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DIAGNOSTIC ACCURACY OF IMMUNOFLUORESCENCE IN GLOMERULO-NEPHRITIS: A SINGLE CENTER EXPERIENCE OF 150 CASES

Safana Sadaf, Saima Batool, Anila Chughtai, Asma Zafar, Omar Chughtai, Akhtar Sohail Chughtai

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ABSTRACT

Objective: The objective of this study was to investigate the diagnostic role of immunofluorescence (IF) in accurate diagnosis of various glomerulonephritis (GN) in Pakistani population.

Material and Methods: Cross sectional study of one-year duration from January, 2019 to December, 2019 was conducted at histopathology department, Chughtai Institute of Pathology, Lahore, Pakistan. A total of 150 cases of GN were included in this study in which light microscopy (LM) and IF findings were reviewed. All clinical and biochemical data was recorded on a proforma for each case.

Statistical methods use to analyze this study included mean ± SD, percentages, ratio, sensitivity, specificity, positive predictive value and negative predictive value.

Results: Of the total cases, 79 (52.7%) were males and 71(47.3%) were females. Mean age of the patients was 24.29±15.07 years. IF helped in changing the diagnosis of LM in 28 cases (18.67%). In this study, the most common pattern of GN diagnosed was membranous GN (30.6%) followed by focal segmental glomerulosclerosis (28%), lupus nephritis (11.3%) and membranoproliferative GN (7.3%). The sensitivity of IF was 96.5% and specificity was 94.4%.

Conclusion: Our study reinforces the fact that IF in conjunction with LM plays crucial role in the diagnosis of GN along with relevant clinical and biochemical data.

Key Words: Light Microscopy, Immunofluorescence, Electron microscopy, Glomerulonephritis, Focal segmental glomerulosclerosis.

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INTRODUCTION

Renal glomerular diseases (glomerulo-nephritis (GN)/ glomerulopathies) claim significant role in human morbidity and mortality [1]. Western data showed that Focal Segmental Glomerulosclerosis (FSGS) is the most common GN in African Americans, while in whites; membranous glomerulonephritis (MGN) represent predominant GN [2,3]. Renal biopsy has been a gold standard in diagnosis approach of medical renal disease from quite a long time but recent advancement in technology has further enhanced its diagnostic significance [1]. These advanced complementary technologies include Immunofluorescence (IF) and Electron Microscopy (EM) which further enhance the diagnostic yield and help rendering a definitive diagnosis. Along with these diagnostic modalities, a thorough clinical data and biochemical/serological findings are imperative for final diagnosis in cases of GN [4,5].

Common and more prevalent types of GN include minimal change disease (MCD), FSGS, MGN, lupus nephritis (LN), IgA nephropathy (IgAN), diabetic nephropathy (DN), membranoproliferative glomerulonephritis (MPGN), post infectious GN (PIGN) and crescentic glomerulonephritis (CGN) [6].

Most common clinical presentations of GN are in forms of combination of various symptoms termed as clinical syndromes. Most common of these clinical syndromes are nephrotic syndrome (NS) and nephritic syndrome (NIS). It is not uncommon for GN to present as isolated symptom not filling the criteria for NS or NIS. These symptoms include proteinuria, macro or micro hematuria and features of acute or chronic renal failure [7, 8].

In developed countries, all three diagnostic modalities Light Microscopy (LM), IF, EM are available and are an integral part of diagnostic approach for GN. However, the facility of EM is not widely available in many developing countries like Pakistan. Therefore, the diagnostic approach for the cases of GN is based mainly upon LM in conjunction with IF in majority of centers across Pakistan [4,5]. It is claimed that even in absence of EM, LM and IF techniques when applied together can establish accurate diagnosis in majority of cases of GN. The role of IF is of crucial significance in establishing the diagnosis along with light microscopic findings in the setting where EM facility is not available as LM alone

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is not sufficient and can lead to misdiagnosis in many cases [9-12]. Hence, the claim for appropriateness of using only IF in combination with LM without further help from EM in diagnosing GN accurately needs strong grounds.

This study aimed to investigate the role of IF in making accurate diagnosis of various GN when used in combination with LM. Correlation with relevant clinical and biochemical/serological findings was also assessed for additional help in diagnostic approach. Furthermore, the spectrum of histological patterns was noted for various GN.

