IJMA

International Journal of Medical Arts

VOLUME 6, ISSUE 6, JUNE 2024

P- ISSN: 2636-4174
E- ISSN: 2682-3780

Available online at Journal Website
https://ijma.journals.ekb.eg/
Main Subject [Internal Medicine]

Original Article

Complement 3a and Complement 5a as Biochemical Markers in Diabetic Nephropathy

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Article information

Received: 12-12-2023

Accepted: 16-01-2024

DOI: 10.21608/IJMA.2024.255133.1887.

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Citation: Ahmed SF, Alsayed AMR, Aref MIAI, Alkot AMF. Complement 3a and Complement 5a as Biochemical Markers in Diabetic Nephropathy. IJMA 2024 June; 6 [6]: 4597-4606. doi: 10.21608/ijma.2024.255133.1887.

ABSTRACT

Background: Diabetes mellitus [DM] is growing into a global epidemic and diabetic nephropathy [DN] is one of its most feared complications. Inflammation is an important mechanism in the pathogenesis of DN and many studies have pointed to anaphylatoxins, complement 3a [C3a] and complement 5a [C5a], being involved in the pathogenesis of DN.

The aim of the work: The aim of this work was validation of C3a and C5a as biomarkers in DN.

Patients and Methods: The study included 96 participants. They were divided into three groups: Group I [32 healthy subjects], Group II [32 diabetic patients without evidence of DN] and Group III [32 diabetic patients with evidence of DN]. All were evaluated by history taking, clinical examination and laboratory tests [blood sugar, HbA1c, urea & creatinine, urine albumin to creatinine ratio, lipid profile and complete blood count]. Estimated glomerular filtration rate was calculated using Modification of Diet in Renal Disease patients' formula. C3a and C5a levels were measured by ELISA.

Results: At C3a was significantly higher in diabetic groups than the controls. However, the difference diabetic and DN groups was non-significant. C5a was increased in both disease groups, but significantly higher in DN. At a cutoff value > 4.27 µg/ml, C3a can be a significant predictor of DM [sensitivity 75%, specificity of 96%, PPV 78.8% and NPV 95.6%]. However, C5a couldn't be a significant predictor of DM. At a cutoff value > 52.129 ng/mL, C5a can be a significant predictor of DN [sensitivity 80.65 %, specificity 73.33 %, PPV 56.4 % and NPV 89.8%].

Conclusion: The anaphylatoxins C3a and C5a, which are important effector molecules of the complement cascades, produce their effects through inducing inflammation participating in the pathogenesis of diabetic kidney disease [DKD].

Keywords: Diabetic nephropathy; Complement; Anaphylatoxins; C3a; C5a.

INTRODUCTION

Diabetes is a dangerous chronic condition that negatively impacts people's lives, families, and society globally. Alarming epidemic levels are being reached. Egypt is among the top 10 countries with highest prevalence of diabetes mellitus. Number of cases in 2019 was estimated to be 8.9 million and it is suspected to rise to 11.4 million in 2030 and 15 million in 2045. It is brought on by either decreased insulin secretion or decreased insulin effectiveness, or most frequently both [1, 2].

Diabetes's long-term consequences can be generally categorized as microvascular and macro-vascular. Nephropathy, retinopathy, and neuropathy are the main microvascular sequelae, while peripheral artery disease, cardiovascular disease, and stroke are the main macrovascular complications [3].

A severe and intensely dreaded consequence of DM is diabetic nephropathy [DN]. The disease is chronic and progresses over time, reaching its peak incidence 10 to 20 years from the onset of diabetes. This microvascular problem affects between 30 and 45 percent of diabetics. Diabetes is currently the world's main leading cause of end-stage renal disease and main reason why people need renal replacement therapy. Dialysis patients are at higher risk of developing cardiovascular disease, where diabetes being the primary diagnosis in 45% of them [4].

