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Evaluation of selected diagnostic biomarkers with cardiovascular and nephrotic complications in diabetic Egyptian males

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A B S T R A C T

Diabetes is well-known disease for its complication and increasing population over time, therefore new methods for complication assessment should be found. The aim of this study is to determine levels of nesfatin-1, von Willebrand factor (vWF) and N-acetyl-β-D-glucosaminidase (NAG) in Egyptian type 2 diabetes (T2DM) with and without complications, their assessment as possible markers in tracing diabetes progression in addition to finding a correlation between these three parameters. Sixty-seven Egyptian male patients were divided into three groups as follows: 25 with T2DM without any complication, 22 with diabetic nephropathy (DN) and 20 patients with diabetic cardiovascular complications (DC). Nesfatin-1, vWF, NAG, HOMA-IR (Homeostasis model assessment of insulin resistance), HOMA-β (Homeostasis model assessment of β-cell function) were all determined using ELISA technique , while the fasting blood glucose (FBG), lipid profile and the kidney parameters were analyzed using the biochemistry system, results were compared to 19 healthy controls with same age. Correlation studies were done between all the studied parameters. All diabetic patients showed a significant increase compared to control in the following parameters: (FBG), glycosylated haemoglobin (HbA_{1C}), HOMA-IR, serum urea, serum creatinine, albumin creatinine ratio (ACR), triglyceride (TG), total cholesterol (TC), atherogenic indices. Significant decrease was seen in HOMA-β, meanwhile LDL-C and HDL-C showed no statistical significance change compared to control. The studied parameters nesfatin-1 and vWF showed statistical significance increase with all diabetic groups, while NAG showed significance increase only with (DN). In conclusions, there was direct correlations between (nesfatin-1 and HOMA-β), (vWF and ACR) and (HbA_{1C} and FBG). No correlation was found between the three studied parameters, however these three markers showed significant increase relative to control where nesfatin-1, vWF can be used in tracing of both complications (DN and DC), and NAG can be used only in tracing of DN.

Introduction

Diabetes mellitus especially type 2 (T2DM) has affected 390 million all over the world and this number is expected to double by year 2030 [1]. Long term complications are most common in T2DM affecting many organs, e.g. cardiovascular disease (CVD) which is a major cause for mortality, thus life expectancy of patients may increase by the management of this complication [2]. The other complication is diabetic nephropathy (DN) affecting 40% of (T2DM) patients which has become the major cause of end stage renal disease [3].

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Nesfatin-1 is a fat-influencing protein [4] and encodes satiety in the hypothalamus, when given intravenously to the hyperglycemic animals; it showed an anti-hyperglycemic effect [5]. According to [6], it might play a role in appetite regulation. Nesfatin-1 is considered as an anorexigenic adipokine [6] it was found in pancreatic cells and it stimulated glucagon secretion [7] thus it may have a relation with diabetes and a role in insulin secretion. There are three types of nesfatin: Nesfatin-1: originated from post translational processing of the N-terminal fragment of nucleobindin 2 (NUCB2). NUCB2 is a 396-amino-acid protein exceptionally conserved across mammalian species; nesfatin-1 consists of 82-amino-acids.

The NUCB-2 post translational cleavage gives nesfatin-2 fragment (85–163) and nesfatin-3 fragment (166–396) [4]. As shown in **Fig. 1**. According to [6] there is no information about the function of nesfatin-2 and 3.

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme present in proximal tubular cells, with very high molecular weight (130-140 kDa), it is normally excreted in urine in low amounts by exocytosis in proximal tubules, but excreted in abnormally higher amounts if the cells are exposed to hyperglycemia [8]. von Willebrand Factor (vWF) is a multimeric glycoprotein synthesized mainly by the endothelial cells and is considered as a diabetic neuropathy and nephropathy marker, the diabetic microangiopathy onset is preceded by endothelial dysfunction [9]. The aim of this study was to investigate the relationship of (nesfatin-1, NAG, vWF) with diabetes complications such as (DN) and (DC) in addition to their correlation between each other in Egyptian (T2DM) patients and to investigate the possibility of taking these three parameters as markers for tracing the progression of diabetes. Possible correlations between the other studied parameters were also evaluated.

Subject and Methods

Ethical considerations

A written consent was given to all patients for participation, and the study was done and approved by the ethics committee of NIDE (National Institute for Diabetes and Endocrinology), with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The ethical approval number accredited at NIDE was **(IDE00231).** All patients were clinically diagnosed with T2DM according to the American Diabetic Association.

Sources of data

The present study comprised 67 Egyptian males diagnosed with "T2DM" recruited from the outpatient clinics of the National Institute for Diabetes and Endocrinology "NIDE", Cairo, Egypt. Male control patients with comparable age (range 45-66 years) were studied. All subjects underwent careful physical examination, detailed clinical and familial history and laboratory investigation.

Types of data:

a-19 Controls with the same age and sex.

b-25 patients with T2DM and no complication.

c-22 patients with T2DM and nephropathy complication (DN).

d-20 patients with T2DM and cardiovascular complication (DC).

Inclusion criteria

Patients with T2DM or with DN or DC. Controls have no acute or clinical conditions involving the endocrine-metabolic system to exclude hormonal effect, not receiving any medications affecting the endocrine-metabolic system (e.g. anti-thyroids, glucocorticosteroids, …etc.).

