

Association of Peripheral Nesfatin-1 with Early Stage Diabetic Nephropathy

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Abstract: Background: It is well known that diabetes mellitus is a major health problem with worldwide spectrum. Diabetic nephropathy is one of its major microvascular complications which is a leading cause for end stage renal disease, hence comes the importance of its early detection even before the development of its triad: albuminuria, hypertension and declining renal function. **Aim of the work:** This study aims to evaluate serum Nesfatin-1 as a potential early biomarker of diabetic kidney disease (DKD) in type 2 diabetic patients. **Patients and methods:** The study enrolled 100 adult patients from Tanta University Hospital and Ahmed Maher Teaching Hospital (Internal medicine inpatient wards and outpatient clinics) over twelve months duration and divided into two groups: Group I which included 50 adult patients with type 2 diabetes and normoalbuminuria (UAE<30) mg/day) and group II which included 50 adult patients with type 2 diabetes and albuminuria (UAE>30 mg/day). Both patients groups were subjected to the following: full history taking, clinical examination, laboratory investigations which included complete blood picture, serum fasting blood glucose, 2 hours post prandial blood glucose, HbA_{1c}, total cholesterol and triglycerides levels, kidney function tests including: blood urea nitrogen and serum creatinine levels, uric acid, ALT, AST, alkaline phosphatase and serum albumin levels, urinary albumin to creatinine ratio, complete urine analysis, determination of serum Nesfatin-1 levels and pelvi-abdominal ultrasonography. **Results:** This study revealed a statistically high significant increase regarding serum Nesfatin-1 levels in group II (diabetics with albuminuria) when compared to group I (diabetics with normoalbuminuria). Serum Nesfatin-1 levels had statistically significant positive correlation with DM duration, serum triglycerides, HBA1c, serum uric acid, serum creatinine, BUN levels and UACR in the studied groups. There was no statistically significant correlation between serum Nesfatin-1 levels and age, SBP, DBP, BMI, ALT, AST, serum cholesterol level, FBS and 2HP.P BG. Serum Nesfatin-1 had an optimal cutoff value > 12.65 mmol/L with area under the ROC curve at 0.841 and at 95% confidence interval (CI) the lower bound was 0.752 and the upper bound was 0.930 and with sensitivity 80.0% and specificity 74.0%. **Conclusion:** In the present study, we concluded that serum Nesfatin-1 can be considered an early biomarker for diabetic nephropathy in type 2 diabetic patients.

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1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn is associated with both microvascular and macrovascular complications.⁽¹⁾

Globally, an estimated 422 million adults are diabetic patients, according to the latest 2016 data from the World Health Organization (WHO).⁽²⁾

Diabetic kidney disease (DKD) is a chronic microvascular complication of diabetes which may lead to end-stage renal disease. It is suggested that in people with diabetes, hyperglycemia, by inducing the production of advanced glycation end-products, leads

to structural alterations in proteins, and these processes are presumed to be the key roots in the pathogenesis of diabetic nephropathy.⁽³⁾

Nesfatin-1 is a newly found neuropeptide consisting of 82 amino acids with predominantly anorectic effects that participates in the regulation of hunger and fat storage and is also characterized as a potent regulator of metabolism. Nesfatin-1 is expressed in several tissues including the pancreatic islet cells and the central nervous system (CNS).⁽⁴⁾

Being mostly generated in the hypothalamus nuclei, nesfatin-1 has the ability to cross the blood-brain barrier without molecular saturation. Through further observations it was shown that nesfatin-1 suppresses food intake after being intracerebroventricularly injected, the injection decreases food intake in a dose-dependent manner

while the injection of a nesfatin-1 neutralizing antibody stimulates appetite.⁽⁵⁾

Nesfatin-1 is expressed in neurons of various brain areas including hypothalamic nuclei such as paraventricular nucleus (PVN), arcuate nucleus (ARC), lateral hypothalamic area (LHA) and in the nucleus of the solitary tract (NTS) and Dorsal motor nucleus of the vagus (DMNX) at the brainstem level. Nesfatin-1 is also secreted in pancreatic islets, gastric endocrine cells and adipocytes.⁽⁶⁾

Aim of the work

This study aims to evaluate Nesfatin-1 as a potential early biomarker of diabetic kidney disease (DKD) in type 2 diabetic patients.

2. Patients and Method

1. Patients:

The study enrolled 100 adult patients from Tanta University Hospital and Ahmed Maher Teaching Hospital (Internal Medicine inpatient wards and outpatient clinics) over twelve months duration.

