Original Article

Assessing urinary IL-18, NGAL levels and albumin creatinine ratio in patients with diabetic nephropathy

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Abstract

Aims

Diabetic nephropathy (DN) is a serious microvascular complication of a longstanding hyperglycemia. This study aims to evaluate whether urinary neutrophil gelatinase-associated lipocalin (NGAL) and urinary Interleukin-18 possess a better diagnostic value than albumin creatinine ratio in assessing the severity of nephropathy in patients with type 2 diabetes mellitus (T2DM).

Material & methods

Ninety participants diagnosed with T2DM were recruited and they were divided into three study groups according to their albumin/creatinine ratio (ACR): (Normoalbuminuria group, Microalbuminuria group, and Macroalbuminuria group). A matching of Ninety healthy subjects were included as controls. Blood and urine samples were collected to measure various markers of glycemic control and kidney function.

Results

IL-18 levels were not changed significantly between all study groups ($P > 0.05$), despite a significant positive correlation between IL-18 and urinary albumin levels.

NGAL levels were significantly increased in Microalbuminuria group and Macroalbuminuria group as compared to the control and Normoalbuminuria groups. NGAL was also positively correlated with urinary albumin and ACR, but negatively correlated with the age and body mass index. Receiver Operating Characteristic curves revealed that for early detection of DN, the best cutoff values to discriminate DN and diabetic without nephropathy groups were ˃ 21.4 ng/ml for NGAL (94.67 sensitivity, 26.67% specificity), ≤0.34 pg/mL for IL-18 (72% sensitivity, 53.33% specificity), and ˃29.8 mg/g for ACR (80% sensitivity, 100% specificity).

Conclusion

We conclude that the urinary ACR is a more accurate individual biomarker of DN when compared to both NGAL and IL-18.

Keywords: Interlukin-18; Neutrophil gelatinase-associated lipocalin; Albumin creatinine ration; Diabetic nephropathy; Type 2 diabetes

1 Introduction

Type 2 Diabetes mellitus (T2DM) is a well-known endocrine and metabolic disease which possess serious effects on human wellbeing [1]. Uncontrolled persistent hyperglycaemia can lead to multiple complications including microvascular and macrovascular complication [2]. Diabetic nephropathy (DN) is a common and serious complication of T2DM associated with adverse outcomes of renal failure, cardiovascular disease, and premature mortality [3], and

is one of the leading causes of end-stage renal disease (ESRD) [4].

Approximately 10–20% of T2DM patients progress to ESRD [5]. DN has been classically defined as an increased protein excretion in urine [6]. Hence, urinary albumin excretion is commonly used as a “non-invasive” biomarker for DN, although it does not fully indicate the disease process. This is because histological deformities may be present before the identification of microalbuminuria. Besides, some subjects may be presented with low glomerular filtration rate (GFR) in-spite of being normoalbuminuric [7], suggesting that albuminuria is not the perfect marker for the early identification of DN [8]. Three major renal morphologic changes observed in the glomerular compartment in DN. This include mesangial expansion, basement membrane thickening and formation of Kimmelstiel-Wilson nodules. The latter eventually progress to glomerulosclerosis. More recently, it has been increasingly reported that the renal tubulo-interstitium plays an integral role in the pathogenesis of DN [9].

Moreover, it was showed that under the stress conditions, renal glomerular, tubular epithelial cells and immune cells produce proinflammatory cytokines [4]. Among these cytokine is interleukin-18 (IL-18) which is a member of the IL-1 family of cytokines and also known as an interferon gamma (IFN-γ) inducing factor [10], with a molecular weight of 18 kDa [11]. IL-18 is generated from activated dendritic cells, monocytes and glial cells [12] and human adipose tissue [13].

It was found that serum levels of IL-18 were increased by acute hyperglycaemia in patients with T2DM as compared to non-diabetics. Furthermore, the elevated levels of urinary or serum IL-18 have been reported in subjects with DN, suggesting that IL-18 is one of the significant pathogenic mediators in the development of diabetic nephropathy [14]. Urinary and serum levels of IL-18 have been described to be raised in subjects with DN, with an independent correlation between these parameters and urinary albumin excretion [15].

