STUDY OF THE EXPRESSION OF CELL ADHESION MOLECULES (ICAM-1 AND VCAM-1) IN PRIMARY GLOMERULAR DISEASES

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ABSTRACT

Background: Glomerulonephritis refers to a collection of primary and secondary renal disorders characterized by inflammation within the glomeruli. Glomerular diseases remain the most common worldwide cause of end stage renal disease (ESRD).

Despite extensive investigations, the mechanism of the initial glomerular injury and disease progression remains mostly unknown. In addition; the efforts to stop or even to slow the progression of chronic renal insufficiency are still unsuccessful.

Knowledge of the molecular mechanisms involved in cell – cell and cell – cell matrix adhesion has increased rapidly in the past decade due to the immense clinical relevance of the adhesion molecules involved in such mechanisms.

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are two members of immunoglobulin-like superfamily of the cell adhesion molecules (CAMs), which are normally expressed by endothelial cells. ICAM-1 and VCAM-1 are also expressed and released by activated T-lymphocytes and macrophages; therefore their increase may also reflect local immune activation.

Aim of the work: The aim of this study was to evaluate the role of cell adhesion molecules (ICAM-1 and VCAM-1) in the pathogenesis and

disease progression of primary glomerular diseases and to correlate between their expression and the expression of markers that indicate locally activated cellular immune reactions including HLA-DR Antigen, CD3, and CD68.

Patients and methods: The present study was carried out on sixty patients with primary glomerulonephritis, who were subjected to full history taking in addition to clinical, laboratory and radiological investigations. Renal biopsies taken from these patients were examined histopathologically and immunohistochemically for the expression of ICAM-1, VCAM-1, HLA-DR, CD3 and CD68 compared to the expression of such markers in the control specimens (Five specimens of normal renal tissue).

Conclusions: The results of the present study revealed statistically significant over-expression of ICAM-1, VCAM-1, HLA-DR Antigen, CD3 and CD68 in the different types of primary glomerulonephritis included in this study compared to their expression in normal renal tissue. There was strong positive correlation between the expression of ICAM-1 and VCAM-1 and the expression of HLA-DR Antigen, CD3 and CD68 in the studied cases. So, cell adhesion molecules (ICAM-1 and VCAM-1) proved to be a reliable factor in the pathogenesis of different types of primary glomerulonephritis, which gives an idea about the important and promising benefit of the use of anti-adhesion molecule therapy in the treatment of different forms of glomerulonephritis.

Key words: Glomerulonephritis, ICAM-1, VCAM-1, HLA-DR, CD3, CD68.

INTRODUCTION

Glomerulonephritis (GN) is considered to be an immunologically mediated disorder with involvement of cellular immunity, humoral immunity, and other inflammatory mediators. [1]

Adhesion molecules and receptors are essential for tissue structural organization. They are unvolved in the processes of cell growth and differentiation, such as embryonic development, inflammation, wound healing, and tumor metastasis. [2]

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are two members of immunoglobulin-like superfamily of the cell adhesion molecules (CAMs) that are normally expressed by endothelial cells. Cytokine activation upregulates dramatically their expression on the cell surface where they support the interaction between leukocytes and endothelial cells. [3]

Elevated levels of circulating soluble adhesion molecules have been detected in disorders where leukocyte / endothelial cell interaction plays a significant role, such as infection, atherosclerosis, neoplasms, chronic inflammatory diseases, and vasculitis. [4, 5]

Some authors suggest that soluble adhesion molecules act as makers for disease activity in autoimmune diseases and vasculitis. [6,7]

Despite extensive investigations, the mechanism of the initial glomerular injury in most renal diseases remains mostly unknown. [8]

The aim of this work was to evaluate the role of cell adhesion molecules (ICAM-1 and VCAM-1) in the pathogenesis and disease progression of primary glomerular diseases and to correlate between their expression and the expression of markers that indicate locally activated cellular immune reactions including HLA-DR Antigen, CD3, and CD68.

PATIENTS AND METHODS

The present study was carried out on sixty patients with primary glomerulonephritis, in The Internal Medicine Department, Tanta University Hospitals, each patient was subjected to:

1-Clinical, laboratory, and radiological testing including:

A detailed full history taking, through clinical examination, and laboratory investigations including: Complete urine analysis, renal function tests including blood urea, serum creatinine, and creatinine clearance, 24 hours urinary protein excretion, plasma proteins, random blood sugar, complete blood picture, erythrocytic sedimentation rate, C-reactive protein, lipid profiles, immunological tests including rheumatoid factor, antinuclear antibody, and hepatitis markers. Abdominopelvic ultrasound was also done.

2-Percutaneous renal biopsy and tissue processing: Two cores of renal tissues were obtained by means of an argovac automated biopsy gun under the guide of ultrasonography and fixed in 10% formalin, subsequently; the specimens were embedded in paraffin. Paraffin-embedded sections were subjected to:

1- Haematoxylin and eosin (H&E) staining for routine microscopy.

2-Immunohistochemistry:

Immunohistochemistry was performed on paraffin-embedded 3-5μ sections according to the avidin-biotin-peroxidase complex (ABC) method ***(Taylor et al., 2002)*** [(9)]. Briefly, sections were deparaffinized with xylene and rehydrated with graded alcohol series. Antigen retrieval was done by immersing the sections in 10 m mol /L citrate buffer (pH 6.0) for 10 minutes at 100˚ C in microwave. Endogenous peroxidase activity was blocked with H_2O_2 (0.6% in methanol). After thorough

washing of the sections with phosphate buffered saline, incubation was done for 30 minutes with non-specific blocking reagent “normal goat serum” to prevent non-specific binding. Subsequently, an overnight incubation of the sections with monoclonal antibodies against: Human Intercellular Cell Adhesion Molecule-1 (ICAM-1) "IHCP-MAB 2130",Chemicon (at a concentration 1:100); Vascular Cell Adhesion Molecule-1 (VCAM-1) "M 7106", Dako (at a concentration 1:200); CD3 "M 7193", Dako (at a concentration 1:50); CD68 "M 0876", Dako (at a concentration 1:50); and HLA-DR Antigen "M 0775"; Dako (at a concentration 1:50), the incubation was done at room temperature in a humidity chamber. The sections were then washed with PBS and the following steps were performed:

1. Incubation with biotinylated secondary antibody for 30 minutes.
2. Incubation with avidin-biotin-peroxidase complex solution for 30 minutes.
3. The reaction products were visualized using 3-3`-diamino-benzidine-tetra-hydrochloride (chromogen).

Sections were then counterstained with Mayer’s haematoxylin, dehydrated in alcohol and mounted in DPX.

Five controls of normal kidney tissue were subjected to immunohistochemical staining by the previous markers for comparison.

Sections from a human tonsil were used as positive controls for immunohistochemical staining of the five used antibodies, while negative controls were prepared by omission of the primary antibody.

Immunohistochemical analysis:

1-Immunohistochemical analysis of ICAM-1 and VCAM-1 immunostaining:

The extent of ICAM-1 and VCAM-1 immunoreactivity was assessed in the glomerular tuft and interstitium on a semiquanatitive basis graded on a four point- scale as follows:

The lesion was scored as **0 (negative)** when immunoreactivity was absent, **1 (mild)** when immunoreactivity was present in <25% of the area examined, **2 (moderate)** when immunoreactivity was present in 25-75% the area examined, and **3 (intense)** when immunoreactivity was present in >75% of the area examined. **(10)**

2-Immunohistochemical analysis of HLA-DR Antigen, CD3 and CD68 immunostaining:

The number of positive cells for HLA-DR Antigens, CD3 and CD68 was expressed as the numbers of the cells per glomerular cross section.

It was scored as **0 (negative)** when immunoreactivity was absent and **1 (positive)** if any number of positive cells for HLA-DR antigen, CD3 and CD68 were present per glomerular cross section examined (X400).

Statistical analysis:

The collected data were organized, tabulated and statistically analyzed using SPSS software statistical computer package version 12. For quantitative data, the range, mean and standard deviation were calculated. The difference between means was statistically analyzed using the F value of analysis of variance (ANOVA). Pearson's correlation coefficient (r) was calculated to test the association between two variables and spearman’s rank correlation was used when one or two variables were non-parametric.

For qualitative data, the number and percent distribution was calculated. Fisher exact test was used as test of significance. Significance was adopted at $p<0.05$ for interpretation of results of tests of significance. [11]

RESULTS

I-Clinical data of the studied cases:

This study included 60 patients suffering from glomerulonephritis, 22 males (36.7%) and 38 females (63.3%). Their ages ranged between 18 and 45 years with a mean of (29.62±7.23).

