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" *The correlation of plasma vaspin levels with microalbuminuria and early diagnosis of diabetic nephropathy in Zagazig University Hospital* "

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ABSTRACT:

Background: Diabetes is an important health condition for the aging population. Diabetic nephropathy stands as a principal contributor to end-stage kidney disease in developed nations. Vaspin, a newly identified adipokine, has been identified as a possible regulator of insulin sensitivity, exhibiting increased expression in adipose tissue among individuals who suffered from obesity plus type 2 diabetes. This research sought to research the possible connection between plasma vaspin levels, anthropometric measurements, and the occurrence of microalbuminuria

Methods: This is a case control study included a total number of 92 subjects (above 65 years old) divided into four groups. Recruitment of patients occurred within the Outpatient Clinic and Nephrology unit of the internal medicine and clinical pathology departments at Zagazig University Hospitals.

Results:

The levels of albumin-to-creatinine ratio, creatinine, and blood urea nitrogen were considerably higher in Group IV as compared to the other groups. However, Group IV had significantly decreased levels of albumin, protein, and glomerular filtration rate. When compared to the other groups, Group III had much lower levels of Vaspin. Additionally, when comparison between Groups I and II, Vaspin levels were much lower in Group IV. Interactions between Vaspin level and weight, BMI (r= 0.869, p= <0.001), WHR, SBP, fasting insulin, HOMA IR, C-Peptide, and TG were positively correlated with statistical significance. In contrast, among Group II, there was an inverse connection between Vaspin level and length of DM. (p = 0.013, r= -0.510). In Group IV, vaspin levels exhibited a statistically significant positive correlation with weight, BMI, WHR, SBP, fasting insulin, HOMA-IR, C-peptide, triglycerides (TG), LDL cholesterol, and GFR (r = 0.892, p < 0.001). Conversely, a significant negative relationship was stated between vaspin levels and disease duration, ACR (r = -0.600, p = 0.002), creatinine, and BUN.

Keywords

microalbuminuria, plasma vaspin, diabetic nephropathy and diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) represents an extensive global health issue due to its complications affecting each of the microvascular and macrovascular systems. The worldwide incidence of diabetes is escalating, currently impacting an estimated 387 million individuals globally, accounting for approximately 8.3% of the world's population [1].

Diabetic kidney disease (DKD) is the foremost reason of end-stage renal disease (ESRD) in industrialized nations. It is commonly referred to as diabetic nephropathy (DNP) or microangiopathy [2].

Several key risk factors promote to the onset of diabetes mellitus, including hyperglycemia, dyslipidemia, a family history of the disease, hypertension, glomerular filtration abnormalities, and proteinuria. Additionally, genetic predispositions, such as polymorphisms in the renin-angiotensin-aldosterone system, compete as a part of disease susceptibility.

Higher concentrations of albuminuria and nephropathy in in each of blood and urine are associated with inflammatory markers, involving interleukin-1 (IL-1), IL-6, IL-18, and tumor necrosis factor-alpha (TNF-α), all of which provide to the advance of DNP. Furthermore, serum amyloid-A (SAA) levels correlate with a rising risk of ESRD, with higher SAA concentrations observed in individuals with heightened glomerular basement membrane (GBM) thickness compared to those with typical and regular GBM structure [3].

Adipose tissue functions as the primary reservoir for energy storage in the human body. The stored energy exists in the form of triglycerides, which can be mobilized into the bloodstream as free fatty acids during periods of hunger or increased demand [4].

Endocrine regulators, including insulin, epinephrine, and norepinephrine, play a crucial role in modulating adipocyte function. Additionally, adipose tissue itself serves as an integral component of the endocrine system and can be categorized into two main classes. The first is the insulin resistance-promoting factors, such as resistin and tumor necrosis factor-alpha (TNF-α). The second is the interleukin-6-inducing factors, which incorporate leptin, adiponectin, and visfatin. These cytokines are fundamental in the onset and cardiovascular diseases advancement [5].

Vaspin, an adipokine secreted by visceral adipose tissue, operates as a serine protease inhibitor. It was primary identified in ,visceral white adipose ,tissue within a type of rat (Otsuka Long-Evans Tokushima Fatty (OLETF)) , a well-established animal model for type 2, diabetes mellitus (T2DM). Structurally, vaspin shares homology with serpins, featuring characteristic beta sheets, alpha helices, and a reactive center.

Research suggests that vaspin may enhance insulin sensitivity within white adipose tissue. This effect is hypothesized to arise from the counteraction of proteases elevated in insulin resistance and obesity. Heiker et al. proposed that vaspin exerts its

beneficial effects via inhibiting the kallikrein 7, a member of the serine protease family [6].

Previous research proposes a probable function for vaspin in chronic inflammation and insulin resistance. This role is confirmed chiefly in diabetic patients. However, the exact stimulus of vaspin on renal utility and proteinuria in individuals with DNP across altered disease stages still uncertain.
By examining these aspects, we sought to understand whether any observed association between vaspin levels and DNP progression a straight impact of vaspin or an indirect consequence of increased inflammation [7].

