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Urinary and Tissue Immunohistochemical Expression of Complement Activation Products among Diabetic Nephropathy Patients in Different Stages

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ABSTRACT

Background: It is currently unclear what role of urinary complement activation products (CAPs) play among diabetic nephropathy (DN) patients. **Objective:** To assess complement activation products (C5a) contribution to diabetic nephropathy development in urine and tissue. **Patients and methods:** A cross-sectional study was conducted at the Nephrology Unit and Pathology Department of Zagazig University Hospitals. This study included sixty-two diabetic patients who had diabetic nephropathy. Urinary C5a levels by ELISA and tissue immunohistochemical expression of C5 a receptor (C5aR) were assessed among all patients. **Results:** Statistically significant difference was found regarding C5a level and its tissue receptors which was higher among the grade IV group and lower among the grade IIa group. Age, HDL, and albumin/ creatinine ratio showed statistically significant correlations with C5a and its receptors. Diabetic duration, nephropathy classes, and C5a, C5aR showed statistically significant correlations with albumin/ creatinine ratio. Age, diabetic duration, HDL, fasting blood glucose level, albumin creatinine ratio, and C5a showed statistical significance as predictors for diabetic nephropathy grades III or IV. At a cutoff value equal to 74.4, C5a had 80.8% sensitivity and 66.7% specificity in the prediction of diabetic nephropathy grade III and IV.

Conclusion: Since urinary and tissue CAPs and the severity of DN are closely correlated, doctors may utilize them as markers to assess the disease's severity and development, particularly in cases when renal tubules are injured.

Keywords: Diabetic nephropathy, Complement Activation Products.

INTRODUCTION

Pathologically, diabetic nephropathy (DN) is frequently marked by glomerular nodular sclerosis formation, expansion of glomerular mesangial matrix, and glomerular basement membrane thickening, in the stages of advanced cases. It is typically characterized by the presence of proteinuria or declining renal function, such as a lower glomerular filtration rate **(1).** Multiple factors contribute to DN's pathophysiology. Recent research revealed that supplements may contribute to the development of DN. But it's not entirely apparent what the implications are or what the underlying mechanism is. Proteinuric kidney disorders, including DN, have been linked to increased CAP s levels in urine and tissue. Changes in CAPs, such as C5a, C3a, and C5b-9, were seen in individuals with various DN stages and were linked to renal tubular injury **(2)**.

Because it triggers the release of TNF-alpha, IL-6, IL-8, and CCL, C5a complement is the most powerful inflammatory mediator among the complement systems **(3)**.

C5aR and C5L2 are two distinct receptors that interact to initiate C5a signaling. The bulk of C5a's functional effects is mediated by C5aR, which is expressed on cell membranes since C5L2 is mainly intracellular and may work as a C5aR negative modulator of signal transduction **(4)**.

The study aimed to investigate the role of urinary and tissue complement activation products (C5a and C5 a receptor) in diabetic nephropathy development.

PATIENTS AND METHODS

A cross-sectional study at the Nephrology Unit and Pathology Departments of Zagazig University Hospitals was performed on sixty-two diabetic patients who had diabetic nephropathy.

Ethical consent:

The research ethics council at Zagazig University approved the study (ZU-IRB#6644/5-1-2021). Every patient signed an informed written consent for the acceptance of participation in the study. The Helsinki Declaration of the World Medical Association was followed when it came to ethical standards for human research.

Inclusion Criteria:

Patients who had one of the study's inclusion criteria were as follows:

- Above 18 years.
- Both sexes.
- Based on clinical and laboratory data, individuals with diabetes mellitus (type II).

Exclusion criteria:

Patients who met one of the following requirements will not participate in the trial:

- Patients with other diabetes mellitus types including type 1 diabetes, gestational diabetes, and young-onset diabetes with maturity,
- Patients suffering from any inflammatory or infectious disorders.
- Patients with abnormal renal biopsy results or concurrent additional renal disorders.

All patients were submitted to:

(A) Clinical examination and history taking.

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(B) Lab investigations:

Routine investigations, fasting as well as 2h postprandial blood sugar, HbA1c, lipid profile and uric acid, renal function tests (urea-creatinine-estimated GFR), urinary albumin/creatinine ratio, and Urinary C5a level using ELISA.

(C) Pathological Investigation:

1- Histopathological examination: All cases were subjected to Hematoxylin & Eosin and PAS stain. Diabetic nephropathy was classified into four classes according to **Tervaert *et al.* [(5)].** Class I, Mild glomerular basement membrane thickening: light microscopy shows only mild changes. Class II, (mesangial expansion), either mild (IIa) or marked (IIb).Class III, Nodular sclerosis (Kimmelstiel–Wilson nodules): at least one glomerulus. Class IV, advanced diabetic glomerulosclerosis: > 50% global glomerulosclerosis (Figure 4).

Patients included were divided into 5 groups according to diabetic nephropathy class:

- Class I: included 13 patients.
- Class IIa: included 16 patients.
- Class IIb: included 7 patients.
- Class III: included 14 patients.
- Class IV: included 12 patients.

2- Immunohistochemistry (IHC):

We deparaffinized the sectioned tissue with xylene then we used grading alcohol for rehydration. After that, we immersed the slides in citric acid buffer (PH 6.0; 0.01 M). Antigen retrieval was performed in an 800 W microwave oven (2 minutes) followed by 200 W (8 minutes). Then we rinsed the slides using (0.01% PBS). 0.3% H2O2 in methanol was used for endogenous peroxidase activity blocking (30 minutes) at room temperature. Then, we applied rabbit polyclonal anti-C5aR1 antibody (1:500 dilution; Abcam, catalog number, ab59390), overnight (4°C) in a moistness chamber. PBS rinses were used multiple times, then we applied the secondary antibodies (Anti-rabbit goat immunoglobulins admixed with horseradish peroxidase). They were applied at 37° C for 30 minutes. Then sections were embedded in hydrogen peroxide with 3-3-diaminobenzidine tetrahydrochloride solution (1 minute). At last, sections were stained by hematoxylin followed by alcohol rehydration and xylene.

