Immunofluorescence and the Spectrum of Glomerular Diseases in Iraq (Single Center Study)

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Abstract

Objective: Immunofluorescent microscopy is an important tool for the diagnosis of glomerular diseases. In this study, we focused on using IF technique together with light microscopy and clinical features in the diagnosis of different types of glomerulonephritis. Spectrum of glomerular disease in Iraq is to be studied and compared with other studies in Iraq and other countries.
Methods: A total of 58 kidney biopsies were taken for routine LM, while IF technique was done in 56 of them. The study started from 1st of June 2010 to the 1st of June 2012.
Results: Focal segmental glomerulosclerosis (17/58=29.3%) topped the list followed by minimal change disease(12/58=20.7%).Immune deposition was observed in (21/56=37.5%) cases and the predominant deposit was immunoglobulin G(20/56=35.7%).The pattern of deposition was granular in most of the cases either in the glomerular basement membrane and/or in the mesangium except one case where immunoflourecent microscopy showed dominant positive staining (3+) for complement factor 1q in the glomerular mesangium and slightly positive staining for complement factor 3 in the same mesangeal areas.(Tow/58=3.4%) cases fulfilled the clinical, serological and histopathological criteria of lupus nephritis .
Conclusion: To obtain a correct diagnosis of glomerulonephritis, we require immunofourescent in parallel with light microscopic examination of renal biopsies and correlation with clinical features, biochemical and serological markers. Clinically, the majority of patients presented as nephrotic syndrome (44/58=75.9%).
Key words: Immunofluorescence,glomerular disease.

الخلاصة

يعتبر مجهر التالق المناعي اداة مهمة في تشخيص امراض الكبيبات الكلوية.لقد ركزنا في هذه الدراسة على استعمال تقنية التالق المناعي مع المجهر الضوئي و العلامات السريرية في تشخيص انواع مختلفة من التهاب الكبيبات الكلوية. لقد درسنا اطياف امراض الكبيبات الكلوية و تمت مقارنتها مع دراسات اخرى في العراق و بعض الدول .لقد اخذنا كل من 58 خزعة لفحصها كلها بالمجهر الضوئي بينما اجرينا تقنية فحص التالق المناعي ل 56 منها .ابتدات الدراسة اعتبارا من الاول من حزيران عام 2010 الى الاول من حزيران 2012.ان التهاب الكبيبات التصلبي البؤري القطعي يغطي قمة شيوع المرض بنسبة 29.3 مؤوية(17 من 58 مريض)يتبعه مرض التبدل الاصغر بنسبة 20.7 مؤوية(12 من 58 مريض). لوحظ الترسب المناعي بنسبة 37.5 مؤوية(21 من اصل 56 حالة) و كان الكلوبيولين المناعي نوع جي هو المترسب السائد و بنسبة 35.7 مؤوية (20 من 56 مريض).كان طراز الترسب حبيبي في اغلب الحالات متركزا في الغشاء القاعدي الكبيبي بالاظافة الى/ او في مسراق الكبيبات ما عدا حالة واحدة كان التالق المناعي للصبغة السائدة فيها هو لعامل التكامل 1 كيو بايجابية ثلاثة و ايجابية قليلة لصبغة عامل التكامل سي 3 في نفس المساريق الكبيبية . حالتان من اصل 58 و بنسبة مؤوية 3.4 استوفتا الصفات السريرية والمصلية وتغيرات الانسجة المرضية الخاصة بالتهاب الكلوة الذؤبي.من الناحية السريرية.الاستنتاج: لغرض الحصول على تشخيص صحيح لالتهاب الكبيبات الكلوية نحتاج الى فحص

التالق المناعي بالتوازي مع فحص المجهر الضوئي لخزع الكلى وما له علاقة من الصفات السريرية و المؤشرات الكيمياحيوية و المصلية, كان غالبية المرضى مصابين بالمتلازمة الكلائية و بنسبة مؤوية 75.9 (44 من اصل 58).

Introduction

Glomerular disorders constitute one of the major causes of morbidity and mortality [1] .In the absence of evidence of systemic disease primary glomerular diseases must be considered in the differential diagnosis. Histologically, these primary diseases are not distinct from the injury pattern seen in systemic diseases. The primary glomerulopathies are recognized by the histologic pattern defined by light microscopy(LM), immunofluorescent(IF) staining for immunogloulins, and the characteristics and locations of immune deposits on electron microscopy. Table 1 listed the types of primary glomerulonephritis (GN), with the prominent histologic findings on biopsy that define the disorder [2].

Table 1. Primary glomerular diseases, defined by histology[2].

Nephritic	Histologic findings	Nephrotic	Histologic findings
Renal-limited vasculitis/microscopic polyangiitis	Necrotizing capillary lesions, crescents; negative IF, EM	Minimal-change disease	Normal light microscopy, effaced foot processes on EM
Anti-glomerular basement membrane disease	Linear IgG staining along glomerular basement membrane	Membranous nephropathy	Subepithelial spikes • on light, IF, EM
Essential cryoglobulinemia	Fibrils on electron microscopy	Membranoproliferative glomerulonephritis	Thickened mesangial matrix, splitting (double contour) of the glomerular basement membrane, C3 granular staining on IF
		Focal segmental glomerulosclerosis	Sclerosis in portions of glomeruli, C3 in areas of sclerosis on IF
		IgA nephropathy	IgA in mesangium on IF
		Fibrillary glomerulonephritis	Fibrillar deposits in mesangium, negative Congo red staining on IF

Immunoflourescent(IF) microscopy provided insight not only into the pathogenesis of glomerular diseases but also very useful in diagnosing primary renal diseases, assessing the nature and severity of renal involvement in various systemic disorders and in addition, yielded important correlations and prognostic features [3] .Facilities for electron microscopic study is not readily available in many institutions. In most cases light microscopy(LM) and IF study are fair enough for definitive diagnosis of GN[4].The finding of characteristic staining

patterns for immunoglobulin and complement indicates that many forms of glomerulonephritis are immune complex diseases and that a few are due to anti-GBM antibodies. On the other hand, in lipoid nephrosis and toxemia of pregnancy, deposits of immunoglobulin and complement are generally absent, indicating that immunologic mechanisms are probably not responsible for these glomerular diseases. The finding of fibrin or other fibrinogen derivatives in glomeruli in toxemia of pregnancy and in certain forms of glomulonephritis supports the interpretation that these substances play a pathogenic role in certain glomerular diseases. The use of immunofluorescence has led to the recognition of two previously unrecognized renal diseases: nephropathy with mesangial IgA-IgG deposits (Berger), and a tubular disorder with deposits of immunoglobulin and complement along the basement membrane[3].