MATERIAL AND METHODS

After taking approval from Institutional Review Board, a cross sectional study was conducted at Histopathology Department of Chughtai Institute of Pathology, Lahore, Pakistan. The study was designed to include all cases reported in span of one year from January, 2019 to December, 2019. A total of 252 cases were reported in designated year, out of which only 150 cases fulfilled the inclusion criteria and were included in the study. Inclusion criteria was set as any histologically adequate medical renal biopsy sent as two cores, one in 10% buffered formalin for LM and other in normal saline for IF along with complete clinical and laboratory data. Patients who presented with NS and NIS were included in the study. A standard histologic adequacy criterion of more than or equal to 10 glomeruli was applied to each biopsy [13]. Exclusion criterions were incomplete clinical or laboratory data, histologically inadequate biopsies and biopsies received as only one core in either formalin or normal saline and biopsies not showing features of GN.

For all 150 cases included in study, the data regarding LM and IF findings along with clinical and laboratory details was recorded on a proforma. Written informed consent was taken from the patients.

For LM evaluation, the core received in 10% buffered formalin was processed in processor (Tissue-tek VIP 6 A1) and then cut at thickness of 3 micron to prepare standard glass slides for microcopy. This was followed by application of special stains including Periodic Acid Schiff (PAS), Gomori Methenamine Silver (GMS) and Trichrome as per standard internationally recommended protocol. For IF evaluation, core received in normal saline was processed in cryostat machine as frozen tissue at -20 degree Celsius. Standard glass slides were prepared after embedding tissue in OCT compound, cutting the tissue at thickness of 3 microns, fixing it in alcohol, air drying it for 10 mints and treating with phosphate buffer saline (PBS) at PH 7.2 for 30 minutes. The slides were then treated with fluorescein isothiocyanate labeled and diluted (1:20) antisera of antibodies IgA, IgM, IgA, C1q and C3 (Dako, Glostrup, Denmark) for three hours in dark humidity chamber. Positive and negative controls were also run. Later, these slides were placed in PBS wash buffer for 5 minutes and mounted with fluorescent mounting media. The slides were viewed under green filter of immunofluorescence microscope by Motic and direct immunofluorescence findings were interpreted based on intensity, pattern and distribution of immune deposits. Each case was evaluated by two consultant histopathologists for LM and IF findings. The final diagnosis was rendered after correlating clinical data and laboratory data with LM and IF findings.

Statistical Analysis: Statistical methods used to analyze this study included mean ± SD, percentages, ratio, range sensitivity, specificity, positive predictive value and negative predictive value.

Sensitivity: True positive (TP)/TP+ false negative (FN)

Specificity: True negative (TN)/False positive (FP) +TN

Positive predictive value: TP/TP+FP

Negative predictive value: TN/FN+TN

RESULTS

Out of the total 150 cases included in the study, 79 (52.7%) cases were males and 71 (47.3%) were females.

The mean age of patients was 24.29±15.07 with age range from 2 years to 75 years. NS was the most common clinical presentation comprising of 74% (n=111) cases, 16.7% (n=25) had NIS and 9.3% (n=14) had both. 24 hours Proteinuria ranged from 1.10 to 12.80 grams/day with mean value of 4.62±2.55. Hematuria was present in 59(39.3%) cases. In Urine examination, severe proteinuria (3+) was seen in 83(55.3%) cases, moderate (2+) in 54 (36.0%) cases, and mild (1+) proteinuria in 13(8.7%) cases.

Creatinine levels <2mg/dl was seen in 119(79.3%) cases, 2-4 mg/dl in 24(16.0%) cases and 7 (4.7%) cases had levels >4mg/dl. Urea levels <50mg/dl were seen in 104 (69.4%) cases, 35(23.3%) had 50 to 150mg/dl and 11 (7.3%) cases showed levels >150mg/dl. ANA was positive in 18 (12.0%) cases, while Anti-ds DNA in 17 (11.3%) cases. Low C3 was

observed in 19 (12.7%) cases while low C4 in 13 (8.7%) cases. IF was positive in 112 (74.7%) cases while negative in 38 (25.3%) cases. In this study, the most common type of GN diagnosed was MGN (30.6%) followed by FSGS (28%), LN (11.3%) and MPGN (7.3%) (Table-I). Of the 150 included cases, IF confirmed the provisional diagnosis of LM in 112 (74.7%) cases while IF was negative in 38 (25.3%) cases. Out of those 112 cases which showed positive IF findings, 28 **(**18.67%) showed clinically significant diagnosis which was not suspected on LM evaluation. Out of these 28 cases, 7 (25%) cases were of IgAN, 2 (7.1%) cases were of stage I (early) MGN, 17 (60.7%) cases were of LN, 1 (3.6%) case was of C1q nephropathy and 1 (3.6%) case was of IgM nephropathy. Our study depicted that IgAN can manifest as a number of morphological patterns, including normal/minimal change pattern (n=1), mesangioproliferative pattern (n=3) (Figure-3), FSGS (n=1) and diffuse glomerulosclerosis (n=2).