Exclusion criteria

Patients who are receiving any insulin or any medications other than oral antihyperglycemic drugs were excluded in addition to patients having nutritional derangement that might affect the study or patients complaining from any acute or chronic illness as confirmed by physical examination and laboratory investigation.

Sample collection:

Sample collection and analysis were done in National Institute for Diabetes and Endocrinology (NIDE). After overnight fasting, blood samples were withdrawn and collected in clotting activator-containing/ serum separating tubes; for investigation of biochemical parameters, centrifuged and serum was separated and divided into four aliquots; one used for immediate determination of all blood biochemical parameters and the other three for ELISA determination (Nesfatin-1, vWF, and insulin). EDTA-containing tubes were used for HbA_{1C} determination and florid-containing tubes were used to determine glucose immediately in the plasma. The BT3500 Chemistry System (Biotechnica instruments Inc., Italy); was used for all blood biochemical parameters including (FBG, serum urea, serum creatinine and ACR, TG, TC, LDL-C, HDL-C). Hemoglobin testing system: Tosoh G8 (Japan); was used for determination of HbA_{1C}.The atherogenic indices were calculated as follows: ratio "1": LDL-C / HDL-C [10], ratio "2": TC / HDL-C [10], ratio "3": Log TG/ HDL-C [11].

Weight and height were measured, and BMI was calculated as {weight (Kg)/height^2 (m^2)}. Insulin was determined by ELISA technique, homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to [12] {(Fasting insulin (μ IU/ml) x (Fasting glucose (mmol/L)/22.5}, Homeostasis model assessment-β cell function (HOMA-β) was calculated according to [12] {(Fasting insulin (μIU/ml) x 20/ (Fasting glucose (mmol/L)-3.5)}. First morning urine void was collected from all subjects on the day of their clinic visit, it was used for the determination of microalbuminuria quantitatively and automatically using ADVIA® 1650 clinical chemistry system, and in determination of NAG by ELISA. Other biochemical parameters including (nesfatin-1, vWF) were determined by ELISA techniques, purchased from Glory Science Co., Ltd (USA).

Statistical analysis

Statistical analysis were done using one way (ANOVA). Parametric data was first tried, followed by post hoc test if Bartlett's test was not significant. When Bartlett's test was significant, logarithmic or reciprocal transformation was performed. Master plex program was used for calculation of nesfatin-1, vWF and NAG. All data were expressed as M ± SEM (mean ± standard error). The correlation co-efficient was calculated using least square method. Data were considered significant if $P < 0.05$.

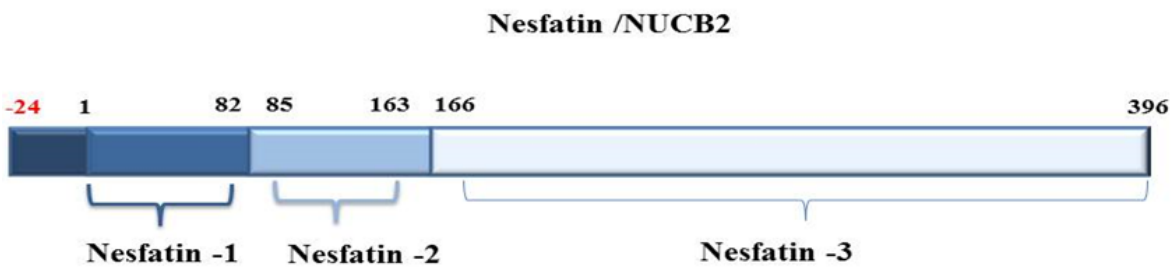

Fig. 1: Molecular structure of nesfatin/NUCB and amino acid sequence in human.

Results

Clinical and demographic variables of the studied groups are shown in **Table 1**, concerning age there was no statistically significant difference between the diabetic groups and the control. Glycemic indices are shown in **Table 2** where FBG, HbA_{1C} and HOMA-IR showed a significant increase $P < 0.001$ in all diabetic patients compared to control. Insulin showed highly significant increase (21.3 ± 2.16 and 20.9 ± 2.04) $P < 0.001$ in DN and DC groups respectively compared to both control and T2DM (9.35 ± 0.92 and 11.27 ± 1.31 respectively) while HOMA-β showed a significant decrease $P < 0.001$ in all diabetic groups relative to control.

Metabolic variables of the studied subjects are shown in **Table 3**. Serum urea showed a highly significant increase (32.99 ± 1.25 and 31.64 ± 0.78 respectively) $P < 0.001$ in DN and DC groups when compared to both control and T2DM (28.2 ± 0.68 and 28.92 ± 0.75 respectively), while serum creatinine showed a significant increase $P < 0.001$ in DN relative to all the studied groups. Micro albuminuria and ACR showed increase in DN and DC compared to the other groups $P < 0.001$. NAG showed increase $P < 0.05$ in DN only (7.9 ± 0.144) when compared to control and T2DM (7.39 ± 0.13 and 7.45 ± 0.135 respectively).

Serum levels of HDL-C and LDL-C showed no significant difference between the diabetic and control group, while TC showed significant increase $P < 0.01$ only due to DC compared to control.TG showed a significant increase $P < 0.001$ in DC (190 ± 8.45) compared with control and T2DM (109.7 ± 9.34 and 133.32 ± 12.49 respectively), and DN (181.09 ± 17.8) compared with control. Ratio "1" showed significant increase $P < 0.05$ only due to DC (4.04 ± 0.23) relative to control (3.15 ± 0.18), ratio "2 and 3" showed significant increase $P < 0.001$ due DC and DN relative to control, and relative to T2DM for ratio "3" only.

