Role of Serum β Trace Protein and Neutrophil Gelatinase Associated Lipocalin in Early Diabetic Nephropathy in Type 2 Diabetes of Iraqi Patients

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ABSTRACT

Background: Diabetic nephropathy (DN) is part of the greatest broadly known diabetic microvascular intricacies inducing around 40% of patients with type 2-diabetes Mellitus (T2DM), Its advancements to end-stage renal disease (ESRD); the primary recognition of DN can be achieved with biomarkers of diabetes. This study evaluates the role of the power of the early biomarker recognition of DN in the T2DM serum.

Design and Methods: A case-control study, it included 90-people, and composed from both genders and age range of individual study was (40-69 years); which divided into 3-groups by using the urinary albumin/ creatinine ratio (ACR). Included 60-patients with T2DM without/with proteinuria, which also divided into 2-groups group I, normoalbuminuria (<30 mg/g) and group II, microalbuminuria (30–300 mg/g); Addition to 30-persons, healthy control group (<30 mg/g), (free from any disease); the assay was applied by turbidimetric/ biochromatic rate. In all groups, β-TP and NGAL which were estimated in serum and both biomarkers having the same methodology by quantitative enzyme immunoassay, (double-antibody sandwich).

Results: The serum β-TP and NGAL have significantly higher levels in diabetic patients with microalbuminuria (group II) as parallel with those with normoalbuminuria (group I) and healthy control, nevertheless their levels have no significant variance between group I and healthy control subjects.

Conclusions: Results of this suggest that serum β-TP and NGAL in diabetic patients with type 2, can be considered a valuable biomarker for early detection of DN.

Keywords: Type 2-diabetes mellitus, Diabetic nephropathy, β-trace protein, Neutrophil gelatinase-associated lipocalin

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INTRODUCTION

Diabetic nephropathy (DN) is part of the greatest broadly known diabetic microvascular intricacies inducing around 40% of patients with type 2-diabetes Mellitus (T2DM), Its advancements to end-step renal disease; in this way affecting the sickness and death of T2DM-patients [1]. Efficient DN treatment includes glycemic management and lowering blood pressure (BP). It's essential to identify DN within early stages as brief treatment can reduce the medical and economic burden of this sickness. At present, microalbuminuria is the most generally explored biomarker for the conclusion of DN; but its indicative value in beginning period DN is constrained as renal injury ordinarily goes before proteinuria. Consequently, there is a desperate requirement for progressively delicate and explicit biomarkers used for finding of DN in early process. It's been nice -hence this leads to glomerular distress, tubular injury, inflammatory reactions and oxidative stress advancement diabetic kidney illness [2].

Beta-trace protein (β-TP) also, Prostaglandin D-type lipocalin synthase (L-PGDS) [3] can be viewed as a protein with a twofold function: First, it works as an enzyme in the creation of PGD2 [4] by this enzyme advances the change of Prostaglandin H2 (PGH2) to prostaglandin D2 (PGD2) [3] and second, after being excretion, it works as an extracellular transporter because of

its lipophilic nature. The β-TP is a low molecular mass glycoprotein and a novel endogenous marker of GFR [5]. It is freely filtered by the glomerulus without secretion or potentially reabsorption in renal tubules and it is excreted by through the kidneys. Elevated the β-TP concentrations in serum reflect diminished clearance of the protein. β-TP half-life is around 1-2 hours and there is minimal extrarenal clearance [6]. In this manner, serum β-TP has been projected as a marker of GFR in both kids and grown-ups [7]. Then again, the investigating of studies Sβ-TP by way of a dependable biomarker in T2DM of renal dysfunction are still exceptionally rare. additionally, there is a paucity of information on the estimation of Serum β-TP in various phases of nephropathy in T2DM patients [8]. In 2004, Kobata et al. [9] recommended that β-TP may foresee the beginning stages of CKD in T2DM-patients.