Patients and Methods

Statement of Ethics

Our research was approved by the institutional review board of Zagazig University (ZU-IRB #10963-1-8-2023). Informed consent was gained from every participant before their attachment in the study, which was carried out in adherence to the ethical guidelines established in the Declaration of Helsinki.

Study Design and sample size

A case-control study was run at the Outpatient Clinic and the Nephrology Unit of the Internal Medicine and Clinical Pathology Departments at Zagazig University Hospitals.

This study included 92 elderly subjects (aged 65 years and older), divided into four groups:
Group I: 23 healthy elderly individuals served as the control group.
Group II: 23 elderly individuals with DM without complications.
Group III: 23 elderly individuals with DM and macrovascular complications.
Group IV: 23 elderly individuals with DM and microvascular complications.

Inclusion criteria were patients of patients diagnosed with type 2 diabetes matching to the American Diabetes Association (ADA) criteria. It is well-defined as fasting blood glucose (FBG) ≥ 126 mg/dL, a 2-hour plasma glucose level ≥ 200 mg/dL, or random plasma glucose ≥ 200 mg/dL accompanied by hyperglycemia symptoms—or those receiving diabetes medication, irrespective of gender, and aged 65 years or older, were included. [8]

Exclusion criteria were patients with type 1 DM, severe infections, acute complications of DM, inflammatory or malignant diseases, genetic or autoimmune disease, liver diseases, kidney diseases other than DNP, heart diseases and pregnant females.

All the participant was subjected to an exhaustive assessment. As part of this, we measured height, weight, blood pressure, and hemoglobin A1c (HbA1c), as well as

ran a complete blood count (CBC), checked the kidneys and liver, analyzed urine, calculated the urinary albumin-to-creatinine ratio (UACR), and ran lipid panels to measure things like serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), and high density lipoprotein (HDL). Evaluation of insulin resistance using the homeostasis model (HOMA-IR), insulin and C-peptide levels measured before and after fasting, this is the estimated glomerular filtration rate (eGFR) that is determined by utilizing the MDRD equation:

eGFR (mL/min) = [(140 - age) × weight / (0.814 × serum creatinine in μmol/L)] × (0.85 if female) [9] Measurement of Serum Vaspin by (ELISA).

This ensures a comprehensive assessment of each participant's overall health status and relevant metabolic parameters.

Statistical analysis

Data obtained from patient history, clinical assessments, laboratory analyses, and outcome measures were initially organized and recorded in Microsoft Excel. Subsequently, the data go through statistical analysis via SPSS software version 21.0.

Frequencies and percentages depicted the categorical variables. For continuous or numerical variables, the mean, standard deviation, and range (minimum-maximum) were computed.

Assessments of the differences among numerous groups for variables with normally distribution were achieved throughout one-way ANOVA test. In contrast, the Kruskal-Wallis test was applied for detecting the differences amongst multiple groups of non-normally distributed variables.

To examine association between categorical variables, the Chi-square (χ^2) test was employed. The strength and direction of relationship between two ranked variables were determined by Spearman's rank correlation coefficient. A positive coefficient means that there is a direct relationship, while a negative coefficient signifies an inverse relationship. The degree of correlation is reflected via the score or the magnitude of the coefficient, which span between -1 to +1. values closer to 1 represents a strong positive correlation, values nearer to 0 indicates a weak association, and values tighter to -1 means that the relation is strongly negative. The independent samples t-test was utilized to recognise statistically significant mean differences between two independent groups for continuous variables, when the assumption of normal distribution is applied.

Receiver operating characteristic (ROC) curve analysis was accomplished to decide the optimal cut-off values of OIF for the most precise diagnosis of early DNP. The optimal cut-off point was identified as the value yielding the highest overall diagnostic accuracy. Statistical significance was evaluated using a threshold of $p < 0.05$. Values of $p < 0.05$ were considered indicative of statistically significant findings, whereas $p > 0.05$ suggested non-significant results.

Results

In terms of age, sex, weight, height, diastolic blood pressure , and statistical significance, no significant distinctions were found between the groups. Conversely, both the body mass index and the waist circumference of Group III were noticeably greater than those of Group II. Groups II and III showed a considerable reduction in hip circumference compared to Group IV. Additionally, as compared to the other groups, Group III had a much greater waist-to-hip ratio (WHR) (Table 1). When comparing Groups I and II, Groups III and IV had significantly higher systolic blood pressure (SBP).

The study groups did not vary also in white blood cells, hemoglobin, ALT, AST, total bilirubin, direct bilirubin, or INR in a statistically significant way. Nonetheless, as compared to the other groups, Group III's platelet counts were much higher. In contrast, when compared to the other groups, Group IV had significantly elevated levels of creatinine, BUN, and ACR and significantly lower levels of albumin, total protein, and GFR.