3-Assessment of immunohistochemical staining: The assessment of C5aR1 staining was performed by semi-quantitative Immunoreactivity Score (IRS) **[(6)].** The positivity was seen in the cell membrane and cytoplasm. The percent of positive cells was assessed as follows: (Zero: No positive cells, One:<10% positive cells, Two:10–50% positive cells, Three: 51–80% positive cells, Four : >80% positive cells). This was multiplied by the intensity as follows: (Zero: no staining, One: mild staining, Two: moderate staining, and three: strong staining). Generating IRS values that ranged from 0 to 12. We classified scores from 0-6 to be a low expression and from 7-12 to be a high expression **(Figure 5).**

Statistical analysis

To analyze the data acquired, Statistical Package of Social Services version 20 was used to execute on a computer (SPSS). Tables and graphs were employed to convey the findings. Quantitative data were presented in the form of the mean, median, confidence intervals, and standard deviation. The information was presented using qualitative statistics such as frequency and percentage. The student's t-test (T) is used to assess the data while dealing with quantitative independent variables. Pearson Chi-Square and Chi-Square for Linear Trend (X^2) were used to assess qualitatively independent data. The significance of a P value <0.05 or less was determined.

RESULTS

The study included sixty-two diabetic patients with a mean age of 46.36±15.6 years and female predominance (58.1%). The patients had a mean duration of diabetes of 5.6±2.3 years. Patients were classified according to renal biopsy results into 4 classes (5 groups) with a semi-equal distribution of the patients. The groups were comparable regarding age and sex distribution. Diabetes duration was higher among greater diabetic nephropathy classes with statistically significant differences between different classes **(p< 0.001) (Table 1).**

Table (1): Demographics and baseline characteristics:

Class	I	IIa	IIb	III	IV	Test of significance	P-value
Number	13	16	7	14	12		
Age (years) mean±SD	38.6±9.9	42.8±13.4	38.5±10.6	46.2±10.6	47±15.2	F= 1.67	0.17
Sex No. (%) Male Female	4 (30.7%) 9 (69.3%)	4 (25%) 12 (75%)	4 (57.1%) 3 (42.9%)	6 (42.9%) 8 (57.1%)	8 (66.7%) 4 (33.3%)	X^2= 4.05	0.4
Diabetes duration (years) mean±SD	1.6±0.4	2.4±0.2	3.2±0.6	6.7±1.4	10.4±2.12	F= 8.5	**<0.001**

Significant differences were found regarding cholesterol (which was higher among the grade IIb group) **(p= 0.002)**, triglyceride (which was higher among the class IV group) **(p= 0.04)**, HDL (which was higher among the class III group) **(p= 0.016)** and LDL (which was higher among grade IV group) **(p= 0.002).** No statistically significant differences were found regarding hemoglobin, fasting blood glucose levels, and hemoglobin A1C.

The different classes had statistically significant differences regarding renal function tests and albumin/ creatinine ratio as serum creatinine, urea increased with increased diabetic nephropathy class **(p<0.001)** and consequently decreased e GFR **(p<0.001).** The albumin creatinine ratio increased with a statistically significant difference with increased diabetic nephropathy class **(p< 0.001)**. A significant difference was found regarding the C5a level which was higher among the class IV group and lower among the class IIa group. There was also a significant difference between the studied groups regarding C5a receptor expression as most of class IIa had low expression (0-6) followed by class I while most of class IV had high expression followed by class III with a significant difference **(p= 0.01) (Table 2).**

Table (2): Laboratory findings:

Class	I	IIa	IIb	III	IV	F	P- value
Number	13	16	7	14	12		
Cholesterol (mg/dL)	74.2±6.2	161.4±38.1 a	203±42.1 a	169.2±5.2 a	186.2±3 a	4.9	**0.002**
Triglycerides (mg/dL)	65.5±4.2	121.21±28.9	131.7±8.2	106±23.8	148.16±33 a	2.5	**0.04**
HDL (mg/dL)	29.2±6.6	49.6±11.9	41.4±8.4	54±12.6	27.6±6.2 d	3.3	**0.016**
LDL (mg/dL)	49.4±4.8	105.5±24.4	120.7±27.2	120.6±24.3a	147.9±34.1 a	5.01	**0.002**
Hemoglobin (g/dL)	12.6±3.1	11.03±1.3	10.6±1.3	10.9±1.6	8.5±1.4	1.09	0.37
FBS	151±33.4	161.3±38.4	129.7±27.7	190.4±5.1	193±32.7	2.1	0.08
HgbA1C	7.4±1.6	7.5±1.3	6.9±0.7	9.6±2.1	8.5±1.3	2.1	0.09
S. creatinine (mg/dL)	0.6±0.13	0.9±0.14	1.09±0.2	2.02±0.4 a, b, c	3.6±0.81 a, b, c, d	62.5	**<0.001**
S. Urea (mg/dL)	34.5±8.1	33.7±7.5	37±5.3	58.4±11.4	21.7±70.1 a, b, c, d	8.02	**<0.001**
eGFR (mg/dL)	137.9±28.6	93.3±21.6a	81.5±18.5 a	41.14±10.1a, b	23.8±5.3 a, b, c	14.5	**<0.001**
Alb/cr (mg/dL)	76.2±7.6	70.7±6.2	114.28±25.7	251.57±58a, b, c	903±136.3 a, b, c	51.8	**<0.001**
C5a	89.9±20.5	59±12.3	89.9±19.3	88.5±20.1	92.6±19.06	2.8	**0.032**
C5a receptor expression No. (%):							
Low expression	5 (38.4%)	13 (81.25%)	3 (42.8%)	5 (35.7%)	2 (16.7%)	X^2=	**0.01**
High expression	8 (61.6%)	3 (18.75%)	4 (57.2%)	9 (64.3%)	10 (83.3%)	13.1	

F: analysis of variance (ANOVA); X^2: Chi-square test: a, b, c, d Post- Hoc analysis; a: Statistically significance against grade I group; b: Statistically significance against group IIa: c: Statistically significance against group IIb: d: Statistically significance against grade III group; level of significance< 0.05

According to C5a receptors expression, patients were divided into a low expression (0-6) group and a high expression (7-12) group. Both groups were comparable regarding age and sex. Diabetes duration was longer among the high expression group with a significant difference **(p<0.001)**.