Distribution of studied groups according the diagnosis (Table 1):

The study groups included 10 patients (16.7%) with minimal change glomerulonephritis, 14 patients (23.3%) with membranous glomerulonephritis, 14 patients (23.3%) with membranoproliferative glomerulonephritis (MPGN), 11 patients (18.3%) with focal segmental glomerulsclerosis (FSGS), 6 patients (10%) with primary crescentic glomerulonephritis, and 5 patients (8.4%) with immunoglobulin-A nephropathy (IgAN).

Frequency of the most common clinical manifestations in the studied patients (Table 2):

1– Lower limb edema, periorbital edema, and sometimes generalized edema with hypertension were the most common presenting manifestations of most types of primary glomerulonephritis (10/10 "100%" in minimal change GN, 14/14 "100%" in membranous GN; 3/6 "50%" in primary crescentic GN, 5/14 "35.7%" in MPGN, and 7/11 "63.6%" in FSGS).

2– Proteinuria and hematuria were the second most common presentation of most types of glomerulonephritis (10/10 "100%" in

minimal change nephropathy, 4/6 "66.7%" in primary crescentic GN, 5/5 "100%" in IgA nephropathy; 9/14 "64.3%" in MPGN, 5/14 "35.7%" in membranous GN, and 4/11 "36.4%" in FSGS).

3 –Impaired renal function was the least observed clinical manifestation (3/14 "21.4%" in MPGN, and 3/6 "50%" in primary crescentic glomerulonephritis).

(II) Histopathological findings:

The histopathological features of the studied cases are illustrated in figures 1, 2, 3, 4, 5 and 6).

(III) Results of the studied immunohistochemical markers:

1-Immunohistochemical analysis of ICAM-1 expression in primary GN compared to normal "control":

There was a significant increase in ICAM-1 expression ($P < 0.05$) in all the studied types of primary GN compared to the control (9/10 "90%" in minimal change disease ranging between mild and moderate expression, 12/14 "85.7%" in membranous GN ranging from mild to intense expression "Fig. 7", 6/6 "100%" in primary crescentic GN ranging from mild to intense expression, 14/14 "100%" in MPGN ranging from mild to intense expression, 10/11 "90.9%" in FSGS ranging between mild and moderate expression, and 4/5 "80%" in IgAN ranging between mild and moderate expression "fig. 8").

The distribution of the studied groups of GN according to the intensity of ICAM-1 immunostaining is illustrated in table 3.

2-Immunohistochemical analysis of VCAM-1 expression in primary GN compared to normal "control":

There was a significant increase in VCAM-1 expression ($P < 0.05$) in all the studied types of primary GN compared to the control (9/10 "90%" in minimal change disease ranging between mild and moderate

expression, 13/14 "92.9%" in membranous GN ranging from mild to intense expression "Fig. 9", 6/6 "100%" in primary crescentic GN ranging between mild and moderate expression, 14/14 "100%" in MPGN ranging from mild to intense expression "Fig. 10", 10/11 "90.9%" in FSGS ranging between mild and moderate expression, and 4/5 "80%" in IgAN ranging between mild and moderate expression).

The distribution of the studied groups of GN according to the intensity of VCAM-1 immunostaining is illustrated in table 4.

3- Immunohistochemical analysis of HLA-DR Antigen expression in primary GN compared to normal "control" (Table 5):

There was a statistically significant increase in the number of HLA-DR positive cells (P< 0.05) in all the studied types of GN (8/10 "80%" in minimal change disease, 10/14 "71.4%" in membranous GN, 5/6 "83.3%" in primary crescentic GN, 10/14 "71.4%" in MPGN "Fig. 11", 9/11 "81.8%" in FSGS "Fig. 12", and 4/5 "80%" in IgAN.

3- Immunohistochemical analysis of CD3 expression in primary GN compared to normal "control"(Table 6):

There was a statistically significant increase in the number of CD3 positive cells (P< 0.05) in all the studied types of GN (8/10 "80%" in minimal change disease, 11/14 "78.6%" in membranous GN, 5/6 "83.3%" in primary crescentic GN, 10/14 "71.4%" in MPGN "Fig. 13", 9/11 "81.8%" in FSGS "Fig. 14", and 4/5 "80%" in IgAN.

4- Immunohistochemical analysis of CD68 expression in primary GN compared to normal "control"(Table 7):

There was a statistically significant increase in the number of CD68 positive cells (P< 0.05) in all the studied types of GN (9/10 "90%" in minimal change disease, 11/14 "78.6%" in membranous GN, 4/6 "66.7%" in

primary crescentic GN, 10/14 "71.4%" in MPGN "Fig. 15", 10/11 "90.9%" in FSGS "Fig. 16", and 4/5 "80%" in IgAN.

Correlation between ICAM-1 expression and the expression of HLA-DR Antigen, CD3, and CD68 *(Table 8)*:

The table shows a statistically significant positive correlation between the increased expression of ICAM-1 and the increased expression of HLA-DR Antigen, CD3, and CD68 in all the studied types of primary GN.

Correlation between VCAM-1 expression and the expression of HLA-DR Antigen, CD3, and CD68" *(Table 9)*:

The table shows a statistically significant positive correlation between the increased expression of VCAM-1 and the increased expression of HLA-DR Antigen, CD3, and CD68 in all the studied types of primary GN.

Diagnosis	Number	%
Minimal change GN	10	16.7
Membranous GN	14	23.3
Primary crescentic GN	6	10.0
Membranoproliferative GN	14	23.3
Focal segmental GS	11	18.3
IgA nephropathy	5	8.4
Total	60	100.0

Table (1): Distribution of the studied groups according to diagnosis.

Studied group of glomerulonephritis	N.	%
Minimal change GN	**10**	
Lower limb edema, and ascites	10	100.0
Proteinuria	10	100.0
General malaise	6	60.0
Membranous GN	**14**	
Periorbital and lower limb edema	14	100.0
Proteinuria	5	35.7
Primary crescentic GN	**6**	
Impaired renal functions	3	50.0
Hypertension	3	50.0
Proteinuria and hematuria	4	66.7
Membranoproliferative GN	**14**	
Proteinuria and hematuria	9	64.3
Generalized edema	5	35.7
Hypertension	5	35.7
Impaired renal function	3	21.4
Focal segmental GS	**11**	
Generalized edema	7	63.6
Hypertension	7	63.6
Asymptomatic proteinuria	4	36.4
IgA Nephropathy GN	**5**	
Proteinuria and hematuria	5	100.0
Fever	3	60.0

Table (2): Clinical data of the studied groups.

Studied groups of glomerulonephritis	ICAM-1 immunoreactivity								p
	0		1		2		3		
	n	%	n	%	n	%	n	%	
Minimal change GN (n=10)	1	10.0	6	60.0	3	30.0	0	0.0	0.030*
Membranous GN (n=14)	2	14.3	4	28.6	5	35.7	3	21.4	0.016*
Primary crescentic GN (n=6)	0	0.0	1	16.7	2	33.3	3	50.0	0.015*
Membranoproliferative GN (n=14)	0	0.0	4	28.6	5	35.7	5	35.7	0.021*
Focal segmental GS (n=11)	1	9.1	4	36.4	6	54.5	0	0.0	0.006*
IgA Nephropathy (n=5)	1	20.0	1	20.0	3	60.0	0	0.0	0.025*

*Significant

Table (3): The distribution of the studied groups of GN according to ICAM-1 immunoreactivity.

Studied groups of glomerulonephritis	VCAM-1 immunoreactivity								p
	0		1		2		3		
	n	%	n	%	n	%	n	%	
Minimal change GN (n=10)	1	10.0	6	60.0	3	30.0	0	0.0	0.030*
Membranous GN (n=14)	1	7.1	5	35.7	5	35.7	3	21.5	0.002*
Primary crescentic GN (n=6)	0	0.0	2	33.3	4	66.7	0	0.0	0.005*
Membranoproliferative GN (n=14)	0	0.0	5	35.7	5	35.7	4	28.6	0.021*
Focal segmental GS (n=11)	1	9.1	4	36.4	6	54.5	0	0.0	0.006*
IgA Nephropathy (n=5)	1	20.0	1	20.0	3	60.0	0	0.0	0.025*

*Significant

Table (4): The distribution of the studied groups of GN according to VCAM-1 immunoreactivity.

Studied groups of glomerulonephritis	HLA-DR Antigen immunoreactivity				p
	0		1		
	n	%	n	%	
Minimal change GN (n=10)	2	20.0	8	80.0	0.007*
Membranous GN (n=14)	4	28.6	10	71.4	0.019*
Primary crescentic GN (n=6)	1	16.7	5	83.3	0.047*
Membranoproliferative GN (n=14)	4	28.6	10	71.4	0.019*
Focal segmental GS (n=11)	2	18.2	9	81.8	0.003*
IgA Nephropathy GN (n=5)	1	20.0	4	80.0	0.049*

*Significant

Table (5): The distribution of the studied groups of GN according to HLA-DR Antigen immunoreactivity.