The Neutrophil Gelatinase-Associated Lipocalin (NGAL) is an individual from the lipocalin protein family. This family incorporates numerous little proteins, the vast majority of which go about as transporters, fundamentally for lipophilic substances. In any case, different jobs for these proteins have been found, for example, the regulation of cell division, differentiation, cell-cell adhesion, and cell survival. The NGAL is utilized as a renal injury biomarker because launch it is quickly as the response of tubular damage [10]. Curiously, among the most encouraging tubular biomarkers in the assessment of acute and chronic renal disease is NGAL, a small protein (25 kDa) estimated in both serum and pee, having a place with the lipocalin protein superfamily and created in renal tubules upon inflammation and tissue injury [11]. It is revealed inside 2-4 hours of renal injury, even before the albumin appearance in the urine [12]. Serum NGAL increments in the early stage of DN and drops down as the ailment progressive. In another word, Serum NGAL relates inversely with the amount of albuminuria [13]. In this study, we evaluate the role of early detection power of serum biomarkers for finding of diabetic nephropathy in T2DM-patients.

TOPICS AND PRACTICES

Topics

This case-control study included 90-subjects as total with age ranging between (40-69 years): 60-patients with T2DM, divided equally into 2-groups, 30-patients-Microalbuminuria (group I) and 30-patients-Normoalbuminuria (group II), in addition to 30-persons-Healthy (control group) were selected as a healthy person during the period from November 2019 to December 2019, the permission to do the research was obtained by the college in consultation unit of diabetes with the laboratory of biochemistry in Al-Zahra teaching hospital in Wasit/ Iraq. T2DM was diagnosed according to criteria of DM, when fasting blood glucose (FBG) level of 126 mg/dL (7.0 mmol/L) at Alternatively blood sugar Around 200 mg/dL (11.1 mmol/L)) or greater in patients with classic hyper blood glucose symptoms or Hemoglobin A1C at just over 6.5 per cent [14]. Accordingly, a suitable regulator of T2DM was well-defined as (HbA1C <6.5%), while HbA1C levels (>6.5%), were used to categorize patients as those with the inadequate regulator of the disease; The grade This was calculated from early DN using the greatest usually used clinical index ACR: (30–300 mg/g) and GFR, estimated using the formulary of chronic kidney diseased epidemiological collaboration (CKD-EPI)) [15].

CKD-EPI eGFR = 141 × min (SCr / κ, 1) α × max (SCr / κ, 1)-1.209 × 0.993age [× 1.018 (if female)] or [× 1.159 (if black)]

where: SCr in μmol/l, κ is 61.9 for females and 79.6 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of SCr/κ or 1, max indicates the maximum of SCr /κ or 1.

The study has approval from the moral committee at the College of Medicine, Baghdad University. Informed consent was attained from each contributor. Both patients and healthy-controls are free from CVD (acceptable within six months), hematological diseases, endocrine illnesses, glucocorticoid Therapy, pregnancy, tumors, acute infections, continuing inflammatory sickness and renal disease other than DN (diabetic patients with macroalbuminuria were excluded from the study).

Biochemical assessments

About 6 ml of blood samples were obtained from veins of healthy control subjects and patients having T2DM obtained from each fasting (8 hours fast) participant in this study. Each blood

samples divided into two parts:

A) First, part 2.5 mL of whole blood retained in ethylenediaminetetraacetic acid (EDTA), tubes for measuring of glycated haemoglobin (HbA1C%) by measuring a boronated affinity assay by using NYCOCARD reader II.

B) Second part 3.5 mL of blood were left for 30 min at temperature of room allow samples to clot in plain tube. After coagulation, sera were separated by centrifugation for 10 min at 3000 rpm. Sera were aspirated and divided into two aliquots in eppendorf tubes for: -

i. Aliquot 1: Immediate measurements of fasting blood sugar (FBG), uric acid (UA), serum creatinine (SCr), blood urea (Urea), serum triglycerides (TG) and serum high-density lipoprotein-cholesterol (HDL-C) were done using assay (colourimetric); and the assay was applied by the automated method by using Abbott Architect C4000.

ii. Aliquot 2: The rest were stored at (-55 to -65 C°) until assayed for β-TP and NGAL and both biomarkers having the same methodology by quantitative enzyme immunoassay (double-antibody sandwich) was measured by an enzyme-linked immunosorbent assay (ELISA) manufactured by (Melsin medical co., limited, china).