Glomerular disorders with a nephritic clinical presentation generally fall into three histologic categories defined by the immunofluorescence patterns of immune deposits within the glomerulus. These are; linear pattern as in Good pasture's and anti-glomerular basement membrane diseases, granular pattern as in SLE, Henoch-Schonlein purpura, IgA nephropathy and cryoglobulinemia, and absent pattern as in ANCA-associated vasculitis, Wegener's granulomatosis, Churg-Strauss syndrome, and microscopic polyangiitis.This categorization is a generalization, since aggressive forms of IgA nephropathy and MPGN may clinically appear nephritic and may be associated with glomerular crescents [2].

Membranous GN(MGN) stage-1 shows no thickening of the GBM and no spikes on LM by silver stain and may not be distinguished from minimal change disease(MCD)[5] .In contrast ,IF can show fine granular deposition of immunoglobulin and complement and can confirm the diagnosis of MGN and distinguish it from anti-GBM nephritis where liner deposits in IF and anti-GBM antibody in serum are seen[5,6].Lupus nephritis (LN) patient of WHO class V also shows immunohistological features of primary MGN so as hepatitis B virus related nephropathy. At the time of biopsy, when systemic lupus erythematosus (SLE) is clinically suspected, the differentiation between hepatitis B virus related MGN and LN may be difficult or impossible[7,8,9].Although sub endothelial and mesangial prominent IgA deposits are suggestive and only tubular basement membrane deposits are specific, correct diagnosis of LN is made by correlating typical clinical features and serological markers[5].Hepatitis B infection may be occult, serum transaminase may be normal and there may be no history of clinical hepatitis. The study of glomerular viral antigens and serological screening is important for the diagnosis of hepatitis B virus-related nephritis[9] .The diagnosis of C1q nephropathy is based on the immunoflourescent finding of intense, mostly mesangial staining for C1q in patients without evidence of SLE or membranoproliferative glomerulonephritis[10,11,12].

Since, GN is a major problem in Iraq. This study was taken to diagnose different types of GN by IF technique in our setting and to show the importance of clinical, biochemical and serological features for correct diagnosis. Spectrum of glomerular diseases in Iraq is to be reviewed and compared with other studies in other countries in different periods.

Methods

This study was carried out at the Iraqi medical center in Karbala city. A total of 58 patients were recruited. The study started from 1st of June 2010 to 1st of June 2012.The indication criteria for kidney biopsy were:nephrotic syndrome, Persistent sub-nephrotic proteinuria, unexplained abnormal kidney function test

when kidney sizes in ultrasound was within normal range, acute kidney injury, acute nephritic presentation, hematuria after excluding urological causes, assessment of serologically confirmed systemic lupus erythematosus with renal involvement, and evaluation of graft dusfunction after kidney transplantation[12].The ages of the patients ranged from 6-50 years. Clinical informations were recorded in a pre-designed proforma before doing the biopsy. All kidney biopsies were performed by the authors themselves after taking the signature of the patients for an informed concept. Of 58 cases, IF examination was done in 56 cases because tissues were received only for routine slide revision in two cases.Fifty five specimens were obtained from native kidneys except one from a transplanted kidney graft. At least two specimens of 12-20 mm in length of renal tissues were taken. One of the specimens was kept in10% formalin and another one in transport media 0f Zeus solution for IF study. Specimens were kept in an iced bag and sent to the laboratory where the formalin-fixed processed tissue section were submitted for LM and were stained for haematoxylin-eosin, Periodic Acid Schiff (PAS), trichromw/Masson, John's and Congo red for histopathologic study. For the IF studies all specimens were stained with the following antibodies (Dako FITC antibodies) to IgG, IgA, and IgM, Kappa, Lambda, C3, and Fibrinogen and C1q.All cases were studied with Zeiss microscope. IF and histopathology of the biopsied specimens were performed by the author herself and thoroughly verified by at least two Histopatholgists[1].

Photographs were taken in both cases of LM and IF study. Adequacy for IF study was considered in the presence of at least one glomerulus under fluorescent microscope. Fluorescein dye conjugated antihuman antibodies (lgG, IgA, IgM,,kappa and lambda light chains,C1q,fibrin and C3) were fixed with the tissue section by ten times diluted anti-sera. Then fall of ultraviolet light into the stained tissue section become excited and emit light of higher wave length to be visible (apple green color) under IF if there is any antigen in the tissue section. Here antibodies and complement lies within the tissue section (if any) act as antigen and artificially prepared antihuman antibodies and complement act as antibody. Total number of glomeruli,GBM,mesangial,endothelial and epithelial cells and infiltration of inflammatory infiltrates,tubules,interstitium and blood vessels were observed under LM.Type (lgG, IgA, IgM ,C1q,fibrin and C3), pattern (granular or linear), site (GBM or mesangium) and intensity of deposition in the glomeruli were observed under IF. The intensity of fluorescein isothiocyanate staining was graded subjectively from (0 to +3) ;0 being negative and +3 maximum intensity (mild +1;moderate ++2; marked +++3)[1].

Adequacy for LM study was considered in the presence of at least 5 glomeruli. The following were the criteria to define different terms and various patterns of GN by LM.Mesangial proliferation: More than 3 mesangial cells embedded in matrix of one segment. Endothelial proliferation: More than 2 nuclei per capillary loops and leukocyte infiltration: More than 5 leukocytes per glomerulus.Crescentic GN: When at least 50% of the glomeruli involved as crescents and MCD when no evidence of any change. Lesions are classified as focal or diffuse proliferative when they involve the minority (<50%) or majority (>50%) of glomeruli respectively. IgA nephropathy: Predominant mesangial deposition of IgA along with C3 detected by IF microscopy[1].FSGS:Segmental (one or two lobules) sclerosis with hyalinosis involving portions of fewer than 50% of the glomeruli in a section. Sclerosis was defined as increase in amount of homogeneous non-febrile extracellular material of similar composition to GBM

and mesangium. MGN: Diffuse thickening of GBM due to sub-epithelial deposits of immune complex without evidence of inflammation or cellular proliferation. Membranoproliferative GN: Diffuse thickening of GBM with predominant proliferation of mesangial cells and extension of matrix often with interposition in between the endothelial cells and GBM causing tram-track appearance under LM. Mesaingioproli-ferative GN(MesPGN): Diffuse increase in glomerular cellularity predominantly due to mesangial cells often with concomitant increase in mesangial matrix[1].