Both nesfatin-1 and vWF showed statistically significant increase $P < 0.001$ in all groups compared to control group. Although nesfatin-1 showed no discriminative ability among diabetic subgroups, vWF showed significant increase $P < 0.001$ comparing all groups with control and comparing DN with T2DM.

Table 1: Clinical, demographic variables (mean ± SEM)

	C(n=19)	T2DM (n=25)	DN (n=22)	DC (n=20)	*P*
Age (years)	53.4±1.3	55±1.4	53.7±1.04	57.1±1.3	-
Weight (Kg)	84.68±3.06	83.6±2.77	96.2±3.25	89.3±2.84	-
Height (m)	1.73±2.3	1.75±1.87	1.78±1.26	1.70±2.69	-
BMI (Kg/m^2)	28.1±0.8	27.07±0.75	30.16±0.87[b]	30.7±0.96[b]	*P*<0.01
T2DM duration (years)	-	6.09±0.55	7.68±0.8	8±0.77	-
Systolic BP (mmHg)	118.15±1.5	131.1±3.7	139±6.06[*]	141.45±4.9[**]	*P*<0.01
Diastolic BP (mmHg)	78.4±1.57	74.2±3.2	82.7±2.47	82±2.57	-

Number "n", Control "C", type 2 diabetes mellitus "T2DM", T2DM + nephropathy "DN", T2DM with cardiovascular complication "DC", body mass index "BMI", BP: Blood pressure, [*]: $P < 0.05$, [**]: $P < 0.01$ compared to "control" group using Kruskal-Wallis ANOVA followed by Dunn's multiple comparisons test. [b]: $p < 0.05$ compared to "T2DM" group using one-way ANOVA followed by Tukey-Kramer multiple comparisons test.

Table 2: Glycemic indices of the studied population (mean ± SEM)

	C (n=19)	T2DM(n=25)	DN (n=22)	DC (n=20)	*P*
FBG (mg/dl)	93.1±2.82	169.7±9.05***	237.4±14.49***	210.4±10.3***	$P < 0.001$
HbA_{1C} (%)	5.68±0.05	8.34±0.33***	10.66±0.41$^{***, \#\#}$	8.92±0.34***	$P < 0.001$
Insulin(µIU/ml)	9.35±0.92	11.27±1.31	21.3±2.16 $^{aaa, bbb}$	20.9±2.04$^{aaa, bbb}$	$P < 0.001$
HOMA-IR	2.19±0.22	4.79±0.7^{aaa}	12.09±1.47$^{aaa, bbb}$	11.2±1.27$^{aaa, bbb}$	$P < 0.001$
HOMA-β	113±11.98	46±6.52 ^{aaa}	49.4±6.5 ^{aaa}	57±7.6^{aaa}	$P < 0.001$

Number "n", Control "C", type 2 diabetes mellitus "T2DM", T2DM+ nephropathy "DN", T2DM with cardiovascular complication "DC", fasting blood glucose "FBG", glycated hemoglobin "HbA_{1C}", homeostasis model assessment of insulin resistance "HOMA-IR", homeostasis model assessment of beta cell function "HOMA-β". ***: $P < 0.001$ compared to "control" group, $^{\#\#}$: $P < 0.01$ compared to "T2DM" group using Kruskal-Wallis ANOVA followed by Dunn's multiple comparisons test. ^{aaa}: $P < 0.001$ compared to "control" group, ^{bbb}: $P < 0.001$ compared to "T2DM" group using one-way ANOVA followed by Tukey-Kramer multiple comparisons test.

Table 3: Metabolic variables of the studied population (mean ± SEM)

	C (n= 19)	T2DM (n= 25)	DN (n= 22)	DC (n= 20)	P
s urea (mg/dl)	28.2±0.68	28.92±0.75	32.99±1.25 ^{aa}	31.64±0.78 ^{bb}	$P < 0.001$
s creat. (mg/dl)	0.95±0.027	1.05±0.04	1.24±0.06$^{aaa, b}$	0.99±0.04 ^{cc}	$P < 0.001$
microalbuminuria(mg/dl)	12±1.8	12.55±1.96	694.2±125.3$^{***, \#\#\#}$	21.20±2.54^{oo}	$P < 0.001$
ACR (mg/gm)	8.26±1.18	9.77±1.43	770±162.35$^{***, \#\#\#}$	18.44±1.42$^{*, \#, oo}$	$P < 0.001$
NAG (U/L)	7.39±0.13	7.45±0.135	7.9±0.144$^{a, b}$	7.7±0.06	$P < 0.05$
TG (mg/dl)	109.7±9.34	133.32±12.49	181.09±17.8*	190±8.45$^{***, \#\#}$	$P < 0.001$
TC (mg/dl)	178±4.99	189.11±7.54	201.62±5.73	211±7.66^{aa}	$P < 0.01$
LDL-C (mg/dl)	117.8 ±5.25	126.52 ±5.96	130.68 ±5.4	138.1 ±6.44	-
HDL-C (mg/dl)	38.26±1.32	35.96±1.88	34.73±0.89	34.9±1.30	-
Ratio "1" :(LDL-C/HDL-C)	3.15±0.18	3.6±0.21	3.7±0.14	4.04±0.23^{a}	$P < 0.05$
Ratio "2" :(TC/HDL-C)	4.75± 0.21	5.44±0.25	5.85±0.17^{a}	6.17±0.28^{aaa}	$P < 0.001$
Ratio "3": (Log TG/HDL-C)	0.43±0.05	0.53±0.05	0.68±0.05 ^{aa}	0.73±0.03$^{aaa, bb}$	$P < 0.001$
Nesfatin-1 (pg/ml)	11.59±0.41	13.95±0.25^{aaa}	14.8±0.28^{aaa}	14.1±0.33^{aaa}	$P < 0.001$
vWF (U/L)	162±8.5	782±45.1^{aaa}	1002±29.8$^{aaa, bbb}$	865±47.2^{aaa}	$P < 0.001$

