Original Article

Immunoperoxidase and Immunofluorescence on Formalin-Fixed, Paraffin-Embedded Tissue Sections versus Immunofluorescence on Frozen Sections in the Assessment of Renal Biopsies

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Abstract

Introduction: There are few published studies comparing immunofluorescence on formalin-fixed, paraffin-embedded (FFPE) tissue sections (IF-P) and immunoperoxidase on FFPE tissue sections (IP-P) with immunofluorescence on frozen sections (IF-F) for evaluation of renal diseases. Also, the accuracy for each method differs greatly. The aim of this study was to evaluate IF-P and IP-P as an alternative to IF-F (gold standard method) in the diagnosis of renal biopsies specimens. **Methods:** In all, 101 renal biopsies were subjected to IF-P, IP-P, and IF-F staining to demonstrate immunoglobulin IgA, IgG, and IgM immune deposits. Sensitivity, specificity, false-positive, and false-negative values were calculated. **Results:** IP-P showed sensitivity of 61.8%, 74.2%, and 64.2%, and specificity of 84.8%, 69.2%, and 66.7% for IgA, IgG, and IgM, respectively. IF-P showed a sensitivity of 45.6%, 69.4% and 52.8%, and specificity of 87.9%, 87.2% and 77.1% for IgA, IgG and IgM, respectively. False-positive cases of IF-P and IP-P were 4, 5, and 11 and 5, 12, and 16 for IgA, IgG, and IgM, respectively. **Conclusion:** Where IF-F lacks glomeruli or fresh renal biopsies are not available, IP-P is a sensitive method, whereas IF-P is a specific method for the evaluation of immune deposits in the renal tissue biopsies. The presence of false-positive cases in both methods deserves further research.

Keywords: *Frozen sections, immunofluorescence, immunoperoxidase, paraffin sections, renal biopsy*

Introduction

For more than 65 years, immunofluorescence on frozen sections (IF-F) has been the gold standard method for the evaluation of renal biopsy specimens along with light and electron microscopy.[1] It is one-step technique, fast, inexpensive, easy to interpret, and preferred by most histopathologists. In this method, fluorescein-labeled antibodies are used to detect immunoglobulin IgA, IgG, IgM, kappa, lambda, C3, C1q, and fibrin on renal frozen sections for the diagnosis of renal diseases.

However, IF-F has many drawbacks. First and the most importantly, transport of fresh samples requires special arrangements to preserve tissue antigenicity for immunofluorescence. If the sample cannot be delivered fresh to the laboratory, then it must either be snap-freezed or placed into Michel's transport media for delivery particularly between hospitals. Such facilities are not always available in clinical areas. The use of special holding fixatives to allow transportation before freezing may lead to loss of antigen reactivity. Second, selecting renal tissue containing glomeruli requires examination with a dissecting microscope. If the biopsy is small, there may be an insufficient number of glomeruli to carry out immunofluorescence. Hence, the formalin-fixed, paraffin-embedded (FFPE) tissue sections are sometimes the only tissue available for the diagnosis of renal diseases. Third, antigen diffusion can also complicate the diagnosis by causing difficulties in the determination of Ig distribution. Fourth, the health and safety risks involved in dealing with infectious renal frozen tissues and exposure to ultraviolet microscopy, albeit small. Fifth, difficulty to store immunofluorescent slides and frozen tissues because of fluorescence fading and possible loss of antigen reactivity, respectively. Sixth, autofluorescence in mammalian cells might confuse with specific IF-F results. Finally, most immunohistochemical methods work on FFPE tissue sections.

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Possible alternatives to IF-F are currently two methods, IF on FFPE tissue sections (IF-P) and immunoperoxidase on FFPE tissue sections (IP-P). In the literature, there are few published studies comparing the two methods with IF-F. Also, the accuracy for each method differs greatly. The aim of this study was to evaluate IF-P and IP-P as an alternative to IF-F in the diagnosis of renal biopsies.

Materials and Methods

Collection of renal specimens

Renal biopsies were collected from Sultan Qaboos University Hospital registry from 2006 to 2015. Cases in which IF-F showed with positive reaction for IgA, IgG, and IgM markers singly or in combination were selected. The corresponding paraffin blocks were retrieved and sectioned for IF-P and IP-P. Very small renal biopsies that cannot be sectioned and blocks with no glomeruli present were excluded. This study was approved by the Medical Research Committee and Ethics Committee at Sultan Qaboos University, College of Medicine and Health Sciences, Oman (MREC# 1108).