They were divided into two groups:

Group I (n: 50):

Included 50 adult patients with type 2 diabetes mellitus and normo-albuminuria (UAE<30 mg/day)

Group II (n: 50):

Included 50 adult patients with type 2 diabetes mellitus and albuminuria (UAE \geq 30) mg/day).

2. Methods

All patients in both groups were subjected to:

1. Full history taking.
2. Clinical examination.
3. Routine laboratory investigations:
 - Complete blood picture.
 - Serum fasting blood glucose, 2H post prandial blood glucose and HbA_{1c}.
 - Lipid profile: Total cholesterol and triglycerides levels.
 - Kidney function tests including: blood urea nitrogen and serum creatinine.
 - Uric acid, ALT, AST, Alkaline phosphatase and serum albumin levels.
 - Urinary albumin to creatinine ratio.
 - Complete urine analysis.
4. Pelvi-abdominal sonography.

5. Specific laboratory investigation:

Determination of serum **Nesfatin-1** levels (by ELISA kit for Nesfatin-1)

3. Inclusion criteria:

Patients with:

- Type 2 Diabetes Mellitus on oral anti-hyperglycemic agents and /or insulin and statins in dyslipidemic subjects.

4. Exclusion criteria:

Patients with:

- Type 1 Diabetes Mellitus.
- Serum creatinine level >2 mg/dl.
- History of renal disease before to the onset of Diabetes Mellitus.
- Any symptoms or signs of inflammatory renal disease.
- Poor compliance to treatment (e.g.: due to side effects of drugs or irregular consumption of medications).
- Cigarette/tobacco or alcohol consumption.
- Obesity (BMI \geq 30 mg/kg²).
- Evidence of renal damage and dysfunction (eGFR < 60 ml/min/1.73 m²).
- Current or recurrent urinary tract infection.
- Active viral/bacterial infection.
- Evidence of fatty liver degenerative disease (non-alcoholic fatty liver disease and non-alcoholic steatohepatitis).
- Use of drugs known to affect serum Nesfatin-1 level as anti-epileptic drugs.
- History of chronic macrovascular complications due to DM.

5. Ethical consideration:

- A written informed consent was obtained from each patient after explanation of risk and benefits in the study.
- Complete data obtained from patients confidentially and every subject had a code number to his/ her file which contained all investigations and clinical data collected.
- The study is approved by the Ethics Committee of Faculty of Medicine, Tanta University.

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6. Sample:

a) 1 ml. of venous blood sample in Wasserman tube was collected from each patient. Serum-coagulation was allowed at room temperature for 10-20 minutes. Centrifugation was done for 20 minutes at the speed of 2000-3000 R.P.M.

b) Assay procedure:

All reagents and samples were brought to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

The essay was performed using the following steps:

1. Prepare all reagents and working standards as directed.
2. Blank well: don't add samples and NES1-antibody labeled
3. With biotin, Streptavidin-HRP, only Chromogen solution A and B, and stop solution are allowed; other operations are the same.
4. Standard wells: add standard 50 μ l, Streptavidin-HRP 50 μ l (since the standard already has

combined biotin antibody, it is not necessary to add the antibody).

5. To be test wells: add sample 40 μ l, and then add both NES1-antibody 10 μ l and Streptavidin-HRP 50 μ l. Then seal the sealing membranes, and gently shaking, incubated 60 minutes at 37 °C.

6. Confection: dilute 30 times the 30 \times washing concentrate with distilled water as standby.

7. 5. Washing: remove the membranes carefully, and drain the liquid, shake away the remaining water.

8. Add chromogen solution A 50 μ l, then chromogen solution B 50 μ l to each well. Gently mixed, incubate for 10 min at 37°C away from light.

9. Stop: Add Stop Solution 50 μ l into each well to stop the reaction (the blue changes into yellow immediately).

10. Final measurement: Take blank well as zero, measure the optical density (OD) under 450 nm wave length which should be carried out within 15min after adding the stop solution.

11. According to standards' concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then

apply the OD values of the sample on the regression equation to calculate the corresponding sample's concentration. It is acceptable to use kinds of software to make calculations.

3. Results

I. Demographic data of the two studied groups:

The study was conducted on 100 adult patients selected from Tanta University Hospital and Ahmed Maher Teaching hospital (Internal Medicine inpatient wards and outpatient clinics). Divided into two groups:

Group I: Included 50 adult patients with type 2 diabetes and normoalbuminuria (UAE<30 mg/day).

Group II: Included 50 adult patients with type 2 diabetes and albuminuria (UAE \geq 30mg/day).