Number "n", control "C", type 2 diabetes mellitus "T2DM", T2DM+ nephropathy "DN", T2DM with cardiovascular complication "DC", serum "s", creatinine "creat.", albumin creatinine ratio "ACR", von Willebrand Factor "vWF", NAG "N-acetyl-β-D-glucosaminidase" TG "Triglyceride", TC "total cholesterol". ^{a}: $P < 0.05$, ^{aa}: $P < 0.01$, ^{aaa}: $P < 0.001$ compared to "control" group, ^{b}: $P < 0.05$, ^{bb}: $P < 0.01$, ^{bbb}: $P < 0.001$ compared to "T2DM" group, ^{cc}: $P < 0.01$ compared to "DN" group using one way ANOVA followed by Tukey-Kramer multiple comparisons test. *: $P < 0.05$, ***: $P < 0.001$ compared to "control" group, $^{\#}$: $P < 0.05$, $^{\#\#}$: $P < 0.01$, $^{\#\#\#}$: $P < 0.001$ compared to "T2DM" group, ^{oo}: $P < 0.01$ compared to "DN" using Kruskal-Wallis ANOVA followed by Dunn's multiple comparisons test. Due to sample size, number of samples for NAG was 18 and 20 in C & DN respectively and for nesfatin-1 and vWF the control was 18 and 20 in C& DN respectively.

Simple linear regression analysis are shown in **Table 4** and revealed that there was a positive direct correlation between the following parameters: FBG vs. HOMA-IR, nesfatin-1 vs. HOMA-β $P < 0.05$ **Fig. 2**, vWF vs. {ACR $P < 0.001$ **Fig. 3**, HbA_{1C} $P < 0.001$ **Fig. 4** and FBG $P < 0.01$ **Fig. 5** }, serum urea vs.{TC $P < 0.01$ and LDL-C $P < 0.05$}, TG vs.{ insulin $P < 0.001$, HOMA-IR $P < 0.05$, HOMA- β $P < 0.05$}, Ratio "2" vs. {insulin $P < 0.05$, HOMA-IR $P < 0.05$}, Ratio "3" vs.{Insulin $P < 0.01$, HOMA-IR $P < 0.01$, HOMA- β $P < 0.05$, ACR vs. HbA_{1C} $P < 0.05$.The correlation between the three parameters was non-significant.

Table 4: Correlations between the investigated parameters

Parameter	R	*P* value	Parameter	r	*P* value
FBG vs. HOMA-IR	0.634	< 0.01	Ratio "3" vs. HOMA-IR	0.3260	< 0.01
HbA_{1C} vs. ACR	0.3791	< 0.05	Ratio "3" vs. HOMA-β	0.3102	< 0.05
TG vs. Insulin	0.3423	< 0.01	Urea vs. TC	0.3395	< 0.01
TG vs. HOMA-IR	0.2838	< 0.05	Urea vs. LDL-C	0.265	< 0.05
TG vs. HOMA-β	0.2671	< 0.05	Nesfatin-1 vs. HOMA-β	0.2794	< 0.05
Ratio "2" vs. insulin	0.2718	< 0.05	vWF vs. ACR	0.3209	< 0.001
Ratio "2" vs. HOMA-IR	0.2853	< 0.05	vWF vs. HbA_{1C}	0.4252	< 0.001
Ratio "3" vs. Insulin	0.3942	< 0.01	vWF vs. FBG	0.397	< 0.01
vWF vs.NAG vs. nesfatin-1		NS			

Pearson rank correlation coefficient (r) assuming Gaussian distributions. All correlations were done in-between all parameters and only the significant correlations were mentioned in the table. FBG "Fasting blood glucose", HOMA-IR "Homeostasis model assessment of insulin resistance", HOMA-β "Homeostasis model assessment of β-cell function", TG "Triglyceride", TC "total cholesterol", ACR "albumin creatinine ratio", vWF "von Willebrand factor", NAG "N-acetyl Beta glycosaminidase", glycated hemoglobin "HbA_{1C}", Ratio 1: LDL-C/ HDL-C, Ratio 2: T-C/HDL-C, Ratio 3: Log10 TAG/ HDL-C, NS: non-significant.