Neutrophil gelatinase associated lipocalin (NGAL) is a marker of urinary tubular damage with (25-kDa) that belongs to the lipocalin protein family [16]. The NGAL generated by neutrophils, which are markedly induced and released in injured epithelial cells, including renal tubular cells [17]. It is manufactured in, pulmonary, hepatic, and intestinal tissues [18]. NGAL is one of the most promised tubular markers in the diagnostic domain of acute and chronic kidney disorders [6]. Recent studies showed that the urinary NGAL levels may be increased in T2DM subjects with normoalbuminuria, indicating that the diabetic tubular injury may evolve prior to the stage of microalbuminuria [3,19]. Recent studies looked on additional, more sensitive, specific, and prognostically accurate biomarkers to assist in the early diagnosis and management of patients at risk of kidney disease. The current study aims to evaluate the diagnostic value of urinary IL-18 and urinary NGAL as biomarkers for early detection of nephropathy as compared to the albumin creatinine ratio in type 2 diabetes mellitus patients in Iraq.

2 Methods

2.1 Subjects

The study was approved by the Health and Medical Human Research Ethics Committee at the Faculty of Medicine, University of Kufa, Iraq, and participants were invited from the Center for Diabetes and Endocrinology, at Al-Sader Teaching Hospital, Al-Najaf, Iraq. Ninety patients, who were clinically diagnosed with T2DM, were included in this study and they were divided into three study groups according to their albumin/creatinine ratio (ACR): Normoalbuminuria group (ACR <30 mg/g), Microalbuminuria group (ACR = 30–300 mg/g), and Macroalbuminuria group (ACR >300 mg/g). A matching of Ninety healthy subjects were also recruited in this study as the control group.

All participants were provided with participant information sheet and they were given the opportunity to ask questions and discuss their concerns. Written consents were taken from them before allowing them to be involved in the study.

2.2 Methods

Anthropometric data were collected from all the participants. In addition, five millilitres of venous blood samples were collected following an overnight fast, to measure glycated haemoglobin, HbA1c (using a quantitative colorimetric determination of glycohaemoglobin in whole blood), fasting plasma glucose (using glucose oxidase method), serum albumin, creatinine, and urea.

Urine samples were also collected to measure urinary albumin and creatinine (using an enzymatic colorimetric methods), as well as urinary IL-18 and NGAL (using Elabscience kit, enzyme-linked immunosorbent assay).

2.3 Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS), version 23, USA. Numerical data were expressed as mean, standard deviation, median and inter-quartile range, whereas, categorical data were expressed as number and percentages. One-way ANOVA test was used to compare mean values among different groups in case of normally distributed variables, whereas, Kruskal Wallis test was used to analyse the non-normally distributed variables. Two-way ANOVA test was used to evaluate the mean difference in the urinary IL-18 and NGAL levels following classification of sample by two factors (albuminuria and treatment). Bivariate correlation was carried out using Pearson's correlation coefficient. P-value was considered significant at $P \leq 0.05$.

3 Results

This study showed no significant difference in the mean values for age, gender and BMI between the control and patients groups ($P > 0.05$), Table 1. However, the mean value of age was significantly lower in the Normoalbuminuria group as compared the other two diabetic study groups. Gender distribution was evenly distributed among all the groups; mean duration of diabetes was significantly highest in the microalbuminuria and macroalbuminuria groups as compared to the normalalbuminuria group ($P < 0.05$), Table 1.

Table 1 Characteristics of the subjects included in this study. Data are explained as (mean ± SD). Significant P value at or <0.05.