Studied groups of glomerulonephritis	CD3 immunoreactivity				p
	0		1		
	n	%	n	%	
Minimal change GN (n=10)	2	20.0	8	80.0	0.007*
Membranous GN (n=14)	3	21.4	11	78.6	0.01*
Primary crescentic GN (n=6)	1	16.7	5	83.3	0.047*
Membranoproliferative GN (n=14)	4	28.6	10	71.4	0.019*
Focal segmental GS (n=11)	2	18.2	9	81.8	0.003*
IgA Nephropathy (n=5)	1	20.0	4	80.0	0.049*

*Significant

Table (6): The distribution of the studied groups of GN according to CD3 immunoreactivity.

Studied groups of glomerulonephritis	CD68 immunoreactivity				p
	0		1		
	n	%	n	%	
Minimal change GN (n=10)	1	10.0	9	90.0	0.004*
Membranous GN (n=14)	3	21.4	11	78.6	0.01*
Primary crescentic GN (n=6)	2	33.3	4	66.7	0.042*
Membranoproliferative GN (n=14)	4	28.6	10	71.4	0.019*
Focal segmental GS (n=11)	1	9.1	10	90.9	0.002*
IgA Nephropathy GN (n=5)	1	20.0	4	80.0	0.049*

*Significant

Table (7): The distribution of the studied groups of GN according to CD68 immunoreactivity.

Variables		CD3 expression	CD68 expression	HLA-DR Antigen expression
ICAM-1 expression	r	0.375*	0.307*	0.351*
	p	0.003*	0.017*	0.006*

*Significant

Table (8): Correlation of ICAM-1 expression with CD3, CD68 and HLA-DR Antigen expression in the studied groups of GN.

Variables		CD3 expression	CD68 expression	HLA-DR Antigen expression
VCAM-1 expression	r	0.349*	0.277*	0.319*
	p	0.006*	0.032*	0.013*

*Significant

Table (9): Correlation of VCAM-1 expression with CD3, CD68 and HLA-DR Antigen expression in the studied groups of GN.

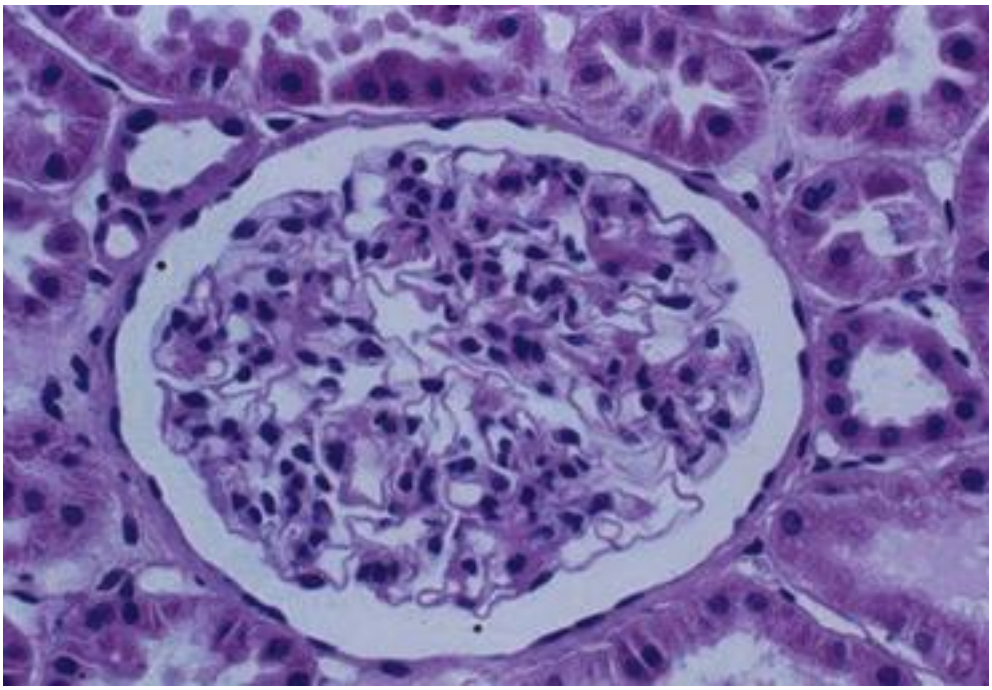

Fig. (1): A case of minimal change glomerulonephritis showing a normally-appearing glomerulus with normal capillary walls and cellularity. The basement membrane is of regular thickness (H & E X200).

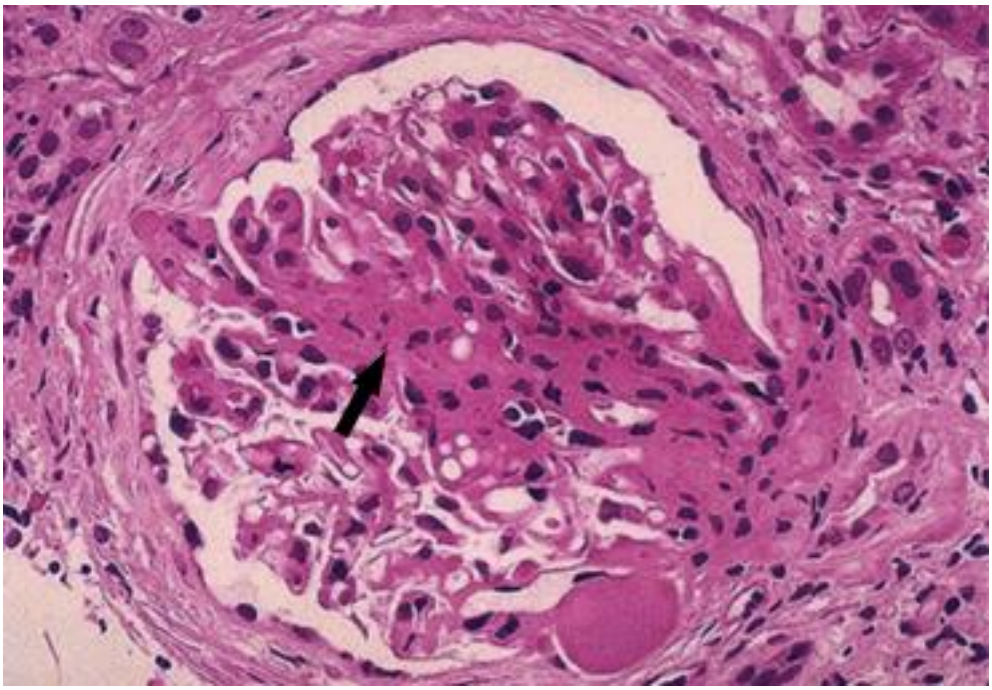

Fig. (2): A case of focal segmental glomerulosclerosis (FSGS) showing a glomerulus with a segmental area of collapse of the glomerular capillaries associated with marked increase in the matrix. An area of collagenous sclerosis running across the middle of this glomerulus is seen "arrow" (H & E X200).

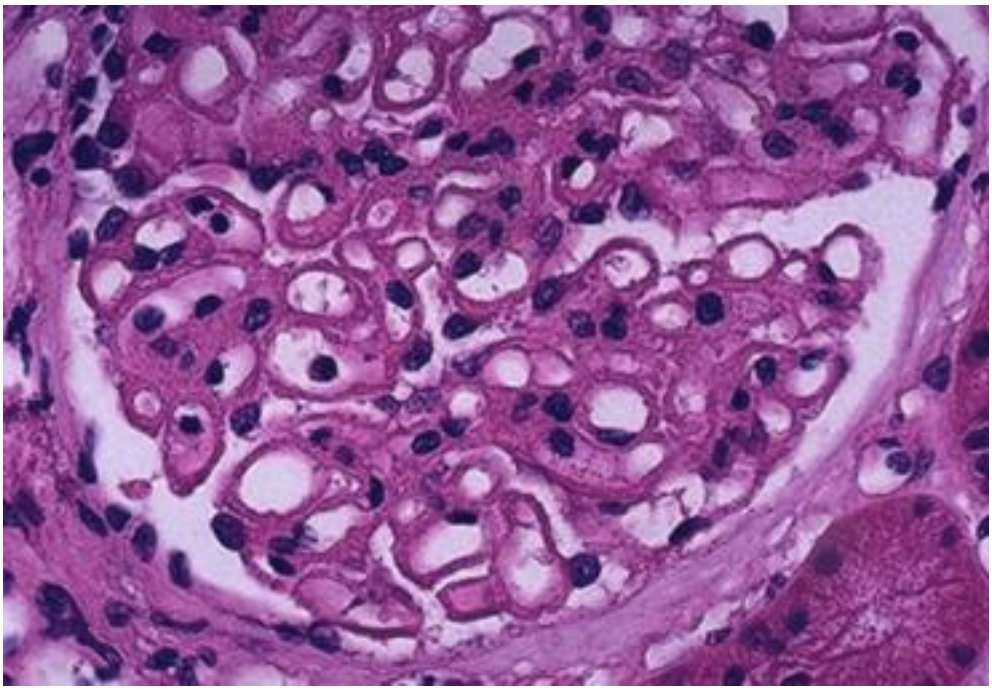

Fig. (3): A case of membranous glomerulonephritis showing that the capillary walls are nearly uniformly thickened, but remain widely patent; the cellularity of the glomeruli is not increased (H & E X400).