About (5-10 ml) of freshly voided morning urine samples were collected into a sterile container and divided into two aliquots.

Aliquot 1: used for general urine examination which includes macro-examination using urine strip kit supplied by Human diagnostics, and microscopic examination to exclude urinary tract infection (UTI).

Aliquot 2: The grade this was calculated from early DN using the greatest usually clinical indices used ACR, the assay was applied by turbidimetric/biochromatic rate and the automated method by using the Dimension clinical system of chemistry.

An analysis of statistics

Analysed results using SPSS V 23. Descriptive statistics (Mean ± SD with tables and figures) and inferential statistics (Chi-square, ANOVA, the multivariate linear regression, and operating receiver curve (ROC)/ Area below Curve (AUC), responsiveness and specificity have been established. used. Statistically important was the Price P of 0.05.

RESULTS

In the Table 1 showed the clinical features of the three research groups. All study groups were identical regarding age, sex, BMI, serum uric

Table 1: Clinical characteristic, baseline investigation and serum level of biochemical parameters in all studied groups.

Clinical Variable	Diabetics		Healthy Control	P-value
	Group I (T2DM-Normoalbuminuria)	Group II (T2DM-Microalbuminuria)		
Age (years)	55.13 ± 7.9	58.13 ± 9	53.7 ± 8.3	0.118
Sex (male: female)	(14/16)	(13/17)	(19/11)	0.25
BMI (kg/m2)	29 ± 4.9	27.7 ± 4.1	28.1 ± 4.2	0.65
SBP (mmHg)	151 ± 23.1**	151.3 ± 15.7**	131 ± 9.6	<0.001
DBP (mmHg)	93.7 ± 17.9*	94.3 ± 20.3*	82 ± 6.7	0.005
Hypertension (yes: no)	(21/9)	(29/1) **††	(19/11)	0.005
FBG (mg/dl)	224.8 ± 85**	218.5 ± 87.7**	101.2 ± 16.8	<0.001
HbA1C (%)	8.8 ± 1.6**	9.4 ± 1.7**	5.2 ± 0.6	<0.001
HDL-C (mg/dl)	58.4 ± 11.1**	54.1 ± 11.3**	80.3 ± 16.3	<0.001
TGs (mg/dl)	195.9 ± 83.8**	203.3 ± 77.3**	104.7 ± 18.1	<0.001
UA (mg/dl)	4.2 ± 0.8	4.2 ± 1.3	4.1 ± 0.7	0.85
Urea (mg/dl)	26.2 ± 6.6	25.5 ± 8.9	29.3 ± 6	0.1
SCr (mg/dl)	0.8 ± 0.17	0.81 ± 0.21	0.84 ± 0.11	0.7
eGFR (ml/min)	93.7 ± 14.2	90.2 ± 17.3	99.4 ± 13.4	0.06
ACR (mg/g)	14.6 ± 8	110 ± 72.8**††	12.4 ± 6.4	<0.001
β-TP (ng/ml)	550 ± 100**	680 ± 150**††	480 ± 60	<0.001
NGAL (ng/ml)	59.6 ± 12.6	88.9 ± 18.2**††	55.4 ± 9.2	<0.001
*P<0.05 against the control group				
**P<0.01 against the control group				
†P<0.05 against the group I				
††P<0.01 against the group I				
ANOVA and Chi-squared test, p ≤ 0.05 considered as significant, Group I: T2DM-Normoalbuminuria, Group II: T2DM-Mmicroalbuminuria.				

acid, urea, creatinine and eGFR. While other variables like systolic and diastolic BP, FBG, HbA1c, HDL-C and TGs was varying greatly in both group of diabetic patients vs. healthy test (p<0.01). However, there was no sizeable difference regarding FBG, HbA1c, HDL-C and TGs for the team I and group II (p>0.05). The systolic and diastolic BP were significantly different in diabetic patients of both groups as compared with the healthy control.

