

Association of significantly elevated serum levels of NGAL and IGFBP4 in patients with diabetic nephropathy

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Abstract

Background: Diabetic nephropathy (DN) is a kidney-related complication affecting approximately 40% of patients with diabetes. Current DN diagnostic criteria predominantly rely on albuminuria and serum creatinine levels; however, the specificity and reliability of both markers are limited. Hence, reliable biomarkers are required to diagnose and effectively manage DN progression.

Methods: Here we investigated the expression level and the association between neutrophil gelatinase-associated lipocalin (NGAL), IGFBP-1, IGFBP-3, and IGFBP-4 in patients with DN and compared it to patients with T2D and control participants. A cohort comprise of 159 individuals (DN = 67) was clinically evaluated and circulatory levels of NGAL, IGFBP1, IGFBP3, and IGFBP4 were determined using ELISA.

Results: Levels of circulating NGAL were significantly higher in people with DN compared to people with T2D and non-diabetic groups (92.76 ± 7.5 , 57.22 ± 8.7 , and 52.47 ± 2.9 mg/L, respectively; $p < 0.0001$). IGFBP4 showed a similar pattern, where it was highest in people with DN (795.61 ng/ml ± 130.7) compared to people with T2D and non-diabetic respectively (374.56 ng/ml ± 86.8 , 273.06 ng/ml ± 27.8 , ANOVA $p < 0.01$). Our analysis presents a significant positive correlation between NGAL and IGFBP4 in people with DN ($\rho = .620$, $p < 0.005$). IGFBP4 also correlated positively with creatinine level and negatively with eGFR, in people with DN supporting its involvement in DN.

Conclusion: Our results report the association between the rise in NGAL and IGFBP4 levels in DN and suggest them as potential markers to aid DN diagnosis.

1. Introduction

Diabetic nephropathy (DN) is a kidney-related complication that affects approximately 40% of patients with type 1 and type 2 diabetes mellitus (T1D and T2D, respectively), and is one of the most common causes of end-stage renal disease (ESRD) worldwide [1, 2]. It is a progressive condition that gradually impairs kidney function. Clinically, DN is defined by persistent elevation in levels of urinary albumin (> 300 g/24 h), and is associated with elevated risk of cardiovascular morbidity and mortality [1]. Due to the progressive nature of DN an early detection is critical to have better treatment outcome, explaining the need for identifying additional diagnostic and prognostic markers [3].

Diagnosis of DN involves measurement of albuminuria, proteinuria and eGFR, however these measurements are not reflective of direct renal injury and are insensitive to small changes in renal function [4]. Levels of microalbuminuria show considerable daily variations caused by other conditions such as exercise, diet, infection, and high blood pressure [5]. Therefore, measuring microalbuminuria was an imprecise predictor of DN progression or regression [4], and the use of microalbuminuria in the early detection of diabetic renal lesions has been questioned [6]. Although the measurement of serum creatinine (sCr) is widely used for diagnosing and monitoring various renal conditions including DN, several studies have questioned its reliability as a diagnostic tool and indicator for acute changes in kidney functions [7]. Furthermore, sCr levels are influenced by age, gender, muscle mass, and hydration

levels, and changes in serum levels may not be observed until a substantial amount of renal function is lost [8–10]. This explains the growing interest in identifying more informative biomarkers to achieve an early diagnosis and better monitoring of kidney disease.

First identified in 1993 and purified from neutrophil granules [11], the neutrophil gelatinase-associated lipocalin (NGAL) is a promising marker for acute kidney injury (AKI) and kidney disease [12]. NGAL is a 25 kDa protein that belongs to the lipocalin superfamily and is encoded by the LCN2 gene. Similar to other members of the lipocalin family, NGAL acts as a transporter for small hydrophobic molecules and is involved in many physiological processes, such as modulation of inflammation, innate immune response, and metabolic homeostasis [13, 14]. Significant increases in baseline levels of NGAL in serum and urine in various renal pathological conditions implicated its potential use as a biomarker for kidney dysfunction [15].