Table (1) demonstrated comparison between the two studied groups regarding age. There was no statistically significant difference between the mean values for age in the two studied groups.

The mean value for age in group I was 58.6 \pm 5.79 years and in group II was 60.74 \pm 5.18 years (t=0.677, P-value= 0.084).

Table (1): Age distribution between studied groups

	Group I (N=50)	Group II (N=50)	t	P
Age (years)	58.6 \pm 5.79	60.74 \pm 5.18	0.677	0.084

P =probability value N. =number **t** =Student's t test on significant: P > 0.05 Significant: P < 0.05

Table (2) demonstrated comparison between the two studied groups regarding the gender. There was no statistically significant difference between genders in the two groups.

In group I the number of females was 16 representing 32% of the group and the number of

males was 34 representing 68% of the group. In group II the number of females was 19 representing 38% of the group and the number of males was 31 representing 62% of the group ($\chi^2=0.39$, P-value=0.52).

Table (2) Gender distribution between groups

		Group		Total	X²	P	
		group I	group II				
Sex	Female	N	16	19	35	0.39	0.52
		%	32.0%	38.0%	35.0%		
	Male	N	34	31	65		
		%	68.0%	62.0%	65.0%		
Total		N	50	50	100		
		%	100.0%	100.0%	100.0%		

P =probability value N. =number **t** =Student's t test Non significant: P > 0.05

* Significant: P < 0.05

Table (3) demonstrated comparison between the two studied groups regarding body mass index (BMI) and diabetes mellitus DM duration. There was no statistically significant difference between the mean values of BMI but there was a statistically significant

difference between the mean values of DM duration in the two studied groups.

The mean value for BMI in group I was 25.53 \pm 2.99 and in group II was 25.72 \pm 2.23 (t=0.363, P-value 0.717). The mean value for DM duration in

group I was 6.56±1.76 years and in group II was 11.42±3.33 years (t=9.102, P-value 0.00).

II. BMI, DM duration and blood pressure distribution of the two studied groups:

Table (3): BMI and DM duration distribution between groups

	Group I (N=50)	Group II (N=50)	t	P
BMI	25.53±2.99	25.72±2.23	0.363	0.717
DM duration (years)	6.56±1.76	11.42±3.33	9.102	0.00**

P =probability value N. =number **t** =Student's t test Non significant: P > 0.05 * Significant: P < 0.05

Table (4) demonstrated comparison between the two studied groups regarding blood pressure. There was no statistically significant difference between the mean values of SBP and DBP in the two studied groups.

Regarding systolic blood pressure (SBP); in group I the mean value was 128.8±8.66 mmHg and in

group II was 129.4±9.5 mmHg (t=0.330, P-value=0.742).

Regarding diastolic blood pressure (DBP); in group I the mean value was 83.8±5.6 mmHg and in group II was 81.8±6.2 mmHg (t=0.682, P-value=0.096).

Table (4): Blood pressure distribution between studied groups

	Group I (N=50)	Group II (N=50)	t	P
SBP (mmHg)	128.8±8.66	129.4±9.5	0.330	0.742
DBP (mmHg)	83.8±5.6	81.8±6.2	0.682	0.096

P =probability value N. =number **t** =Student's t test Non significant: P > 0.05 * Significant: P < 0.05

III. Comparison between the laboratory parameters of the two studied groups:

Table (5) demonstrated comparison between the two studied groups regarding CBC. There was no statistically significant difference between the mean values of HB, TLC and PLT in the two studied groups.

In group I the mean value of Hb was 12.8±1.7gm/dl and in group II was 12.52±1.7gm/dl (t=0.791, P-value=0.431).

In group I the mean value of TLC was 7.28±1.46×10³ cells/mm³ and in group II was 7.01±1.47×10³ cells/mm³ (t=0.926, P-value=0.357).

In group I the mean value of PLT was 228.7±69.9×10³ μl and in group II was 226.82±71.4×10³ μl (t=0.133, P-value=0.894).

