope and do not require a special microscope (fluorescent microscope) which is costlier.
- * Tissue morphology preserved for study and thus the site of Igs deposits pin-pointed and its relation with other structural elements easily studied . This is relatively difficult in immunofluorescence method, where the tissue morphology cannot be seen and which requires sufficient training to interpret the results.
- * The stain serves as a good record for several months, can be easily photographed with ordinary film and no special arrangements are required. This is not possible in immunofluorescence where immediate photographic recording is essential, otherwise the fluorescence will fade quickly, and it requires a special film and an automatic exposure meter designed for those films.
- * Can be selectively used in cases which show glomeruli on H&E and thus wastage of stain and efforts can be prevented. This is not possible in immunofluorescence ordinarily unless and until a special microscope is available to confirm the presence of glomeruli in tissue specimens used for it. With sp many advantages, it also has following disadvantages:
- * At present the cost is high i.e., about one hundred and forty rupees per case. The cost is likely to come down with increase in demand. Also, with further experience the wastage in working will also be reduced and so it will become more economical.
- * The time of staining which is about six hours. However, the use of “Stain-Plate” can reduce the time to about two hours. Also experiments are underway to reduce the time of staining.
- * The major problem, however, encountered was of nonspecific staining of serum proteins, which hindered the proper visualisation of Igs deposits. To overcome this, we suggest the additional step of “proteolytic digestion” as established by various workers^{3,4,11}. In this study an attempt was also made to see how far the demonstration of igs is of help in the diagnoses of various kidney lesions. It was found that PAP stain alongwith methenamine silver stain was very useful in histopathological classification. Many workers who have used this technique^{6,7,5,15} have reported similar findings and advantages of PAP technique over the Immunofluorescence microscopic technique.

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