Complement system, a very complex network of proteins presents in plasma and on cell surfaces, can be activated via three pathways. The classic pathway is triggered by binding of antigen–antibody complexes to C1q. This will then activate C1r and C1s, which cleave C4 and C2 forming the C3 convertase "C4b2a". The Lectin pathway which begins with signal recognition by Mannose binding lectin [MBL], ficolins, or collectins [can recognize particular pathogen-associated molecular patterns] activating their serein protease activity which in turn cleaves C2 and C4 with subsequent formation of C3 convertase "C4b2a" [5].

In Alternative pathway, the situation is rather different. The internal thioester bond of C3 is highly reactive and undergoes spontaneous hydrolysis resulting in a molecule known as C3 [H_2O] which resembles C3b. This can then bind to factor B. Factor B is then cleaved by factor D. The larger fragment of factor B remains bound to C3b to form the alternative pathway C3 convertase C3bBb that can generate more C3b. The presence of complement regulators in healthy cells ensures that spontaneous hydrolysis of C3 is kept in check. If this C3 [H_2O] binds to a nearby surface that is incapable of inactivating it [such as bacteria, yeast cells or damaged host tissues], this then leads to amplification of the alternative pathway. C3 [H_2O] is able to create new C3 convertase in the presence of Factors B and D, thus acting as an 'amplification loop' for other pathways, as well as the alternative pathway [6].

All three pathways ultimately result in the cleavage of C3 and C5, leading to the formation of membrane attack complex [MAC], and liberation of C3a and C5a which are potent mediators of inflammation [7].

Chronic metabolic and hemodynamic stresses cause cellular damage stimulating innate system of immunity, particularly complement system, in diabetic kidney. Complement homeo-stasis is a delicately balanced state that can elicit a remarkable inflammatory response when it is disrupted or overexcited [8].

In a large cohort of diabetic cases with and without DKD, it was found that the complement activation marker C4d was correlated with severity of microvascular, interstitial lesions, and lower eGFR suggesting that complement activation might play a role in the development of DN [9]. One study evaluated urinary levels of soluble MAC in patients with overt DN, found that it was strongly intercorrelated with biomarkers of inflammation, fibrosis, proteinuria, and the rate of renal function decline [10]. Several studies have pointed out that the anaphylatoxins [C3a & C5a], formed during complement cascade activation, are involved in the pathogenesis of DN and might be a target for future therapies [11].

Well-defined cytokine-like polypeptides referred to as anaphylatoxins [C3a and C5a] are produced when the complement system is activated [7]. C3a and C5a are strong mediators of inflammation and believed to participate in the pathogenesis of DN [12].

Potential complement-targeted therapeutics for DKD in red boxes include C1-INH [the recombinant form of human C1 esterase inhibitor which is a complement regulating protein that halts the classical pathway], OSM721 [a monoclonal antibody targeting MBL-associated serine protease], C3a receptor inhibitors, C5a receptor inhibitors, and Eculizumab [monoclonal antibody that binds with high-affinity C5 preventing its cleavage and the generation of

C5a with subsequent prevention of MAC formation] [7]. Dipeptidyl peptidase-4 inhibitors block also complement-activating serine proteases and inhibit in particular the lectin pathway [13].

The aim of this work was validation of complement 3a [C3a] and complement 5a [C5a] as biomarkers in diabetic nephropathy.

PATIENTS AND METHODS

Study design: This cross-sectional study was conducted on 96 participants [64 patients presented with type 2 DM, randomly enrolled, from the inpatient units and outpatient clinics of internal medicine department, Al-Azhar University hospitals and 32 healthy control subjects] from May 2021 to October 2021. The soft war Epi Info was used initially to calculate the appropriate sample size for the study. Prior to their inclusion in the study, every subject provided informed consent. Al-Azhar Faculty of Medicine's ethical committee gave approval to the protocol. They were divided into three groups:

Group I: included 32 normal healthy subjects.

Group II: included 32 diabetic patients without laboratory evidence of diabetic nephropathy [normal urine albumin creatinine ratio [UACR < 30 mg/g], normal serum creatinine level [0.5-1.1 mg/dL in females & 0.6-1.2 mg/dL in males] and normal estimated glomerular filtration rate [eGFR ≥ 90 ml/minute /1.73m^2] [14].

Group III: included 32 diabetic patients with laboratory evidence of diabetic nephropathy.