alt-text: Table 1

Parameter	Control (N = 90)	Diabetic patients (N = 90)		
		Normoalbuminuria (30)	Microalbuminuria (N = 38)	Macroalbuminuria (N = 22)
Age	51 ± 8.9	45.3 ± 6.9[c]	53.6 ± 10.4	52.8 ± 11.2
Gender (male/female) ratio	39/51	16/14	10/28	11/11
Duration of diabetes(years)	–	5.3 ± 7.2[c]	7.7 ± 6.2	7.2 ± 5.4
Body mass index (kg/m^2)	27.5 ± 5	29.2 ± 3.1	28.3 ± 4.4	26.9 ± 3.4
Fasting Plasma Glucose (mg/dL)	99.5 ± 10.4[d]	166.4 ± 69.8[c]	240.1 ± 85.4[b]	272.5 ± 110.6[a]
Glycated hemoglobin (%)	5.3 ± 0.4[d]	8.5 ± 1.4	8.9 ± 1.3	8.1 ± 1.5[a]
Serum albumin (mg/dL)	4.9 ± 1.3[d]	4 ± 1	3.9 ± 0.6	4.4 ± 0.7[a]
Serum creatinine (g/dL)	0.7 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.3
Serum urea (mg/dL)	25.6 ± 9.3[b]	34.3 ± 8.8	36.8 ± 7.3	44.2 ± 11.1[a]
Urine albumine (g/dL)	2.6 ± 1.8	3.5 ± 3.4	6.2 ± 5.5[b]	42.6 ± 37.6[a]
Urinary creatinine (mg/dL)	165.5 ± 86.3	160.7 ± 135.6	89.6 ± 74.1[b]	63.1 ± 40.3[a]
ACR	15.2 ± 8.3	19.7 ± 7.4	74.4 ± 41.3[b]	1077.2 ± 171.1[a]
Urinary IL-18 (pg/mL)	0.4 ± 0.2	0.4 ± 0.03	0.3 ± 0.1	0.3 ± 0.03
Urinary NGAL (ng/mL)	40.5 ± 19.1[d]	58.7 ± 38.7[c]	64 ± 29.7[b]	72.8 ± 21.6[a]

Values are expressed as mean ± SD; tests are one-way ANOVA and Post-hoc LSD.

[a] Significant difference between the Macroalbuminuria and the other study groups.

[b] Significant difference between the Microalbuminuria and the other study groups.

[c] Significant difference between the Normoalbuminuria and the other study groups.

[d] Significant difference between the control group and the other study groups.

The Mean fasting plasma glucose levels were significantly positively correlated with the severity of albuminuria. However, the mean HbA1c levels were highest in the microalbuminuria and normoalbumiuria groups as compared to the macroalbuminuria group and control group, Table 1.

The urinary ACR was significantly elevated in the macroalbuminuria group as compared to the other groups, ($P < 0.05$), Table 1. In contract, the mean urinary IL-18 was not significantly different among all study groups, ($P > 0.05$), Table 1.

The mean urinary NGAL levels were significantly increased in the macroalbuminuria group and the microalbuminuria group as compared to the other study groups, ($P < 0.05$), Table 1.

Table 2 represents a comparison between the output data of receiver operating characteristic (ROC) curves for urinary NGAL, IL-18, and ACR levels. A cut-off value of urinary NGAL, IL-18 and ACR levels were found to be > 21.4 ng/ml, ≤0.34 pg/ml and >29.8 mg/g, respectively. The urinary levels of NGAL >21.4 ng/ml predicted the presence of nephropathy with a 94.67% sensitivity, 26.67% specificity, and AUC = 0.543 with 16.7% accuracy. The urinary levels of IL-18 ≤ 0.34 pg/ml predicted the presence of nephropathy with a 72.00% sensitivity, 53.33% specificity, and AUC = 0.595 with 68.9% accuracy. On the other hand, the urinary ACR ration of >29.8 mg/g predicted the presence of nephropathy with a 80% sensitivity, 100% specificity, and AUC = 0.900 with 83.3% accuracy, Table 2.

Table 2 Comparison between the output data of ROC curves for urinary NGAL, IL-18, and ACR levels, in patients with and without diabetic nephropathy.

alt-text: Table 2

Variable	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC (95% CI)
NGAL (ng/ml)	>21.4	94.67%	26.67%	50.0%	15.9%	16.7%	0.543 (0.486–0.697)
IL-18 (pg/ml)	≤0.34	72.00%	53.33%	88.5%	27.6%	68.9%	0.595 (0.434–0.648)
ACR (mg/g)	>29.8	80.00%	100.0%	100.0%	50.0%	83.3%	0.900 (0.819–0.953)

PPV: Positive predictive value; NPV: Negative predictive value.

Table 3 shows the true negative (TN), false positive (FP), true positive (TP), and false negative (FN) values for urinary NGAL, IL-18, and ACR.