Similarly, 2 cases of early stage MGN, diagnosed upon IF finding of strong granular IgG depositing along basement membranes, showed normal/minimal change pattern on LM. These cases could have easily been missed if only LM had been taken into consideration. Among Lupus Nephritis, 2 cases showed mesangioproliferative pattern (WHO class II), 5 cases showed FSGS (WHO class III), 4 cases showed diffuse proliferative pattern (WHO class IV) (Figure-2), 5 cases showed membranous pattern (WHO class V) and 1 case showed diffuse glomerulosclerosis (WHO class VI). One case of C1q Nephropathy showed morphological pattern of FSGS and one case of IgM nephropathy showed mesangioproliferative pattern. In our study, direct immunofluorescence provided significant help in confirming the diagnosis of 20 cases of FSGS, 46 cases of MGN (see figure 1), 1 case of DN, 4 cases of PIGN, 11 cases of MPGN and 3 cases of CGN. The statistical analysis of our study is shown in Table-II.

Table-I: Division of all glomerulonephritis cases and associated direct immunofluorescence findings.

Histopathological Diagnosis	No of cases 'n'	%	DIF positive	DIF negative
Membranous Glomerulonephritis	46	30.6	46 (moderate to strong granular IgG & C3 positivity along glomerular basement membrane)	0
Focal Segmental Glomerulosclerosis	42	28	20 (Granular IgM in 20 cases, granular C3 in 4 cases)	22
Lupus Nephritis	17	11.3	17 (Full house pattern)	0
Membranoproliferative Glomerulonephritis	11	7.3	11 (Granular IgG, C3, IgM along glomerular basement membrane)	0
Diffuse Glomerulosclerosis	8	5.3	3 (Nonspecific trapping in sclerosed areas)	5
IgA Nephropathy	7	4.7	7 (Granular IgA in mesangium)	0
Minimal Change Disease	5	3.3	0	5
Crescentic Glomerulonephritis	4	2.7	3 (Immune complex mediated, Granular IgG & C3)	1 (Pauci-immune glomerulonephritis, Granulomatosis with polyangitis).
Post Infectious Glomerulonephritis	4	2.7	4 (granular IgG and C3 positivity in mesangial pattern)	0
Diabetic Nephropathy	3	2.0	1 (Linear IgG)	2
IgM Nephropathy	1	0.7	1 (Granular IgM)	0
C1q Nephropathy	1	0.7	1 (Granular C1q)	0
Amyloidosis	1	0.7	0	1

Table-II: Statistical analysis of role of immunofluorescence in glomerulonephritis.

Statistics	Percentage
Sensitivity	96.5%
Specificity	94.4%
Positive Predictive Value	98.2%
Negative Predictive Value	89.5%

Table-III: Comparison of light microscopic diagnosis and changed diagnosis after immunofluorescence study (n=28).

Light microscopy	Immunofluorescence diagnosis
1. Mesangioproliferative GN	LN class II (n=2),
2. Focal segmental glomerulosclerosis	LN class III (n=5),
3. Diffuse proliferative glomerulonephritis	LN class IV (n=4),
4. Membranous glomerulonephritis	LN class V (n=5),
5. Global Glomerulosclerosis	LN class VI (n= 1)
1. Mesangioproliferative glomerulonephritis (n=3) 2. Minimal change disease (n=1) 3. Focal segmental glomerulosclerosis (n=1) 4. diffuse glomerulosclerosis (n=2)	IgA nephropathy (n= 7)
Minimal change disease	Early membranous glomerulonephritis (n=2)
Focal segmental glomerulosclerosis	C1q Nephropathy (n=1)
Mesangioproliferative glomerulonephritis	IgM nephropathy (n=1)

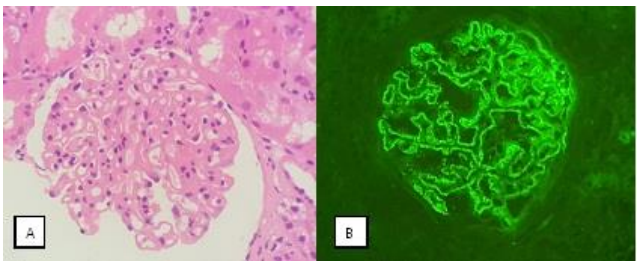

Figure-I: (A) Membranous Glomerulonephritis showing diffuse glomerular basement membrane thickening. Glomerular capillary loops appear round and rigid (H & E, x400). (B) Direct immunofluorescence of membranous glomerulonephritis showing diffuse strong granular IgG (3+) deposits along capillary walls (x400).