Inclusion criteria: Patients who fulfil the criteria of American Diabetic Association [ADA] for diagnosis of diabetes mellitus, patients with or without diabetic nephropathy and at least their age ≥ 35 years were included.

Exclusion criteria: Subjects with any of the following; age < 35 years, presence of other causes of renal impairment, clinical evidence of any associated autoimmune disease, active urinary tract infection, obstructive uropathy, patients on other non-diabetic medications that may disturb blood sugar levels or pregnant females were excluded.

Clinical evaluation: All subjects were evaluated as following: Complete history taking with special stress on date of onset and duration of DM, presence of any related diabetic complications and current medications. Physical examination with special stress on presence of hypertension, clinical evidence of nephropathy, CNS and cardio-vascular complications.

Routine laboratory tests that usually done for diabetic patients that include; fasting and two hours post prandial blood sugar [15], HbA1c [16], Urea [17], creatinine [18], lipid profile including cholesterol [19], triglycerides [TGs] [20], low density lipoprotein [LDL] [21], high density lipoprotein [HDL] [22], complete blood count [CBC] and urine albumin to creatinine ratio [UACR] [23].

GFR was calculated by using Modification of Diet in Renal Disease patients [MDRD] formula. According to this formula, estimated GFR [eGFR] [mL/min/1.73 m^2] = 175 × [serum creatinine] -1.154 × [Age] -0.203 × [0.742 if female] × [1.212 if black] [24].

Samples collection: Using a sterile vein puncture, seven milliliters of venous blood were extracted during the fasting state; two milliliters were then placed into a sterile tube with EDTA for CBC analysis. After transferring the remaining 5 milliliters into a dry, sterile centrifuge tube, the entire blood was spun for 10 minutes at 4000 rpm after being left to clot for 30 minutes at room temperature. After separating the clear supernatant serum, 300 µl was frozen at -20°C until serum C3a and C5a were analyzed. Conversely, additional biochemical studies were conducted using the leftover serum. An aseptic midstream random urine sample collected to measure the albumin/creatinine ratio.

Measurement of serum C3a and C5a concentrations: Two sets of ELISA kits, one for human C3a and the other for human C5a from Bioassay technology laboratory, Korain Biotech Co., Ltd. 419 Harborne Road, Edgbaston, Birmingham, England were used in the analysis. This assay employs enzyme-linked immune-sorbent assay [ELISA] based on double antibody sandwich technology. The measurement was done according to the instructions included with the kits.

Statistical analysis of the results: The collected data were tabulated and analyzed using SPSS version 23. Quantitative data were expressed as means ± standard deviations [SD]. On the other hand, qualitative data were expressed as percentages from the total number. Quantitative data were then tested for normality using "Shapiro – Wilk" test, assuming normality at P > 0.05. "One-way ANOVA" and the post hoc multiple comparison "Tukey" tests were used to compare means of the studied groups if data were normally distributed.

Whereas the non-parametric tests "Kruskal Wallis" and "Mann Whitney U" were used to compare means of the studied groups if the data weren't normally distributed. On the other hand, "Chi square [X^2]" test was used to analyze qualitative data to detect if there is any significant difference between studied groups. The accepted level of significance when comparing different groups was stated at or less than 0.05 [$P \leq 0.05$ was considered significant]. The software was used to build ROC [Receiver Operator Characteristic] curves which were used to determine cutoff value of serum C3a and C5a with optimum sensitivity and specificity in prediction of occurrence of DM and DN.

RESULTS

In the current work, patient age was significantly increased in the DN group than the diabetic and control groups [63.2±9.6 vs 56.1±8.3 and 42.2 ± 7.5 years, respectively]. In addition, group 3 had significantly longer duration of DM and hypertension than the group 2. However, none in control group had DM or hypertension. Males were significantly higher in DN than diabetic and control groups. In addition, DM, hypertension, neuropathy, retinopathy, CNS and CVS complications were significantly prevalent in DN than DM and control groups [Table 1].