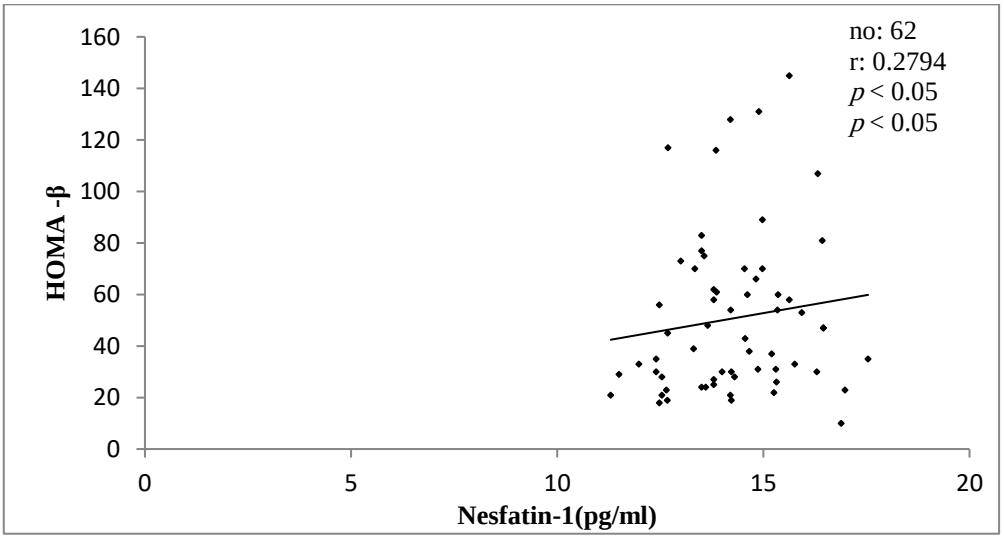

Fig. 2: Linear regression between nesfatin-1 (pg/ml) and HOMA-β "Homeostasis model assessment of of β-cell function". Pearson rank correlation coefficient (r), total number of diabetic patients (no). Direct, significant correlation.

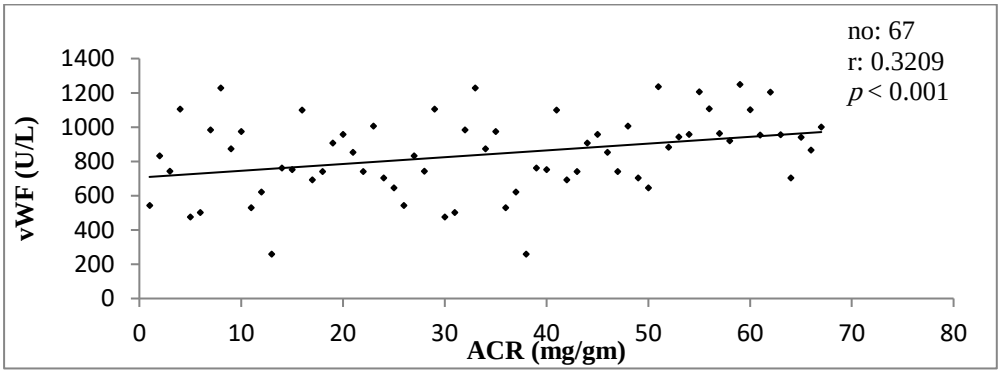

Fig. 3: Linear regression between ACR "albumin creatinine ratio" (mg/gm) and vWF "von Willebrand factor" (U/L). Pearson rank correlation coefficient (r), total number of diabetic patients (no). Direct, significant correlation.

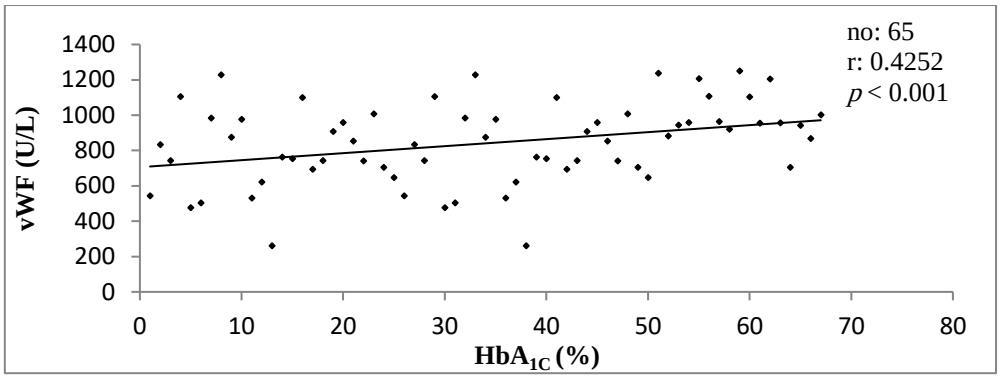

Fig. 4: Linear regression between HbA_{1C} "glycated haemoglobin" (%) and vWF (U/L). Pearson rank correlation coefficient (r), total number of patients (no). Direct, significant correlation.