Cases of AKI presented NGAL as a sensitive and rapid marker of kidney injury [15]. NGAL was also elevated in patients with autosomal dominant polycystic kidney disease [16, 17]. In patients with nephropathy and T1D or T2D, NGAL levels were significantly elevated in serum and urine, and it inversely correlated with estimated glomerular filtration rate (eGFR) [18, 19]. Patients with diabetes and kidney dysfunction had elevated levels of NGAL but normal albuminuria, implicating the potential role of NGAL as a diagnostic biomarker for DN [19]. Additionally, due to the involvement of the IGF system in kidney growth, structure, and function, the role of carriers and modulators for IGF-1 in the renal system such as insulin-like growth factor-binding proteins (IGFBPs) was investigated in patients with diabetic kidney syndrome [20]. IGFBP-1, -2, -4, and -5 are predominantly produced and expressed in the glomerulus, whereas IGFBP-3 and -6 are chiefly expressed in the kidney cortex [20]. Patients with DN showed a significant increase in IGFBP-1 serum levels and low IGFBP-3 levels compared with control subjects [21, 22]. This suggested IGFBPs as promising biomarkers for the diagnosis of DN.

The present study aimed to analyze the difference in NGAL and IGFBPs concentrations comparing patients having T2D with and without nephropathy to examine the association (if any) between DN and these marker in our cohort. We further investigated the possible association between NGAL and IGFBPs and whether they could serve as sensitive biomarkers for DN.

2. Materials And Methods

2.1. Study population

A total of 159 participants were enrolled in this study. The study involved three main groups; 67 participants with diabetic nephropathy (DN), 50 with Type 2 diabetes (T2D) and 42 non-diabetic individuals that were both age and body mass index (BMI) matched (Table 1). Clinical diagnosis of T2D involved having persistent hyperglycemia (fasting glucose level > 7 mmol/L and 2-hr FBG > 11 mmol/L) with normal kidney function. People with DN showed pronounced T2D and persistent elevation in ACR >30 mg/g and were clinically diagnosed with DN by a nephrologist according to the American Diabetes Association criteria [23]. This study excluded people with T1D, renal transplant or End-stage renal

disease. Healthy participants had no history and were not diagnosed with medical conditions such as T2D or DN.

Table 1
Patient Demographic and Clinical Biochemistry findings of study population

Marker	Non-diabetic group (\pm SEM) N = 42	T2D group (\pm SEM) (n = 50)	DN group (\pm SEM) (n = 67)	ANOVA (<i>P</i> value)	Multiple comparisons with post hoc Bonferroni (<i>P</i> value)		
					T2D vs. DN	T2D vs. Healthy	DN vs. Healthy
Age in years	57.74 \pm 1.32	58.96 \pm 1.02	59.09 \pm 1.38	0.015	1.000	0.104	0.014
Gender (M % / F%)	43 / 57	44 / 56	41 / 59				
Body Mass Index	33.20 \pm 0.69	33.94 \pm 0.88	34.23 \pm 0.85	0.001	1.000	0.006	0.001
SBP (mmHg)	122.50 \pm 2.25	132.98 \pm 3.88	132.03 \pm 3.41	0.087	1.000	0.139	0.162
DBP (mmHg)	73.76 \pm 1.52	69.72 \pm 2.26	68.78 \pm 1.98	0.21	1.000	0.574	0.261
Fasting Glucose (mmol/l)	5.52 \pm 0.12	8.27 \pm 0.36	9.61 \pm 0.48	< 0.001	0.050	0.0001	0.0001
HbA1C (%)	5.66 \pm 0.09	9.53 \pm 1.73	8.09 \pm 0.22	0.031	0.816	0.027	0.236
T chol (mmol/l)	4.79 \pm 0.15	4.15 \pm 0.013	4.02 \pm 0.012	< 0.001	1.000	0.006	0.0001
TG (mmol/l)	1.07 \pm 0.08	1.41 \pm 0.16	1.77 \pm 0.11	< 0.001	0.102	0.202	0.001
HDL (mmol/l)	1.44 \pm 0.06	1.25 \pm 0.005	1.13 \pm 0.003	< 0.001	0.207	0.026	0.0001
LDL (mmol/l)	3.57 \pm 0.73	2.28 \pm 0.11	2.1 \pm 0.10	0.01	1.000	0.050	0.011
VLDL (mmol/l)	0.43 \pm 0.03	0.56 \pm 0.06	0.71 \pm 0.04	< 0.001	0.105	0.196	0.0001
Serum Creatinine (μ mol/l)	75.69 \pm 2.89	79.42 \pm 3.54	118.36 \pm 6.57	< 0.001	0.0001	1.000	0.0001
BUN (mmol/l)	5.02 \pm 0.20	5.10 \pm 0.29	7.53 \pm 0.52	< 0.001	0.001	1.000	0.0001

Data are mean \pm standard error mean; SBP systolic blood pressure; DBP diastolic blood pressure; BUN blood urea nitrogen; eGFR glomerular filtration rate.