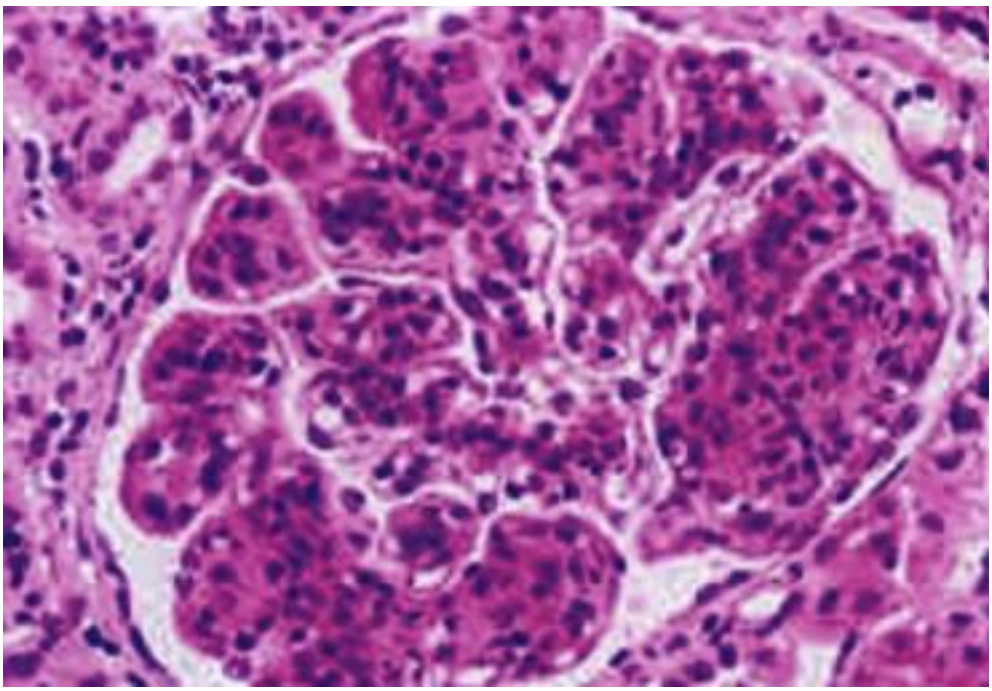

Fig. (4): A case of membranoproliferative glomerulonephritis (MPGN) showing a glomerulus displaying the typical lobulated appearance of this disease, with markedly increased mesangial cells and matrix in lobules, the capillary loops are poorly defined (H & E X 200).

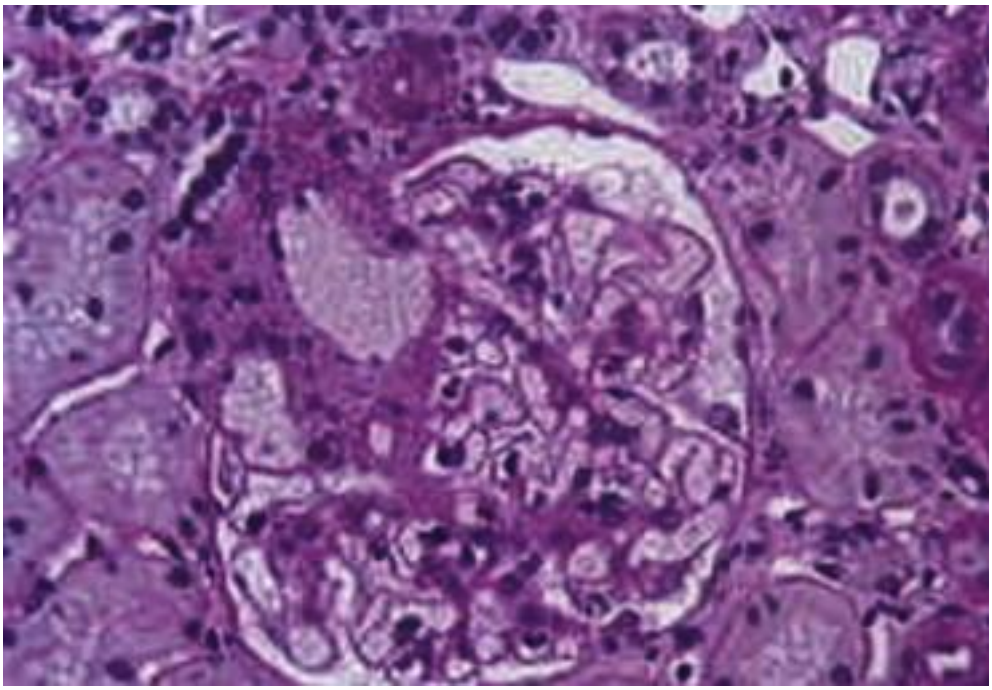

Fig. (5): A case of immunoglobulin A nephropathy (IgAN) showing a glomerulus displaying diffuse mesangial thickening with mesangial hypercellularity (H&E X200).

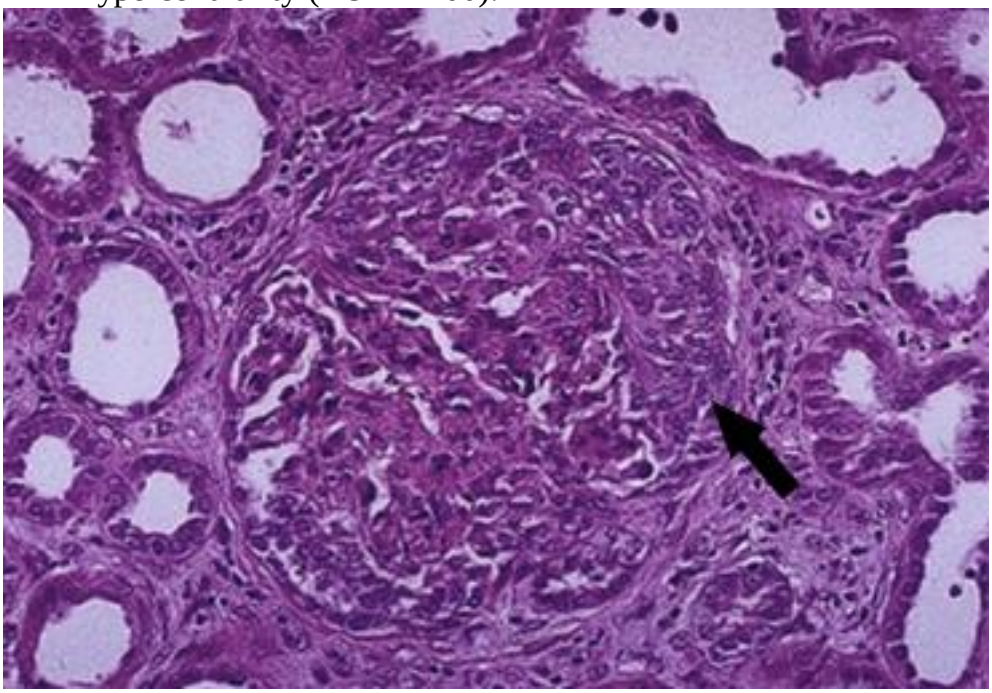

Fig. (6): A case of primary crescentic glomerulonephritis: The crescent is composed of proliferating epithelial cells; and is present in the periphery of the glomerulus "arrow" (H & E X 100)