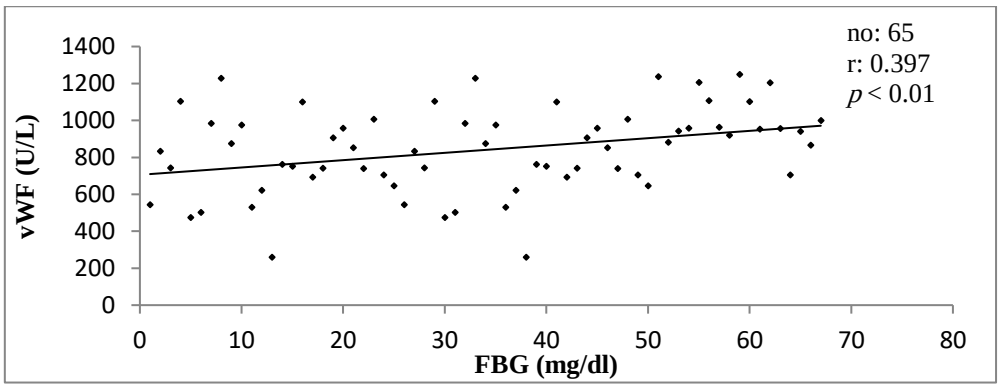

Fig. 5: Linear regression between FBG "fasting blood glucose" (mg/dl) and vWF "von Willebrand factor" (U/L). Pearson rank correlation coefficient (r), total number of diabetic patients (no). Direct, significant correlation.

Discussion

Nesfatin-1, vWF and NAG are known for their relation with diabetes, so a correlation between them was proposed, as well as suggesting them as possible markers in tracing the diabetes complication progression. In the present study both FBG and HbA_{1C} showed an extremely significant increase in all the diabetic groups, the same as recoded by [13]. Insulin and HOMA-IR showed statistically significant increase than control, thus proving the increase resistance in insulin with increasing complication and this was in accordance with [14] who reported also that insulin resistance (IR) has a strong relation with cardiovascular disease. HOMA-β showed significant decrease thus showing pancreatic dysfunction with the progression of diabetic complication, the same finding was recorded by [15]. Thus, both HOMA-IR and. HOMA-β can also be taken as possible markers for diabetes and its complications.

In the current study, serum urea showed a statistically significant increase in DN and DC, the same finding was recorded by [16], who found significant increase in Egyptian T2DM patients with and without complications. Serum creatinine showed a slight significant increase in DN and DC, while T2DM showed no difference. Micro albuminuria and ACR both showed increase due to DN and DC, this was in agreement with [13] who found a significant increase in ACR when comparing 30 Egyptian controls to 38 albuminuric diabetic patients. In this study NAG showed a significant increase only due to DN this was in accordance with [17] who found correlation with NAG and diabetic nephropathy, he also declared that NAG can be taken in early atherosclerosis detection much more than the damage in the glomeruli in T2DM patients. In contrast to our finding [18] NAG showed a weak prognostic ability for detection of kidney disease in T2DM.

In the current study LDL-C and HDL-C showed no significant difference between the diabetic groups and control group, this result was in accordance with[14], while [19] found decrease in HDL-C and attributed this to the mild hyperglycemia which causes a significant decrease of HDL function. In this study TG and TC showed a significant increase due to DC, but TG alone showed an increase in DN with no significance in T2DM, these results are matched with [14] who found no significant change in TG of T2DM.

In the present study the three ratios showed significant increase in DC thus indicating the increase risk of heart diseases with increasing diabetic complications, while ratios "2 and 3" showed a significant increase in DN. Opposite to our results [20], found a significant increase $P < 0.0001$ in TC/HDL-C ratio of T2DM patients compared to control.

Nesfatin-1 showed a significant increase in all the diabetic groups relative to control, which was in accordance with [21] who found increase in nesfatin of newly T2DM, while the opposite finding was found by [22] where nesfatin-1 showed decrease in T2DM compared to control. This discrepancy in results were attributed to the difference in the design of study which includes selection of patients (e. g., lean vs. obese, glycemia level, type of diet) and experimental conditions [22]. Another explanation was that some of the patients had concomitant obesity, thus can be accompanied by adipocytokine level changes. Also, nesfatin-1 production by adipose tissue increases obesity and varies due to the type of food [6]. So, nesfatin-1 circulation may be affected by T2DM duration, and that nesfatin-1 might be a good indicator for T2DM progression and a target for the treatment of antidiabetic [23]. Also [24] reported that nesfatin-1 could be taken as biomarker for assessment of diabetes progression. It was declared by [25] a possible role of nesfatin-1 as potential novel biomarker for early detection and prediction of gestational diabetes mellitus.

In the present study vWF also showed a significant increase in all the diabetic groups relative to control. This was in line with [26] where the plasma levels of vWF were significantly elevated in patients with T2DM complicated by CVD, he stated that vWF could be characterized in prognosis of cardiovascular complications in T2DM patients. Our data was in line too with the Egyptian study of [16] showing significant increase in T2DM patients with and without microalbuminuria.

According to [27] the increased vWF levels, may reflect the damage or the activation to the endothelial cells, and it is involved in platelet aggregation and adhesion thus acting as a carrier of coagulation factor VIII in plasma [28]. It was proved by [29] that vWF has direct relation with diabetic patients above 60 years, and that the duration of T2DM can influence the endothelium causing vWF secretion. Thus, both nesfatin-1 and vWF could be taken as possible markers not only for diabetes but for the complications (DN and DC).

Correlation study showed indirect correlation of nesfatin-1 with HOMA-β suggesting a relation with pancreas deterioration. Also, direct significant correlation is found between HOMA-IR (FBG, TG, Ratio "2 and 3"). These data were in line with [30] who found a correlation between HOMA-IR and TG and in line with [31] who found significant positive correlation between HOMA-IR and both TG and FBG. This was contrary to the findings of [32] who found no association between HOMA-IR (FBG and lipid parameters).