Using a dissecting microscope, fresh renal biopsy is usually dissected into three pieces; one portion for light microscopy, the other for IF-F, and the third portion for electron microscopy examination. In this study, the findings of electron microscope examination were not included. For each of the following methods, known positive and negative controls were run with each set.

IF-F

The portion of renal biopsy was snap-frozen in liquid nitrogen and cut at 5 μm thickness using a cryostat (serial no. 0325; Thermo Scientific, Cheshire, UK). Slides were fixed in acetone at 4°C for 10 min and then air-dried at room temperature for 5 min. Slides were circled using a pen (code no. S2002; Dako, Glostrup, Denmark). Slides were then washed in phosphate-buffered saline (PBS) at pH 7.2, three changes for 5 min each. Slides were incubated with fluorescein isothiocyanate (FITC)-labeled antibody in the dark at room temperature as in Table 1. Excess FITC-labeled antibodies were drained off. Slides were then washed in PBS at pH 7.2, three changes for 5 min each. Finally, slides were mounted in glycerol medium and examined by immunofluorescence microscope (BX50F4; Olympus, Tokyo, Japan).

IF-P

FFPE tissue blocks were cut at 3 μm using a rotatory microtome (Leica RM2135; Nussloch, Germany). Slides were deparaffinized with two changes of xylene for 3 min each, rehydrated with 100% twice for 3 min each, 95% alcohol for 1 min, and finally with 70% alcohol for 1 min. Slides were then washed with tap water for 3 min and then the tissues in the slides were marked with a Dako pen. Slides were washed with PBS three times, each time for 10 min. Then, slides were incubated with proteinase K (ready to use, code no. S3020; Dako, CA, USA) for 1 h. Slides were washed again with PBS three times, each time for 10 min. Slides were incubated with primary antibody as in Table 1. Then, they were washed with PBS three changes, each time for 10 min. Finally, slides were mounted in glycerol medium and examined by immunofluorescence microscope (BX50F4; Olympus).

Table 1: Antibodies used for immunoperoxidase, immunofluorescence on formalin-fixed, paraffin-embedded tissue sections, and immunofluorescence on frozen sections staining methods

Staining methods	Antibodies	Code No.	Antibody (g/L)	Dilution	Incubation time (min)
IF-F	IgA, FITC	F0316	1.5	1:20	60
	IgG, FITC	F0315	1.1	1:40	60
	IgM, FITC	F0203	4.1	1:20	60
IF-P	IgA, FITC	F0316	1.5	1:20	60
	IgG, FITC	F0315	1.1	1:40	60
	IgM, FITC	F0203	4.1	1:20	60
IP-P	IgA	A0262	5.0	1/6000	30
	IgG	A0423	5.7	1/7000	30
	IgM	A0425	4.1	1/5000	30

FITC: Fluorescein isothiocyanate; IP-P: Immunoperoxidase on formalin-fixed, paraffin-embedded tissue sections; IF-F: Immunofluorescence on frozen sections; IF-P: Immunofluorescence on formalin-fixed, paraffin-embedded tissue sections. All antibodies were obtained from Dako (Glostrup, Denmark). All antibodies were diluted with antibody diluent (code no. S0809; Dako)

IP-P

Paraffin blocks were cut, deparaffinized, and washed in PBS as in IF-P method. After washing, slides were incubated with 3% hydrogen peroxidase for 10 min, and then washed once for 5 min in PBS. Slides were incubated with proteinase K for 30 min followed by three times washing in PBS each for 5 min. After that, slides were incubated with primary antibody as in Table 1. Slides were then washed with PBS three times each for 5 min followed by incubation for 30 min with secondary antibody (EnVision + System-HRP-labeled polymer anti-Rabbit; Dako) and then washed with PBS three times each for 5 min. After that, the reaction was visualized using diaminobenzidine (code no. K3468; Dako) for 2 min. Slides were counterstained with Mayer's hematoxylin for 2 min and then washed for 2 min in running tap water. Finally, slides were dehydrated, cleared, mounted in DPX, and examined by light microscope (BX51; Olympus).