Marker	Non-diabetic group (± SEM) N = 42	T2D group (± SEM) (n = 50)	DN group (± SEM) (n = 67)	ANOVA (<i>P</i> value)	Multiple comparisons with post hoc Bonferroni (<i>P</i> value)		
					T2D vs. DN	T2D vs. Healthy	DN vs. Healthy
eGFR MDRD (mL/min /1.73 m ²)	81.07 ± 2.14	79.22 ± 3.19	59.7 ± 3.00	< 0.001	0.0001	1.000	0.0001
Albumin (g/l)	40.5 ± 0.52	37.94 ± 0.50	37.28 ± 0.42	< 0.001	0.927	0.002	0.0001
Urine Creatinine (mg/l)	14.75 ± 1.22	11.94 ± 0.86	9.08 ± 0.77	0.049	0.071	0.140	0.001
Microalbumin (mg/l)	14.82 ± 2.0	14.35 ± 1.61	490.72 ± 186.62	< 0.001	0.003	1.000	0.005
ACR (mg/g)	9.77 ± 1.2	11.32 ± 1.07	953.48 ± 327.78	0.004	0.013	1.000	0.020
NGAL (mg/l)	52.47 ± 2.9	57.22 ± 8.7	92.76 ± 7.5	< 0.001	0.002	1.000	0.001
IGFBP1 (mg/l)	19.01 ± 2.2	31.38 ± 4.7	34.85 ± 3.3	0.0091	1.000	0.080	0.008
IGFBP3 (mg/l)	66.01 ± 32.4	64.99 ± 27.8	69.65 ± 24.8	0.425	0.661	1.000	1.000
IGFBP4 (ng/ml)	273.06 ± 27.8	374.56 ± 86.8	795.61 ± 130.7	< 0.001	0.013	1.000	0.003
Medications:	None	85.3	76.2				
– ARBs (%)		14.7	23.8				
– ACEi (%)							

Data are mean ± standard error mean; SBP systolic blood pressure; DBP diastolic blood pressure; BUN blood urea nitrogen; eGFR glomerular filtration rate.

This study was approved by the Ethical Review Committee of Dasman Diabetes Institute and were in accordance with the guidelines of the Declaration of Helsinki. All participants were recruited at Dasman Diabetes Institute (Dasman, Kuwait) and gave written informed consents before enrollment in the study.

2.2. Sample collection and biochemical measurements

Blood and urine samples were obtained from participants following an overnight fast. Blood samples were collected in vacutainers containing Ethylenediaminetetraacetic acid (EDTA) and centrifuged to

separate the plasma that was aliquoted and stored at -80°C for further analysis. First void urine samples were collected in 120 mL urine collection tubes in the morning. Blood pressure readings present the average of three consecutive measurements, with a 10-min rest period between each reading (digital sphygmomanometer, Omron HEM-907XL Digital). Quantification of biochemical molecules concentration, such as fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) was done by a chemistry analyzer (Siemens Dimension RXL; Diamond Diagnostics, Holliston, MA). Concentrations of urinary albumin “spot” and creatinine in addition to urinary albumin-to-creatinine ratio were measured using an automated analyzer (CLINITEK Novus Automated Urine Chemistry Analyzer; Siemens Healthineers, Erlangen, Germany). Fully automated renal function tests (RFTs) were performed using a VITROS 250 automatic analyzer, and estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease study (MDRD) equation [24].

2.3. Measurement of NGAL and IGFBPs

Stored plasma samples were thawed and centrifuged at $10,000 \times g$ for 5 min at 4°C to remove any debris. NGAL concentration was quantified by an ELISA kit (Wuhan EIAAB Science Co. Cat. No. M1388h), following manufacturer’s protocol. No significant cross-reactivity with other proteins was noted. Serum levels of IGFBP-1, -3, and -4 were measured using an R&D Magnetic Luminex Assay (R&D Systems Europe, Ltd, Abingdon, UK) following manufacturer’s protocol.