Table [2] presented the results of laboratory workup among study groups. This revealed that, blood sugar [fasting and postprandial], and glycated hemoglobin were significantly higher in DN than DM and control groups and in DM than control group. However, urea and creatinine were significantly increased, while estimated GFR was significantly reduced in DN than DM groups and control groups. However, the difference between DM and control groups was statistically non-significant. In addition, albumin/creatinine ratio, hemoglobin, red blood cells, triglycerides, HDL, C3a and C5a showed variance between study groups. C3a was significantly increased in diabetic groups [DM and DN] than the control group. However, the difference between both groups did not reach statistical significance. On the other side, C5a was significantly increased in DN than DM and control groups and in DN than DM groups.

ROC curve analysis of C3a in both control and diabetic groups showed that at a cutoff value > 4.27 µg/ml, C3a can be a significant predictor of T2DM in comparison with normal subjects having sensitivity of 75% and specificity of 96%. Whereas +ve predictive value at this cutoff limit is 78.8% and –ve predictive value is 95.6%. Whereas ROC curve analysis of C5a in both control and diabetic groups showed that C5a couldn't be a significant predictor of T2DM when compared with normal subjects [P= 0.282] [Table 3, Figure 1].

ROC curve analysis of C3a in both diabetic and DN groups showed that C3a couldn't be a significant predictor of diabetic nephropathy when compared with diabetic subjects [P=508]. Whereas ROC curve analysis of C5a in both diabetic and DN groups showed that at a cutoff value > 52.129 ng/mL, C5a can be a significant predictor of diabetic nephropathy in comparison with normal subjects having sensitivity of 80.65 % and specificity of 73.33 %. Whereas +ve predictive value at this cutoff limit is 56.4 % and –ve predictive value is 89.8%. From ROC curves and their analysis, it could be noticed that the performance of C3a is better when comparing between control and diabetic subjects whereas C5a performance is better when comparing diabetic and diabetic nephropathy subjects [Table 4, Figure 2].

Table [1]: Demographic and clinical data results expressed as means ± standard deviation "SD" [for quantitative data] and as percentage [for qualitative data] in the three studied groups

	Group1 [control]	Group2 [Diabetic]	Group3 [DN]
Quantitative data			
Age [years]	42.2 ± 7.5	56.1 ± 8.3	63.2 ± 9.6 §
DM onset [years]	0	7 ± 2.5 *	14.6 ± 3.9 * §
HTN onset [years]	0	2.03 ± 2.6 *	6.7 ± 5 * §
Qualitative data			
Male ratio	52%	87.5% *	96.8% *
Female ratio	48%	12.5% *	3.2% *
Hypertensive	0%	56.25% *	84.4 % * §
Neuropathy	0%	56.25 *	96.9% * §
Retinopathy	0%	3.3%	51.6% * §
CNS complications	0%	9.4%	21.9% *
CVS complications	0%	18.75% *	62.5% * §

* Significant difference [$P \leq 0.05$] when compared with group 1; § Significant difference [$P \leq 0.05$] when compared with group 2

Table [2]: Laboratory data results expressed as means ± SD in the three studied groups

	Group1 [control]	Group2 [Diabetic]	Group3 [DN]
FBS [mg/dl]	76.4 ± 11.1	215.8 ± 54.8 *	166.1 ± 53.5 * §
PP BS [mg/dl]	106.5 ± 11.6	300.4 ± 73.5 *	242.1 ± 66.5 * §
HbA1c [%]	4.9 ± 3.1	9.7 ± 2.1 *	8.3 ± 1.6 * §
Urea [mg/dl]	19.8 ± 4.4	30 ± 6.8	90.7 ± 41.7 * §
Creatinine [mg/dl]	0.77 ± 0.1	0.8 ± 0.1	3.2 ± 1.8 * §
eGFR [ml/min/1.73m^2]	104.01 ± 7.5	102.7 ± 21.3	28.6 ± 15.3 * §
Alb/Creat [mg/g]	15.5 ± 5	14.9 ± 5.7	791.1 ± 638.3 * §
Hb [g/dl]	13.4 ± 1.5	13.6 ± 1.8	11.2 ± 1.5 * §
RBCs [million/mm^3]	5.04 ± 0.4	5.04 ± 0.7	4.2 ± 0.6 * §
WBCs [1000/mm^3]	6.5 ± 1.3	7.03 ± 2	7.2 ± 2.04
Platelets [1000/mm^3]	224.2 ± 47.2	259.4 ± 89.7	239.3 ± 104.5
Cholesterol [mg/dl]	166.4 ± 16.3	179.9 ± 31.1	178.3 ± 50.3
TGLs [mg/dl]	108.4 ± 24.9	163.1 ± 73 *	228.5 ± 117 * §
LDL [mg/dl]	103.02 ± 20	109.3 ± 23.6	93.4 ± 40.2
HDL [mg/dl]	41.7 ± 9.9	38.1 ± 9.8 *	39.2 ± 18.7 *
C3a [µg/ml]	3.25 ± 0.81	7.6 ± 6.6 *	6.6 ± 5.1 *
C5a [ng/ml]	44.5 ± 8.1	48.5 ± 13	63.1 ± 20.9 * §