Evaluation

Each method was assessed separately without knowledge of IF-F results by a renal pathologist. The intensity of staining in renal tissues was scored on a scale of 0 (no reaction), +1 (weak reaction), +2 (moderate reaction), and +3 (strong reaction).

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 23 (SPSS Inc., Chicago, IL, USA). Calculations for sensitivity, specificity, positive predictive value, negative predictive value, kappa (κ) test, and *P* -value were used for analysis of the IF-P and IP-P compared to the gold method (IF-F). For κ test, a value above 0.80 was considered perfect agreement. A kappa value between 0.61 and 0.80 was regarded as good agreement, from 0.4 to 0.6 moderate agreement, from 0.21 to 0.40 fair agreement, and less than 0.2 poor agreement. A *P* value of less than 0.05 was considered statistically significant.

Results

A total of 160 biopsies were retrieved. Glomeruli were present for evaluation in paraffin blocks in 101 (63.13%) cases. The rest were excluded due to inadequate tissue. Males accounted for 36.6% of the cases and females for 63.4%. The mean age was 25.53 years, ranging from 9 months to 66 years. Among those 101 cases, there were 35 lupus nephritis, 20 focal and segmental glomerulosclerosis (FSGS), 13 membranous glomerulopathy (MN), 10 membranoproliferative glomerulonephritis (MPGN), 9 IgA nephropathy (IgAN), 3 mesangioproliferative glomerulonephritis (MesPGN), and 11 others [pauci-immune vasculitis, (1), acute tubular injury (3), diffuse mesangial proliferation (6), chronic immune complex mediated glomerulonephritis (1)].

IP-P

IgA, IgG, and IgM showed specificity of IP-P in 84.8%, 69.2%, and 66.7%, respectively. Whereas, sensitivity of IgA, IgG, and IgM was 61.8%, 74.2%, and 64.2%, respectively [Table 2]. There was a moderate agreement between the two staining methods for IgA and IgG markers and a fair agreement for IgM marker as the kappa values were 0.40, 0.43, and 0.31, respectively, for positive and negative staining and the *P* values for all were >0.05. The staining pattern of IP-P is similar to that of IF-F [Figures 1a and b].

IF-P

IgA, IgG, and IgM showed a specificity of 87.9%, 87.2%, and 77.1%, respectively. Whereas, the sensitivity of IgA, IgG, and IgM was 45.6%, 69.4%, and 52.8%, respectively [Table 3]. There was a fair agreement between the two staining methods for IgA and IgM markers and a moderate agreement for IgG marker and the kappa values were 0.30, 0.27, and 0.53 respectively, for positive and negative staining and the *P* values for all were >0.05. The staining pattern of IF-P is similar to that of IF-F [Figures 1a and c].

Discussion

Despite the improvement in antigen retrieval methods, pure qualitative antibodies, good detection system, and highly automated immune-machines, most histopathology laboratories still use direct IF-F for the evaluation of immune deposits in glomerular diseases.

Table 2: Comparison between IF-F and IP-P findings for IgA, IgG, and IgM in 101 renal biopsy samples

IP-P	IF-F	Positive	Negative	Sensitivity	Specificity	PPV	NPV
IgA	Positive	42	5	61.8	84.8	89.4	51.9
	Negative	26	28				
IgG	Positive	46	12	74.2	69.2	79.3	62.8
	Negative	16	27				
IgM	Positive	34	16	64.2	66.7	68	62.7
	Negative	19	32				

IP-P: Immunoperoxidase on formalin-fixed, paraffin-embedded tissue sections; IF-F: Immunofluorescence on frozen sections; PPV: Positive predictive value; NPV: Negative predictive value