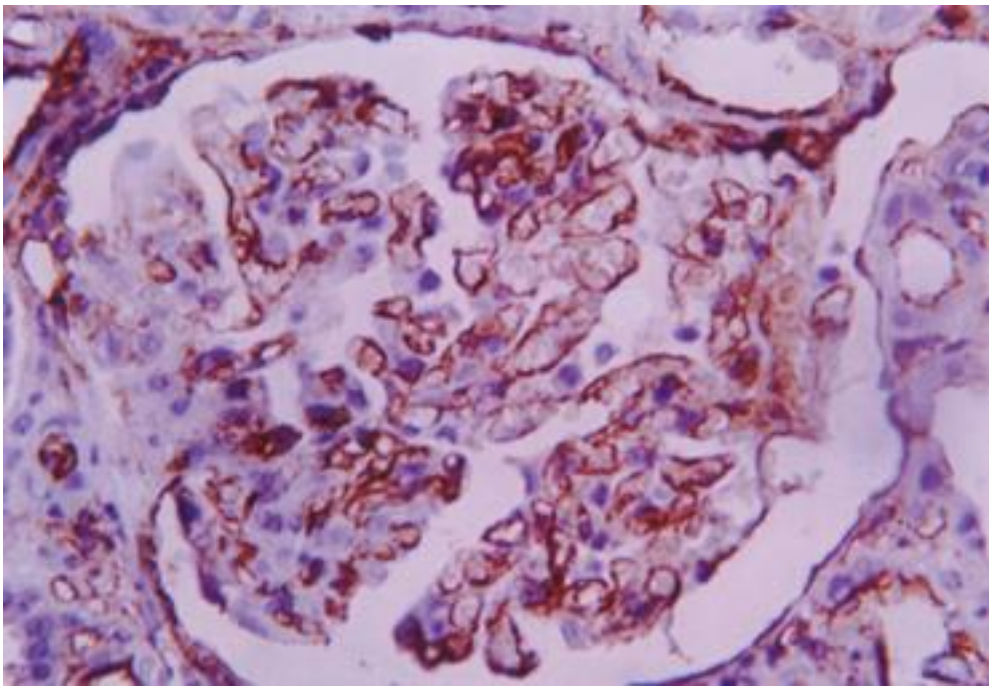

Fig. (7): A case of membranous glomerulonephritis showing intense ICAM-1 expression observed in the glomerular endothelial and parietal epithelial cells especially in the area of hypercellularity (Immunoperoxidase X200).

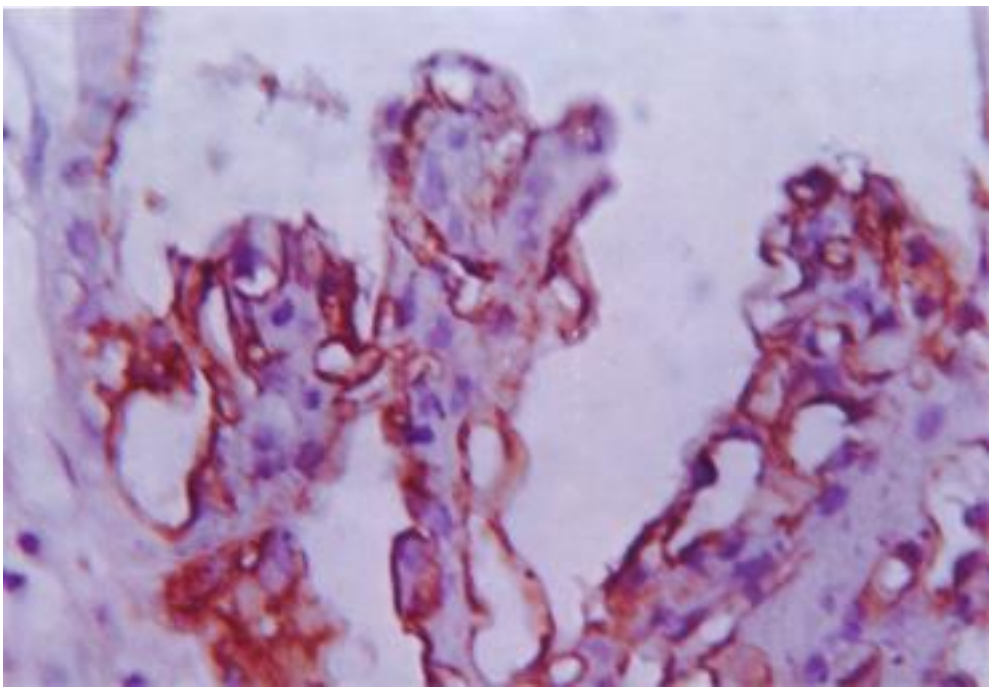

Fig. (8): A case of IgA nephropathy showing moderate glomerular and mesangial expression of ICAM-1 (Immunoperoxidase X400).

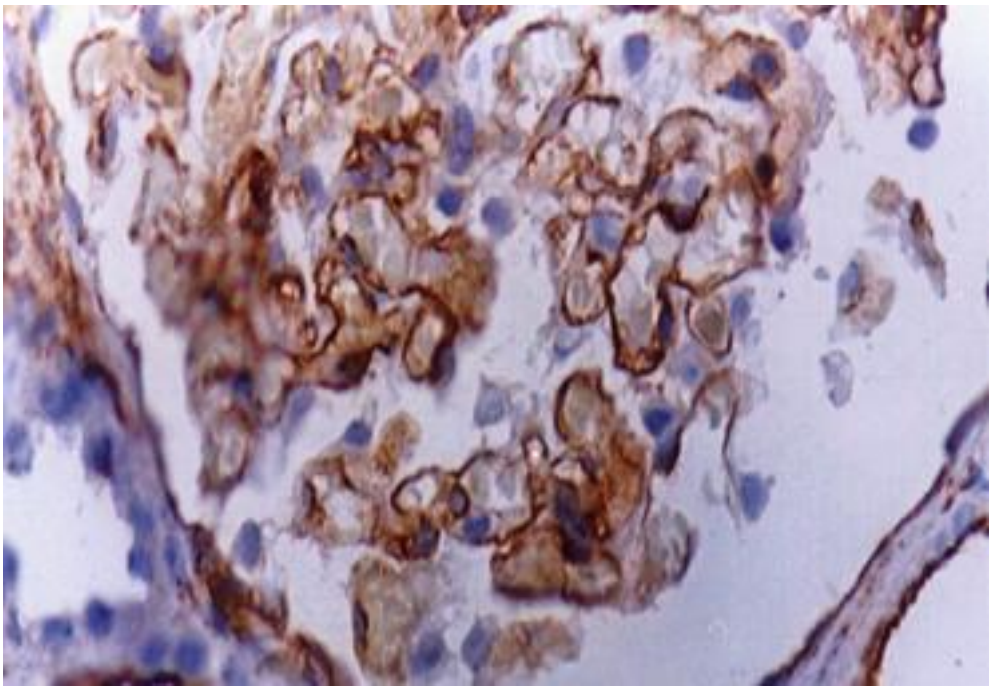

Fig. (9): A case of membranous glomerulonephritis showing moderate VCAM-1 expression in the glomerular endothelial and the parietal epithelial cells (Immunoperoxidase X 400).

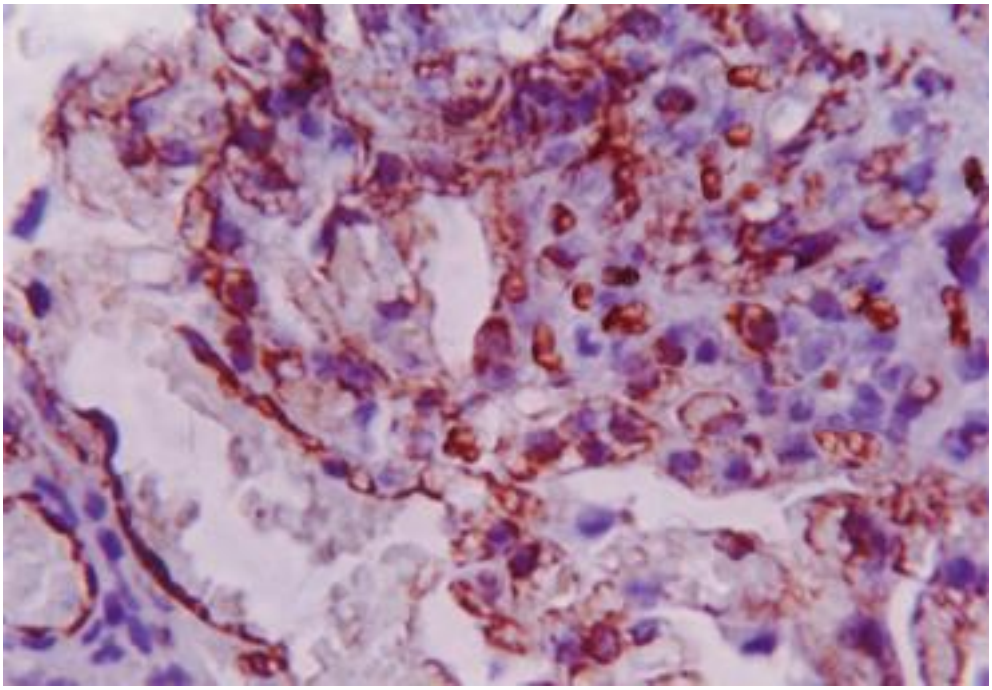

Fig. (10): A case of membranoproliferative glomerulonephritis showing intense VCAM-1 expression observed in the glomerular endothelial, parietal epithelial and mesangial cells (Immunoperoxidase X 400).