* Significant difference [P ≤ 0.05] when compared with group 1; § Significant difference [P ≤ 0.05] when compared with group 2

Table [3]: C3a and C5a performance in differentiating control from diabetic patients

	C3a	C5a
Area under the curve [AUC]	0.824	0.584
Significance [P value]	< 0.001*	= 0.282
Optimum cutoff level	> 4.27 µg/mL	> 47.766 ng/mL
Sensitivity	75%	46.67%
Specificity	96%	76%
+ve predictive value	78.8%	25.5%
-ve predictive value	95.6%	89%

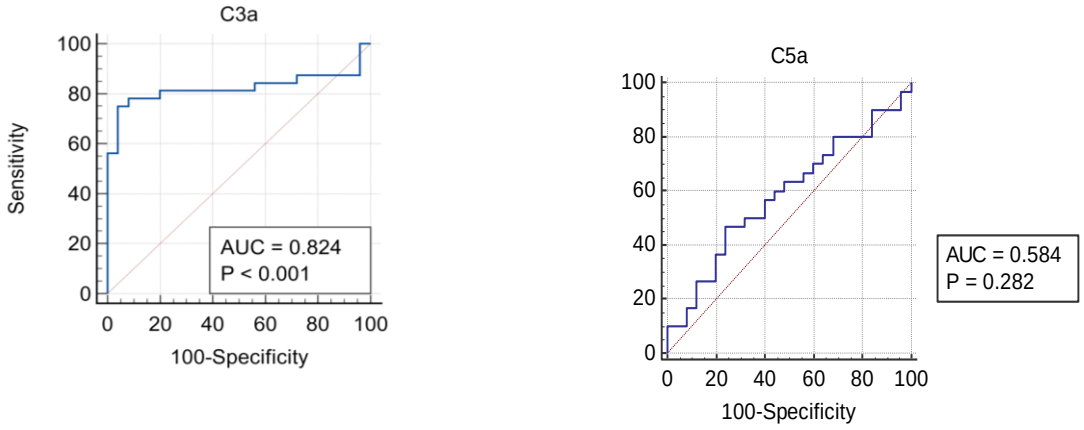

Figure [1]: C3a [left curve] and C5a [right curve] performance in differentiating control from diabetic patients

Table [4]: C3a and C5a performance in differentiating diabetic from DN patients

	C3a	C5a
Area under the curve [AUC]	0.538	0.770
Significance [P value]	0.608	**< 0.001***
Optimum cutoff level	≤ 5.3772 ng/mL	> 52.129 ng/mL
Sensitivity	68.75%	80.65%
Specificity	46.88%	73.33%
+ve predictive value	18.6%	56.4%
-ve predictive value	89.5%	89.8%

