Original Article

Assessment of the role of urinary complement activation in diabetic nephropathy

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Abstract

Background: Increased urinary complement activation products (CAPs) have been reported in proteinuric kidney diseases including DN. The changes of urinary CAPs, including C3a, C5a and C5b-9, were reported in patients at different stages of DN. Aim of work: To study the changes of urinary complement activation products C5a and their pathological significance in the development of DN and correlate urinary and tissue expression of C5a with histopathological stages of diabetic nephropathy. Subjects and methods: This cross-sectional study was conducted, consisted of 62 cases with Diabetic nephropathy (DN) attend at the Internal medicine department, Zagazig University Hospital in 6 months. All patients were subjected to full medical history. Periodic thorough clinical examination was done. Routine laboratory investigations were performed involving measurement of Human Complement fragment 5a (C5a) by using the renal biopsy specimen and the urine samples from patients with different stages of DN. Results: We found a statistically significant difference among different DN stages of the studied group regarding Ca5 marker, which markedly increased among cases of class III and IV. At cut-off value 81.8, the diagnostic performance of urinary Ca5 as a diagnostic predictor for severe diabetic nephropathy stages presented with sensitivity 80.6% and specificity 74.1% with 0.795area under ROC curve, 77.4% test accuracy. Conclusion and recommendations: In summary, by including patients at different stages of DN, the present study has provided a full view of the change of urinary CAPs in DN patients. As there is a close correlation of urinary CAPs with the severity of DN, the urinary CAPs might be used as a marker for clinicians to evaluate the severity and progression of the disease, especially for the injury of renal tubules.

Keywords: Complement, Diabetes, Nephropathy.

INTRODUCTION

Diabetic nephropathy (DN) affects about 40% of diabetes mellitus (DM) patients and is the leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) all over the world, especially in high- and middle-income countries. Most DN has been present for years before it is diagnosed. DN, also known as “diabetic kidney disease (DKD)”, is one of the most important diabetic microvascular complications, affecting 30–45% patients with either type 1 DM (T1DM) or type 2 DM (T2DM), with a peak incidence in the 10–20 years duration of DM (1).

DN, pathologically, is often characterized by glomerular basement membrane (GBM) thickening, glomerular mesangial matrix expansion, and formation of glomerular nodular sclerosis in its advanced stages, and clinically, is usually defined by proteinuria occurrence or declined renal function, e.g., reduced glomerular filtration rate (GFR). DN patients exhibiting modest, or no albuminuria may progress to ESRD. DN is also a single strong predictor of mortality in patients with DM. Even worse, the absolute number of DN patients continues to increase and the incidence of ESRD from DN keeps expanding, consistent with the global DM pandemic (2).

The pathogenesis of DN is multifactorial. Recent studies suggested that complement might be involved in the progression of DN. However, the exact significance and underlying mechanism are far from clear. Increased urinary complement activation products (CAPs) have been reported in proteinuric kidney diseases including DN. The changes of urinary CAPs, including C3a, C5a and C5b-9, in patients at different stages of DN were reported (3).

Among the complement systems, complement C5a is the most effective mediator in inflammation by inducing secretion of interleukin 6 (IL-6), IL-8, tumor necrosis factor alpha (TNF-α) and CCL. C5a signaling is activated by interacting with two different receptors, C5aR and C5L2. C5aR is expressed on cell membrane that mediates most of the functional effects of C5a, whereas C5L2 is predominantly intracellular and may be a negative modulator of C5aR-mediated signal transduction (4).

AIM AND OBJECTIVES

To study the changes of urinary complement activation products C5a and their pathological significance in the development of DN and correlate urinary and tissue expression of) C5a(with histopathological stages of diabetic nephropathy.

SUBJECTS AND METHODS

Technical design: This cross-sectional study was conducted, consisted of 62 cases with Diabetic nephropathy (DN) attend at the Internal medicine department, Zagazig University Hospital in 6 months. Inclusion criteria included patients of both sexes, aged above 18 years with proven type 2 diabetes mellitus patients based on clinical and laboratory evidence while exclusion criteria involved patients with other types of diabetes mellitus, such as maturity onset diabetes of the young, gestational diabetes mellitus and type 1 diabetes mellitus, any infective or inflammatory diseases or patients with renal biopsy inconsistent with DN or with concomitant other renal diseases.