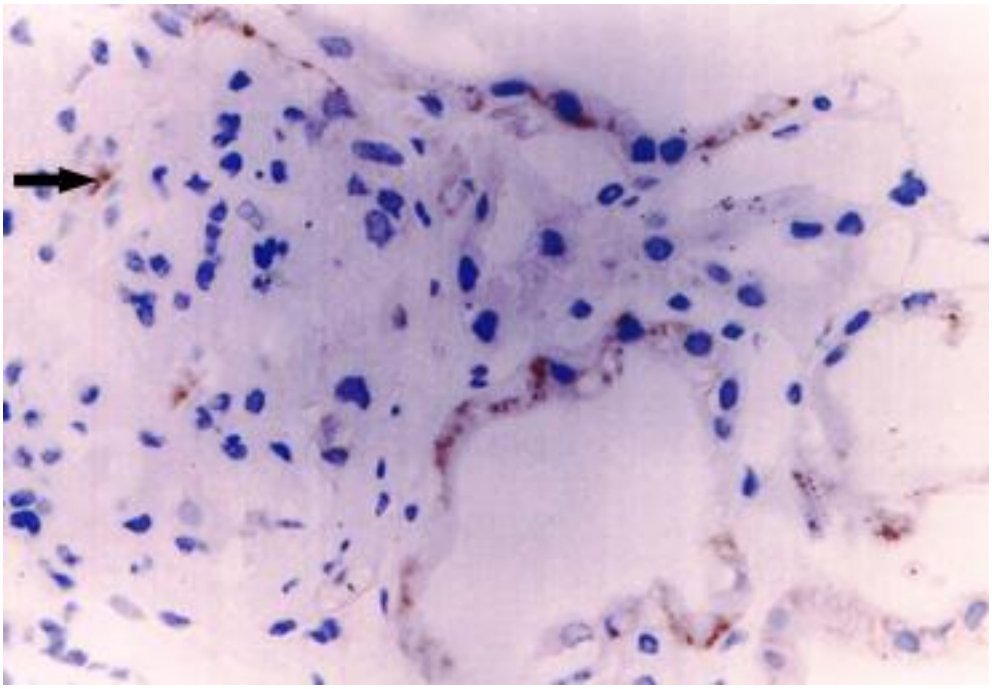

Fig. (11): A case of membranoproliferative GN showing glomerular "arrow" and interstitial tubular expression of HLA-DR Antigen (Immunoperoxidase X 400).

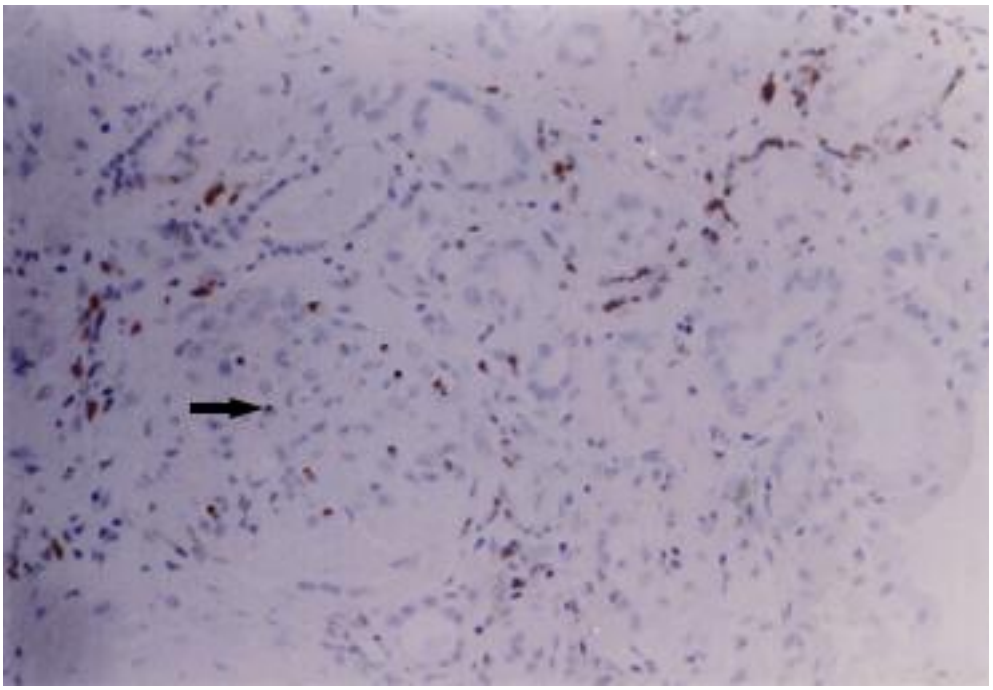

Fig. (12): A case of focal segmental glomerulosclerosis showing HLA-DR positive cells detected within in the glomeruli "arrow". Interstitial expression of HLA-DR antigen is also seen (Immunoperoxidase X 100).

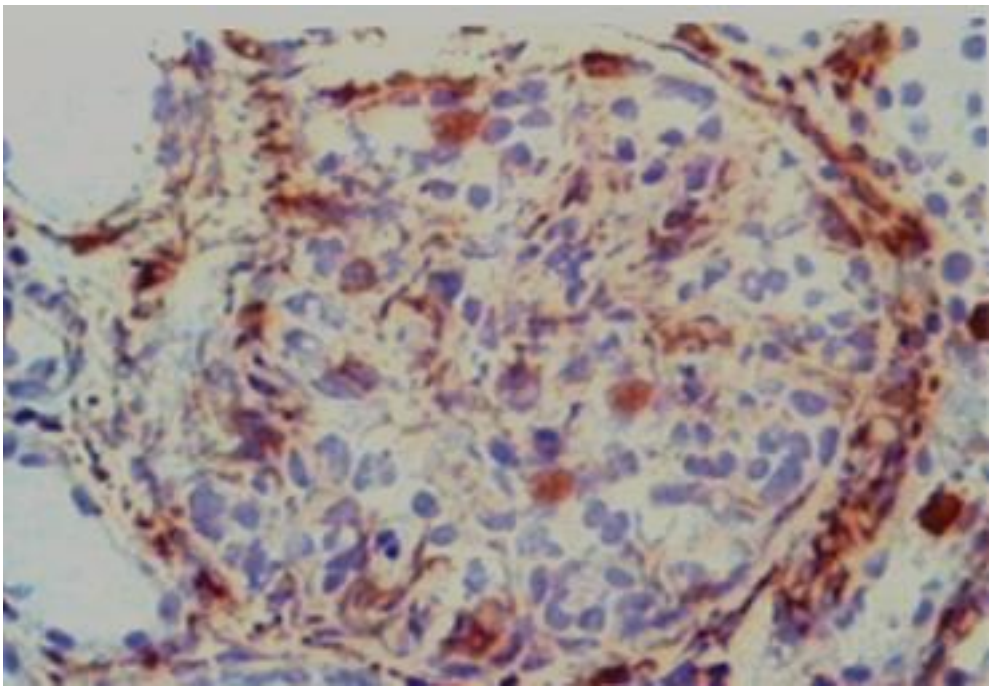

Fig. (13): A case of membranoproliferative GN showing CD3 positive cells observed within the glomeruli and in the interstitial areas (Immunoperoxidase X400).

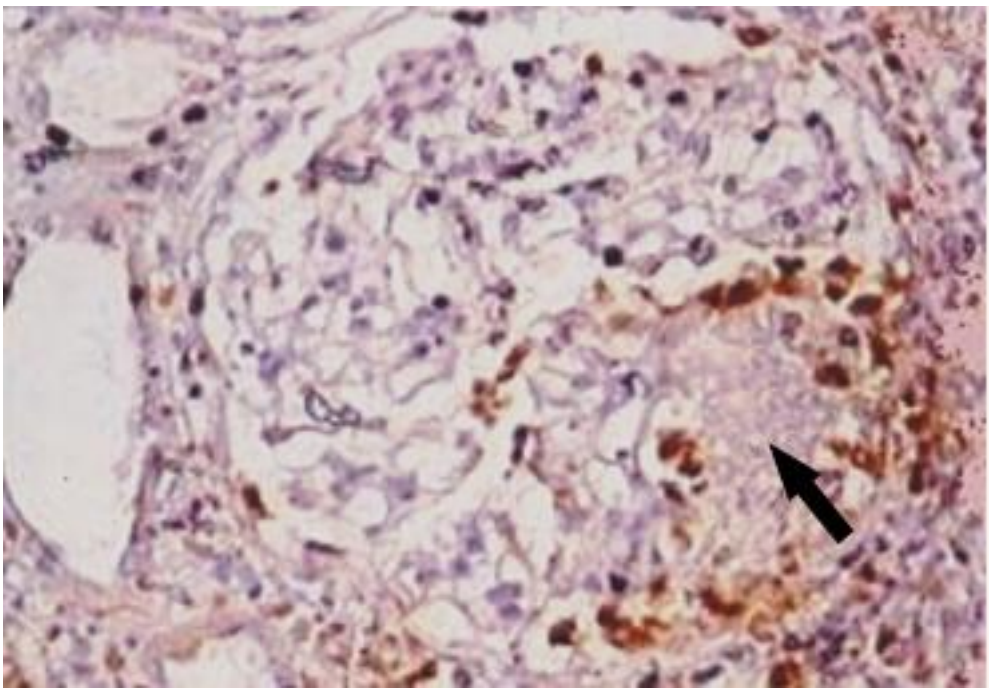

Fig. (14): A case of focal segmental glomerulosclerosis showing CD3 expression in the cells surrounding a segmental area of necrosis "arrow" (Immunoperoxidase X400).