Clinical information regarding age, sex, duration of onset of disease, presentation, urine analysis,biochemical,virological and serological parameters, past medical therapy, were collected from data submitted along with biopsy data.Mean values and percentages were used in this study.

Results

Of the 58 biopsies obtained from clinically suspected cases of glomerular diseases 30 (51.7%) were male and 28 (48.3%) were female and male female ratio was 1.07:1.Their ages ranged from 6-50 years(mean=26.11).FSGS(17/58=29.3%) topped the list in this study followed by MCD (12/58 =20.7%); MGN (8/58=13.8%) and other types of renal disorders (21/58=36.2%) as shown in table 2.

Table2: the prevalence og GN according to the histopathology.

Diagnosis	No. of patients	Percentage (%)
FSGS	17	29.31%
MCD	12	20.69%
MGN	8	13.80%
GGS	5	8.62%
MesaP GN	4	6.89%
Am	3	5.17%
HNS	2	3.45%
immune GN (ANCA+ve)	2	3.45%
LN	2	3.45%
IgAN	2	3.45%
TR	1	1.72%
total	58	100%

HNS=hypertensive nephrosclerosis, IgAN=IgA Nephropathy, GGS=global glomerulo-sclerosis, Am=amyloidosis, TR=transplant rejection, IGN=immune GN[12], IgAN= immunoglobulin A nephropathy, No=number, ANCA= antineutrophil cytopla-smic antibody.

Definitive diagnosis was possible in all cases because the biopsy represented renal cortices. All specimens were considered adequate due to more than five glomeruli were present for light and two for IF microscopic studies.

Among 56 cases (21=37.5%) were IF positive and (35=62.5%) were negative. Table 3 shows frequency of immune deposits in common glomerular diseases for our patients. Among 12 cases of MCD,one showed mild increased cellularity and matrix in the mesangium by LM and mesangial deposits of C_3 and C1q in IF. Others were normal in IF and LM.Two (3.45%) specimens fulfilled clinical, morphological and immunofluorescent criteria for lupus nephritis, one was male and other was

female. The predominant deposits in IF of LN was C_3 (2=3.45%) , IgG (2=3.45%)followed by C_1q,and fibrin in the crescent of one case (Table III)..Biopsies from one case of renal allograft patient found no immune deposits only C_4D lymphocyte and was diagnosed as acute transplant rejection. MGN was diagnosed in 8 cases with IgG and C3 deposition in all and C1q in 2 of them. Among the 56 patients in this study, IgG deposition was found in 34.48% followed by C_3 (31.03%).most of these deposits were fine granular at the GBM as shown in figure 1,2,3,and 4.

Table 3:Numbers of our patients according to the type of glomerular deposits by IF examination.

Disease	Fibrin	IgG	IgM	IgA	C_1q	C_3	Kappa LC	Lambda LC
FSGS(n=17)	0	2	0	0	1	2	0	0
MCD(n=12)	0	1	1	0	0	1	0	0
MGN(n=8)	0	8	0	0	2	8	0	0
GGS(n=5)	0	0	0	0	0	0	0	0
MesPGN(n=4)	0	4	0	0	1	2	1	1
Am (3)	0	0	0	0	0	0	0	0
HGS(n=2)	0	0	0	0	0	0	0	0
LN (n=2)	1	2	0	0	1	2	2	2
IgAN(n=2)	0	0	0	2	0	1	1	1
Immune GN ANCA + ve (n=2)	0	2	0	0	1	2	2	2
TR(1)*	0	0	0	0	0	0	0	0
total	1	20	1	2	6	18	6	6**

F=Fibrin,LC=light chain IgM=immunoglobulin M,*=C_4D lymphocytes cells positive.**some patients were taken more than 1 time according to the type of IF deposits in their kidney biopsies

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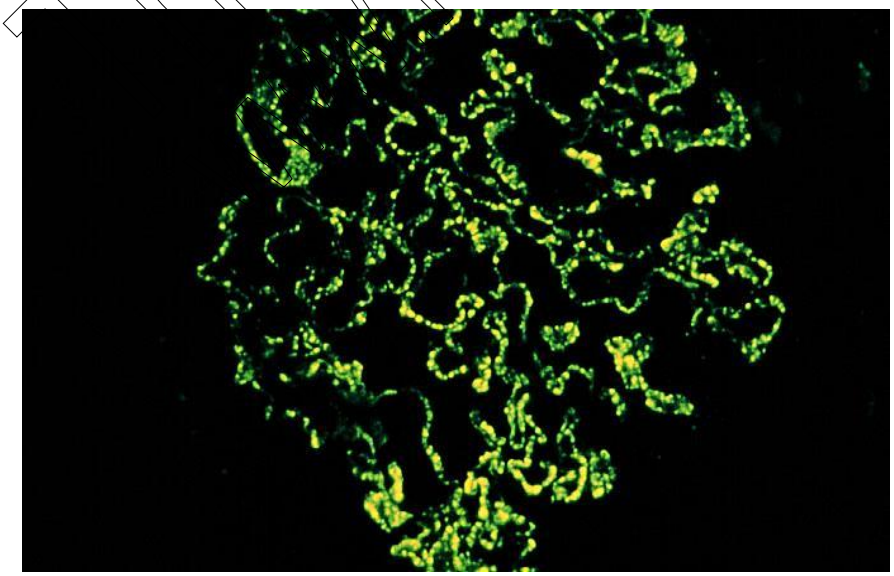

Figure 1: MGN showing diffuse granular deposition of IgG and C3 along the glomerular basement membrane in a 30 year old female student of oncology presented with postpartum generalized edema with positive serological markers of SLE.she responded to weekly doses of rituximab (two doses).Her CD 20 lymphocytes were negative on follow up .