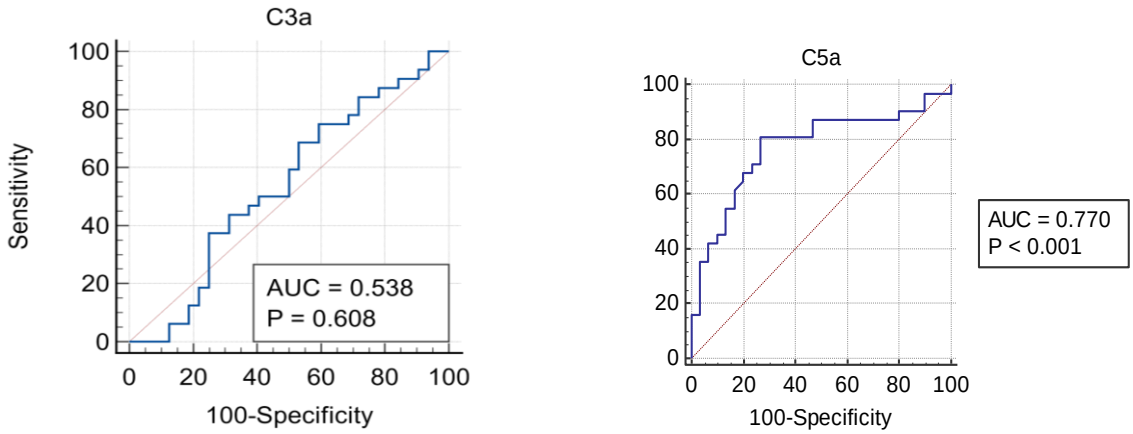

Figure [2]: C3a [left curve] and C5a [right curve] performance in differentiating diabetic from DN patients

DISCUSSION

Current study results showed that the duration of DM from its first diagnosis was significantly higher in DN group when compared to diabetic group with significantly higher age in DN group. This is consistent with **Gregg *et al.*** [25], who observed that, in general, kidney damage may begin 10 to 15 years after diabetes starts [usually between the ages of 45 and 64. In another word the risk of chronic diabetes complications rises with diabetes duration. This is also in line with a study done by **Ufuoma *et al.*** [26], they concluded that diabetic patients with DN had considerably older average ages and longer average diabetes durations than diabetic patients without nephropathy.

Shahwan *et al.* [27] in his collective multi-center study reported that: earlier researchers found a strong correlation between the duration of diabetes and onset of DN. The longer the duration of diabetes the greater the risk for development of diabetic nephropathy.

In addition, these results were consistent with **Wang *et al.*** [28], who asserted that the probability of developing and deteriorating DN increased with increasing the time that DM had been present.

The current study showed that the incidence and duration of hypertension were significantly higher in DN group than diabetic group. The two most prevalent chronic medical conditions that regularly occur together are diabetes and hypertension. Patients with diabetes are roughly twice as likely to have hypertension as people without the disease. Diabetes is characterized by insulin resistance, dyslipidemia, and hyperglycemia. By promoting inflammation, coagulation, endothelial dysfunction, and platelet defragmentation, all of these variables contribute to the onset and progression of atherosclerosis. This narrowing of blood vessels and rise in peripheral vascular resistance ultimately result in hypertension [29].

The current study showed that male sex predominates in diabetic and DN groups as compared to the control one. This finding may be related to the random method used for enrollment or to social issue in our community. However, because males without diabetes are often more insulin resistant than women, men may need to acquire less weight than women in order to develop type 2 diabetes. Therefore, throughout the age range, men may develop diabetes at a lower average body mass index [BMI]. Middle-aged men have a significantly higher risk of developing diabetes and related complications than women [30].

De Hauteclocque *et al.* [31] reported that, male sex is an important independent risk factor associated with renal dysfunction in Type 2 diabetes. Also, this is concordant with **Kautzky-Willer *et al.*** [32] who stated that men progress rapidly to DN and more often undergo dialysis therapy. Men have unhealthy lifestyles compared to women, and they are also more susceptible to type 2 diabetes and abdominal obesity at smaller body mass indexes [33, 34].