There were no statistically significant differences between both groups regarding cholesterol, triglycerides, and HDL, however, LDL was higher with a statistically significant difference among the high expression group **(p= 0.015)**. Hemoglobin was higher with a statistically significant difference among the low expression group **(p<0.001)**. Both groups were comparable regarding fasting blood sugar while HgbA1c was higher significantly among the high expression group. A statistically significant difference was detected between both groups regarding s. creatinine and urea which were higher among the high expression group **(p<0.001)**.

Subsequently, eGFR was lower significantly among the high expression group **(p<0.001)**. Albumin/ creatinine was higher among the high expression group **(p<0.001)**. C5a levels were higher significantly among the high C5a receptors expression group **(p= 0.04)**. High C5a receptor expression was more frequent among advanced diabetic nephropathy classes (class III & IV) while low expression was shown more frequently among class I and II with significant difference **(p= 0.01) (Table 3).**

Table (3): Comparison between low and high C5a receptors expression:

	Low expression (n= 28) mean ± SD	High expression (n= 34) mean ± SD	Test of significance (t)	P-value
Age (years)	46.8±11.3	47.3±9.8	0.18	0.8
Sex No. (%) Male Female	 12 (42.8%) 16 (57.2%)	 14 (42.2%) 20 (75.8%)	0.018	0.9
Diabetes duration (years) mean ± SD	2.8±0.5	7.4±1.7	7.68	**<0.001**
Cholesterol (mg/dL)	166.98±23.9	172.4±21	0.95	0.35
Triglycerides (mg/dL)	122.8±28.5	149.02±37.1	1.78	0.08
HDL (mg/dL)	33.87±5.1	37.98±5.4	0.98	0.32
LDL (mg/dL)	110.65±23.65	129.54±30.98	2.4	**0.015**
Hemoglobin (g/dL)	11.5±1.2	9.9±0.9	-5.9	**<0.001**
FBS	164.34±38.1	167.9±39.8	0.8	0.3
HgbA1C	7.3±1.5	8.4±0.8	3.7	**0.005**
S. creatinine (mg/dL)	0.9±0.21	2.1±0.4	5.9	**<0.001**
S. Urea (mg/dL)	34.87±5.2	59.87±10.2	4.8	**<0.001**
eGFR (mg/dL)	88.43±10.87	39.43±5.3	6.1	**<0.001**
Alb/cr (mg/dL)	90.21±12.3	830.21±21.1	19.87	**<0.001**
C5a	81.3±19.5	92.4±22.5	2.05	**0.04**
Diabetic nephropathy class No. (%): I IIa IIb III IV	 5 (17.9%) 13 (46.4%) 3 (10.7%) 5 (17.9%) 2 (7.2%)	 8 (23.5%) 3 (8.8%) 4 (11.8%) 9 (26.5%) 10 (29.4%)	X^2= 13.1	**0.01**

t: student t- test; X^2: Chi square test; level of significance<0.05; HDL: High density lipoprotein; LDL: Low density lipoprotein; FBS: Fasting blood sugar; HgbA1C: Hemoglobin A1C; eGFR: estimated glomerular filtration rate; Alb/cr: Albumin creatinine ratio.

Linear regression analysis for factors affected C5a levels revealed that all factors were entered in 5 steps of multivariate linear regression analysis to be adjusted for confounders (R: 0.68; adjusted R: 0.29; F: 2.6; p= 0.005). Age, HDL, albumin/ creatinine ratio and C5a receptors expression showed statistically significant correlations with C5a **(Figure 2).**

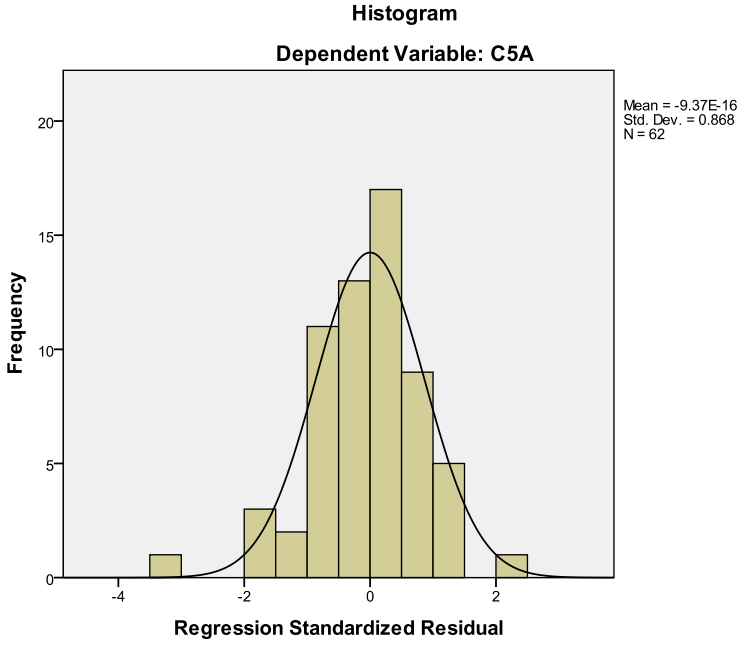
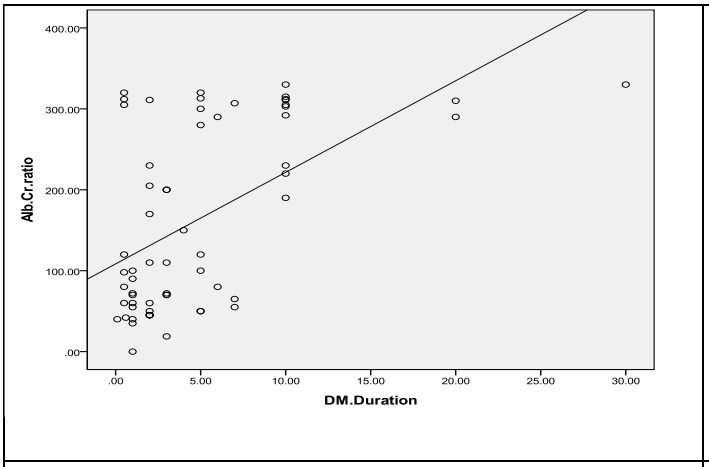
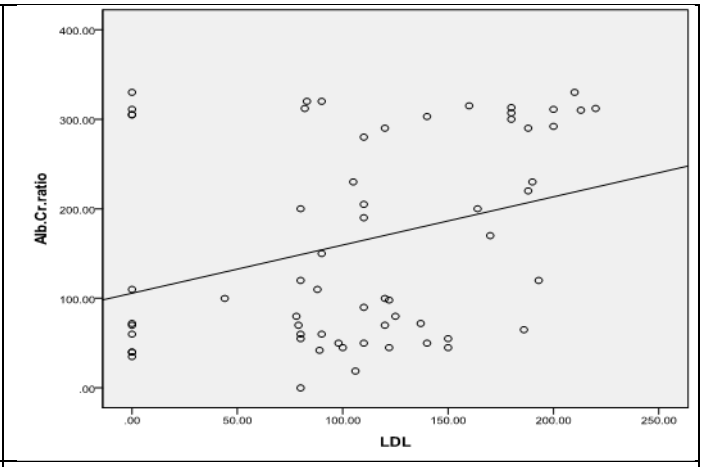