Table (5): Comparison between the two studied groups regarding complete blood picture

	Group I (N=50)	Group II (N=50)	t	P
Hb (gm/dl)	12.8±1.7	12.52±1.7	0.791	0.431
TLC×10³ cells/mm³	7.28±1.46	7.01±1.47	0.926	0.357
PLT×10³ μl	228.7±69.9	226.82±71.4	0.133	0.894

P =probability value N. =number **t** =Student's t test Non significant: P > 0.05 * Significant: P < 0.05

Table (6): Liver function tests in the two studied groups

	Group I (N=50)	Group II (N=50)	t	P
ALT (IU/L)	13.36±4.71	13.18±2.7	0.234	0.815
AST (IU/L)	17.36±4.78	17.72±3.25	0.358	0.227
Alkaline Phosphatase (IU/L)	71.62±12.72	75.76±15.92	0.247	0.154
Serum albumin (gm/dl)	4.07±0.38	4.01±0.51	0.640	0.524

P =probability value N. =number **t** =Student's t test Non significant: P > 0.05 * Significant: P < 0.05

Table (6) demonstrated comparison between the two studied groups regarding liver function tests. There was no statistically significant difference between the two studied groups regarding ALT, AST, Alkaline phosphatase and serum albumin.

In group I the mean value of ALT was 13.36±4.71 IU/L and in group II was 13.18±2.7 IU/L (t=0.234, P-value=0.815)

In group I the mean value for AST was 17.36±4.78 IU/L and in group II was 17.72±3.25 IU/L (t=0.358, P-value=0.227).

In group I the mean value for alkaline phosphatase was 71.62±12.72IU/L and in group II was 75.76±15.92 IU/L (t=0.247, P-value=0.154).

In group I the mean value for serum albumin was 4.07±0.38gm/dl and in group II was 4.01±0.51gm/dl (t=0.640, P-value=0.524).

Table (7) demonstrated comparison between the two studied groups regarding lipid profile. There was no statistically significant difference between the two

studied groups regarding serum cholesterol and triglycerides levels.

In group I the mean value for serum cholesterol was 188.82±20.9 mg/dl and in group II was 189.18±22.8 mg/dl (t=0.082, P-value=0.935).

In group I the mean value for serum triglycerides was 110.34±30.7 mg/dl and in group II was 120.5±36.67 mg/dl (t=0.508, P-value=0.135).

Table (7): Lipid profile in the two studied groups

	Group I (N=50)	Group II (N=50)	t	P
Serum cholesterol (mg/dl)	188.82±20.9	189.18±22.8	0.082	0.935
Serum triglycerides (mg/dl)	110.34±30.7	120.5±36.67	0.508	0.135

P =probability value N. =number **t** =Student's t test Non significant: P > 0.05 * Significant: P < 0.05

Table (8) demonstrated comparison between the two studied groups regarding glycemic profile. There were no statistically significant differences between the two studied groups regarding FBS and 2HP.P but there was statistically significant difference between the two studied groups regarding HBA1c.

In group I the mean value for FBS was 128.7±16.7 mg/dl and in group II was 131.8±25.86 mg/dl (t=0.483, P-value=0.382).

In group I the mean value for 2H P.P was 208.44±25.09 mg/dl and in group II was 211.4±29.09mg/dl (t=0.548, P-value=0.585).

In group I the mean value for HBA1c was 6.29±0.63% and in group II was 7.65±0.64% (t=1.060, P-value=0.032).

Table (8): Glycemic profile in the two studied groups

	Group I (N=50)	Group II (N=50)	t	P
Fasting blood sugar (mg/dl)	128.7±16.7	131.8±25.86	0.483	0.382
Post prandial blood glucose (mg/dl)	208.44±25.09	211.4±29.09	0.548	0.585
HBA1c (%)	6.29±0.63	7.65±0.64	1.060	0.032*

P =probability value N. =number **t** =Student's t test Non significant: P > 0.05 * Significant: P < 0.05

Table (9) demonstrated comparison between the two studied groups regarding kidney functions tests. There were statistically significant differences between the two studied groups regarding serum creatinine, BUN, ACR and uric acid.

In group I the mean value for serum creatinine was 0.95±0.24mg/dl and in group II was 1.35±0.3 mg/dl (t=7.151, P-value=0.00).

In group I the mean value for BUN was 11.6±3.02 mg/dl and in group II was 13.2±3.0 mg/dl (t=2.652, P-value=0.009).

In group I the mean value for ACR was 25.48±2.62 mg/g and in group II was 467.69±294.82 mg/g (t=7.9, P-value=0.00).

In group I the mean value for serum uric acid was 4.39±0.61mg/dl and in group II was 6.25±0.57 mg/dl (t=1.672, P-value=0.004).

Table (9): Kidney function tests in the two studied groups

	Group I (N=50)	Group II (N=50)	t	P
Creatinine (mg/dl)	0.95±0.24	1.35±0.3	7.151	0.00**
BUN (mg/dl)	11.6±3.02	13.2±3.0	2.652	0.009*
ACR (mg/g)	25.48±2.62	467.69±294.82	7.9	0.00**
Uric acid (mg/dl)	4.39±0.61	6.25±0.57	1.672	0.004*

P =probability value N. =number **t** =Student's t test Non significant: P > 0.05 * Significant: P < 0.05

Table (10) demonstrated comparison between the two groups regarding serum Nesfatin-1 levels. There was a statistically significant difference between the two studied groups regarding serum Nesfatin-1 level.