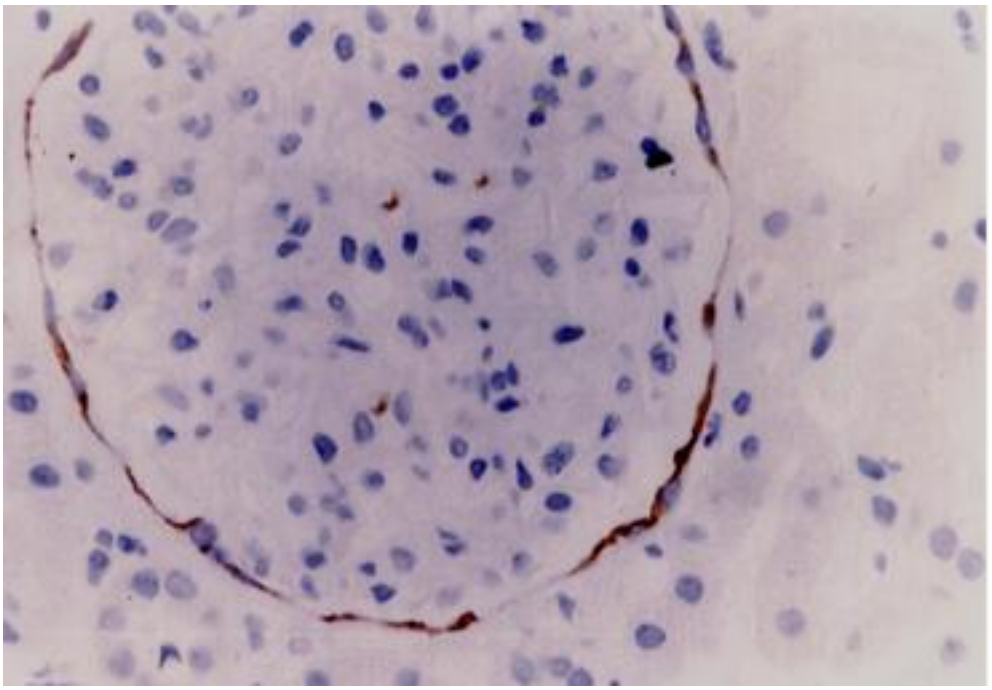

Fig. (15): A case of membranoproliferative GN showing CD68 expression in the parietal epithelial cells and within the glomeruli (Immunoperoxidase X 400).

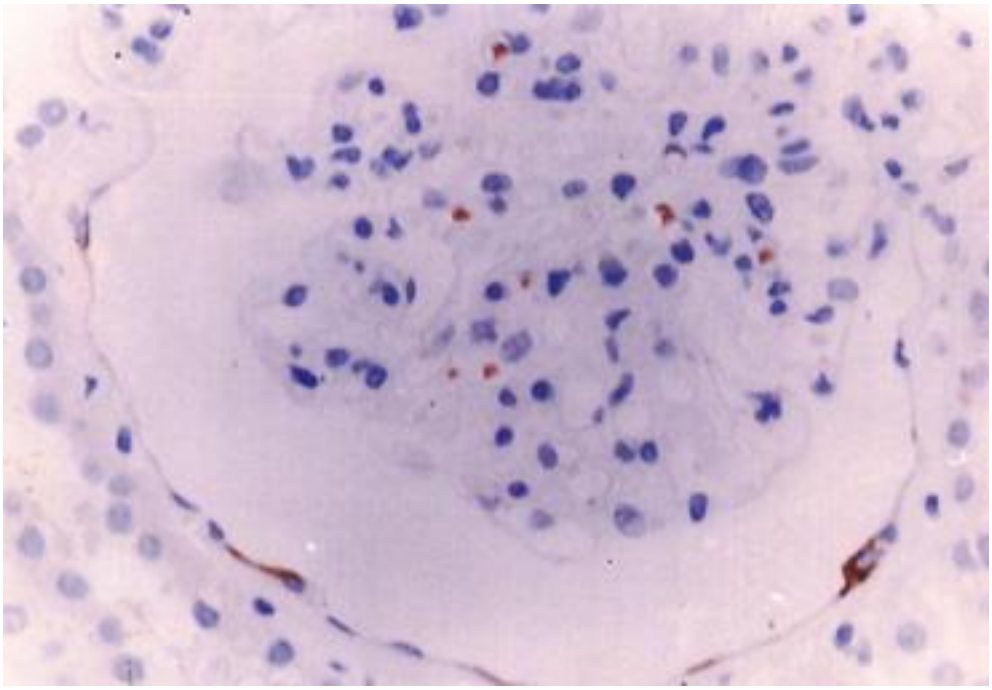

Fig. (16): A case of focal segmental glomerulosclerosis showing CD68 expression in the parietal epithelial cells and within the glomeruli (Immunoperoxidase X 400).

DISCUSSION

Glomerulonephritis refers to a collection of primary and secondary renal disorders characterized by inflammation within the glomeruli. [12] Glomerular diseases remain the most common worldwide cause of end stage renal disease "ESRD" [13].

Despite extensive investigations, the mechanism of the initial glomerular injury and disease progression remain mostly unknown. In addition; the efforts to stop or even to slow the progression of chronic renal insufficiency are still unsuccessful. [14]

A major advance has been made in understanding the cellular and molecular mechanisms which mediate these disorders. [15] Knowledge of the molecular mechanisms involved in cell–cell and cell–matrix adhesion has increased rapidly in the past decade due to the immense clinical relevance of the adhesion molecules involved in such mechanisms. [16&17]

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1(VCAM-1) are two members of immunoglobulin-like superfamily of the cell adhesion molecules (CAMs) that are normally expressed in the glomeruli. ICAM-1 is expressed on the glomerular endothelium and parietal epithelium, while VCAM-1 is detected only on the glomerular parietal epithelium. ICAM-1 and VCAM-1 are also expressed and released by activated T-lymphocytes and macrophages; therefore their increase may also reflect local immune activation. [10]

The present study was performed in a trial to study the expression patterns of cell adhesion molecules ICAM-1 and VCAM-1 by immunohistochemical examination of renal biopsies from patients with different types of primary glomerulonephritis compared to the

expression of such markers in normal renal tissue, in order to evaluate the role of these adhesion molecules in the pathogenesis and disease progression of primary glomerular diseases. It was also intended to correlate between the expression of these cell adhesion molecules and the expression of other immunohistochemical markers of locally activated cellular immune reactions including HLA-DR Antigen, CD3, and CD68.

In this study, twenty two out of sixty patients were males (36.7%), while the rest of the patients were females (63.3%), with the mean age of (29.62±7.23). This is nearly in concordance with the age and gender distribution of different types of primary glomerulonephritis reported by ***Glasscock et al., (2002)*** [18].

The frequencies of the clinical manifestations in the different forms of glomerulonephritis included in this study were nearly in concordance with the previous published studies. [19, 20, 21, 22, 23]

As regards to the expression of ICAM-1 and VCAM-1, the results of present study showed significant over-expression of both markers in all the types of primary glomerulonephritis included in this study compared to their expression in normal renal tissue.

These results were in harmony with those of ***Park et al., (2000)*** [24], who reported that the expression of ICAM-1 and VCAM-1 was pronounced in primary renal diseases with or without autoimmune disorders. ***Arrizabalaga et al. (2008***) [25] reported similar results and stated that upregulation of glomerular expression of ICAM-1 and VCAM-1 might reflect a higher histologic activity in patients with glomerulonephritis.

Mackinnon et al., (2005) [26] also stated that there was a significant association between endothelial activation reflected by

increased glomerular expression of ICAM-1 and VCAM-1, and increasing proteinuria in patients with primary GN.

Similar results were also documented by ***Hauser et al., (1997)*** [27], who reported that focal expression of ICAM-1 and VCAM-1 in the renal tubules occurred in various primary renal diseases and correlated with the severity of tissue damage and intensity of the inflammatory reactions.