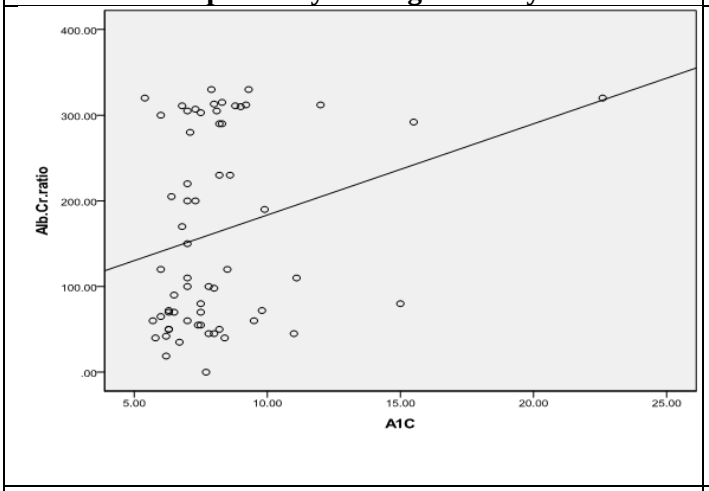
Figure (1): Histogram for the frequency of every factor affecting C5a using linear regression analysis.



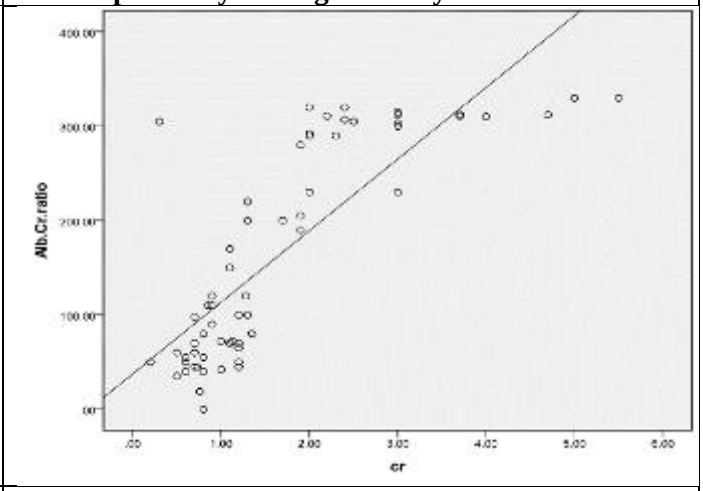
(A) Albumin creatinine ratio and diabetes duration were positively and significantly correlated



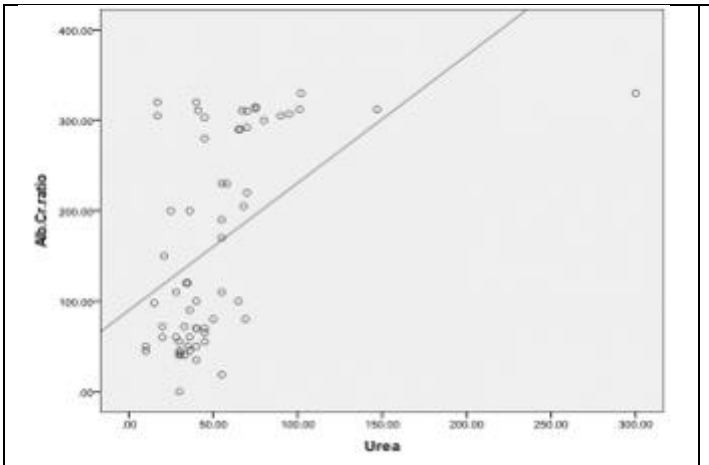
(B) Albumin creatinine ratio and LDL were positively and significantly correlated



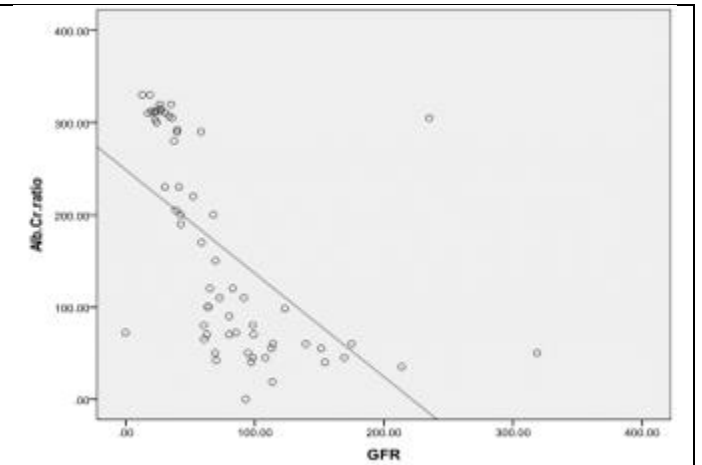
(C) Albumin creatinine ratio and hemoglobin A1c were positively and significantly correlated



(D) Albumin creatinine ratio and s. creatinine were positively and significantly correlated