In the current study urea showed a direct correlation between (TC, LDL-C, serum creatinine and ACR), while the ACR showed a weak direct relationship with (s creatinine and HbA_{1C}). vWF showed a direct relationship with ACR, HbA_{1C} and FBG.

Correlation study didn't show any relationship between nesfatin-1, vWF and NAG.

Conclusions

In conclusion, the current study demonstrated that both nesfatin-1 and vWF may be used as diagnostic biomarkers in the clinical follow up of DN and DC, whereas, NAG may be used only in DN.

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Conflicts of interest: There are no conflicts of interest.

References

1) **International diabetes federation (2017).** IDF Diabetes Atlas, 8th edn. Brussels, Belgium.
2) **Olafsdottir, E., Aspelund, T., Sigurdsson, G., Benediktsson, R., Thorsson, B., Harris, T. B., Launer, L. J., Eiriksodottir, G. and Gudnason, V. (2013).** Similar decline in mortality rate of older persons with and without type 2 diabetes between 1993 and 2004: the Icelandic population-based Reykjavik and AGES-Reykjavik cohort studies. BMC Public Health, 13-36.
3) **Hasslacher, C., Ritz, E., Wahl, P. and Michael, C. (1989).** Similar risks of nephropathy in patients with type 1 or type 2 diabetes mellitus. Nephrol. Dial. Transplant., **4**: 859-863.
4) **Oh-I, S., Shimizu, H., Satoh, T., Okada, S., Adachi, S., Inoue, K., Eguchi, H., Yamamoto, M., Imaki, T., Hashimoto, K., Tsuchiya, T., Monden, T., Horiguchi, K., Yamada, M. and Mori, M. (2006).** Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature, **443**: 709-712.
5) **Shimizu, H., Ohsaki, A., Oh-I, S., Okada, S. and Mori, M. (2006).** A new anorexigenic protein, nesfatin-1. Peptides, **30**(5): 995-998.
6) **Ramanjaneya M., Chen, J., Brown, J. E., Tripathi, G., Hallschmid, M., Patel, S., Patel, S., Kern, W., Hillhouse, E. W., Lehnert, H., Tan, B. K. and Randeva, H. S. (2010).** Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. Endocrinology, **151**: 3169-3180.
7) **Riva, M., Nitert, M. D., Voss, U., Sathanoori, R., Lindqvist, A., Ling, C. and Wierup, N. (2011).** Nesfatin-1 stimulates glucagon and insulin secretion and beta cell NUCB2 is reduced in human type 2 diabetic subjects. Cell Tissue Res., 346-393.
8) **Kavukçu, S., Soylu, A. and Türkmen, M. (2002).** The clinical value of urinary N-acetyl-beta-D-glucosaminidase levels in childhood age group. Acta Med. Okayama., **56** (1): 7-11.
9) **Gaede, P., Vedel, P., Parving, H. H. and Pedersen, O. (2001).** Elevated levels of plasma von Willebrand factor and the risk of macro- and microvascular disease in type 2 diabetic patients with microalbuminuria. Nephrol. Dial. Transplant., **16**: 2028-2033.

10) **Jia, L. Q., Long, S. Y., Fu, M. D., Yan, B. Y., Tian, Y., Xu, Y. H. and Gou, L. T. (2006).** Relationship between total cholesterol/high-density lipoprotein cholesterol ratio, triglyceride/high-density lipoprotein cholesterol ratio, and high-density lipoprotein subclasses. Metabolism, **55**:1141-1148.

11) **Dobiásová, M. and Frohlich, J. (2001).** The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apo B-lipoprotein-depleted plasma (FERHDL). Clin. Biochem., **34:** 583-588.

12) **Seo, H. L., Byung-Wan, L., Hee, K. W., Jae, H. M., Kwang, J. K., Eun, S. K., Bong, S. C. and Hyun, C. L. (2001).** Postprandial triglyceride is associated with fasting triglyceride and HOMA-IR in Korean subjects with type 2 diabetes. Diabetes Metab. J., **35**: 404-410.

13) **Matboli, M., Azazy, A., Adel, S., Bekhet, M. and Eissa, S. (2017).** Evaluation of urinary autophagy transcripts epression in diabetic kidney disease. Journal of Diabetes and its Complications, **31**: 1491-1498.

14) **Kashyap, S. R., Osme, A., Ilchenko, S., Golizeh, M., Lee, K., Wang, S., Bena, J. F., Previs, S. F., Smith, J. D. and Kasumoy, T. (2018).** Glycation reduces the stability of Apo AI and increases HDL dysfunction in diet-controlled type 2 Diabetes. J. Clin. Endocrinol. Metab., **103**(2): 388-396.

15) **Chandrakanth, M. V., Das, S., Rout, S. B., Mohanty, R., Singh, S. C., Pattnaik, M. and Sethy, B. (2012).** Study of markers of atherosclerosis including insulin resistance in patients with chronic renal failure. Journal of Diabetes Mellitus, **2**(2): 208-213.

16) **Ibrahim, H. A., El-Meligi, A. A., Abdel-Hamid, M. and Elhendy, A. (2004).** Relations between von Willebrand factor, markers of oxidative stress and microalbuminuria in patients with type 2 diabetes mellitus Med. Sci. Monit., **10**(3): 85-89.