The ongoing study showed that the glycemic indices were significantly higher in both diabetic and DN groups when compared with the control group, and this in fact logic. Among diabetics, diabetic control is much poorer in diabetic patients when compared to DN group. Many assumptions were put to explain this situation. First, diabetic patients without diabetic nephropathy lag in seeking and follow medical advices. Second, glucose metabolism differs between diabetic kidney disease [DKD] patients and DM patients with no kidney affection. In patients with chronic kidney disease

[CKD], there is a reduction in the renal clearance of insulin and the peripheral tissue breakdown of insulin. Third, reduced renal gluconeogenesis commonly happens in these patients as a result of the increasing loss of renal mass. Forth, in individuals with CKD, the majority of antidiabetic medications have a longer half-life. Fifth, due to reduced gluconeogenesis in sick kidneys, poor nutrition from uremia, change in insulin metabolism, and the existence of other comorbidities, individuals with late stages of DKD may potentially be susceptible to hypoglycemia. After dialysis initiation, one-third of diabetic hemodialysis patients spontaneously have their hyperglycemia normalize even frequent hypoglycemic events, a phenomenon termed "burnt-out diabetes" [35].

Albuminuria is an early marker of diabetic nephropathy, and correlated significantly with loss of glomerular filtration rate and increased cardiovascular risk [36]. In this work urinary albumin/creatinine ratio was high in DN group - as planned in the protocol- if compared to control or diabetic groups.

The current study showed that the RBCs count and levels of hemoglobin were significantly lower in DN group when compared either with the control or with the diabetic group. This appears logic, as settled for long in the literature, anemia of CKD progress with the progression of kidney disease. Erythropoietin-deficiency related anemia can develop in diabetics in early stages of renal disease. Nutritional deficits and some medications for diabetic patients, such as ACE inhibitors, fibrates, metformin, and thiazolidinedione, also increase the risk of anemia [37].

Consistent with this current study findings, **Shi *et al.*** [38] reported that Hypertriglyceridemia and low levels of high-density lipoprotein [HDL] cholesterol are common manifestations of dyslipidemia in diabetics. Diabetic Dyslipidemia [DD] is common among patients with DM [prevalence rate 72-85%]. DD has an important role in the development of atherosclerosis and is associated with a substantially increased risk of diabetic vascular complications [39].

The current study showed that both micro-vascular complications [retinopathy and neuro-pathy] and macro-vascular complications [CNS and CVS] were significantly higher in DN group when compared with diabetic groups. Macro-vascular complications predominate in diabetic group despite some of them have diabetic retinopathy.

Umanath and Lewis [40] stated that the majority of DN patients will pass away from cardiovascular-related causes even before their condition progresses to end-stage kidney disease with substantial correlation between DN and cardiovascular illness.

Evidences are evolving showing a significant role of the innate immune system, notably the complement system, in the development of DN. Results from both experimental and clinical studies provide evidence for a relationship between kidney malfunction and the complement system. Complement is hypothesized to participate in the pathogenesis of diabetic nephropathy via two major pathways. First, in the context of diabetes mellitus, glycated proteins that are exposed to high sugar levels trigger the lectin pathway. Secondly, it is believed that hyperglycemia causes complement regulatory proteins to become glycated, which impairs their ability to regulate [11].

Current study showed that the mean ± SD of C3a in the control group was 3.25 ± 0.81 with 95% confidence interval [CI] 2.91 – 3.58 in diabetic group, the mean ± SD of C3a was 7.6 ± 6.6 with 95% CI 5.2 – 10.1, whereas in DN group the C3a mean ± SD was 6.6 ± 5.1 with 95% CI 4.7–8.5 [all measured in µg/ml]. C3a was significantly higher in both diabetic groups when compared to controls [$P < 0.00001$] but with no significant difference in the levels between diabetics and DN groups.

Based on western blot analysis, **Li *et al.*** [41] concluded that the activation of the complement system was indicated by the elevated serum levels of C3 and its fragments C3a in diabetic's serum. He and his coworkers added there is a strong correlation between complement C3 and insulin resistance with an increased risk of developing diabetes. Several clinical studies, both cross-sectional and longitudinal, confirmed this association in an independent cohort.

Shim *et al.* [42] in his study observed that anaphylatoxin C3a plays a role in metabolic disorders such as diabetes, obesity, and athero-sclerosis. **Wlazlo *et al.*** [43] observed a significant association of C3a with DM. They suggested that adipocytes may be the site of production and activation of C3 and active form C3a. Compelling preclinical evidence demonstrated the role of C3 and its cleavage product C3a in adipose tissue inflammation and insulin resistance.