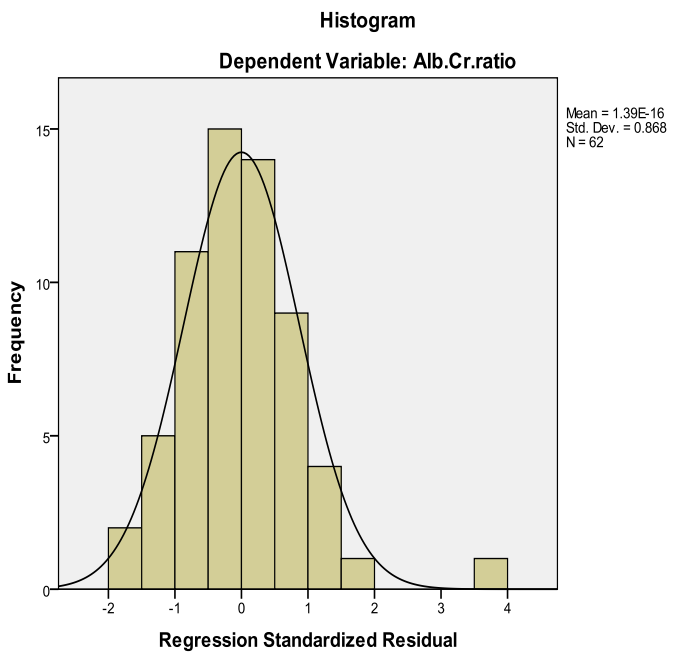


(E) Albumin creatinine ratio and s. urea were positively and significantly correlated

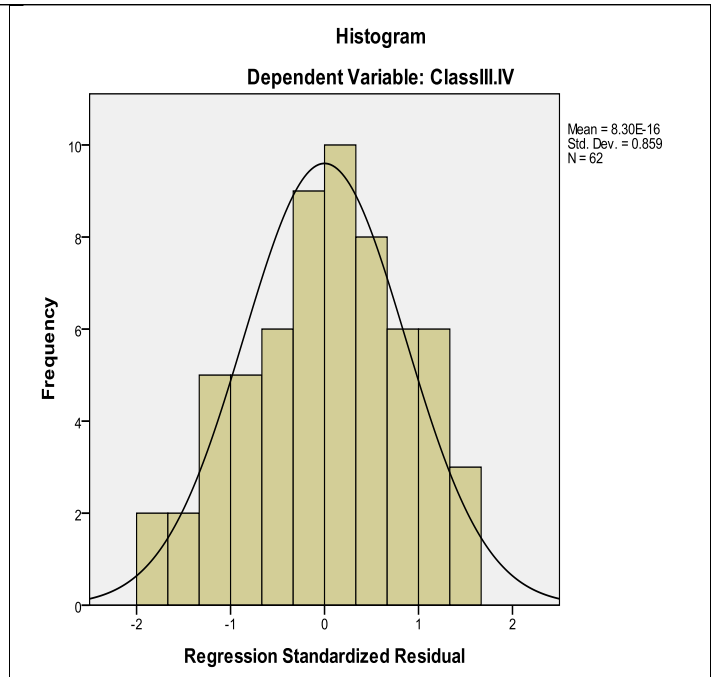


(F) Albumin creatinine ratio and eGFR were positively and significantly correlated

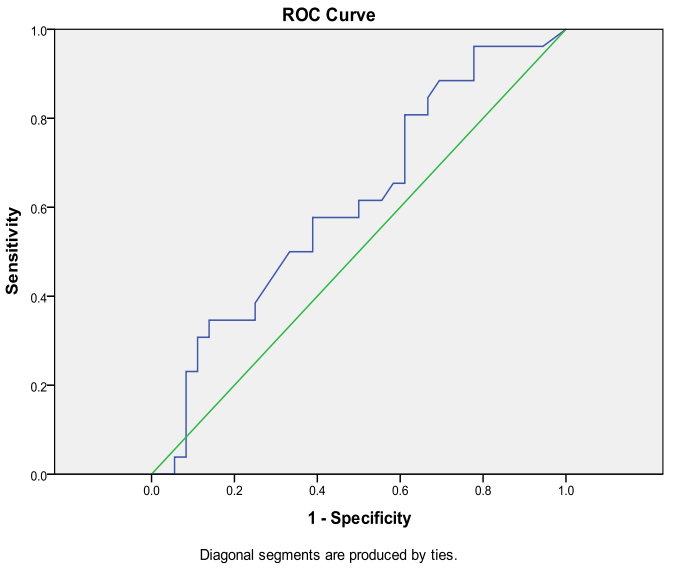
Figure (2): Correlations between albumin/ creatinine ratio and other parameters. Diabetes duration and albumin creatinine ratio were shown to be significantly positively related **(r: 0.4; p< 0.001) (Figure 2A)**. LDL and albumin/creatinine ratio were strongly positively correlated. **(r: 0.3; p= 0.014) (Figure 2B)**. The relationship between albumin/creatinine ratio and hemoglobin A1c was shown to be significantly positive **(r: 0.25; p= 0.04) (Figure 2C).** The ratio of albumin to creatinine and the level of serum creatinine showed a statistically significant positive correlation **(r: 0.8; p<0.001) (Figure 2D)** as well as urea **(r: 0.5; p<0.001) (figure 2E)**. The albumin/creatinine ratio and eGFR were significantly inversely correlated **(r: -0.58; p<0.001) (Figure 2F).**



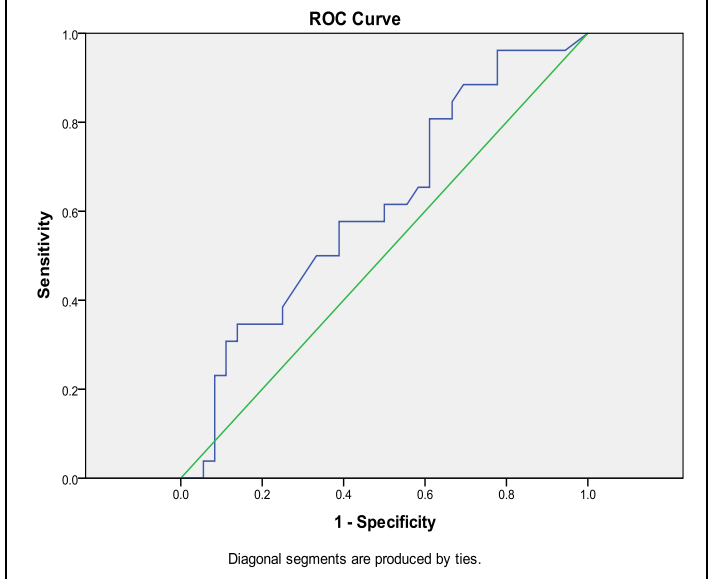
A Histogram for the frequency of every factor affecting albumin creatinine ratio using linear regression analysis