2.4. Statistical analysis

Data of all study groups, people with T2D, DN, and healthy control subjects, was compared by one-way analysis of variance (ANOVA) with Bonferroni *post hoc* test for multiple comparisons to determine pair-wise statistical significance. All data presented as mean \pm standard deviation (SD). Spearman correlation analysis was used to evaluate the univariate association between NGAL and other biomarkers. The analysis was also used to evaluate the correlation between IGFBPs and renal function. Statistical assessment was considered significant with $p < 0.05$. Data was statistically analyzed with IBM Corp. (2017) IBM SPSS Statistics for Windows, version 25.0 (Armonk, NY: IBM Corp.). Received operating curve (ROC) analysis was performed to study the utility of NGAL and IGFBP4 as markers for people with DN. To obtain statistical measures of the NGAL and IGFBP4 the scales were categorized based on the elevated serum creatine or ACR to assess the AUC analysis of ROC curves. We calculated 95% CIs for from the ROC analysis results based on cut-off points established. The ROC analysis results were interpreted as follows: $\text{AUC} < 0.70$, low diagnostic accuracy; AUC in the range of $0.70\text{--}0.90$, moderate diagnostic accuracy; and $\text{AUC} \geq 0.90$, high diagnostic accuracy.

3. Results

The study involved three groups of Kuwaiti participants: 50 patients with T2D, 67 patients with DN and 42 non-diabetic participants. All groups were statistically evaluated showing no significant difference in systolic and diastolic blood pressure (Table 2). There was a significant difference in FBG levels between people with T2D, DN and non-diabetic control (Table 2, $p < 0.001$), with the highest values presented in

people with DN. Levels of glycated hemoglobin (HbA1c) were higher in people with T2D and DN compared to the non-diabetic group (Table 2, $p < 0.05$). In general, there was a significant difference in lipid profile parameters between the study groups (Table 2). People with T2D and DN showed a significant increase in both TG and vLDL ($p < 0.001$), while a significant reduction in levels of TC, LDL and HDL. (Table 2, $p < 0.01$).

Table 2

Spearman's rank correlation between IGFBPs and renal markers in the non-diabetic, T2D and DN groups.

Study group	Markers	IGFBP-1		IGFBP-3		IGFBP-4	
		ρ	<i>P</i> value	ρ	<i>P</i> value	ρ	<i>P</i> value
Non-diabetic group	Serum Creatinine	-0.039	0.807	-0.176	0.270	0.302	0.052
	BUN	0.047	0.769	0.212	0.183	-0.038	0.809
	eGFR	-0.010	0.948	0.177	0.267	-0.306	0.049
T2D group	Serum Creatinine	0.006	0.966	-0.034	0.814	0.269	0.059
	BUN	0.160	0.267	0.002	0.991	-0.013	0.927
	eGFR	-0.141	0.334	-0.036	0.794	-0.271	0.059
DN group	Serum Creatinine	0.249	< 0.05	0.178	0.150	0.397	< 0.001
	BUN	0.371	< 0.001	0.194	0.116	0.327	< 0.05
	eGFR	0.171	0.166	-0.206	0.095	-0.448	< 0.001
Spearman coefficient (ρ)							

3.1 People with DN show higher levels of NGAL and IGFBPs

Our data showed a significant increase in plasma NGAL levels in people with DN compared to people with T2D ($p = 0.002$, Fig. 1A, Table 2) and non-diabetic individuals ($p = 0.001$, Table 2). There was a significant elevation in levels of both IGFBP-1 and IGFBP-4 in people with DN compared to other participants (Fig. 1B and D). IGFBP-4 was significantly higher in people with DN (795.61 ± 130 ng/ml) compared to both, people with T2D (374.56 ± 86.8 ng/ml, $p = 0.013$) and non-diabetic group (273.06 ± 27.8 ng/ml, $p = 0.003$). On the other hand, the increase in IGFBP-1 (34.85 ± 3.3 mg/l) was significant

compared to people from the non-diabetic group (19.01 ± 2.2 , $p = 0.008$), but it showed no significance in comparison to people with T2D (31.38 ± 4.7 mg/l). IGFBP-3 expression levels showed no significant differences between the three groups (Fig. 1C).