In group I the mean value for serum Nesfatin-1 was 10.57 ± 3.4 mmol/L and in group II was 16.65 ± 5.5 mmol/L ($t=6.595$, $P\text{-value}=0.00$).

Table (10): S. Nesfatin-1 level distribution between the two studied groups

	Group I (N=50)	Group II (N=50)	t	P
Nesfatin-1 (mmol/L)	10.57±3.4	16.65±5.5	6.595	0.00**

P =probability value N. =number t =Student's t test Non significant: $P > 0.05$ * Significant: $P < 0.05$

IV. Correlation between serum Nesfatin-1 level and the study parameters:

Table (11) demonstrated correlations between serum Nesfatin-1 level and study parameters in the two studied groups.

There were statistically significant positive correlations between **serum Nesfatin-1** and **DM duration** ($r=0.648$, $P\text{-value}=0.00$), **serum triglycerides** ($r=0.563$, $P\text{-value} 0.005$), **HBA1c** ($r=0.525$, $P\text{-value}=0.004$), **serum uric acid** ($r=0.521$, $P\text{-value} 0.002$), **serum creatinine** ($r=0.787$, $P\text{-value}=$

0.00), **BUN** ($r=0.856$, $P\text{-value}=0.00$) and **ACR** ($r=0.625$, $P\text{-value}=0.003$) in the studied groups.

But there was no statistically significant correlation between **serum Nesfatin-1** and **age** ($r=0.217$, $P\text{-value}=0.528$), **SBP** ($r=0.379$, $P\text{-value}=0.070$), **DBP** ($r=0.163$, $P\text{-value}=0.141$), **BMI** ($r=0.257$, $P\text{-value}=0.481$), **ALT** ($r=0.361$, $P\text{-value}=0.354$), **AST** ($r=0.322$, $P\text{-value}=0.457$), **serum cholesterol** ($r=0.297$, $P\text{-value}=0.105$), **FBS** ($R=0.199$, $P\text{-value}=0.130$) and **2HP.P** ($r=0.149$, $P\text{-value}=0.145$) in the studied groups.

Table (11): Correlations between serum Nesfatin-1 level and the study parameters:

	S.Nesfatin-1	
	r	P
Age	0.217	0.528
SBP	0.379	0.070
DBP	0.163	0.141
BMI	0.257	0.481
DM duration	0.648	0.00**
ALT	0.361	0.354
AST	0.322	0.457
Cholesterol	0.297	0.105
Triglycerides	0.563	0.005*
FBS	0.199	0.130
2HP.P BG	0.149	0.145
HBA1c	0.525	0.004*
Uric acid	0.521	0.002*
Creatinine	0.787	0.00**
BUN	0.856	0.00**
ACR	0.625	0.003*

P =probability value N. =number r =Correlation coefficient Non-significant: $P > 0.05$ * Significant: $P < 0.05$

Table (12): detection of serum Nesfatin-1 levels cutoff value according to ROC curve regarding the studied groups

Area Under the Curve				
Test Result Variable (s): Serum Nesfatin-1				
Area	CUTOFF	P	95% Confidence Interval (CI)	
			Lower Bound	Upper Bound
0.841	>12.65	0.00**	0.752	0.930

P =probability value Non-significant: $P > 0.05$ * Significant: $P < 0.05$

V. Serum Nesfatin-1 levels cutoff value and its association and agreement in the two studied groups:

Tables (12, 13) Demonstrated that serum Nesfatin-1 had an **optimal cutoff value** > 12.65 mmol/L with area under the ROC curve at 0.841 and at 95% confidence interval (CI), the lower bound was 0.752 and the upper bound was 0.930.

Serum Nesfatin-1 had **Sensitivity** of 80.0% and **Specificity** of 74.0% and statistically high significant difference in the studied groups (P-value 0.000) which proved that serum Nesfatin-1 could be considered as biomarker for early detection of diabetic kidney disease in type 2 diabetic patients.