Based on the description of hypertension according to 2017 Core of American society/Guidelines for the American college of cardiology, T2DM-Patients-Microalbuminuria were significantly more hypertensive when compared with T2DM-Patients-Normoalbuminuria and healthy control [16]. The study also showed that serum NGAL rates were substantially elevated in the group II patients as compared to group I and healthy controls, while the levels of β-TP were big increased in both collections of diabetics as Safe monitoring contrasted, with significantly higher group II levels as a group I levels (p<0.01).

The Person correlation coefficient between Serum β-TP and NGAL biomarkers in diabetic patients with all group studied were listed in the Table 2.

In this table, the biomarkers have correlations with each other. In all studied subjects (patients and healthy control) β-TP, NGAL and ACR were found to be significantly correlated with each other (P<0.001), while within the group of diabetic patients (normoalbuminuria and microalbuminuria) β-TP was significantly correlated with NGAL (P<0.04) and ACR (P<0.002), Still, and NGAL was more associated with the ACR (P<0.001). There was no significant correlation among β-TP, NGAL and ACR in subjects without nephropathy (normoalbuminuria and healthy control).

In the Table 3, ROC curve for assessment of β-TP and NGAL in T2DM-Patients for diagnosis of early-stage DN. Serum β-TP produced an AUC of 0.764 (95% CI, 0.646–0.883; P<0.001). The top value cut-off of β-TP was used for early detection DN is 635 ng/ml with sensitivity 56.7%, specificity 83.3%, PPV 77.3%, NPV 65.8% and accuracy 70%.

Serum NGAL produced an AUC of 0.957 (95% CI, 0.891–1.000; P<0.001). The top value cut-off of NGAL was used for early detection DN is 77.8 ng/ml with sensitivity 93.3%, specificity 96.7%, PPV 96.6%, NPV 93.5% and accuracy 95%.

Combined β-TP and NGAL has an AUC of 0.954 (95% CI, 0.902–1.000; P<0.001) with sensitivity 50% and specificity 100%, with PPV 100% and NPV 66.7%, and accuracy 75%.

To determine the efficiency of (β-TP, NGAL, β-TP with NGAL, and ACR) in early detection of DN, the most sensitive biomarkers for the early stage of DN is NGAL (Sensitivity 93.3%), followed by β-TP (Sensitivity 56.7%). While the combination of both β-TP and NGAL has a sensitivity of 50% but it is highly specific for early detection of DN (Specificity 100%). As described in the Figure 1.

DISCUSSION

Most of the patient with the diabetic illness will create renal infection [17]. Around half of the people with T2DM and 33% of individuals with T1DM create DKD, a microvascular complication

Table 2: Correlations of biomarkers with each other's.

Groups	Number of patients	Biomarkers	β-TP		NGAL		ACR	
			R	P-value	R	P-value	R	P-value
Diabetic and control subjects	90	β-TP	/	1	0.369	< 0.001	0.521	<0.001
		NGAL	0.369	< 0.001	/	1	0.583	<0.001
Group I and group II (Diabetic patients)	60	β-TP	/	1	0.267	0.04	0.404	0.002
		NGAL	0.267	0.04	/	1	0.71	<0.001
Group I and control (Without nephropathy)	60	β-TP	/	1	-0.05	0.705	0.199	0.132
		NGAL	-0.05	0.705	/	1	-0.194	0.14

Table 3: The area under operator characteristic ROC curve for assessment NGAL and β-TP in the diagnosis of early-stage DN in T2DM-Patients.