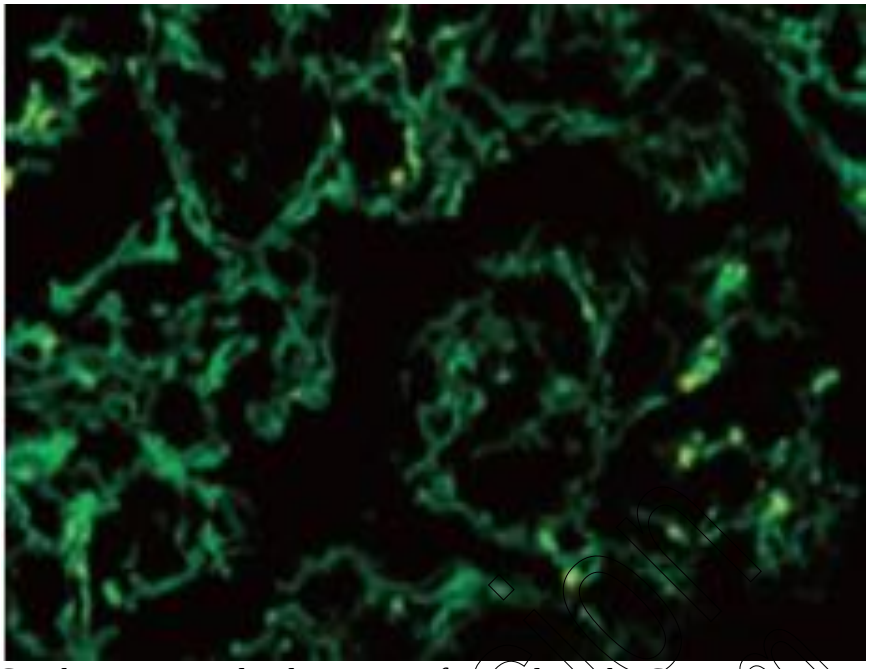

Figure 2: MCD showing granular deposition of IgM along the GBM in 11 year old boy with 6 years history of nephrotic syndrome.

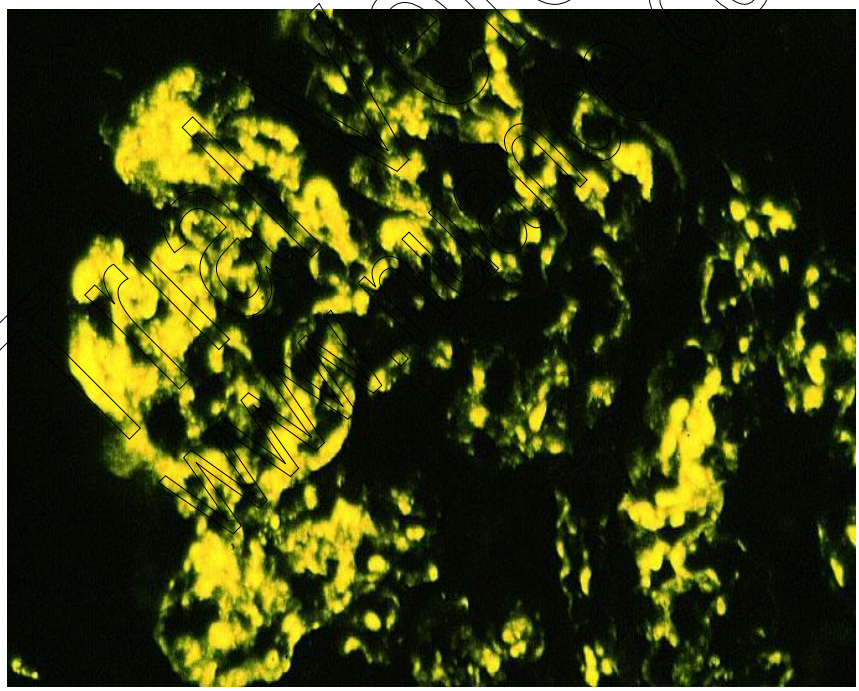

Figure 3:The granular deposits of C3 scattered along capillary walls and in the mesangium by IF microscopy in a 17 year old male patient with MPGN.

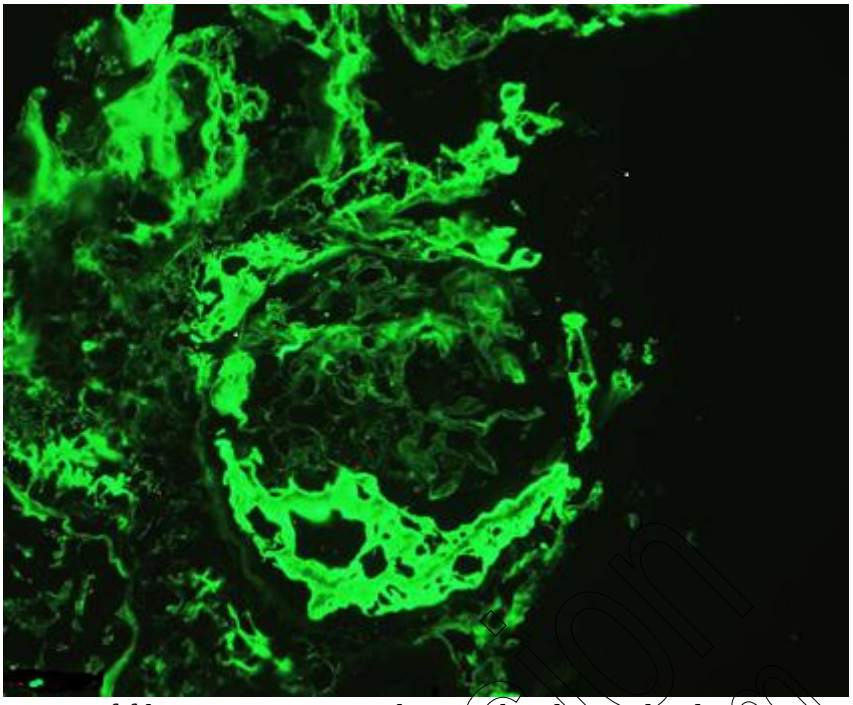

Figure 4: deposits of fibrin in a patient with LN ;The glomerular damage is so severe that fibrin leaks into Bowman's space, leading to proliferation of the epithelial cells and formation of the bright crescent shown here.this was a 23 year old female patient with proved SLE and she didn't respond to treatment of pulse steroid and cyclophosphamide and progressed to chronic kidney failure.

Whereas staining for linear deposits of IgG and IgM were slightly positive, however, these staining were not on mesangial lesion, see figures 5.