Table (13): Association and agreement between marker cutoff value and Serum Nesfatin-1 levels in the studied groups

		Group		Total	X ²	P	Kappa agreement	
		Group I	Group II					
Marker	<12.65	N	37	10	47	29.26	0.00**	0.54
		%	74.0%	20.0%	47.0%			
	>12.65	N	13	40	53			
		%	26.0%	80.0%	53.0%			
Total	N	50	50	100				
	%	100.0%	100.0%	100.0%				

P = probability value No. = number χ^2 = chi square test non-significant: P > 0.05 * Significant: P < 0.05

4. Discussion

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn is associated with both microvascular and macrovascular complications. ⁽¹⁾

Diabetic kidney disease (DKD) is one of the most common microvascular complications of DM, greatly affecting the life quality and survival of the patients. As global prevalence of diabetes is steadily increasing, the number of patients with DKD is expanding day by day. DKD is now the leading cause of ESRD, a disease that is described as a worldwide medical catastrophe. ⁽⁷⁾

The routine classical evaluation of DKD includes appearance of albuminuria, decreased creatinine clearance and increased serum creatinine. But, it has been reported that a decline in the renal function of patients with diabetes was not always accompanied by an increased ACR. About 20%-30% of patients with T2DM, accompanied by renal insufficiency, showed normoalbuminuria, which is a condition referred to now as non-proteinuric DKD ⁽⁸⁾, hence the need for new biomarkers for earlier detection of DKD has emerged.

Nesfatin-1 is a newly discovered hypothalamic neuropeptide that regulates appetite. It is an 82 amino-acid peptide originating from the cleavage of nucleobindin2 NUCB2. Nesfatin-1 is expressed in

neurons of various brain areas including PVN, ARC and LHA in hypothalamic nuclei and in the NTS and DMNX at the brainstem level. It has the ability to cross blood brain barrier without molecular saturation. It is also expressed in pancreatic b-cells, where it is co-localized with insulin in secretion vesicles. ^(4, 9)

The middle segment covering the amino acids from 23 to 53, which is called M30 is the responsible for the dose-dependent inhibition of food intake. The amino acid sequencing of this segment is similar to that of alpha-MSH and Agouti-related peptides (AgRP). ⁽¹⁰⁾

Nesfatin-1 has been reported to possess an anti-hyperglycemic effect which is peripheral and time, dose and insulin dependent. Recent experimental studies have also linked nesfatin-1 to enhanced peripheral and hepatic insulin sensitivity, through promoting peripheral glucose uptake and decreasing gluconeogenesis via different pathways. ^(10, 11)

The aim of our study was to evaluate serum Nesfatin-1 as a potential early biomarker of diabetic kidney disease (DKD) in type 2 diabetic patients. It was conducted on 100 adult patients selected from Tanta University Hospital and Ahmed Maher Teaching hospital (Internal Medicine inpatient wards and outpatient clinics). Divided into two groups:

Group I:

Included 50 adult patients with type 2 diabetes and normo-albuminuria (UAE < 30 mg/day).

Group II:

Included 50 adult patients with type 2 diabetes and albuminuria (UAE \geq 30 mg/day).

In our study, there was a statistically significant difference between the mean values of DM duration in the two studied groups (P-value 0.00).

Our results were in agreement with the results reported by **Zeng et al** ⁽¹²⁾ in 2017 who reported that there was a statistically significant difference between the two studied groups regarding DM duration (P-value 0.00).

In our study, there was no statistically significant difference between the mean values of BMI in the two studied groups (P-value 0.717).

Our results were in agreement with the results obtained by **Wei et al** ⁽¹³⁾ in 2018 who reported that there was no statistically significant difference between the studied groups regarding BMI (P-value =0.08).

On the other hand our results were in disagreement with the results reported by **Abd-Elaaty et al** ⁽¹⁴⁾ in (2017) who reported that there was a statistically significant difference between the studied groups regarding BMI (P-value<0.001).

The previous study carried out by **Abd-Elaaty et al** ⁽¹⁴⁾ may have differed from ours regarding BMI distribution as they hadn't exclude obese patients with BMI \geq 30 mg/kg² as we had excluded them in our study.

In our study, there were no statistically significant differences between the two studied groups regarding the mean values of HB, TLC and PLT (P-value=0.431, 0.357 and 0.894 respectively).

Our results regarding HB, TLC and PLT were comparable with the results reported by **Kahraman et al** ⁽¹⁵⁾ in 2016 who reported that there were a statistically significant difference between the three studied groups regarding HB (P-value=0.006) but there was no statistically significant difference regarding TLC and PLT between the studied groups (P-value= 0.846 and 0.104 respectively).

The results obtained by **Kahraman et al** ⁽¹⁵⁾ regarding HB level may be differed from our study as anemia were recorded in the macro-albuminuric group in contrary to our study which may be due to the progression of DKD causing decreased erythropoietin secretion with the interstitial affection of the kidneys.