Biomarker	AUC	95% CI	Cut-off	Sensitivity %	Specificity %	p-value	PPV	NPV
β-TP	0.764	0.646–0.883	635	56.7	83.3	< 0.001	77.3	65.8
NGAL	0.957	0.891–1.000	77.8	93.3	96.7	< 0.001	96.6	93.5
β-TP /NGAL	0.954	0.902–1.000	/	50	100	< 0.001	100	66.7
ACR	100	1.000–1.000	29.7	100	100	<0.001	100	100

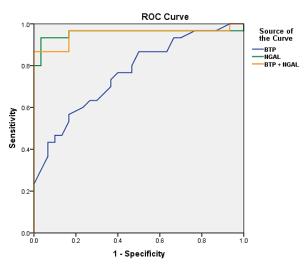**Figure 1: ROC curve meant for the diagnosis early-stage of DN using serum β-TP, NGAL and combined β-TP/NGAL.**

that is presently the main source of CKD and ESKRD world [18]. In this academic work, we have tried to create the role of circulating β-TP and NGAL levels in patients with T2DM to predict DN in early-stage even if the kidney function was normal.

There are several mechanisms shared between DM and hypertension, of these abnormal activations of angiotensin aldosterone system, excessive production of reactive oxygen species, sympathetic nervous system activation and abnormal renal sodium handling [19]. In this case-control study, no significant difference was noted in SBP and DBP in diabetic patients with normo- and microalbuminuria, this is in agreement with Motawi et al. [20]. Quite the same, Mahfouz et al. [21] Important disparity found in both SBP and DBP between diabetic patients within normoalbuminuria and microalbuminuria. However, according to the definition of hypertension based on 2017 US College of cardiology/American heart association whelton guidelines Whelton et al. [16], the study showed an elevated frequency of hypertension among others diabetic customers with microalbuminuria compared with normoalbuminuria and group control. The Unified Kingdom potential diabetes schoolwork (UKPDS) found that the hypertension is a significant hazard factor for the advancement of microalbuminuria and retinopathy and powerful systolic pulse control was altogether connected with the diminishing of long-haul complications of DM [22,23].

No substantial difference could be found in the FBG and HbA1C levels between groups of diabetic patients (I, II); while their levels were significantly superior in diabetic patients compared with the control group. Our finding is in an agreement with the Motawi et al. [20]. Other studies showed a significant difference in FBG in patients with microalbuminuria compared with normoalbuminuria Zhang et al. [2] and Mahfouz et al. [21], there is combined proof that diabetic nephropathy is emphatically connected with poor glycemic control [24].

The currently underway study showed lipid patterns the serum has was level from TG was significantly higher in both groups of diabetes than in the control group; HDL-C in diabetics, on the other side, was slightly lower patients' groups compared to the control group. This finding is consistent with other studies of dyslipidemia in diabetic patients Fouad et al. [25]. There is no sizeable difference in TG and HDL-C levels between diabetic patients of both groups, a finding that is inconsistent with what was demonstrated in previous studies Motawi et al. [20] and Zhang et al. [2].

The adjustments in raised degrees of TG and diminished HDL-cholesterol might be discovered quite a while before the beginning of overt diabetes [26]. Albeit low degrees of HDL related with cardiovascular sicknesses, this change is an autonomous hazard factor for the advancement of DM itself [27].

The β-TP is a low-atomic weight glycoprotein and it's currently considered as a novel marker for evaluating GFR. β-TP is predominantly found in cerebrospinal liquid, with lower focuses in serum [28]. It is unreservedly separated by the glomerulus and broadly reabsorbed and debased by the proximal tubule, with just modest quantities discharged in the pee under typical conditions [29]. β-TP helps in transformation of prostaglandin H2 (PGH2) to PGD2 the later assumes a significant job in platelet conglomeration, vasodilation, irritation, adipogenesis, and bone rebuilding [28]. This research revealed that the serum was β-TP level was significantly advanced in diabetic patients compared with control group and the elevation was more pronounced in micro- than normoalbuminuria group. this finding is an agreement with Motawi et al. [20] studies

which found that β-TP was developed in the DN group compared with a diabetic patient without nephropathy and control. Also, the result is consistent with Hebah et al. [8] that found that the serum of β-TP is higher in diabetic group than the control group which related to kidney dysfunction in T2DM patient as β-TP elimination limited to the kidney.