Methods: All patients were subjected to full medical history. Periodic thorough clinical examination was done. Routine laboratory investigations were performed involving measurement of Human Complement fragment 5a (C5a) by using the renal biopsy specimen and the urine samples from patients with different stages of DN.

STATISTICAL ANALYSIS

Once data was collected, a code sheet was developed. Organization, tabulation, presentation, and analysis of data were performed using SPSS V21 (Statistical Package for Social Studies version 21). Quantitative data was described using number and percent. Quantitative data was described using number and percent. Quantitative data was presented as mean, range and standard deviation (SD). Parametric tests were applied for normally distributed data.

RESULTS

Table (1) shows basic characteristics of the studied group. This table shows that 69.4% of the studied group were females with mean age of 42.6 years ranged from 18 years up to 64 years, duration of diabetes ranged from suddenly discovered cases at time of study up to 30 years, 59.5% of them had DN of class II and 19.4% was of class IV. Laboratory data of the studied group is demonstrated in Table (2). Table (3) demonstrates the relation between diabetic nephropathy and lipid profile of the studied group. We found a high statistically significant difference among different DN stages of the studied group regarding lipid profile tests, that markedly changed among cases of class IV. The relation between diabetic nephropathy and CBC data of the studied group was cleared in Table (4). This table shows a high statistically significant difference among different DN stages of the studied group regarding hemoglobin level, that markedly decreased among cases of class IV. Table (5) clears the relation between diabetic nephropathy and kidney function tests of the studied group. This table shows a high statistically significant difference among different DN stages of the studied group regarding kidney function tests, which markedly changed among cases of class IV. Table (6) reveals the relation between diabetic nephropathy and Ca5 marker level of the studied group. This table shows a statistically significant difference among different DN stages of the studied group regarding Ca5 marker, which markedly increased among cases of class III and IV. Furthermore, Table (7) and Figure (1) show a statistically significant positive correlation between urinary Ca5 level with disease duration, creatinine, and albumin\ creat. Ratio, while there was a statistically significant negative correlation with HDL and GFR. Table (8) and Figure (2) demonstrate the receiver operating curve (ROC) analysis for the urinary Ca5 levels as a diagnostic predictor of class III and IV of diabetic nephropathy. At cut-off value 81.8, the diagnostic performance of urinary Ca5 as a diagnostic predictor for severe diabetic nephropathy stages presented with sensitivity 80.6% and specificity 74.1% with 0.795area under ROC curve, 77.4% test accuracy.

Tables

Table (1): Basic characteristics of the studied group.

	Studied group N=62			
	Minimum	Maximum	Mean	S.D
Age\ years	18	64	42.6	12.6
Disease duration\ months	0.00	360	59.2	64.7
Sex Male Female	N (%) 19 (30.6%) 43 (69.4%)			
Diabetic nephropathy class I II IV	N (%) 13 (21%) 37 (59.5%) 12 (19.4%)			

Table (2): Laboratory data of the studied group.

	Studied group N=62			
	Minimum	Maximum	Mean	S.D
Cholesterol	98	290	184.1	57.1
TG	70	324	134.1	56.8
HDL	18	100	50	18.6
LDL	44	220	130.2	45.7
Hemoglobin	5.3	15	10.4	1.7
Creatinine	0.2	5.5	1.66	1.19
Urea	10.1	300	52.2	40.8
GFR	12.8	318.8	77.4	57.9
FBG	90	409	171.2	56.96
HbA1c	5.4	22.6	8.11	2.67
Alb\creat. Ratio	18.8	330	166.1	111.8
C5a	0.00	137.4	82.1	33.9

Table (3): Relation between diabetic nephropathy and lipid profile of the studied group.