B: Histogram for the frequency of every factor affecting albumin creatinine ratio using binary logistic regression analysis

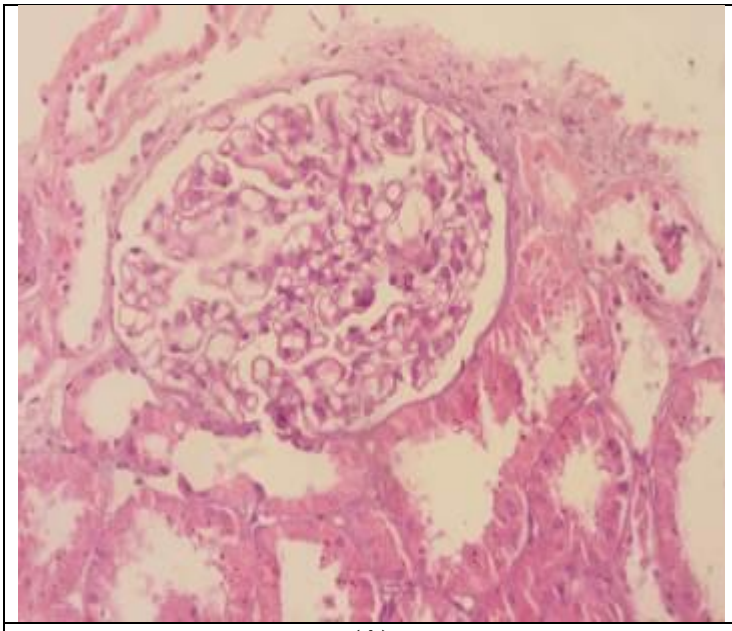


C: Accuracy of C5a in the prediction of diabetic nephropathy classes III and IV

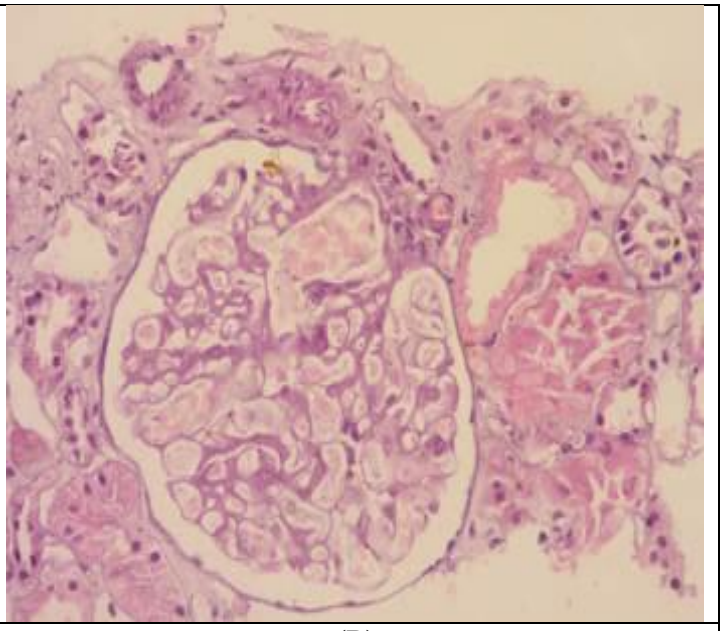


D: Accuracy of C5a receptors expression in predicting classes III and IV diabetic nephropathy

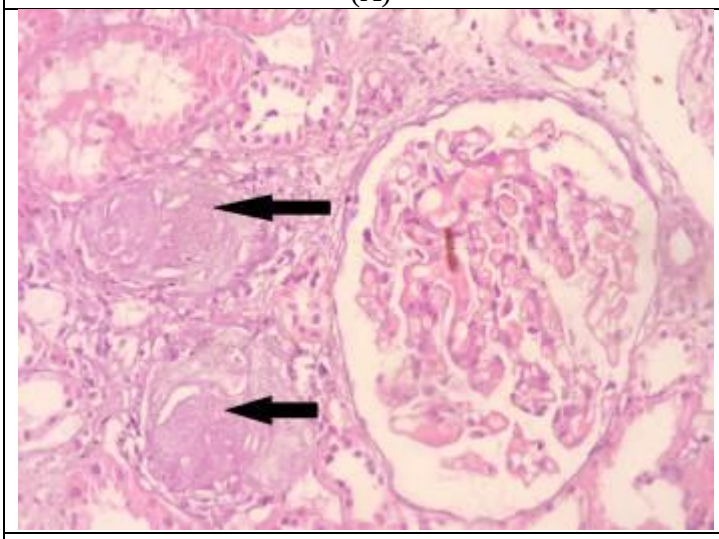
Figure (3): Linear regression analysis (multivariate analysis) for factors affecting albumin creatinine ratio revealed that All factors were entered 5 steps of multivariate linear regression analysis to be adjusted for confounders (R: 0.88; adjusted R: 0.78; F: 11.4; p< 0.001). Diabetes duration, diabetic nephropathy classes, C5a, and C5a receptor expression showed statistically significant correlations with the albumin/ creatinine ratio **(Figure 3A).** Binary logistic regression analysis (multivariate analysis) for factors predicting diabetic nephropathy class III and IV revealed that all factors were entered 5 steps multivariate linear regression analysis to be adjusted for confounders (R: 0.94; adjusted R: 0.86; F: 24.9; p< 0.001). Age, Diabetes duration, HDL, fasting blood glucose level, albumin creatinine ratio, C5a, and C5a receptor expression showed statistical significance as predictors for diabetic nephropathy grades III or IV **(Figure 3B).** At a cutoff value equal to 74.4, C5a had 80.8% sensitivity and 66.7% specificity in the prediction of diabetic nephropathy grade III and IV **(Figure 3 C).** At a cutoff value equal to 8, C5a receptors expression had 84% sensitivity and 78% specificity in the prediction of diabetic nephropathy **(Figure 3 D).**