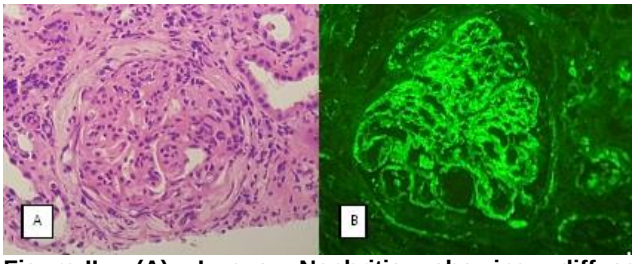

Figure-II: (A) Lupus Nephritis showing diffuse proliferative glomerulonephritis (WHO Class IV). Glomeruli showing global proliferative lesion comprising of a fibrous crescent, wire loop lesions in thickened capillary walls and glomerular endocapillary and mesangial cells proliferation (H & E, x400). (B) Direct immunofluorescence showing mesangial and sub endothelial granular immune deposits of IgG (along with IgM, IgA, C1q and C3, Full house) (x400).

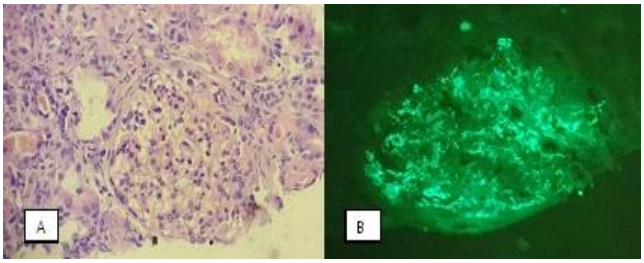

Figure-III: (A) IgA nephropathy showing mesangial cells proliferation (mesangioproliferative pattern). Tubules showing RBC's cast (H & E, x200). (B) Direct immunofluorescence of IgA nephropathy showing diffuse strong granular IgA (3+) deposits in mesangium (x400).

DISCUSSION

Percutaneous needle biopsy of the kidney is the most important tool used by nephrologist to determine the cause of suspected GN. Role of renal biopsies is imperative in evaluation of patients having hematuria, proteinuria, generalized body swelling and renal insufficiency clinically presenting as either NS or NIS [8]. The proposed diagnostic regimen for diagnosis of GN includes light microscopic, immunofluorescence and electron microscopic evaluation of renal biopsy. All these diagnostic modalities are mostly available in developed countries. However, the situation in developing countries is different and mostly only LM and IF facilities are available. In Pakistan, majority of centers only have facility for LM while only a few specialized centers have facility of LM combined with IF. Light microscopic evaluation alone is not sufficient to diagnose GN and can potentially lead to misdiagnosis, hence, is not recommended. It is recommended that at least IF evaluation is performed along with LM if no further help from EM is available [14].

In this study, the most common type of GN diagnosed was MGN (n=46, 30.6%), followed by FSGS (n=42, 28%) and LN (n=17, 11.3%). Nasir *et al* [14] study also showed MGN (24%) to be the most common type followed by FSGS (18.4%) and MCD (16%). A study conducted at SIUT (Sindh Institute of Urology and Transplantation) Karachi, Pakistan reported prevalence of common types of GN as MCD (43.8%), FSGS (38.14%) and MGN (7.96%) [4]. Another study reported the FSGS to be the most common diagnosis on renal biopsies [15].

Out of all 46 cases of MGN, 44 (95.65%) were diagnosed as stage II MGN as they showed diffuse glomerular basement membrane (GBM) thickening with subsequent spike pattern of GBM on JMS stain and moderate to strong (2+ or 3+) granular IgG & C3 along GBM upon IF evaluation. While 2 (4.35%) out of total 46 cases of MGN did not reveal any GBM thickening on LM with no spike pattern of GBM on

JMS stain but subsequent strong granular IgG & C3 along GBM on IF evaluation, hence, labeled as early/stage I MGN. Our findings are compatible with other studies [16,17]. These two cases of early MGN which have not showed classical LM and special stain findings of MGN have revealed the diagnostic importance of IF evaluation in such subtle cases of GN which can potentially be either missed or misdiagnosed as MCD otherwise. MCD and MGN both have different treatment regimen and prognosis hence it is crucial to rightly diagnose these two conditions [18].