17) **Kim, S. R., Lee, Y., Lee, S., Kang, E. S., Cha, B. and Lee, B. (2017).** The renal tubular damage marker urinary N-acetyl-β-d-glucosaminidase may be more closely associated with early detection of atherosclerosis than the glomerular damage marker albuminuria in patients with type 2 diabetes. Cardiovasc. Diabetol., **16**: 16.

18) **Qin, Y., Zhang, S., Shen, X., Zhang, S., Wang, J., Zuo, M., Cui, X., Gao, Z., Yang, J., Zhu, H. and Chang, B. (2019).** Evaluation of urinary biomarkers for prediction of diabetic kidney disease: a propensity score matching analysis. Ther. Adv. Endocrinol. Metab. Dec 2;10:2042018819891110. doi: 10.1177/2042018819891110. eCollection 2019.

19) **Ebtehaj, S., Gruppen, E. G., Parvizi, M., Tietge, U. J. F. and Dullaart, R. P. F. (2017).** The anti-inflammatory function of HDL is impaired in type 2 diabetes: role of hyperglycemia, paraoxonase-1 and low grade inflammation. Cardiovasc. Diabetol., **16**: 132.

20) **Zhang, M., Zhou, J., Liu, Y., Sun, X., Luo, X., Han, C., Zhang, L., Wang, B., Ren, Y., Zhao, Y., Liu, X. and Hu, D. (2018).** Risk of type 2 diabetes mellitus associated with plasma lipid levels: The rural Chinese cohort study. Diabetes research and clinical practice, **135:** 150-157.

21) **Zhang, Z., Li, L., Yang, M., Liu, H., Boden, G. and Yang, G. (2012).** Increased plasma levels of nesfatin-1 in patients with newly diagnosed type 2 diabetes mellitus. Exp. Clin. Endocrinol. Diabetes, **120:** 91-95 .

22) **Li, Q. C., Wang, H. Y., Chen, X., Guan, H. Z. and Jiang, Z. Y. (2010).** Fasting plasma levels of nesfatin-1 in patients with type 1 and type 2 diabetes mellitus and the nutrient treated fluctuation of nesfatin-1 level in normal humans. Regul. Pept., **159:** 72-77.

23) **Zhai, T., Li, S., Fan, X., Tian, Z., Lu, X. and Dong, J. (2017).** Circulating nesfatin-1 levels and type 2 diabetes: A systematic review and meta-analysis. Journal of Diabetes Research, **2017:** 8pages

24) **Akour, A., Kasabri, V., Boulatova, N., Bustanji, Y., Naffa, R., Hyasat, D., Khawaja, N., Bustanji, H., Zayed, A. and Momani, M. (2017).** Levels of metabolic markers in drug-naive prediabetic and type 2 diabetic patients. Acta. Diabetol., **54**: 163-170.

25) **Mierzyński, R., Poniedziałek-Czajkowska, E., Dłuski, D., Patro-Małysza, J., Kimber-Trojnar, Z., Majsterek, M. and Leszczyńska-Gorzelak, B. (2019).** Nesfatin-1 and Vaspin as Potential Novel Biomarkers for the Prediction and Early Diagnosis of Gestational Diabetes Mellitus. Int. J. Mol. Sci., **20**(1): 159.

26) **Peng, X., Wang, X., Fan, M., Zhao, J., Lin, L. and Liu, J. (2020).** Plasma levels of von Willebrand factor in type 2 diabetes patients with and without cardiovascular diseases: A meta-analysis. Diabetes Metab. Res. Rev., **36**(1): e3193.

27) **Vischer, U. M., Emeis, J. J., Bilo, H. J., Stehouwer, C. D., Thomsen, C., Rasmussen, O., Hermansen, K., Wollheim, C.B. and Ingerslev, J. (1998).** von Willebrand factor (vWF) as a plasma marker of endothelial activation in diabetes: improved reliability with parallel determination of the vWF propeptide (vWf: AgII). Thromb. Haemost., **80**: 1002-1007.

28) **Kessler, L., Wiesel, M. L., Attali, P., Mossaed, J. M., Cazenave, J. P. Pinget, M. (1998).** von Willebrand Factor in diabetic angiopathy. Diabetes Metab., **24**: 327-336.

29) Chen, S. F., Xia, Z. L., Han, J. J., Wang, Y.T., Pan, S. D., Wu, Y. P., Zhang, B., Li, G. Y., Du, J.W., Gao, H.Q., de Groof, P. G., de Laat, B. and Hollestelle, M. J. (2013). Increased active von Willebrand factor during disease development in the aging diabetic patient population. AGE, **35**(1): 171–177.

30) Vangipurapu, J., Stancˇa´kova, A´., Kuulasmaa, T., Soininen, P., Kangas, A. J. and Ala-Korpela, A. (2012). Association between liver insulin resistance and cardiovascular risk factors. Journal of Internal Medicine, **272:** 402-408.

31) Banerji, M. A., Faridi, N., Atluri, R., Chaiken, R. and Lebovitz, H. E. (1999). Body composition, visceral fat, leptin, and insulin resistance in Asian Indian Men. J. Clin. Endocrinol. Metab., **84**: 137-144.

32) Snehalatha, C., Yamuna, A. and Ramachandran, A. (2008). Plasma adiponectin does not correlate with insulin resistance and cardiometabolic variables in nondiabetic Asian Indian teenagers. Diabetes Care, **31**: 2374-2379.