In our study, there were no statistically significant differences regarding serum cholesterol and triglycerides between the two studied groups (P-value=0.935, 0.135 respectively).

We were in agreement with the results reported by **Khandare et al** ⁽¹⁶⁾ in 2017 who reported that there were no statistically significant differences between the two studied groups (group 1: T2DM patients with normo-albuminuria and group 2: T2DM patients with

albuminuria) regarding serum cholesterol and triglycerides (P-value=0.742 and 0.48 respectively).

On the other hand our results regarding serum cholesterol and triglycerides were in disagreement with the results reported by **Mahendran et al** ⁽¹⁷⁾ in 2016 who reported that there were statistically significant differences between the three studied groups regarding serum cholesterol and triglycerides.

Our results regarding serum cholesterol and triglycerides may have differed from the results obtained by **Mahendran et al** ⁽¹⁷⁾ as obese patients with BMI \geq 30 and patients with uncontrolled dyslipidemia weren't excluded.

In our study, there were no statistically significant differences between the two studied groups regarding FBS and 2HP.P (P-value 0.382 and 0.585 respectively) while there was a statistically significant difference regarding HBA1c (P-value= 0.032).

Our results were in agreement with the results reported by **Amer et al** ⁽¹⁸⁾ in 2018 who reported that there was no statistically significant difference regarding FBS (P-value=0.082) but there was a statistically significant difference regarding HBA1c (P-value<0.01) between the studied groups.

Our results regarding glycemic profile were in agreement regarding FBS and disagreement regarding HBA1c with the results reported by **Wang et al** ⁽¹⁹⁾ in 2015 who reported that there were no statistically significant differences between the three studied groups regarding FBS and HBA1c (P-value =0.541 and 0.328 respectively).

Our study results regarding HBA1c may have differed from the results obtained by **Wang et al** ⁽¹⁹⁾ as tighter control of DM were achieved especially in our normo-albuminuric group while patients with poor compliance on treatment or uncontrolled DM weren't excluded from the other study.

Also our results regarding 2HP.P BG were in agreement with the results reported by **Khandare et al** ⁽¹⁶⁾ who reported that there was no statistically significant difference between the two studied groups regarding 2HP.P BG (P-value=0.342).

In our study, there was a statistically significant value regarding serum uric acid between the two studied groups (P-value=0.004).

Our results were in agreement with the results reported by **Irannejad et al** ⁽²⁰⁾ who reported that there was a statistically significant difference between the two studied groups regarding serum uric acid (P-value=0.001).

In our study, there was a statistically significant difference between the two studied groups regarding serum creatinine (P-value=0.003).

Our results were in agreement with the results reported by **Wu et al** ⁽²¹⁾ in 2017 who reported that there was a statistically significant difference

regarding serum creatinine between the studied groups (P-value<0.001 for each).

On the other hand, our results were in disagreement with the results obtained by **Kocak et al**⁽²²⁾ in 2018 who reported that there were no statistically significant differences between the two groups regarding serum creatinine (P-value 0.059).

In our study, there was a statistically significant difference between the two studied groups regarding BUN (P-value=0.009).

Our results were in disagreement with the results obtained by **Khandare et al**⁽¹⁶⁾ who reported that there was no statistically significant difference between the two studied groups regarding serum urea (P-value=0.105).

Our study results regarding serum creatinine and BUN levels may have differed from the results obtained by **Kocak et al**⁽²²⁾ and **Khandare et al**⁽¹⁶⁾ as it has been reported that a decline in the renal function of patients with diabetes was not always accompanied by an increased ACR. About 20%-30% of patients with T2DM, accompanied by renal insufficiency, showed normoalbuminuria, which is a condition referred to now as non-proteinuric DKD⁽⁸⁾.

In our study, there was a statistically significant difference between the two studied groups regarding ACR (P-value=0.00)

Our results were in agreement with the results reported by **Zeng et al**⁽¹²⁸⁾ in which there was a statistically significant difference between the two studied groups regarding ACR (P-value=0.00).

In our study, there was a statistically significant difference between the two studied groups regarding serum Nesfatin-1 level (P-value 0.00).

Our results were in agreement with the results reported by **Irannejad et al**⁽²⁰⁾ in which there was a statistically significant difference between the two studied groups (P-value<0.001).

Also our results were in agreement with the results reported by **Sonbol et al**⁽²³⁾ in 2018 in which there was a statistically significant difference between the studied groups regarding serum Nesfatin-1 (P-value<0.001).