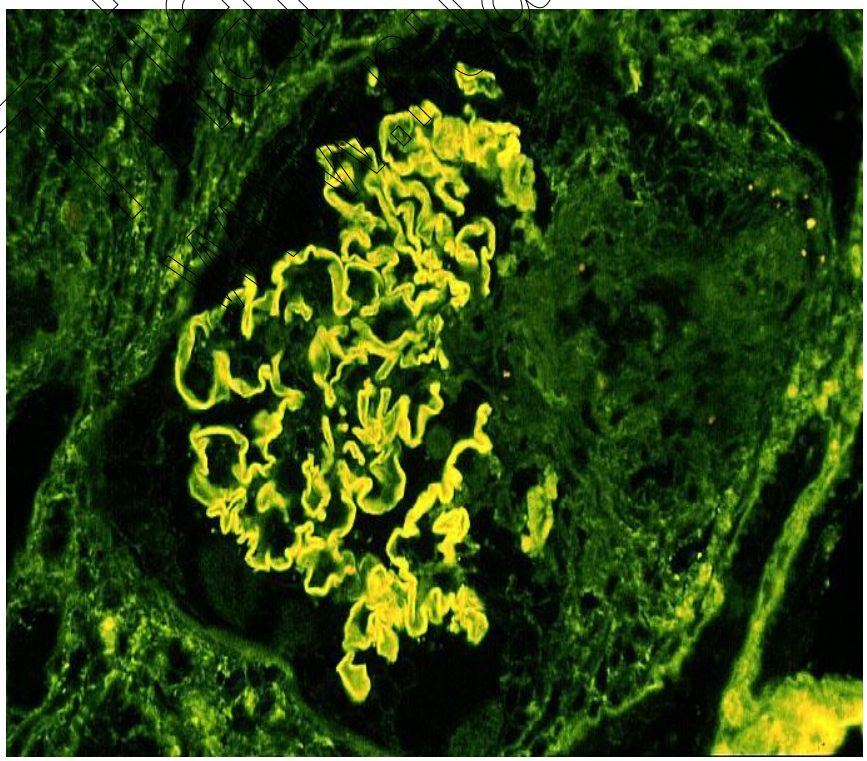

Figure 5:shows deposits of IgG in a smooth, diffuse, linear pattern in a 26 female patient who was proved to have ANCA positive vasculitis with secondary GN.

Figure 6 shows diffuse granular mesangeal and paramesangeal deposits of mainly IgA,and slight positive staining of C3 and Kapa,Lamda deposits with sparing of the GBM.This is a patient with IgAN.

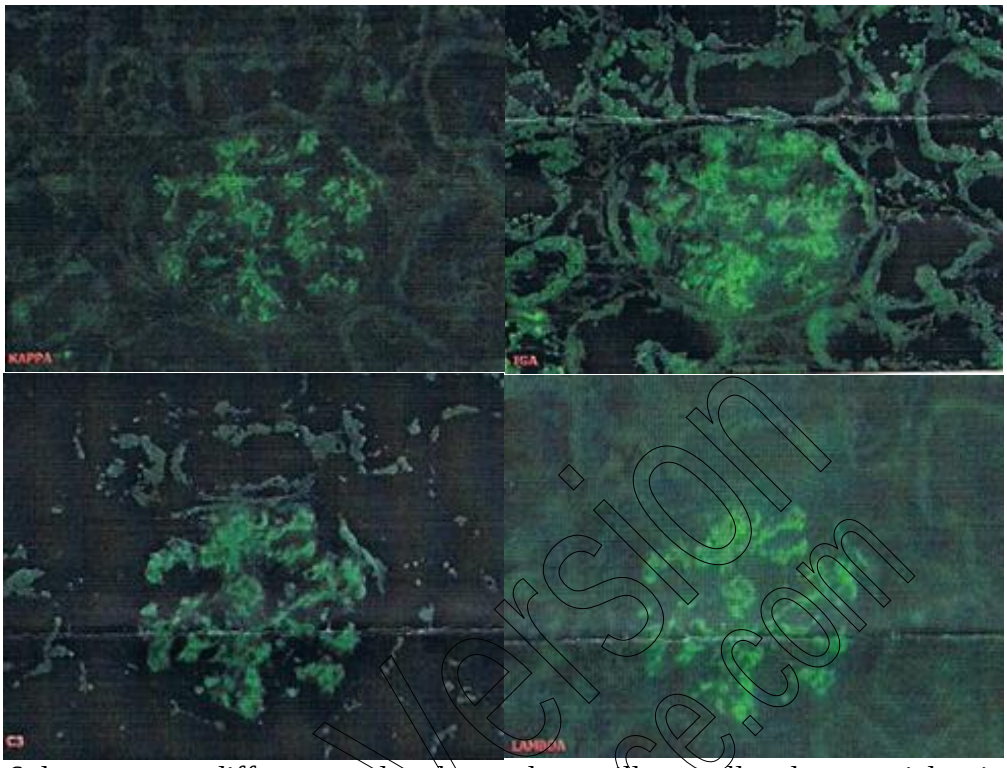

Figure 6:demonstrates diffuse granular glomerular capillary wall and mesangial staining for IgA (mainly),C3,lamda and kappa. This is a 40 year old male patient with IgA N with proteinuria and no edema .

Except in one case where IF show dominant positive staining (3+) for C1q in the glomerular mesangium and slightly positive staining for C_3 in the same mesangeal area, see figures 7,8.This patient 11 years boy with 7 years history of steroid resistant nephrotic syndrome with negative investigations of SLE.

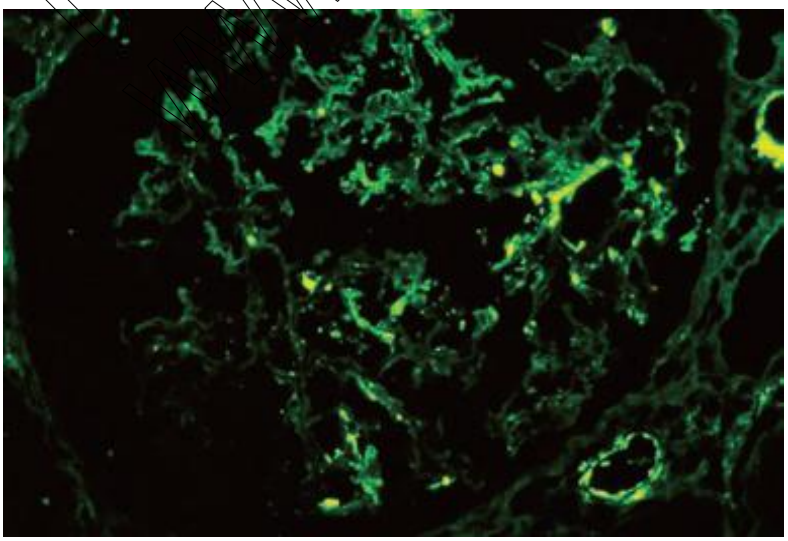

Figure 7: C1q nephropathy showing dominant positive staining (3+) for C_3 in the glomerular mesangium in an 8 year old boy with frequent relapsing nephrotic syndrome . He had a poor response to systemic steroid and show a better response to cyclosporine oral therapy.