		N	Mean	S.D	Test	P value
Cholesterol	I	7	137.9a	36.6	3.61	0.02 S
	II	22	182b	54.8		
	III	13	182.2	56.9		
	IV	10	223.4	53.2		
TG	I	7	121.7a	91.5	2.96	0.04 S
	II	22	130b	56.9		
	III	13	114.2c	31.3		
	IV	10	177.8	31.5		
HDL	I	7	54.1a	11.3	4.57	0.007 S
	II	21	51.6b	16.8		
	III	13	58.1c	21.2		
	IV	10	33.1	13.04		
LDL	I	7	91.9ad	13.1	7.68	<0.001 HS
	II	21	120.6b	39.2		
	III	13	129.9c	43.2		
	IV	10	177.5	41.7		

a: Significant difference between group I & IV S: P-value<0.05 is significant
b: Significant difference between group II & IV HS: P-value<0.001 is high significant
c: Significant difference between group III& IV
d: Significant difference between group I & III

Table (4): Relation between diabetic nephropathy and CBC data of the studied group.

		N	Mean	S.D	F test	P
Hemoglobin	I	13	10.9a	1.47	8.84	<0.001 HS
	II	23	10.9b	1.33		
	III	14	10.9c	1.61		
	IV	12	8.5	1.42		
FBG	I	13	151.1	46.95	2.16	0.102 NS
	II	22	158.6	39.1		
	III	14	190.4	39.6		
	IV	12	193.5	32.7		
HbA1c	I	13	7.42	1.61	2.77	0.05 NS
	II	23	7.35	1.17		
	III	14	9.65	2.81		
	IV	12	8.53	1.34		

a: Significant difference between group I & IV S: P-value<0.05 is significant

b: Significant difference between group II & IV
c: Significant difference between group III& IV

NS: P-value>0.05 is not significant

Table (5): Relation between diabetic nephropathy and kidney function tests of the studied group.

		N	Mean	S.D	F test\ KW*	
Creatinine	I	13	0.65ad	0.23	84.2	<0.001 HS
	II	23	0.995be	0.23		
	III	14	2.02c	0.44		
	IV	12	3.6	1.04		
Urea	I	13	34.5ad	12.8	29.9*	<0.001 HS
	II	23	34.7be	12.8		
	III	14	58.4c	20.4		
	IV	12	97.8	70.1		
GFR	I	12	149.4ad	74.6	47.4*	<0.001 HS
	II	23	89.7be	29.6		
	III	14	41.1	10.34		
	IV	12	23.8	6.15		
Alb\ creat. Ratio	I	12	82.6ad	75.8	41.6*	<0.001 HS
	II	23	84be	42.9		
	III	14	251.6c	67.6		
	IV	12	307	25.6		

a: Significant difference between group I & IV
b: Significant difference between group II & IV
c: Significant difference between group III& IV
d: Significant difference between group I & III
e: Significant difference between group II & III

HS: P-value<0.001 is high significant

Table (6): Relation between diabetic nephropathy and Ca5 marker level of the studied group.

		N	Mean	S.D	KW test	P value
C5a	I	13	65.2ad	33.8	17.3	0.004 S
	II a	16	59.1be	38.9		
	II b	7	82.8	38.9		
	III	14	98.1	20.1		
	IV	12	109.4	37.6		

a: Significant difference between group I & IV
b: Significant difference between group II a & IV
d: Significant difference between group I & III
e: Significant difference between group II a & III

S: P-value<0.05 is significant

Table (7): Correlation between Ca5 levels and basic parameters of the studied cases.

	Cases (N=62)	
	r*	P-value
Age	-0.05	0.814 (NS)
Disease duration	0.257	0.04 (S)
Cholesterol	0.248	0.07 (NS)
TG	0.147	0.45 (NS)
HDL	-0.315	0.02 (S)
LDL	0.247	0.08 (NS)
Hemoglobin	-0.193	0.13 (NS)
FBG	0.201	0.125 (NS)
HbA1c	0.143	0.31 (NS)
Creatinine	0.379	0.002 (S)
GFR	-0.361	0.006 (S)
Alb.\ creat. ratio	0.405	0.002 (S)
Urea	0.126	0.33 (NS)

r* Pearson correlation

Figure (1): Negative correlation between urinary Ca5 level and GFR of the studied cases.