While our results were in disagreement with the results reported by **Abd-Elaaty et al**⁽¹⁴⁾ in which there was no statistically significant difference between the studied groups regarding serum Nesfatin-1 level (P-value 0.564).

The results obtained by **Abd-Elaaty et al**⁽¹⁴⁾ regarding serum Nesfatin-1 level may have differed from ours as patients on drugs which may affect serum Nesfatin-1 level as anti-epileptic drugs or patients with special habits of smoking or alcohol consumption weren't excluded while we did exclude them.

In our study, There was no correlation between serum Nesfatin-1 and BMI in the studied groups (r=0.257, P-value=0.481).

Our results were in disagreement with the results reported by **Guo et al**⁽²⁴⁾ in 2014 who reported that serum Nesfatin-1 level had statistically negative correlation with BMI which differed from our study probably because they included cases with only a BMI ≥ 28 and excluded patients with IGT and DM.

While we were in agreement with the results reported by **Kuyumcu et al**⁽²⁵⁾ in 2018 who reported that there was no correlation between serum Nesfatin-1 level and BMI (r=0.06, P-value=0.70).

In our study, there was a statistically positive correlation between serum Nesfatin-1 and HBA1c in the studied groups (r=0.525, P-value=0.004).

We were in agreement with the results reported by **Zhang et al**⁽²⁶⁾ in 2012 who reported that plasma Nesfatin-1 level correlated positively with HBA1c in both IGT and newly diagnosed T2DM patients groups.

But we were in disagreement with the results reported by **Fupeng et al**⁽²⁷⁾ in 2014 who reported that plasma Nesfatin-1 level had a statistically negative correlation with HBA1c in both T2DM and IGT patients groups.

Our results regarding the positive correlation between serum Nesfatin-1 level and HBA1c may have differed from the results obtained by **Fupeng et al**⁽²⁷⁾ as the patients in their study groups had thyroid dysfunction with high TSH level which had a negative correlation with serum Nesfatin-1 level in their study and may have a greater influence on serum Nesfatin-1 level than DM.

In our study, serum Nesfatin-1 had statistically positive correlations with serum creatinine (r=0.787, P-value= 0.00) and ACR (r=0.625, P-value=0.003) in both studied groups.

Our results were in agreement with the results reported by **Sonbol et al**⁽²³⁾ who reported positive correlations between serum Nesfatin-1 level and both serum creatinine (r=0.640, P-value<0.001) and ACR (r=0.511, P-value<0.001) in normo-albuminuric, micro-albuminuric and macro-albuminuric groups. Also we agreed with the results reported by **Irannejad et al**⁽²⁰⁾ which showed positive correlations between serum Nesfatin-1 level serum creatinine (r=0.282, P-value=0.008) and ACR (r=0.595, P-value>0.001) in both normo-albuminuric and micro-albuminuric groups.

In our study, serum Nesfatin-1 had an optimal cutoff value > 12.65 mmol/L with area under the ROC curve at 0.841 with Sensitivity 80.0% and Specificity 74.0% in the studied groups for association with DM nephropathy in T2DM patients.

To the best of our knowledge, there was no previous study which discussed a ROC curve for the

relation between serum Nesfatin-1 and DM nephropathy in T2DM patients.

Our study had no control group consisting of healthy subjects without T2DM but the assay range for serum Nesfatin-1 stated by the manufacturer of the kits used in our work was 0.2mmol/L. The normo-albuminuric group had a mean value of serum Nesfatin-1 level of 10.57 ± 3.4 mmol/L, while the albuminuric group had a mean value of 16.65 ± 5.5 mmol/L which showed significant increase in serum Nesfatin-1 with the presence of DM and the progression of DKD.

Finally, we concluded that serum Nesfatin-1 level could be considered as an early marker for diabetic nephropathy in T2DM patients.

Recommendations

1. Further large scale studies are needed to investigate the role and the relation of Nesfatin-1 to diabetic nephropathy in type 2 diabetic patients.

2. Serum Nesfatin-1 can be used as an early biomarker for detection of DKD before the development of albuminuria which may appear late after the pathological renal changes are well established.

3. Our study had some important limitations; first, our research was carried out in a cross-sectional design and the causal associations could not be addressed. Second, the practice gold standard to diagnose diabetic nephropathy is renal biopsy, whereas we used UAE classification of albuminuria as a non-invasive marker and substitute of this diagnostic gold standard. Third, the relatively small sample size of patients included in this study. Lastly, lack of a control group consisting of disease-free subjects without T2DM was another important limitation of this preliminary work.

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