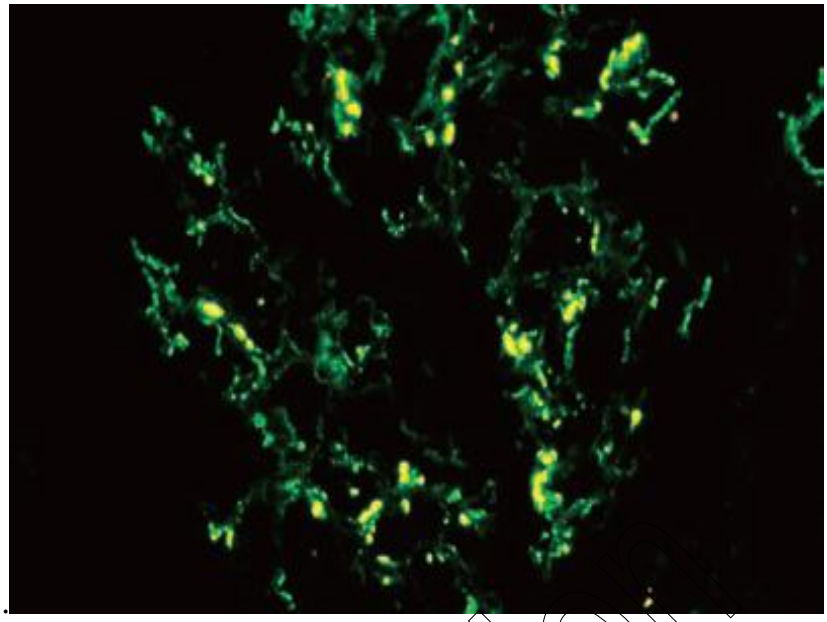

Figure 8:C1q nephropathy showing dominant positive staining (3+) for C_1q in the glomerular mesangium.this is the same patient in figure 7.

Table 4 shows the clinical presentation of common GN. The commonest presentation was nephrotic syndrome (44=75.86%) followed by proteinuria (37=36.79%), systemic hypertension (17=29.31%).Nephritic syndrome was the characteristic presentation of acute diffuse proliferative GN.

Table 4:The numbers of patients in different GN groups according to mode of presentation.

Mode of presentation	Mes.GN (n=4)	HNS (n=2)	MGN (n=8)	LN (n=2)	IgAN (n=2)	GGS(n=5)	Am. (N=3)	MCD(n=12)	FSGS(n=17)	TR (n=1)	IGN ANCA+ ve(n=2)	Total
Nephrotic Edema (75.86%)	4	1	8	2	2	4	3	8	12	0	0	44
Subnephrotic proteinuria (36.97%)	2	0	8	1	0	3	3	8	12	0	0	37
SHT (32.75%)	3	2	4	2	1	3	2	1	0	0	1	17
Anemia (17.24%)	2	1	0	2	1	1	1	0	2	0	0	10
CKF (17.24%)	1	0	2	1	0	1	1	0	1	1	2	10
HAEM (10.34%)	2	1	0	1	2	0	0	0	0	0	0	6
Nephritic syndrome (10.34%)	2	1	0	1	0	1	0	0	0	0	0	5
SLE (3.44%)	0	0	0	1	0	0	0	0	1	0	0	2

AKF=acute kidney failure,HAEM=Haematuria,SHT=systemic hypertension,CKF=chronic kidney failure.

Discussion

Since GN IS immunologically mediated, IF microscopy has no alternative for diagnosis of glomerular diseases. Yet,

combined analysis of LM and IF findings and correlation with clinical, biochemical and serological features are essential for accurate diagnosis.our results showed positive IF deposits in 37.5% which is very important in the diagnosis of primary glomerular dieases and its prevalence in Iraq.FSGS (17= 29.31%) was the most common pattern of glomerulonephritis followed by MCD.

There is a great variation regarding the commonest pattern of GN in different studies [13,14]. We found many similarities when we compared our results with studies see table 5. It shows the spectrum of glomerular diseases in different studies in Iraq in different places and times.FSGS topped the list in all Iraqi studies except in Mahassin study where MesPGN is the commonest lesion. MCD and MesPGN was more frequent in the period 1986-1996,while FSGS is the predominant during the period 1994-2012.lack of IF staining in these studies[13] is one of the causes of the difference in the spectrum of glomerular diseases in Iraq.

Table 6 presents renal biopsy data from 36 countries worldwide.In 19 countries there are data available to enable readers to study the patterns of renal biopsies between the 2 periods(at the beginning and about 30 years later).We compare the data in Iraq or other individual countries in different period ; as well as we compare our data with other countries. It provides an analysis of evolving trends among the various countries and the surrounding regions. The data also show the common features in histological profile among Asian countries in contrast to other countries in the East as well as the West. We have presented a comprehensive table and have incorporated as much data as possible from the original publications in nephrology journals during the past 30 years. Based on data in the various papers from different countries we have focused on the various primary GN, removing secondary GN (like lupus nephritis and diabetic nephropathy) from the total numbers biopsied in order to obtain comparable data for various types of primary GN. Most countries included IgA nephritis as a separate group of primary GN.It topped the spectrum of glomerular diseases in Australia, Portugal, Noerthern, Ireland, Japan, France, Finland, China, and Czech Republic. All the other mesangial proliferative GN that were non-IgA nephritis were classified as mesangial proliferative GN.This is the case in Iraq as shown by Mahassina and Al Habal studies[13],while in our study and in Ikdam et al ,FSGS is the commonest in the spectrum of glomerular diseases.Similerly FSGS was more frequent in Belgium,Barazil,India,Saudia Arabia, and Senegal.

The socioeconomic change affecting our country after the war reflecting the degree of development of the living standards and housing facilities. In the early years with exposure to parasitic, bacterial and other infective agents predisposing to mesangial proliferative GN,that is already decreasing in keeping with urbanization and better housing and other amenities.The exposure to various types of dietary and other industrial allergens that entered our country without governmental control has risen. This explain why we now witness a rising prevalence of membranous GN in Iraq 13.8% which has a T helper 2 dominance.

A few countries including Iraq, however, grouped IgA nephritis under mesangial proliferative GN in the initial years, but subsequently in later years also had separate categories for IgA nephritis and mesangial proliferative GN.

Table 5: Spectrum of Glomerular Diseases in Different Iraqi Studies[13].