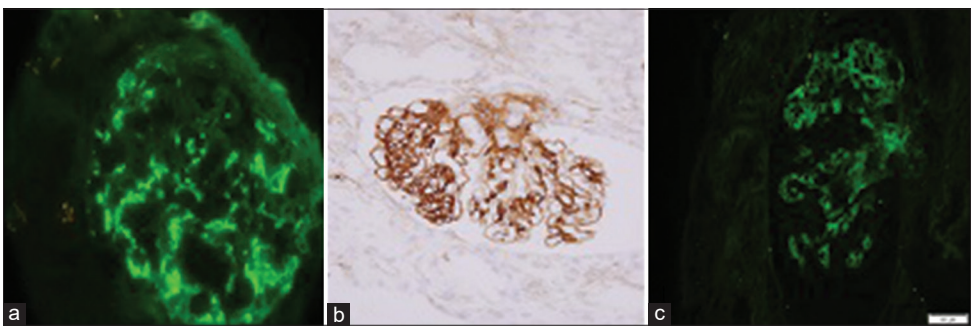

Figure 1: Representative renal biopsy specimens using various staining methods of IgA in mesangioproliferative glomerulonephritis. (a) Immunofluorescence on frozen sections (×600). (b) Immunoperoxidase on formalin-fixed, paraffin-embedded tissue sections (×400). (c) Immunofluorescence on formalin-fixed, paraffin-embedded tissue sections (×400)

Table 3: Comparison between IF-F and IF-P findings for IgA, IgG, and IgM in 101 renal biopsy samples

IF-P	IF-F	Positive	Negative	Sensitivity	Specificity	PPV	NPV
IgA	Positive	31	4	45.6	87.9	88.6	43.9
	Negative	37	29				
IgG	Positive	43	5	69.4	87.2	89.6	64.2
	Negative	19	34				
IgM	Positive	28	11	52.8	77.1	71.8	59.7
	Negative	25	37				

IF-F: Immunofluorescence on frozen sections; IF-P: Immunofluorescence on formalin-fixed, paraffin-embedded tissue sections; PPV: Positive predictive value; NPV: Negative predictive value

Our standard immunofluorescence panel includes IgA, IgG, IgM, C3, C1q, kappa, and lambda in keeping with international guidelines.[2] Our study, however, was limited to three classes of Igs (IgA, IgG, and IgM) due to the limited financial resources available for the project. In this study, the results of IP-P revealed a sensitivity of 61.8%, 74.2%, and 64.2% for IgA, IgG, and IgM, respectively. Among the three renal markers, IgG showed the highest sensitivity, as the false-negative cases were only 16. IgA and IgM showed false-negative cases of 26 and 19, respectively. IP-P showed a specificity of 84.8%, 69.2%, and 66.7%, for IgA, IgG, and IgM, respectively. Among the three renal markers, IgA showed the highest specificity, as the false-positive cases were only five. IgG and IgM showed false-positive cases of 12 and 16, respectively.

Our finding for IP-P staining is higher than those reported by a similar study.[3] In their 70 renal biopsy specimens, it was found that IgA, IgG, and IgM had a sensitivity of 49%, 42%, and 49% and a specificity of 39%, 31.3%, and 46%, respectively. Subsequently, they concluded that IP-P method is not suitable for evaluation of renal biopsies.

Other similar studies show different findings. In a study which used 48 renal biopsies to compare between IF-F and IP-P using a citrate buffer as an antigen retrieval method, a sensitivity of 76.47%, 93.75%, and 95.45% for IgA, IgG and IgM, respectively, was found.[4] However, the specificity in their study for IgG (54.54%) and IgM (57.14) was lower than in our study. The specificity for IgA (96%) is higher than in our study.

The study by Shubham *et al.* revealed a much higher rate. In their study of 100 renal biopsy specimens, the sensitivity for IgA, IgG, and IgM was 92%, 95.1%, and 86.5% and specificity was 100%, 79.5%, and 87.3, respectively. They concluded that IP-P can be used as a primary method or alternative method for IF-F, where tissue for IF-F is not adequate.[5] Another study which used 398 renal biopsies to compare between IF-F and IP-P showed a sensitivity of 80%, 72%, and 98% and a specificity of 94%, 89%, and 24% for IgA, IgG, and IgM, respectively. They concluded that glomerular deposits of Igs and complement in the assessment of renal specimens can be detected by IP-P.[6]

In this study, the results of IF-P staining method showed a sensitivity of 45.6%, 69.4%, and 52.8% and specificity of 87.9%, 87.2%, and 77.1% for the evaluation of IgA, IgG, and IgM immune deposits in the renal biopsy specimens, respectively. Another study revealed a sensitivity of 56.5%, 73.9%, and 44.4% for IgA, IgG, and IgM immune deposits, respectively, in the assessment of 70 renal biopsy specimens. Their conclusion was that IF-P cannot replace IF-F in the assessment of renal biopsies and must be interpreted with great caution.[7]