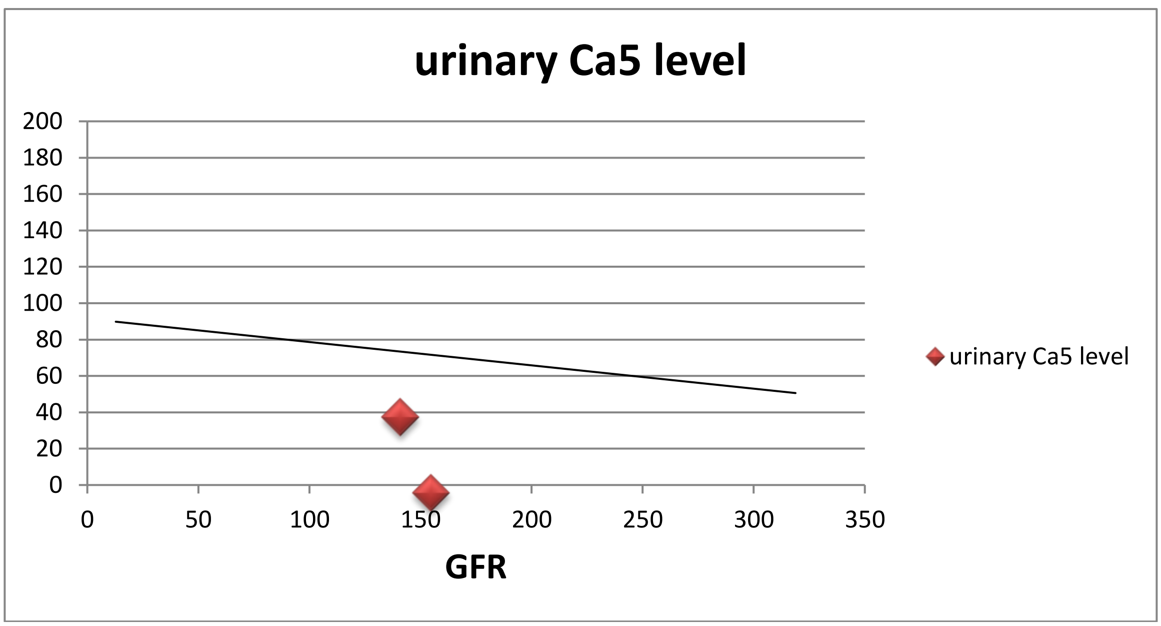


Table (8): Receiver operating curve (ROC) analysis for the urinary Ca5 levels as a diagnostic predictor of class III and IV of diabetic nephropathy.

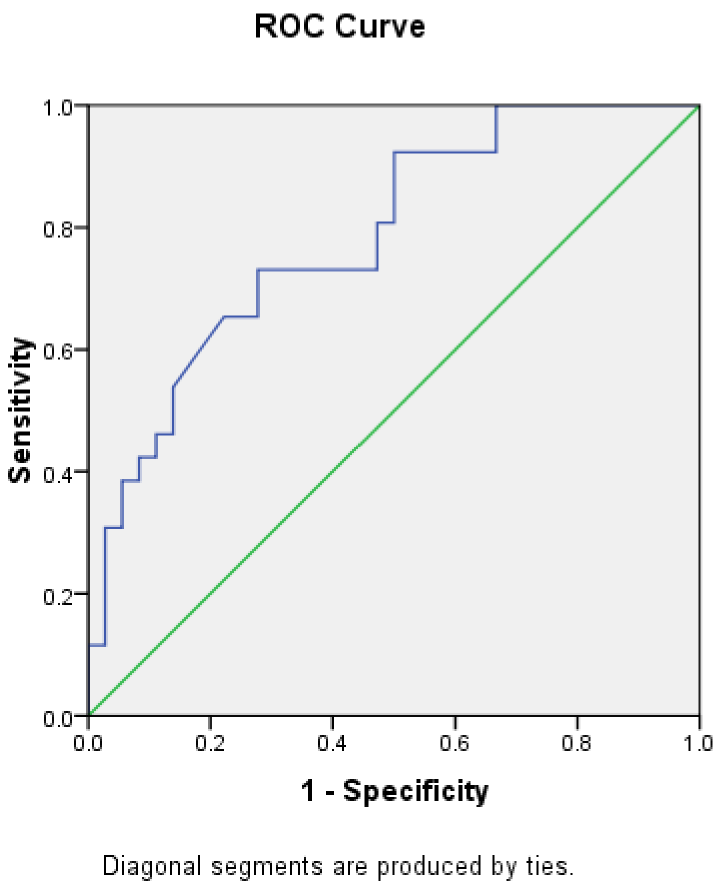
Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	P-Value
≥ 81.8	80.6%	74.1%	70%	84.4%	77.4%	0.795	<0.001 (HS)