3.2. IGFBP-4 is significantly correlated with kidney function parameters

Performing Spearman rank correlation analysis showed a significant correlation between IGFBP-4 and indicators of renal activity. This was presented through a significant positive correlation with serum creatinine ($\rho = 0.39$, $p < 0.001$), BUN ($\rho = 0.32$, $p < 0.05$), and a negative correlation with eGFR ($\rho = -0.44$, $p < 0.001$). In the case of IGFBP-1, our analysis showed a significant negative correlation with both serum creatinine ($\rho = -0.249$, $p < 0.05$) and BUN ($\rho = -0.371$, $p < 0.001$) in people with DN. Whereas eGFR showed no association with IGFBP-1 in the same group (Table 3). We found no association between IGFBP-3 and parameters reflecting kidney function in people with DN (Table 3). Moreover, we have assessed the relationship between IGFBP4 and urinary protein excretion (urine creatinine, microalbumin and ACR). IGFBP4 was found to associate significantly only with urine Creatinine in the DN group ($\rho = -0.289$, $p = 0.021$, Supplementary table 1).

Table 3
Spearman's rank correlation between NGAL with listed renal markers and IGFBPs

Markers	Non-diabetic group		T2D group		DN group	
	ρ	<i>P value</i>	ρ	<i>P value</i>	ρ	<i>P value</i>
Serum Creatinine	0.381	< 0.05	0.631	< 0.001	0.530	< 0.001
BUN	0.195	0.221	0.577	< 0.001	0.608	< 0.001
eGFR	- 0.430	< 0.001	- 0.707	< 0.001	- 0.552	< 0.001
IGFBP1	0.010	0.950	0.167	0.256	0.195	0.120
IGFBP3	0.005	0.976	- 0.113	0.450	0.122	0.331
IGFBP4	0.019	0.906	0.252	0.085	0.620	< 0.001
Spearman coefficient (ρ)						

3.3 NGAL manifests a significant association with renal markers in people with DN

Spearman's correlation analysis showed a significant correlation between NGAL and markers of kidney function (Table 4). Our data showed that NGAL was positively correlated with serum creatinine ($\rho = .53$, $p < 0.001$, Fig. 2B), and blood urea nitrogen (BUN) ($\rho = .608$, $p < 0.001$, Fig. 2D), while it correlated negatively with eGFR ($\rho = -.552$, $p < 0.001$, Fig. 2C). Interestingly, our analysis revealed a significant correlation between NGAL and IGFBP-4 in people with DN ($\rho = .62$, $p < 0.001$). In a similar manner, NGAL showed a significant association with parameters of kidney function in people with T2D (Table 4). The correlation

was positive with serum creatinine ($\rho = .63, p < 0.001$), and BUN ($\rho = .577, p < 0.001$) but negative with eGFR ($\rho = -.707, p < 0.001$). There was no significant correlation between NGAL and IGFBP-4 in people with T2D (Table 4). Furthermore, no significant association between NGAL and urinary proteins was found.

Table 4
ROC Analysis optimized for Serum Creatinine and ACR

	Group	AUC	P value	Cut-off	95% CI	Sensitivity	Specificity
Serum Creatinine	NGAL	0.79	< 0.001	43917.41	0.72–0.87	52.2	91.8
	IGFBP4	0.74	< 0.001	213.50	0.66–0.83	51.1	84.0
Albumin Creatinine Ratio (ACR)	NGAL	0.70	< 0.001	43789.01	0.62–0.79	41.7	76.1
	IGFBP4	0.68	< 0.001	240.0	0.59–0.77	44.0	75.4

3.4. Ngal Is Positively Correlated With Igfbp-4

The Spearman rank correlation coefficient showed a significant positive association between NGAL and IGFBP-4 (Table 4). The correlation between NGAL and IGFBP-4 was exclusive to people with DN ($\rho = .62, p < 0.001$, Fig. 3C). A similar correlation was not found in people with T2D or non-diabetic participants (Table 4). Additionally, NGAL did not correlate with other IGFBPs (i.e. IGFBP-1 and -3) in our study population (Fig. 3A, B).