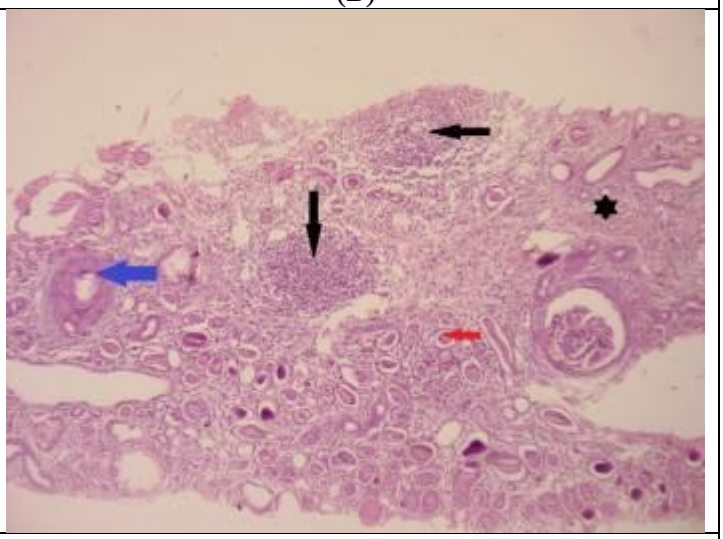
(A)



(B)



(C)



(D)

Fig. (4): Representative samples of morphological lesions in DN : (A) Renal biopsy from class I patient showing only mild changes(x 400). **(B)** Renal biopsy from class II patient showing mesangial expansion (x 400). **(C)** Renal biopsy from class IV patient showing glomerulosclerosis: Kimmelstiel–Wilson lesions (black arrows)(x 400). **(D)** Example of vascular (blue arrow) and inflammatory (black arrow) changes and interstitial fibrosis (star) in DN patients (x 200).

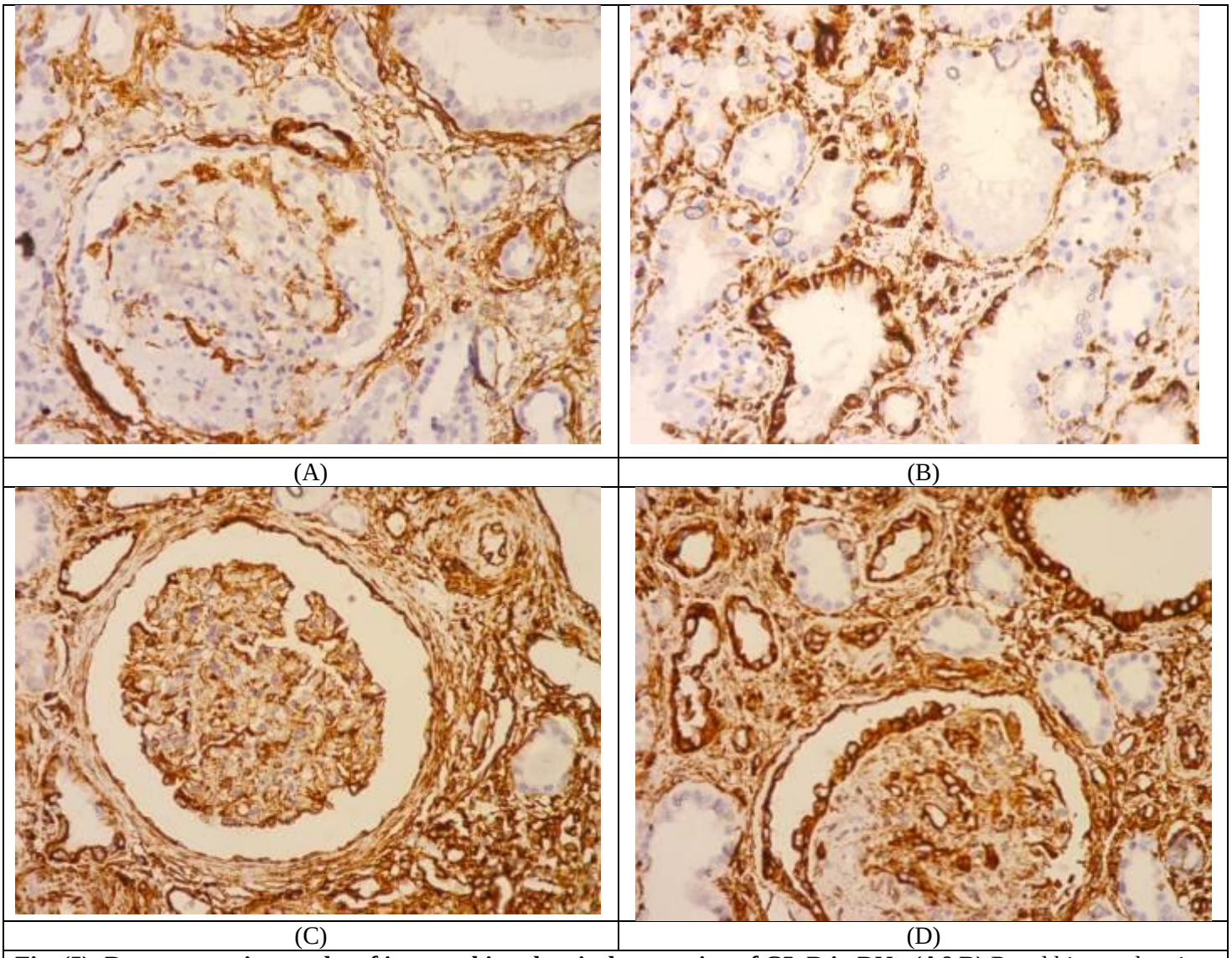


Fig. (5): Representatvie samples of immunohistochemical expression of C5aR in DN : (A&B) Renal biopsy showing low C5aR immunohistochemical expression (x 400).. **(C&D)** Renal biopsy showing high C5aR immunohistochemical expression (x 400)

DISCUSSION

In the world, diabetic nephropathy (DN), which affects 25–30% of people with diabetes, is the main contributor to end-stage renal disease. The pathogenic mechanism of DN is still poorly understood despite substantial research. There is currently no effective therapy alternative available to manage that serious illness [(7)].