PPV: Positive Predictive Value

NPV: Negative Predictive Value

AUC: Area under curve

Figure (2): ROC curve analysis for urinary Ca5 levels.



DISCUSSION

Increased urinary complement activation products (CAPs) have been reported in proteinuric kidney diseases including DN. However, the exact significance of urinary CAPs in DN is still unclear. Of note, previous studies examined urinary CAPs mainly in DN patients at an advanced stage of the disease. No observation of urinary CAPs in the earlier stages of the disease has been made. As DN is a highly heterogeneous disease, the extent and significance of urinary complement activation might differ among patients in different conditions; for example, patients of different pathological types and patients at different stages of the disease (5).

Among the complement systems, complement C5a is the most effective mediator in inflammation by inducing secretion of interleukin 6 (IL-6), IL-8, tumor necrosis factor alpha (TNF-α) and CCL. C5a signaling is activated by interacting with two different receptors, C5aR and C5L2. C5aR is expressed on cell membrane that mediates most of the functional effects of C5a, whereas C5L2 is predominantly intracellular and may be a negative modulator of C5aR-mediated signal transduction (6).

The aim of this study was to study the changes of urinary complement activation products C5a and their pathological significance in the development of Diabetic nephropathy (DN). In the present study, 69.4% of the studied group were females with mean age of 42.6 years ranged from 18 years up to 64 years, duration of diabetes ranged from suddenly discovered cases at time of study up to 30 years, 59.5% of them had DN of class II and 19.4% was of class IV. The mean of Cholesterol level was 184.1 ± 57.1, the mean of TG was 134.1 ± 56.8, the mean of HDL was 50 ± 18.6, the mean of LDL was 130.2 ± 45.7, the mean of Hemoglobin was 10.4 ± 1.7, the mean of Creatinine was 1.66 ± 1.19, the mean of Urea was 52.2 ± 40.8, the mean of GFR was 77.4 ± 57.9, the mean of FBG was 171.2 ± 56.96, the mean of HbA1c was 8.11± 2.67, the mean of Alb\creat. Ratio was 166.1 ± 111.8 and the mean of C5a was 82.1 ± 33.9.

In a published study, Li et al. (2019) reported urinary CAP levels in 50 biopsy-proven DN patients. However, with the mean eGFR being 44.28 mL/min/1.73 m2 (44.28 ± 26.79 mL/min/1.73 m2) and mean proteinuria being 6.33 g/24 h (6.33 ± 4.35 g/24 h)12, most of the patients included in the study by Li et al. still belonged to the advanced stage of DN (7). In addition, Yiu et al. (2018) found that mean age of patients 58 years; mean haemoglobin A1c 6.6%; mean serum creatinine 364 mg/dl; mean proteinuria 5.80 g/24 h (8).

In the current study, there was a high statistically significant difference among different DN stages of the studied group regarding lipid profile tests, that markedly changed among cases of class IV. In agreement to our study, Palazhy and Viswanathan, (2017) found that dyslipidemia was present among 56.52% of control subjects and 75.28% of nephropathy subjects (P=0.012) (9).

In the present study, there was a high statistically significant difference among different DN stages of the studied group regarding hemoglobin level, that markedly decreased among cases of class IV. Sonmez et al. (2010) reported that endothelial function is inversely associated with Hb levels in diabetic patients with stage 1 to 2 CKD, and proteinuria is an effect modifier of this association (10).