Study/ period	Primary glomerular disease					
	FSGS	MesPGN.	MCD	MPN	MGN	RPN
Present study 2010-2012	17 (29.31%)	4 6.89%)	12 (20.6%)	0	8 13.8%)	2 (3.4%)
Ikdam K.Shakir et al 1994-2001	117 (26.3%)	100 (22.5%)	76 (17.1%)	72 (16.2%)	65 (14.5%)	15 (3.4%)
Habal M.G.1996-1998	3 (7%)	0	8 (19%)	11 (26%)	9 (21%)	4 (9.5%)
Mahassin SS 1986-1996	51 (13.8%)	98 (26.6%)	62 (16.8%)	48 (13%)	50 (13.5%)	0
Abbas A.M.et al 1991-1994	24 (17.6%)	23 (16.9%)	27 (19.7%)	19 (16.9%)	21 (15.4%)	0

Study/ period	Secondary glomerular disease						
	LN	AM	DM	Herid N	HNS	IgAN	TR
Present study 2010-2012	2 (3.4%)	3 (5.17%)	0	0	2 (3.4%)	2 (3.4%)	1 (1.7%)
Ikdam K.Shakir et al 1994-2001	25 (45.5%)	15 (27.3%)	8 (14.5%)	6 (10.9%)	1 (1.8%)	0	0
Habal M.G.1996-1998	3 (7%)	1 (2%)	1 (2%)	0	0	0	0
Mahassin SS 1986-1996	27 (7.3%)	25 (6.8%)	0	0	0	0	0
Abbas A.M.et al 1991-1994	0	0	0	0	0	0	0

*Five patients with GGS excluded in this table.

These countries also have a high prevalence of IgA nephritis and are mainly in the Asian region.The high prevalence of IgA nephritis in some countries, just as the low prevalence In others, could also reflect their policy of biopsying more patients with asymptomatic hematuria and proteinuria in contrast to those which biopsy relatively less patients with asymptomatic hematuria;more over IF staining was not performed in most of the old studies.

Some countries showed different prevalence of primary glomerular disease in different studies during different periods as shown in Hong Kong, India, Iraq, Italy, Sudan, and UK.

The prevalence of primary GN in various countries throughout the world varies depending on the genetic profile of the population as well as their environmental exposure, hence the different patterns of GN amongst the various countries. As the countries evolve, the social environment and other factors in these countries may also change in keeping with improvements in living and other conditions.These changes may explain why the patterns of GN change over a period of time, some of this may be in response to environmental antigens, though in reality there are many and varying factors in each country which influence the pattern of GN. Among these many factors, one which may influence the pattern of GN in various countries could be the hygiene hypothesis.According to this hypothesis,overcrowding and poor hygiene in early life may protect from atopic diseases because exposure to microbes favors the development of a T helper 1 dominant response. On the other hand, dominance of a T helper 2 subset could be responsible for the increasing incidence of allergies.The allergens involved could be from the diet, other putative antigens as well as from an industrialized environment in the more developed countries. The hygiene hypothesis proposes that early and frequent exposure to bacterial and other antigens occurring in less developed or developing countries leads to a T helper 1 phenotype response, but better public hygiene and less infections lead to a T helper 2 phenotype response with

increased risk for developing allergies. We reviewed the prevalence of GN in relation to the hygiene hypothesis and explained why certain types of GN Like membranoproliferative GN And mesangial proliferative GN Are associated with poorer or developing Countries(T helper 1 dominance), whereas membranous GN, minimal change disease and IgA nephritis are more common in industrialized countries (T helper 2 dominance) 15.Some countries have a separate group classified under mesangiocapillary GN (MCGN) as it is still prevalent in their population, whilst other countries have very few patients with MCGN, like Singapore (<1%), and have included them under 'others'. Where MCGN is separately classified we have included a separate heading named MCGN. Those countries with a high prevalence of MCGN usually have a low prevalence of mesangial proliferative GN. Both MCGN and mesangial proliferative GN are related to infections. Post-infectious GN or diffuse endocapillary GN is very uncommon in many countries and their biopsy registries do not classify such biopsies as a separate group, and in the few that do so, we have included these under 'others' as well. As far as possible we have strived towards the presentation of data which would enable us to make appropriate comparisons for the distribution of primary GN among the various countries.

Table 6: An overview of the prevelance (%)of primary glomerulonephritis in 36 different countries

Country	Period year	Number of biopsies	MCD %	MesPN %	IgAN %	MGN %	FSGS %	MesCN %	Others%
Australia[14].	1976–1978	623	6	18	23	8	7	5	33
	1986–1993	634	3	16	37	13	15	3	14
	1995–1997	1,147	6	0	49	15	21	3	6
Austria[14].	1980–1990	449	13	14	12	6	6	3	47
Belgium [15].	1991-1996	326	19.1	4	21.2	15.1	30.3	7	3.3
Brazil[14].	1979–1989	943	0	0	12	15	30	12	33
	1990–1993	206	5	0	10	20	43	14	7
	1999–2005	1,131	9	4	18	21	30	0	19
China[14].	1980–1985	8,852	5	20	15 40	7	11	7	34
	1979–2000	7,101	1	30	45	10	6	7	7
	1979–2002	9,278	1	26		10	6	0	12
Czech Republic[14].	1994–2000	2,217	13	11	35	9	11	0	22
Denmark[14].	1985–1997	2,380	18	26	0	12	14	5	26
Egypt[15].	1998-1999	1234	16.5	4	0	12.7	22.6	13.5	15.7*
Finland[15].	1976-2000	3310	5	11.6	34.9	11.6	3.9	3.8	30.2
France[14].	1976–1985	663	10	0	27	12	0	6	45
	1976–1980	179	13	5	29 36	12	12	0	28
	1981–1985	170	10	6	37	18	12	0	19
	1986–1990	131	11	7		25	6	0	15
Hong Kong[14].	1974–1978	215	21	10	8	11	15	9	26
	1993–1997	871	14	11	39	13	10	4	8
Hungary[14].	1990–2002	798	8	0	15	14	7	0	56
India[14].	1964–1973	369	23	14	12	20	1	20	23
	1986–2002	3,845	16	0	0	14	24	0	34
	1990–2001	2,673	18	12	14	16	28	0	12
Indonesia[14]	1976–1980	94	10	33	0	6	6	7	37
	1985–1986	459	16	35	0	4	7	0	38
Iran[14].	1981–1994	623	13	2	12	18	8	0	47
	1998–2001	364	11	3	15	26	12	0	34