3.5. Roc Analyses For Ngal And Igfbp4

The cut-off points based on ROC curve analyses showed significant predictive power of the ACR and serum creatinine for NGAL and IGFBP4 markers, with Serum creatinine exhibiting high accuracy (Fig. 4A and B) and ACR exhibiting low accuracy (Fig. 4C and D). The AUCs, p-values, cut-off points optimized for sensitivity and specificity, 95% CIs, sensitivities, and specificities obtained for Serum Creatinine and ACR are reported in Table 4.

3.6 Assessing the levels of ANGAL and IGFBP4 in normal creatinine group

We have stratified our 3 groups including DN cases based on creatinine levels into normal (Male $< = 119.3$; Female $< = 91.9$) and high (Male > 119.3 ; Female > 91.9). Serum levels for both NGAL and IGFBP4 seem to be increased in both subgroups. However, their increased in levels were more pronounced in the high creatinine subgroup (Supplementary table 2). Moreover and within the normal creatinine subgroup, it was evident the increased of both NGAL and IGFBP4 levels in the DN group compared to both DM and non-diabetic ones supporting the utility of both markers for early diagnosis of DN.

4. Discussion

During the past decade, the prevalence of diabetes has escalated, affecting 415 million people worldwide. DN is a common complication associated with T1D and T2D, and it is regarded as the leading cause of chronic kidney disease (CKD) worldwide [25]. In addition to the progress in the available management and treatment options, finding biomarkers with higher sensitivity would greatly improve the prevalence of DN and incidence of diabetes related ESRD. Currently, microalbuminuria and serum creatinine are the diagnostic markers for DN, however they exhibit some limitations that affect their diagnostic efficiency [5, 8–10]. Indicating the need for identifying additional diagnostic and prognostic markers to achieve better management of DN. In the present study we showed a significant increase in NGAL levels in a group of people with DN. The rise in NGAL levels was significantly associated with elevated levels of IGFBP-4 and renal indicators in people with DN. Only people with DN showed significant increases in NGAL and IGFBP-4 levels, suggesting them as potential diagnostic and prognostic markers for DN.

Due to the involvement of the IGF system in CKD pathology [20], where IGF1 is one of the main factors involved in the development of DN. We investigated the involvement of other IGFBPs and their contribution to the pathology of a diabetic kidney. IGFBP-4, which is typically expressed in the kidneys, has been suggested as a marker for autoimmune diseases, including chronic lupus nephritis [26]. Wu et al. showed a positive correlation between circulating IGFBP-4 and serum Cr levels and an inverse correlation with eGFR in patients with lupus nephritis. In our cohort, people with DN showed a significant elevation in levels of IGFBP-4 compared with the control or people with T2D [27]. The rise in IGFBP4 levels demonstrated a significant association with renal markers including, serum Cr, BUN and eGFR. This came in agreement with previous reports and it supports the potential involvement of IGFBP4 in diabetic kidney disease [28].

The pathophysiology of DN has been attributed to multifactorial interactions between metabolic and hemodynamic factors, including glucose-dependent pathways and the renin–angiotensin system [29]. Such molecular abnormalities may damage the glomerulus and renal tubulointerstitium, which contribute to elevated levels of NGAL and other renal markers, including serum Cr and BUN. Compared to the routinely used renal markers, NGAL is a sensitive biomarker for AKI that increases significantly in the blood and urine within 2 hours of injury [30]. As such, elevated NGAL levels marks the occurrence of AKI [31]. Increased levels of plasma NGAL showed a predictive power for CKD progression and was reflective of renal disease severity [32, 33]. In our study, a rise in NGAL and IGFBP4 levels was evident in people with DN even in those demonstrating normal creatinine levels (Supplementary table 2), the increase in circulating NGAL in people with DN reflected its importance as an indicator of DN. This was accentuated by the significant positive correlation with IGFBP4 in people with DN, a marker that has been linked with DN [27, 28]. Conventional renal markers like Cr and microalbumin are important for the diagnosis and staging of kidney disease. However, a significant change in conventional markers is usually detected after the occurrence of a substantial glomerular damage. Although, NGAL is a rapid marker for the detection of an acute renal injury, changes in NGAL levels were not reflective of a kidney functional deficit

[15]. Nonetheless, others found that changes in NGAL levels are predictive of CKD progression and severity [32].