The mean ± SD of C5a in the control group was 44.5 ± 8.1 with 95% confidence interval [CI]

41.1 – 47.8. In diabetic group, the mean ± SD of C5a was 48.5 ± 13 with 95% CI 43.9 – 53.2. Whereas in DN group the C5a mean ± SD was 63.1 ± 20.9 with 95% CI 55.14 – 71.02 [all measured in ng/ml]. C5a was significantly higher in DN group when compared to control group [P < 0.00001] and when compared to diabetic group [P = 0.001] but with no significant difference in the levels between control and diabetic groups.

The significantly high C5a in DN patients in the current study is concordant with the findings of **Huang *et al.*** [44] who stated that many experimental and preclinical studies support the role of complement activation in diabetic nephropathy, yet few clinical correlates are present. The same authors reported that the onset and advancement of DN are significantly influenced by the activation of the complement system. The complement system is an important activator of inflammatory responses and increasing amount of research has shown how important inflammation is to the onset and course of DN [44].

In the current study C5a was found to be increased in both disease groups, but significantly higher in DN group when compared with DM group. In contrast C3a showed no significant difference between both diabetic groups. As discussed before, fatty tissue is one of the sites where C3 can be formed and converted to C3a [43]. A significantly decreased appetite and an increase in the break-down of fat and lean body mass will occur in patients with CKD as a result of elevated pro-inflammatory cytokines brought on by reduced renal function, volume overload, oxidative stress, low antioxidant levels, increased susceptibility to infections, and related comorbidities [45]. Depletion of body fat leads to reduction in one of the sources of C3 formation and activation. This may explain why C3a showed no significant difference between diabetic and DN groups due to loss of adipose tissue with establishment of CKD.

ROC curve analysis showed that C3a could be used as a significant predictor of DM when compared with normal subjects with a sensitivity of 75% and a specificity of 96%. However, the differentiating ability of C3a between DM and DN is insignificant. It should also be considered that increase in serum C3a level has been recorded in other conditions including for example, progressive primary open angle glaucoma [46], systemic lupus erythematosus [SLE] patients positive for anti-dsDNA [47], covid-19 infection [48] and also, other nephropathies including IgA nephropathy, membranous nephropathy and lupus nephritis [49] reducing its diagnostic potentialities.

Concerning C5a, ROC curve analysis showed that, C5a could be used as a significant predictor of DN when compared with DM with a sensitivity of 80.65% and a specificity of 73.33%. However, the differentiating ability of C5a between normal subjects and DM is insignificant. Similarly, to C3a, increase in serum C5a level has been recorded in other conditions for example SLE, IgG4 related disease [50], covid-19 infection [51], patients with abdominal aortic aneurysm [52] and Neuromyelitis Optica [53].

Conclusion: The anaphylatoxins C3a and C5a are important effector molecules of the complement cascades exerting their effects through inducing inflammation. Being mediators of the inflammatory response, C3a and C5a, participate in the pathogenesis of DKD and mediate kidney damage.

Study limitations: Rarely is a study perfect. This study has limitations include the rather limited resources and the difficulties in involving the patient for longer durations so as to it is possible to follow up the correlation between the anaphylatoxins C3a and C5a as diabetes progress to diabetic nephropathy and in different stages for diabetic nephropathy.

Recommendations: We have focused on detecting the differences in these molecules between control, diabetic and diabetic nephropathy patients and our results can be considered as bases for future longitudinal studies involving larger samples of population of concern with follow up in different stages of diabetic nephropathy with recruitment of essential resources. Further researches, particularly on larger scale of population, are still needed to understand these molecules comprehensively and to outline their possible diagnostic and therapeutic potentials

Conflict of interests: there are no conflicts to declare.

Funding: No funding was given to the authors in order for them to do the research, write, or publish this paper.

Acknowledgments: The authors thank the employers of inpatient units and outpatient clinics of Internal Medicine Department, Al-Azhar University hospitals.

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IJMA

International Journal of Medical Arts

VOLUME 6, ISSUE 6, JUNE 2024

P- ISSN: 2636-4174
E- ISSN: 2682-3780