The second most common type of GN observed in this study was FSGS constituted 42 out of 150 cases (28%). All cases of FSGS showed segmental sclerosis. Out of these 42 cases of FSGS, granular positivity of IgM was noted in 20 (47.62%) cases while granular positivity of C3 was noted in 4 (9.52%) cases. Our results are compatible with findings of Nasir *et al* [14] study which showed IgM deposition in 40% cases while C3 deposition in 20% cases of FSGS. A total of 22 cases (52.38%) of FSGS did not show any immune deposition on IF. IF can be negative in FSGS cases, because of segmental nature of lesion in only few glomeruli within whole kidney [19].

The third most common type of GN observed in this study was LN comprising 17 (11.3%) out of total 150 cases included in the study. These cases were stratified further according to WHO histological classification of LN as class II (n=2), class III (n=5), class IV (n=4), class V (n=5), class VI (n= 1). No case of class I lupus nephritis was observed in this study. All these cases of LN showed full house pattern of IF positivity for IgG, IgA, IgM, C1q and C3.

One of the most significant finding in this study was the diagnostic role of IF in 28 (18.67%) out of 150 cases included in the study in which LM findings alone were non-diagnostic. These cases included LN (n=17, 60.7%), IgAN (n=7, 25%), early MGN (n=2, 7.1%), C1q nephropathy (n=1, 3.6%) and IgM nephropathy (n=1, 3.6%). These findings are similar to results observed in studies by Abbas *et al* [4], Nasir *et al* [14] and Buch *et al* [20]. In all of these cases, the LM findings were either non-specific or subtle, hence, in absence of IF examination these cases would have been misdiagnosed.

A total of 7 (4.7%) cases of IgAN were observed in this study, all of which showed deposition of IgA in mesangium and along GBM. All these cases showed a variation in histology patterns on LM including mesangioproliferative pattern (n=3, 42.8 %), minimal change pattern (n=1, 14.3%), FSGS pattern (n=1, 14.3%) and diffuse glomerulosclerosis pattern (n=2, 28.6%). IgAN is one of the most common types of GN worldwide and it most commonly presents with hematuria. If only LM was taken into account, this entity can be misdiagnosed. It's important to diagnose this disease correctly as it has bad prognosis and can lead to end stage renal disease in 30% of the cases in 10 years [21].

The only case of IgM nephropathy (n=1, 0.7%) observed in this study showed mesangioproliferative pattern on LM and strong IgM deposition on IF. IgM nephropathy is renal disease that can progress to FSGS. IgM nephropathy has idiopathic etiology and clinically presents with complaint of proteinuria without any other associated systemic disease [22].

Out of 150 total cases included in the study only 1 (0.7 %) case was of C1q nephropathy which showed FSGS like pattern on LM. In case of no further evaluation by IF, this case might have wrongly been diagnosed as FSGS based on LM findings; however, it showed diffuse granular C1q deposition on IF leading to definitive diagnosis.

In the present study, all eleven cases of MPGN showed coarse granular moderate to strong IgG and C3 deposition in the glomerular capillary walls along with IgM. This finding is similar to study of Zucchelli *et al* [23].

Out of 8 cases of diffuse glomerulosclerosis in the present study, 3 cases showed nonspecific trapping of antibodies in sclerosed glomeruli while 5 were negative on IF.

All 5 cases of MCD in our study showed no immune deposits on IF and were diagnosed solely on LM findings. Our data showed 4 cases of CGN, 3 cases showed deposition of antibodies while 1 case was negative for antibody deposition on IF helped in sub categorization of CGN into immune-mediated CGN and Pauci-immune CGN. One case that was negative on IF was finally diagnosed as Pauci-immune glomerulonephritis (Granulomatosis with polyangitis).

Similarly, all four cases of PIGN showed granular IgG and C3 positivity in mesangial pattern, thus correlating with the findings of previous studies [24, 25].

Out of 3 cases of DN 1 case showed linear IgG along GBM while 2 cases showed negative findings on IF.

One case of amyloidosis was diagnosed on LM findings with help from special stain as it showed apple green birefringence on Congo red stain. No immune deposition was noted in this case.

CONCLUSION

We concluded that with the combination of LM and IF, majority of the cases of common types of GN can be accurately diagnosed. Furthermore, the input from IF is imperative in some special types of GN which can have varied morphological patterns and in absence of IF evaluation can potentially be misdiagnosed for example IgAN, LN, IgM nephropathy and C1q nephropathy. We also concluded that appropriate clinical and laboratory data is also mandatory for final diagnosis.

AUTHOR CONTRIBUTIONS

Safana Sadaf: Designed and executed the project, draft preparation.
Saima Batool: Literature review, statistical analysis.
Anila Chughtai: Data collection, data Analysis, Proof reading.
Asma Zafar: Study design, proof reading.
Omar Chughtai: Supervised the project.
Akhtar Sohail Chughtai: Supervised the project, proof reading.

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