Collectively, here we are reporting significantly increased levels of two biomarkers, NGAL and IGFBP4, in people with DN implicating their involvement in renal pathology. This elevation was not prominent in people with T2D and was not present in the non-diabetic group. A previous report highlighted NGAL as a marker for the progression and severity of CKD [33], while IGFBP4 indicated the chronicity of renal pathology [26]. The potential link of these proteins in DN was accentuated by the significant positive correlation between IGFBP4 and NGAL in people with DN, which suggest their use as biomarkers aiding an early detection of DN. Our study is limited by the cross-sectional design that should be considered upon data interpretation. Thus, future studies are required to elucidate the mechanism through which NGAL and IGFBP4 are contributing to a state of DN and the potential usefulness of using them as additional markers of DN. One of the main limitations of this study is the lack of a group of CKD patient caused by other than diabetes. This would have allowed us to decipher whether these markers are DN specific or can detect other kidney impairments. Another limitation of the study is that we could not assess the inflammatory role within the DN group in our cohort.

Conclusion

Here we report the importance of two circulating proteins, NGAL and IGFBP4, as potential biomarkers for DN. Our observational study presented a positive link between NGAL and IGFBP4 in people with DN, thus implicating their potential use as indicators of renal pathology. In the field of nephrology, DN continues to be a major condition that is best managed by early detection to prevent the occurrence of irreversible kidney damage. Here, our data presented circulating markers as potential diagnostic tools for DN. Future studies should further investigate their feasibility and usefulness as early markers of DN.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all participants before enrollment to the study. The study and methods were approved by the Ethical Review Committee of the Dasman Diabetes Institute and were in accordance with the guidelines of the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HA: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. MA: data analysis and interpretation and critically revised the manuscript. EA: patient coordination and sample collection. SD: data analysis, management and statistical analysis. YB: patients' recruitment and data interpretation. IK: performed the ELISA assay. PC: performed the ELISA assay. ZA: patient coordination and sample collection. VV: Blood processing, storage and data analysis. FM: Data interpretation and critically revised manuscript. AA: Study design, data interpretation and management. JA: Study design, data interpretation, wrote and critically revised the manuscript. All authors have seen and approved the final manuscript.

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Figures

Figures 1 - 4 are not included with this version of the Manuscript.

Figure 1. Circulatory levels of NGAL, IGFBP-1, IGFBP-3, and IGFBP-4 in our cohort. (A) Quantification of NGAL levels showed a significant increase in circulating NGAL in people with DN (97.76 ± 7.5 mg/l) compared with the non-diabetic group (52.47 ± 2.9 mg/l, $p = 0.001$) and people with T2D (57.22 ± 8.7 mg/l, $p = 0.002$) (B) IGFBP-1 levels were elevated in people with T2D (31.38 ± 4.7 mg/l) and DN (34.85 ± 3.3 mg/l) compared with the non-diabetic group (19.01 ± 2.2 mg/l, $p = 0.008$). (C) Levels of IGFBP-3 showed no difference between the three groups. (D) Quantification of IGFBP-4 levels showed a significant increase in people with DN (795.61 ± 130.7 mg/l) compared with non-diabetic people (273.06 ± 27.8 mg/l, $p = 0.003$), and people with T2D (374.56 ± 86.8 , $p = 0.013$).

Figure 2. Correlation analysis between NGAL and clinical variables associated with DN. Spearman's rank correlation coefficient showed a significant; (A) positive correlation between serum creatinine and NGAL ($r = .530$, $p < .0005$), (B) a negative correlation between eGFR and NGAL ($r = -.552$, $p < .0005$) and (C) a positive correlation between BUN and NGAL ($r = .758$, $p < .0001$).

Figure 3. Correlation analysis between NGAL and IGFBPs in people with DN. Spearman's rank correlation coefficient showed; (A) IGFBP-1 is not correlated with NGAL ($r = .195$, $p = .12$), (B) IGFBP-3 is not correlated with NGAL ($r = .122$, $p = .331$), and (C) a positive correlation between IGFBP-4 and NGAL ($r = .620$, $p < .0005$).

Figure 4. Receiver Operating Characteristic curves for the NGAL and IGFBP4 using both serum creatinine and ACR diagnostic curve markers. A) and B) are the ROC analyses for NGAL and IGFBP4 using serum creatinine; C) and D) the ROC analyses for NGAL and IGFBP4 using ACR.

Supplementary Files

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