Other study reported a higher accuracy rate for IF-P and concluded that IF-P is a valuable salvage technique for renal biopsies lacking glomeruli for IF-F. In fact, they also reported that IF-P is superior to IF-F in detecting positivity of the proximal tubular intracytoplasmic crystalline inclusions for kappa light chain in cases of light-chain Fanconi syndrome (LCFS). Kappa immunostaining was positive in only four cases of LCFS by IF-F, whereas it was positive in all 10 cases by IF-P.[8] Both studies did not report the specificity for IF-P. This study reports a high specificity for IF-P. Another study, which used three different antigen retrieval methods, namely, Tris buffer, heat induced using citrate buffer, and pronase, showed that the sensitivity and intensity of IF-P staining were less when compared with the IF-F. Despite their low sensitivity, they concluded that it is possible to establish the diagnosis in most cases of immune complex-mediated glomerular diseases with IF-P.[9]

In this study, proteinase K was used for antigen retrieval. Several optimization trials for different incubation times were tested to achieve the optimum results. Microwave treatment with and without enzyme and pretreatment link were also tested. We found that treatment with proteinase K for 30 and 60 min in IP-P and IF-P, respectively, was satisfactory. Other studies also used the same enzyme.[5-7,10-12]

It is important to note that fixation can mask the epitope and can no longer bind to the primary antibody. Masking of the epitope can be caused by cross-linking of amino acids within the epitope, cross-linking unrelated peptides at or near an epitope, altering the conformation of an epitope, or altering the electrostatic charge of the antigen.[13] Shi *et al.* used dual microwave retrieval of EDTA (0.0M, pH 8) solution in FFPE tissue sections and they achieved a high accuracy rate in demonstrating IgA, IgG, IgM, kappa,

lambda, C3, and C1q in renal biopsy specimens.[14] Other study used a combination of both microwave treatment and protease digestion and found it to be an effective method for the unmasking of antigens in paraffin sections.[15] On the other hand, Rathore *et al*. used different antigen retrieval methods including enzyme digestion, microwave oven, and heating by pressure cooker and obtained a low accuracy rate.[3] In general, the choice of antigen retrieval method depends on the target antigen, the antibody used, the type of tissue, and the type and duration of fixation.[16] In this study, we used a high detection system which is EnVision HRP. It permits high dilutions with less background. Other study used the same system.[6]

In comparison to IF-F, IP-P has many advantages including one sample is needed for both light microscopy and IP-P, production of thin sections, more accurate antigen location, storage for longer time, retrospective studies can be performed, does not require cryostat and immunofluorescence microscope,[14] most antibodies are available to work on FFPE tissue blocks, and interpretation of IP-P findings can be easily analyzed by a histologist. In comparison to IF-F, the advantages of IF-P are similar to those previously mentioned for IP-P except that IF-P needs an expensive immunofluorescence microscope to interpret the findings as well as it does not show tissue structure.

Interestingly, a number of false-positive cases was noticed in both methods. In IF-P, 4, 5, and 11 false-positive cases were seen in IgA, IgG, and IgM, respectively. Higher false-positive cases were also seen in IP-P with 5 (IgA), 12 (IgG), and 16 (IgM). This finding is in agreement with other reported study which found in their IP-P method almost similar false-positive cases of 3, 5, and 29 in IgA, IgG, and IgM, respectively.[6] This finding might indicate that some antigens might be lost or not preserved during the process of IF. Whereas with IP, antigens are preserved usually by 10% neutral buffered formalin fixative.[17,18] It has been reported that false-positive staining pattern in IF-P staining method could be due to under-digested tissue.[12] This finding deserves further research.

In IP-P and IF-P methods, we have observed in some cases of IgG an increased background, but it did not interfere with the diagnosis. This background was also seen with IF-F method. We tried several optimization methods to reduce the background but with little success. Some researchers refer this high background to thick sections or the presence of endogenous activity.[5,6]

As a limitation of this study, we point out that although the interpretation of IP-P and IF-P staining methods is almost similar to IF-F, serum proteins might deposit in the glomerular capillaries leading to nonspecific staining. It was suggested to carefully observe the location of those artifacts.[10] Also, the use of image analysis software might give a better accuracy rate. IgA, IgG, IgM, kappa, lambda, C3, and C1q renal markers were determined using an image analysis software. They scored a very high accuracy rate for almost all markers.[14]