The increased expression of ICAM-1 and VCAM-1 in primary glomerular diseases was explained by ***Ley (2001)*** [28] ***and Hicks & Bullard (2006)*** [29], who stated that one of the hallmark features of the inflammatory response is the recruitment of leucocytes to the inflammatory site. Leukocyte recruitment requires capture, rolling, activation, adhesion, and transmigration. Cell adhesion molecules and chemoattractants have been identified to mediate each of these steps. Moreover, ***Amin et al., (2005)*** [30] stated that these adhesion molecules amplify the inflammatory process by allowing the ingress of leucocytes into the diseased tissue.

Daniel (2002) [31] ***and Nagao et al., (2007)*** [32] stated that, for leucocytes to move from rapidly flowing blood to enter to the site of inflammation, they must undergo a sequence of dynamic interaction with endothelial cells lining the microscopic blood vessels in the inflamed tissues. This process depends on proteins termed adhesion molecules expressed by leucocytes and endothelial cells. Endothelial cells act as active participants in the inflammatory process occurring within the glomeruli. [33,34,35]

In addition to the previous role of cell adhesion molecules, ***Sujata et al., (2003)*** [36] reported that ICAM-1 and VCAM-1 are essential for T-cell activation by acting as co-stimulatory signals on target cells and may mediate the infiltration process.

Other studies [37, 38, 39] reported that ICAM-1 appears to play a major role in cell mediated cytolysis, antigen presentation and lymphocyte aggregation. Moreover, induction of ICAM-1 on the mesangial cells and their interaction with phagocytic leukocytes leads to the production of cytokines and chemokines by mesangial cells which are capable of exacerbation of glomerular inflammation.

The results of present study showed also significant expression of HLA-DR Antigen, CD3, and CD68 in all the types of primary glomerulonephritis included in this study, and there was a significant correlation between the expression of ICAM-1 and VCAM-1 and the expression of HLA-DR Antigen, the numbers of glomerular T-lymphocytes (indicated by CD3 positivity), and the numbers of glomerular monocytes/ macrophages (indicated by CD68 positivity).

Park et al., (2000) [24] reported similar results as he found that HLA-DR Antigen, CD3, and CD68 were co-expressed with ICAM-1 and VCAM-1 in the glomerular endothelium of their studied renal biopsies.

Muller et al., (1991) [40] also reported that significant expression of HLA-DR Antigen has been shown in the proximal tubular epithelial cells (PTECs) and within the glomeruli in absence of interstitial cellular infiltrates in various forms of glomerulonephritis, and it was frequently associated with abnormal expression of ICAM-1.

Sullen et al., (2001) [41] reported that HLA-DR Antigen is important in antigen presentation, and that its expression leads to accumulation of regional T- lymphocytes, which play an important role in the inflammatory process in different forms of glomerulonephritis by secretion of growth factors like interleukins-4, tumor necrosis factor-α (TNF- α), and transforming growth factor-B (TGF-B), which leads to fibroblast proliferation with subsequent deposition of extracellular

matrix, these growth factors also stimulate the proliferation and migration of more T-lymphocytes.

In the present study, it was found that the number of CD68-positive cells was significantly increased in various types of GN. ***Takashi et al., (1999) and Nikoli et al.,*** **(2001)** [42,43] found that glomerular and interstitial macrophage accumulation is a prominent feature in most types of human glomerulonephritis. They stated that macrophages accumulate in the kidney from the peripheral blood in response to variety of chemokines and adhesion molecules. Macrophage-derived proinflammatory cytokines such as TNF-α and IL-1 induce mesangial cells to secrete chemokines and express adhesion molecules such as ICAM-1 which facilitate further infiltration and accumulation of macrophages into the glomeruli. Monocytes /macrophages may also be reactivated by T-lymphocytes.

Norman et al., (2008) [44] stated that the implementation of anti-adhesion molecule therapies in many inflammatory diseases is taking part in the recent years, so, clear understanding of the role of cell adhesion molecules will contribute not only to understand the pathogenesis of some glomerular disorders, but also to search for new methods for diagnosis of such diseases and new treatment modalities, however, further studies are needed for proper evaluation of the benefit of anti-adhesion molecule therapy in the treatment of different forms of glomerulonephritis.

CONCLUSIONS

In conclusion, The present study clearly indicates that cell adhesion molecules (ICAM-1 and VCAM-1) proved to be a reliable factor in the pathogenesis of glomerulonephritis, this was indicated by their

significant over-expression in the different types of primary glomerulonephritis included in this study, as well as the concomitant significant correlation between the expression of ICAM-1 and VCAM-1; and the expression of HLA-DR Antigen, CD3, and CD68 (markers of locally activated cellular immune reaction).

The clinical applications of the results of the present study include the possible use of cell adhesion molecule blockade in cases of primary glomerulonephritis, as it proved to be a useful therapeutic strategy in many idiopathic illnesses.

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الملخص العربي

دراسة تعبير الجزيئات اللاصقة للخلايا (ICAM-1 و VCAM-1) فى الأمراض الأولية للكبيبات الكلوية

المقدمة: تضم التهابات الكبيبات الكلوية مجموعة من الأمراض الكلوية الأولية والثانوية, والتي تتميز بحدوث الالتهاب داخل الكبيبات الكلوية، وتعد من أهم أسباب الفشل الكلوي في جميع أنحاء العالم. وبالرغم من تقدم البحث العلمي إلا أنه لم يتوصل إلى تفسير نهائي للمسببات الأولية لهذه الأمراض وكذا كيفية أو ميكانيكية حدوثها وتطورها وكذلك لا يوجد علاج محدد وشافي لهذه الأمراض.

ولقد تزايدت خلال العقد الماضي معرفة كيفية حدوث الالتصاق بين الخلايا وبعضها وكذلك بين الخلايا والمواد المحيطة بها لما للدور الذي تقوم به الجزيئات اللاصقة المسئولة عن ذلك من أهمية إكلينيكية قصوى.

تعد الجزيئات اللاصقة بين الخلايا (ICAM-1) والجزيئات اللاصقة الوعائية (VCAM-1) من عائلة الجزيئات اللاصقة الخلوية التي توجد بصورة طبيعية في الخلايا المبطنة لجدران الأوعية الدموي كما يتم إفرازها من الخلية الليمفاوية تى والخلايا الآكلة الكبيرة ،لذلك تعكس زيادتها حدوث تنشيط محلى للجهاز المناعي.

الهدف من البحث:

تقييم دور الجزيئات اللاصقة (ICAM-1 و VCAM-1) في حدوث الأمراض الأولية للكبيبات الكلوية و تطورها ومعرفة العلاقة بينها وبين بعض الدلالات الأخرى التي تعبر عن نشاط مناعي خلوي محلى مثل HLA-DR, CD3 و CD68.

المرضى و طرق البحث:

شملت هذه الدراسة 60 مريضاً يعانون من الأمراض الأولية للكبيبات الكلوية تم تشخيصهم اعتماداً على التاريخ المرضى و الأعراض الإكلينيكية والفحوصات المعملية المشخصة والأشعات ، كما تم عمل فحص هستولوجى لعينات الكلى المأخوذة مهم بالإضافة إلى فحص مناعي هستوكيميائى لهذه العينات لدراسة تعبير الجزيئات اللاصقة بين الخلية (ICAM-1) والجزيئات اللاصقة الوعائية (VCAM-1) بالإضافة إلى دراسة تعبير CD68, CD3, HLA-DR ومقارنتها بعدد 5 من العينات الضابطة المأخوذة من نسيج كلوي طبيعي.

النتائج:

وقد خلصنا من هذا البحث إلى الوصول للنتائج التالية:

- وجد ارتفاع ذو دلالة إحصائية في تعبيرالجزيئات اللاصقة بين الخلايا (ICAM-1) والجزيئات اللاصقة الوعائية (VCAM-1) وكذلك في تعبير الصبغات المناعية (HLA-DR , CD3 , CD68) في جميع أنواع الأمراض الأولية للكبيبات الكلوية بالمقارنة بتعبيرهم في نسيج الكلى الطبيعي.
- وكذلك وجدت علاقة ايجابية قوية ذات دلالة إحصائية بين ارتفاع تعبير الجزيئات اللاصقة بين الخلايا (ICAM-1) والجزيئات اللاصقة الوعائية (VCAM-1) وزيادة ارتفاع تعبير (HLA-DR, CD3 , CD68) فى الحالات المدروسة .

الاستنتاج:

أثبتت الدراسة أن الجزيئات اللاصقة بين الخلايا (ICAM-1) والجزيئات اللاصقة الوعائية (VCAM-1) هى عوامل هامة في حدوث أمراض الكبيبات الكلوية الأولية مما يعطى

فكرة عن الأهمية و الفائدة الواعدة لاستخدام مضادات الجزيئات اللاصقة في علاج الصور المختلفة لهذه الأمراض.