Continue table

Iraq[13].	1986-1996	369	16.8	26	0	13.5	13.5	13	17.2
	1991-1994	196	13	11.3	0	10.7	12.2	9.6	53.2
	1994-2001	500	15.2	20	0	13	23.7	14.4	13.2
	1996-1998	42	19	0	0	21.4	7	26.1	26.5
	2010-2012	58	20.6	6.8	3.4	13.8	29.3	0	26.1
Italy [14].	1987–1993	8,287	8	0	35	21	12	0	25
	1970–1979	449	5	0	15	14	11	0	55
	1980–1989	840	5	0	27	22	8	0	38
	1990–1994	637	8	0	32	23	9	0	29
Japan[14].	1980–1985	1,850	16	15	27	11	6	4	21
	1976–1985	622	0	34	44	7	4	2	3
	1986–1995	397	6	32	46	8	5	2	1
	1996–2000	290	8	16	51	8 0	9	0	8
Korea [14].	1973–1988	1,412	40	10	29 27	13	4	0	5
	1973–1995	1,732	32	6	29 38	14	7	0	15
	1988–1995	1,561	34	1		16	9	0	12
	1987–2006	1,346	21	0		17	8	5	11
Kuwait[14].	1995–2001	584	13	0	8	5	18	0	56
Macedonia[14].	1975–2001	716	7	4	12	14	10	0	53
Malaysia[14].	1970–1981	1,000	26	25		6	5	0	39
	1982–1991	1,000	21	16	19	6	3	0	36
	1994–2000	281	41	15	14	6	7	0	16
Noerthern Ireland [15].	1976-2005	1844	9.8	0	38.8	29.4	5.7	11.2	5.1
Peru[14].	1985–1995	731	6	15	2	20	24	4	30
Portugal[14].	1977–2003	1,030	14	17	31	11	7	5	15
Romania[15].	1995-2004	401	8.5	O*	28.9	11.2	11.5	29.4	10.5
Russia [14].	1978–1983	852	7	56	0	9	11	10	8
	1970–1999	2,746	6	49	0	13	8	18	6
Saudi Arabia[14].	1989–1994	147	1	21	13	13	41	11	2
	1994–1999	127	9	25	10	4	35	16	0
Senegal[15].	1993-1998	115	7	2	2	10	54	5	0
Singapore [14].	1976–1986	1,127	9	32	42 45	3	5	0	9
	1987–1997	666	13 19	17	40	6	6	0	13
	1998–2008	786		7		11	15	0	8
South Africa [15].	2000-2009	1284	6	19.2	5.8	18.5	10.5	20.4	19.6
Spain[14].	1994–1999	4,824	10 10	0	24 14	14	15	0	37
	1994–2001	4,157		5		13	11	0	49
Sudan[14].	1972–1974	572	0	40	0	5	10	1	44
	1982–2005	2,154	0	46	18	16	0	5	15
	2001–2004	506	0	0	31	13	25	0	31
United Arab Emirates[14].	1978–1996	490	18	0	6	20	18	0	38
UK[14].	1972–1973	746	32	15	0	9	6	14	23
	1978–1984	2,806	18	19	14	29	6	5	28
	1976–2005	903	10	0	39	0	6	10	7
USA[14].	1975–1979	73	4	0	11 25	38	14	4	29
	1980–1984	96	7	0	13 11	23	9	8	26
	1985–1989	143	11	0	27 25	20	10	5	41
	1990–1994	304	11	0		15	25	9	30
	1986–2002	208	9	0		17 1	38	0	9
	1994–2003	195	5	12		0	20	8	20

*included in IgAN.

** include chronic GN,secondary GN.

***41 patients in our study have secondary GN and we exclude diabetic patients.

Of the 12 cases of MCD in our study eleven showed no significant alteration in LM.in our studt the diagnosis of MCD was made by absence of glomerular alteration in LM and lack of immune deposits in IF.Minor degrees of mesangial matrix increment and/or hyper cellularity also form an integral part of MCD, but when it is more prominent, it is difficult to segregate from MesPGN.[16, 17]

Linear deposit of IG is the typical character of anti-GBM disease.[18] Anti-GBM disease comprises about 2-5% of GN .our explanation for the linear deposition of IgG and C3 along GBM and IgG deposition in the mesangium observed in our patient is due to superimposition of anti-GBM disease on preexisting MGN. This is consistent with the hypothesis that intermingling of immune complexes with newly formed GBM material may alter the antigenicity of GBM, leading to the formation of anti-GBM antibody or reverse sequence i.e. Superimposition of immune complex disease on anti-GBM nephritis might be the cause [19].The predominant immune deposits in MesPGN was IgG and C3 in the mesangium and along the GBM.Similar findings were also observed by other studies.[7, 8, 19, 20]

In our study we found 5 patients with positive deposition of C1q complement component in the glomerular basement membrane in addition to IgG, kappa, lambda light chains and C3.we found one had mesangeal deposition in 3+ without glomerular basement membrane deposition. This patient was 21 year old female with histopathological findings of FSGS in light microscopy with negative serology for SLE .Electron microscopy was not feasible for this patient and she was treated as primary C1q nephropathy with cyclosporine A with good response. This is consistent with Jennette and Hipp who first proposed that C_1q nephropathy was a distinct clinical entity that caused GN in the absence of SLE.[21, 22] with deposition of C_1q predominantly in the mesangial area. In these early reports, C_1q nephropathy had specific histopathological patterns of mainly focal or diffuse mesPGN. In renal biopsies, several patterns have subsequently been reported, ranging from minor glomerular abnormalities or mesPGN to focal glomerulosclerosis with most recent reports describing C_1q nephropathy of the focal glomerulosclerosis type.[22]. Davenport et al. and Nishida et al. reported cases of C_1q nephropathy of the membrano proliferative glomerulonephritis and MNP types.[21, 22,23,24,25]. All other four patients with positive C1q had positive serological markers for SLE and three of them progressed to chronic kidney failure.

Electron microscopic study is still very important especially in certain cases; however because of the lack of the centers that carry out this type of study and because of the extra financial burdens on our patients whom mostly pay for such a test out of their pockets, we reached the right diagnosis in most of the our cases by good LM and IF studies along with meaningful correlation between biopsy findings, clinical presentation and serology.

Nephritic syndrome was the most frequent presentation of mesPGN and nephrotic syndrome was the common mode of presentation in MNP. The clinical presentation of the study correlated with the findings of other studies. [1, 2, 8, 9, 15, 26].

In conclusion, IF microscopy in combination with LM, clinical, biochemical and serological markers make the diagnosis of glomerular disease feasable.FSGS is the commonest histopathological type of adult GN in Iraq. Nephrotic syndrome is the predominant mode of clinical presentation in the studied patients.

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