Table 3 The true negative, true positive, false negative and false positive values of urinary NGAL, IL-18 and ACR ratio in patients with & without diabetic nephropathy.

alt-text: Table 3

Variable	Cutoff	Patients without nephropathy		Patients with nephropathy	
		TN	FP	TP	FN
NGAL	>21.4	14	1	1	74
IL-18	≤0.34	8	7	54	21
ACR	>29.8	15	0	60	15

Abbreviations**:** NGAL: urinary neutrophil gelatinase-associated lipocalin; IL-18: Interleukin-18; ACR: Albumin creatinine ratio; TN: true negative; FP: false positive; TP: true positive; FN: false negative.

Pearson correlation analyses were done between the urinary NGAL and IL-18 and ACR among participants with diabetic nephropathy. No significant correlation was seen among the selected markers, Table 4.

Table 4 Pearson Correlations between the urinary NGAL, IL-18 and ACR ratio in diabetic patients with nephropathy.

alt-text: Table 4

		NGAL (ng/mL)	IL-18 (pg/mL)	ACR
NGAL (ng/mL)	Pearson Correlation	1	0.133	0.009
	Sig. (2-tailed)		0.229	0.932
	N	90	84	90
IL-18 (pg/mL)	Pearson Correlation	0.133	1	0.127
	Sig. (2-tailed)	0.229		0.251
	N	84	84	84
ACR ratio	Pearson Correlation	0.009	0.127	1
	Sig. (2-tailed)	0.932	0.251	

	N	90	84	90

4 Discussion

Diabetic nephropathy is a very devastating condition, significantly affecting the quality of life and causes substantial increase in morbidity and mortality if not managed early [20]. This study was conducted in order to assess the correlation and to illustrate the diagnostic value of urinary IL-18 and NGAL with various stages of albuminuria in T2DM patients with nephropathy.

Findings from the current study showed that the rate of DN is increased with the increase of the age and with increase in the duration of the disease. Something which has already been addressed in previous studies [21–23]. Moreover, the patients in the normoalbuminuria group have the lower mean age value as compared to the microalbuminuria group and the macroalbuminuria group. This result can be attributable to the fact that the patients in this group constitute a proportion of new cases with diabetes mellitus [22,23].

Gender was not a significant variable in the selected study groups. There is a lot of controversy about the gender effects on the progression of DN [24,25].

The severity of DN as manifested by the degree of albuminuria was positively correlated with the HbA1c levels, ($p < 0.05$). This may suggest that hyperglycaemia may be the driving force for the development of DN [26,27]. This is because diabetic participants with persistent hyperglycaemia (high HbA1c) may develop some sort of proteinuria or renal anaemia at the early stage of DN. Later one, due to the persistent renal anaemia, there is a falsely reduction in the HbA1c levels, resulting in patients with DN. This reason explains why we cannot rely on HbA1c levels in patients with severe stage renal disease [28].

We had controversial results when comparing serum urea, serum creatinine levels among the three study groups and the control group. The levels of serum urea positively correlated with the albuminuria stage, however, serum creatinine was not significantly associated with the disease stage, concluding that microalbuminuria may not be associated with an abnormal serum creatinine or creatinine clearance, but can be an important warning signal which if ignored can result in irreversible renal damage [29].

Inflammation plays an essential role in the progression of DN. Recent evidence indicates that innate immunity, rather than adaptive immunity, is the major driving factor in the inflammatory response in DN. IL-18 is a potent proinflammatory cytokine that induces IFN-ᵧ. The IL-18 leads to production of other proinflammatory molecules, including IL-8, IL-1β, TNF-α, and intercellular adhesion molecule-1, from mononuclear cells and macrophages. These molecules are known to increase in T2 DM and may contribute to maintain microinflammation in renal tissues of patients with T2 DM [30–34].