It should be noted that earlier research focused primarily on DN patients with advanced disease when looking at urinary CAPs. There has never been a report of urinary CAPs in early disease stages [(8)].

Two different receptors, C5aR and C5L2, work together to start the C5a signaling process. C5aR, which is expressed on cell membranes, mediates the bulk of the functional effects of C5a because C5L2, which is largely intracellular and may work as a negative modulator of C5aR- signal transduction [(9)].

The present study included sixty-two diabetic patients with a mean age of 46.36±15.6 years and female predominance (58.1%). The patients had a mean diabetes duration of 5.6±2.3 years. Patients were classified according to renal biopsy results into 4 classes (5 groups) with a semi-equal distribution of the patients. The groups were comparable regarding age and sex distribution. Diabetes duration was higher among greater diabetic nephropathy classes with statistically significant differences between different classes **($p< 0.001$).**

Pelletier *et al.* [(10)] showed that the group contained a total of 83 patients. 80 percent of the participants were men and 87 percent had type 2 diabetes, all but two, their age mean value was of 69 ± 10 years., and mean pressure was 94 ± 12 mm Hg as well as GFR was 25 ± 9 ml/min per 1.73 m^2. The participants' median HbA1C was 7.3 percent, with 46% having an HbA1C below that level and 13% having a mean HbA1C above it. The average albumin to creatinine ratio was 0.13 (0.05–0.32) g/mol. The yearly eGFR on average was 2.93 ml/min per 1.73 m^2.

In our study, Significant differences were found regarding cholesterol (which was higher among the grade IIb group) (p= 0.002), triglyceride (which was higher among the class IV group) (p= 0.04), HDL (which was higher among class III) group) (p= 0.016) and LDL (which was higher among the grade IV group) (p= 0.002), According to **Palazhy and Viswanathan's** [11] research, dyslipidemia was seen in 56.52 percent of control patients and 75.28 percent of nephropathy subjects (P=0.012), which is consistent with our study's findings.

In the current study, the different groups had statistically significant differences regarding renal function tests and albumin/ creatinine ratio as serum creatinine, urea increased with increased diabetic nephropathy class (p<0.001) and consequently eGFR decreased (p<0.001). The albumin creatinine ratio increased with a statistically significant difference with increased diabetic nephropathy class (p< 0.001). A significant difference was detected regarding C5a urinary level and C5a R immunohistochemical expression which was higher among the grade IV group and lower among the grade IIa group. This coincides with **Zheng *et al.*** [12] who discovered that the RIG showed significant alterations in indicators of renal function impairment, including serum urea nitrogen, creatinine, and eGFR level, and they also revealed that Urinary CAPs were shown to significantly increase as the renal insufficiency stage of proteinuria proceeded. Urine levels of C5a, C3a, and C5b9 had a strong positive correlation with DN patients.

This study showed that Age, HDL, and albumin/ creatinine ratio showed statistically significant correlations with C5a and C5a R immuno-expression, significant correlations between albumin/creatinine ratio, and all of (diabetes duration, LDL, hemoglobin A1c, s. creatinine , and urea : (r: 0.4; p< 0.001), (r: 0.3; p= 0.014), (r: 0.25; p= 0.04), (r: 0.8; p<0.001), (r: 0.5; p<0.001) respectively**.** While significant inverse relation was seen between the albumin/ creatinine ratio and eGFR was found (r: -0.58; p<0.001).

Li *et al.* [13] demonstrated a substantial correlation between urinary levels of C3a and C5a, urinary protein, serum creatinine, and estimated glomerular filtration rate, which confirmed the findings of our investigation. While **Wendt and colleagues** [14] discovered that the majority of peptides generated from CFB had a positive connection with eGFR. C3-glomerulonephritis, minimal change disease, lupus nephritis, focal segmental glomerulosclerosis, and membranoproliferative glomerulonephritis were the disorders with the greatest amounts of measurable C3 excretion compared to controls.

In the current study at a cutoff value equal to 74.4, C5a had 80.8% sensitivity and 66.7% specificity in the prediction of diabetic nephropathy grade III and IV. **Ogrodowski *et al.*** [15] elevated urine C5b9 in patients with renal disorders was first described. The study involved six DN patients. Those who had glomerulopathies typically had higher UMAC levels: 18 of the 38 individuals had UCr values between 200 and 20,000 ng/mg UCr. Patients with MN were not distinguished from those with other types of glomerulopathy based on UMAC values. In actuality, individuals with focal glomerulosclerosis or diabetic glomerulosclerosis, which caused nephrotic syndrome, had the highest UMAC levels. Supporting our study's premise, biopsy samples from people with diabetic glomerulosclerosis and elevated UMAC levels revealed many deposits of SC5b-9 in the tubular epithelial cells, but little to no deposits of SC5b-9 in glomeruli.

Tan *et al.* [16] demonstrated that the potent pro-inflammatory mediator's complement C5a and C3a, which are results of complement activation, may drive a range of metabolic responses in the kidney in the context of diabetes, including, disrupted mitochondrial respiratory function, altered energy utilization as well as reactive oxygen species generation.

Similar to our findings, **Yiu *et al.*** [17] showed a robust and positive relation between renal C5a levels and the development of DN, with the degree of interstitial fibrosis and tubular damage being related to an increase in renal C5a levels in individuals with DN.

While Morita *et al.* observed higher urine C3b, Bb, and MAC in proteinuric individuals in 2000—in contrast to our study—they also included 17 DN patients. Due to the small number of DN patients included in this research, it is impossible to link CAPs to the development of the illness. Furthermore, most patients in these trials had marked renal insufficiency, so it is uncertain how CAPs alter in DN patients at the stages of micro-albuminuria and proteinuria.

CONCLUSION

The current study has provided a thorough picture of how CAPs vary in DN patients at different stages. We looked at the connection between urine complement activation C5a and C5a R immunohistochemical expression and different stages of diabetic nephropathy, focusing especially on individuals with advanced disease stages.

Since CAPs and the severity of DN are closely correlated, doctors may utilize them as a marker to assess the disease's severity and development, particularly in cases when renal tubules are injured.

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