In conclusion, where IF-F lacks glomeruli or fresh renal biopsies are not available, IP-P is a sensitive method, whereas IF-P is a specific method for the evaluation of immune deposits in the renal tissue biopsies. The presence of false-positive cases in both methods deserves further research.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Coons AH, Kaplan MH. Localization of antigen in tissue cells, improvements in a method for the detection of antigen by means of fluorescent antibody. J Exp Med 1950;91:1-13.
2. Mayo Clinic/Renal Pathology Society Consensus Report on Pathologic Classification, Diagnosis, and Reporting of GN. Sethi S, Haas M, Markowitz GS. J Am Soc Nephrol 2016;27:1278-87.
3. Rathore M, Khadim M, Mushtaq S, Atique M. Paraffin-based immunohistochemistry in the evaluation of glomerular diseases in renal biopsies. J Coll Phys Surg Pakistan 2012;22:353-7.
4. Jafari M, Monsef-Esfahani A, Solimani B. Diagnostic value of immunoperoxidase staining and immunofluorescence in the study of kidney biopsy specimens. Iran J Kidney Dis 2015;9:286-90.
5. Shubham S, Bhardwaj M, Mahapatra HS. Comparative evaluation of immunoperoxidase versus immunofluorescent techniques in interpretation of kidney biopsies. Indian J Pathol Microbiol 2016;59:305-9.
6. Molne J, Breimer ME, Svalander CT. Immunoperoxidase can replace immunofluorescence in the assessment of human renal biopsies. Am J Kidney Dis 2005;45:674-83.
7. Wagrowska-Danilewicz M, Danilewicz M. Immunofluorescence on paraffin-embedded sections in evaluation of immune complex deposits in renal biopsy specimens. Pol J Pathol 2009;60:3-9.
8. Nasr SH, Galgano SJ, Markowitz GS, Stokes MB, D'Agati VD. Immunofluorescence on pronase-digested paraffin sections: A valuable salvage technique for renal biopsies. Kidney Int 2006;70:2148-51.
9. Mubarak M, Kazi Javed I, Kulsoom U, Ishaque M. Detection of immunoglobulins and complement components in formalin fixed and paraffin embedded renal biopsy material by immunofluorescence technique. J Nephropathol 2012;1:91-100.
10. Messias NC, Walker PD, Larsen CP. Paraffin immunofluorescence in the renal pathology laboratory: More than a salvage technique. Mod Pathol 2015;28:854-60.
11. Nada R, Kumar A, Kumar VG, Gupta KL, Joshi K. Unmasking of complements using proteinase-K in formalin fixed paraffin embedded renal biopsies. Indian J Nephrol 2016;26:182-7.
12. Singh G, Singh L, Ghosh R, Nath D, Dinda AK. Immunofluorescence on paraffin embedded renal biopsies: Experience of a tertiary care center with review of literature. World J Nephrol 2016;5:461-70.
13. Werner M, Chott A, Fabiano A, Battifora H. Effect of formalin tissue fixation and processing on immunohistochemistry. Am J Surg Pathol 2000;24:1016-9.

14. Shi S, Cheng Q, Zhang P, Wang N, Zheng Y, Bai XY, *et al*. Immunofluorescence with dual microwave retrieval of paraffin-embedded sections in the assessment of human renal biopsy specimens. Am J Clin Pathol 2013;139:71-8.
15. Chowdhury AR, Ehara T, Higuchi M, Hora K, Shigematsu H. Immunohistochemical detection of immunoglobulins and complements in formaldehyde-fixed and paraffin-embedded renal biopsy tissues; an adjunct for diagnosis of glomerulonephritis. Nephrology (Carlton) 2005;10:298-304.
16. Matos LL, Trufelli DC, de Matos MG, da Silva Pinhal MA. Immunohistochemistry as an important tool in biomarkers detection and clinical practice. Biomark Insights 2010;5:9-20.
17. Larsen CP, Ambuzs JM, Bonsib SM, Boils CL, Cossey LN, Messias NC, *et al*. Membranous-like glomerulopathy with masked IgG kappa deposits. Kidney Int 2014;86:154-61.
18. Nasr SH, Fidler ME, Said SM. Paraffin immunofluorescence: A valuable ancillary technique in renal pathology. Kidney Int Rep 2018;3:1260-66.