Urinary IL-18 levels were not significantly different between different study groups. This result came in contract to findings from other studies who showed that the level of urinary (or serum) IL-18 was significantly elevated in the diabetic groups when compared to a matching control group [30–32]. A systemic review and meta-analysis study showed that urinary IL-18 holds promise as a biomarker in the prediction of acute kidney injury (AKI) but has only moderate diagnostic value, and given the modest clinical value of IL-18 and its high cost, serum creatinine concentration might still be a good indicator rather than IL-18 [33]. The large cohort study of Nisula et al. [34] showed that IL-18 had poor to moderate ability to predict AKI, renal replacement therapy (RRT), and 90-day mortality. Thus, it should be used with caution for diagnostic or predictive purposes in the critically ill patients [34].

The low levels of IL-18 in the current results can be attributed to multiple factors: one of them is that IL-18 has been shown in various cross-sectional analyses to reveal a positive correlation with blood glucose levels, insulin or insulin resistance and the metabolic syndrome. A decrease in IL-18 was also observed to be an independent predictor of improvement in insulin sensitivity in patients treated with antidiabetic medications [30–34].

Moreover, nearly about 80 of diabetic patients enrolled in this study were treated with metformin. This treatment is a widely prescribed as first-line therapy for T2DM. Metformin has been shown to reduce cardiovascular events and all-cause mortality in The UK prospective diabetes study (UKPDS), incidence of lactic acidosis, also it was shown beneficial effects beyond its hypoglycemic effect, such as anti-inflammatory, anti-cancer and anti-aging effects [35]. Metformin was found to have a highly and selectively action to reduce pro-inflammatory cytokine secretion from activated macrophages [36]. Furthermore, the low level of IL-18 can be attributable to the strict inclusion criteria in this study i.e. all patients enrolled in this study were without hypertension, while it was found that individuals with hypertension had significantly higher levels of IL-18 than those without hypertension. Although there are some suggestions that IL-18 is a marker of DN in T2DM patients, its predictive power remains uncertain [37].

In kidney disease and diabetes, different mechanisms have been suggested to explain the increased urinary excretion of NGAL. Initially it has been proposed in animal studies that increased urinary NGAL levels are primarily a result of reduced reabsorption in the injured tubules. In contrast, it was found that the cause of elevated levels of urinary NGAL was increased NGAL production in the tubules in a mouse model of cisplatin-induced nephrotoxicity. This may explain the reason behind the use of urinary NGAL as a marker of acute tubulointerstitial damage by some studies [38].

In this study, the levels of urinary NGAL was significantly correlated with the degree of renal damage, being much higher in the macroalbuminuria group ($p < 0.05$). Similar observation was found in other studies which concluded that there is a significant increase in both serum and urinary NGAL in T2DM as compared to control subjects and the level was increased further with increasing albuminuria [24,38]. This result agrees with other researchers who's stated that NGAL increases in patients with type one diabetes even before diagnosis of microalbuminuria representing an early biomarker of normoalbuminuric DN [26].

5 Conclusion

In conclusion, when assessing the diagnostic performance of urinary NGAL and IL-18 and whether they are more sensitive and specific than ACR using the ROC and Pearson Correlation, we found that the urinary ACR has an excellent diagnostic power, and it is a precise biomarker with premium specificity, very good sensitivity and acceptable accuracy, and it is more specific than urinary NGAL and IL-18 in predicting the severity of nephropathy in patients with type 2 diabetic patients.

Conflicts of interest

None.

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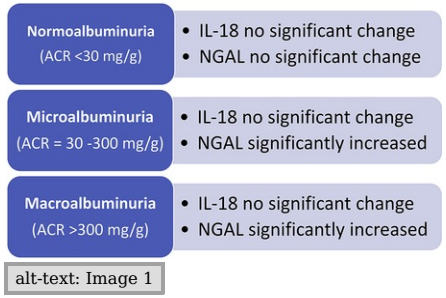
Graphical abstract

Three study groups of type 2 diabetes mellitus patients according to their albumin/creatinine ratio.



alt-text: Image 1

Highlights

- Diabetic nephropathy is a serious complication of persistent hyperglycaemia.
- Under stress condition, renal glomerular, tubular epithelial cells and immune cells produce proinflammatory cytokines.
- IL-18 were increased by acute hyperglycaemia in patients with T2DM as compared to non-diabetics.
- NGAL generated by neutrophils, which are markedly induced and released in injured renal tubular cells.
- Urinary ACR is a more accurate individual biomarker of DN when compared to both NGAL and IL-18.

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