In our study, there was a high statistically significant difference among different DN stages of the studied group regarding kidney function tests, which markedly changed among cases of class IV. In agreement to our study, Zheng et al. (2019) found that significant changes in indices reflecting renal function injury, such as serum creatinine, serum urea nitrogen and eGFR level, were observed in the RIG (11).

In the present study, there was a statistically significant difference among different DN stages of the studied group regarding Ca5 marker, which markedly increased among cases of class III and IV. In agreement to our study, Zheng et al. (2019) reported that the urinary CAPs were found to increase significantly as the proteinuria stage increased further in the renal insufficiency stage. There were strong positive correlations between urinary levels of C3a, C5a and C5b-9 in DN patients($r = 0.861$, $P < 0.001$ between C3a and C5a; $r = 0.857$, $P < 0.001$ between C3a and C5b-9; and $r = 0.816$, $P < 0.001$ between C5a and C5b-9). No significant change in the levels of urinary CAPs was found in the diabetes mellitus control group and MG compared with the normal control group (11).

In the current study, there was a statistically significant positive correlation between urinary Ca5 level with disease duration, creatinine, and albumin\ creat. Ratio, while there was a statistically significant negative correlation with HDL and GFR. There was no statistically significant correlation between urinary Ca5 level with age, Cholesterol level, TG, LDL, Hemoglobin, FBG, HbA1C and urea.

In agreement to our study, Li et al. (2019) supported our study by showing that urinary levels of C3a and C5a were significantly correlated with serum creatinine, urinary protein and estimated glomerular filtration rate (7). While Wendt et al. (2021) found that the majority of peptides derived from CFB demonstrated positive association with eGFR. Highest levels of significant C3 excretion relative to controls were seen in minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), membranous glomerulonephritis (MGN), lupus nephritis (LN), diabetic kidney disease (DKD), IgAN, membranoproliferative glomerulonephritis (MPGN), and C3-glomerulonephritis (12).

The present study shows that at cut-off value 81.8, the diagnostic performance of urinary Ca5 as a diagnostic predictor for severe diabetic nephropathy stages presented with sensitivity 80.6% and specificity 74.1% with 0.795 area under ROC curve, 77.4% test accuracy. Ogrodowski et al. (1991) first reported increased urinary C5b-9 in patients with renal diseases. Six DN patients were included in the study. patients with glomerulopathies tended to have elevated UMAC levels: 18 of 38 patients had levels that ranged from 200 to 20,000 ng/mg UCr. UMAC levels did not distinguish patients with MN from those with other causes of glomerulopathy. Indeed, the highest UMAC levels were seen in patients with nephrotic syndrome due to focal glomerulosclerosis or diabetic glomerulosclerosis. In such patients the ratio: urine protein (UPr)/UCr was correlated with the logarithm of the ratio UMAC/UCr ($r = 0.85$, $P < 0.001$). Biopsy specimens of patients with diabetic glomerulosclerosis or focal glomerulosclerosis and elevated UMAC levels showed heavy deposits of SC5b-9 in tubular epithelium but little or no SC5b-9 in glomeruli, suggesting that in these patients UMAC resulted from SC5b-9 formed on tubular epithelium and this supported our study (13).

In agreement to our study, Yiu et al. (2018) revealed that the renal C5a level increased with the severity of tubular injury and interstitial fibrosis in patients with DN, suggesting a strong and positive correlation between C5a signaling and the progression of DN (8). While in contrast to our study, in 2000, Morita et al. reported increased urinary iC3b, Bb and MAC in proteinuric patients, and 17 DN patients were included. Because of the small number of DN patients included in these studies, association analysis of urinary CAPs with the progression of the disease is impossible (14).

CONCLUSION AND RECOMMENDATIONS

In summary, by including patients at different stages of DN, the present study has provided a full view of the change of urinary CAPs in DN patients. We analyzed the association of urinary complement activation with different stages of diabetic nephropathy especially for the patients at a later stage of the disease. In addition, as there is a close correlation of urinary CAPs with the severity of DN, the urinary CAPs might be used as a marker for clinicians to evaluate the severity and progression of the disease, especially for the injury of renal tubules.

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