The NGAL is a 25 kDa protein of 178 amino acids and is predominantly generated in kidney tubules light of structural tumours of the kidneys. As opposed to serum NGAL, creatinine or blood urea nitrogen, is considered a proxy for systemic renal damage [30].

The serum level of NGAL in the current study was increased especially in patients with DN '' microalbuminuria'' compared with diabetic patients without nephropathy ''normoalbuminuria'' and control; Suggesting that the serum NGAL as a tubular injury marker precedes the presence in albuminuria as a glomerular injury marker, the study also found a highly significant difference in serum NGAL levels among the studied groups and was corresponding to the severity on kidney damage and being lower in the healthy control group and increased as ACR increase. Our results are in consent with the Motawi et al. [20] The one who finds the serum levels of NGAL were in T2DM with microalbuminuria group significantly higher than in T2DM with normoalbuminuria and control teams with a strong positive association with DM age and period. Kaul et al. [31] found that diabetes patients with normoalbuminuria had significantly higher levels of serum NGAL than control group suggested that tubular dysfunction in early DN is not secondary to albuminuria. On the other hand, Kim et al. [32] suggested in their trial that differences in NGAL value between control, normoalbuminuria, and microalbuminuria were nonsignificant, contradicting NGAL as an early marker of DN.

Wajda et al. [33], also, found that β-TP a significant correlation with SCr, NGAL and age in a patient with kidney disease. When comparing diabetic patients of both groups with healthy control β-TP, NGAL and ACR were positively correlating with each other. The same correlation also found when a patient with DN was compared with the diabetic patient without nephropathy, suggested that the β-TP and NGAL is a useful and sensible

calculation predictor to be interested early of the renal in diabetes.

The ROC curves were used to test the diagnostic efficiency of β-TP and NGAL, and whether they were can be used as diagnostic tools instead of ACR with almost the same sensitivity and specificity of ACR. The present study found that NGAL has more sensitivity and specificity than β-TP in early detecting diabetic patients with nephropathy than β-TP. but when these two biomarkers are combined and used to detect early DN they lead to most specific than each other but they have the lowest sensitivity; these results made NAGL more sensitive and specific than β-TP in the predication of DN. these results made NGAL more useful than β-TP and combined β-TP /NGAL in detecting early DN. These results are in consent with Mahfouz et al. [21] Who noticed that serum NGAL in micro- and macro-albuminuric diabetics increased significantly compared to the control group. Other studies also found comparative results Sueud et al. [34] and Motawi et al. [20]. On the other hand, Kobata et al. [9] stated that there were no big differences in the mean levels of β-TP between diabetic patients with normoalbuminuria and analbuminuria, however, β-TP was a good marker for the identification of early renal dysfunction in type 2-diabetes.

These biomarkers correlate with the developing of renal impairment via changing different concentrations according to the degree of the nephron damage. Part of it's due to its low molecular weight these biomarkers the formed glomerulus is filtered and reabsorbed at the proximal tubule. If the renal tubule is scratched, the reabsorption declines of biomarkers while its creation from epithelial cells increases, resulting in increases their levels in the blood and urine; as it is secreted within a few hours of nephropathic damage, even before glomerular involvement. Therefore, these biomarkers are a promising biomarker to detect DN in the earliest stages.

CONCLUSION

Through increased levels of serum β-TP and NGAL as endogenous filtration biomarkers in diabetic patients with type 2, it considered as a predictive biomarker which forces linked in early detection of diabetic nephropathy.

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