Case groups HbA1c, FBS, fasting insulin, HOMA-IR, and C-peptide values were all significantly higher than control. Moreover, when contrasted with the control group, all case groups established a significant rise in 2-hour postprandial blood sugar.

The lipid profile was significantly higher in Group III than in any of the another groups. As seen in Table 2, Group IV also had significantly higher levels of cholesterol and LDL cholesterol when compared to Groups I and II.

There were statistically significant variations in Vaspin levels across the groups, as shown in Table 3. When compared to the other groups, Group III had much lower levels of Vaspin. Vaspin levels were also noticeably lower in Group IV compared to Groups I and II. In addition, compared to Group I, Group II had much reduced Vaspin levels.

Vaspin levels were positively correlated with weight, body mass index (BMI), waist circumference (WH), systolic blood pressure (SBP), fasting insulin (HOMA-IR), C-peptide, total cholesterol (TG), low-density lipoprotein (LDL) cholesterol (LDL), and glomerular filtration rate (GFR) in Group IV (DM+Microalbuminuria). Statistical analysis revealed an inverse relationship between Vaspin levels and ACR, BUN, creatinine, and illness duration. Table 4 and Figure 1 display these results.

Table 5 showed that Vaspin had a significant validity in prediction of macro complication of DM among complicated DM cases with sensitivity, specificity and accuracy 87%, 78.3% and 82.6% respectively.

Table 6 showed that weight, BMI, HbA1c, FBS, Cholesterol and TG were significant positive predictors for Vaspin and disease duration and HDL were significant negative predictors.

Table (1): Demographic data and Anthropometric measures among the groups studied:

Variable		Group I (Control) (*n*=23)	Group II (DM) (*n*=23)	Group III (DM+Macro) (*n*=23)	Group IV (DM+Micro) (n=23)	F	P
Age: (years)	*Mean ± SD* *Range*	75.87±6.55 66-88	75.09±6.72 66-88	75.26±6.83 65-86	74.83±6.4 65-86	0.10	0.96
Sex:	*Female N(%)* *Male N(%)*	10 (43.5%) 13 (56.5%)	12 (52.2%) 11 (47.8%)	13 (56.5%) 10 (43.5%)	10 (43.5%) 13 (56.5%)	χ^2 1.17	0.76
Weight: (Kg)	*Mean ± SD* *Range*	75.78±14.25 56-110	80.61±13.31 62-106	82.48±16.92 60-115	79.22±12.52 62-106	0.89	0.45
Height: (cm)	*Mean ± SD* *Range*	172±7.99 155-187	177.17±8.58 165-189	168.35±9.28 155-187	174.04±1055 165-189	2.76	0.06
BMI: (Kg/m^2)	*Mean ± SD* *Range*	26.39±4.31 20-35	25.17±3.66 21-36	29±4.7 [b] 20.8-37.7	26.12±3.92 21.6-36.6	**3.55**	**0.02***
WC: (cm)	*Mean ± SD* *Range*	98.09±16.27 75-131	93.39±17.93 74-145	111.61±19.88 [b] 78-158	101.43±20.11 78-145	**3.96**	**0.01***
HC: (cm)	*Mean ± SD* *Range*	107±14.69 89-136	100.22±12.96 86-137	99.96±11.6 83-125	113.78±16.67 [b,c] 88-152	**4.98**	**0.003***
WHR:	*Mean ± SD* *Range*	0.90±0.08 0.75-1.08	0.92±0.08 0.81-1.16	1.12±0.18 [a,b] 0.82-1.41	0.88±0.07 [c] 0.75-1.1	**20.65**	**<0.001 ****
SBP: (mmHg)	*Mean ± SD* *Range*	125±11.58 100-140	123.04±10.74 110-140	132.61±7.37 [a,b] 120-145	131.09±6.73 [a,b] 120-145	**5.65**	**0.001***
DBP: (mmHg)	*Mean ± SD* *Range*	78.91±7.38 70-90	78.90±7.68 70-90	82.61±7.37 70-90	83.04±7.03 70-90	2.18	0.10

SD: Stander deviation, F: ANOVA test χ^2: Chi square test. NS: Non-significant (P>0.05) *: Significant (P<0.05) **: highly significant (P<0.001)
a: Significant versus Group I b, Significant versus Group II c: Significant versus Group III

Table (2): hematological results among studied groups:

Variable		Group I (Control) (*n*=23)	Group II (DM) (*n*=23)	Group III (DM+Macro) (*n*=23)	Group IV (DM+Micro) (n=23)	F	P
WBC: ($x10^3/mm^3$)	*Mean ± SD* *Range*	7.17±1.3 5-9	7.52±1.04 6-9	7.43±1.12 6-9	7.70±1.33 6-10	0.75	0.53
HB: (gm/dl)	*Mean ± SD* *Range*	12.87±1.29 11-15	12.17±1.07 11-14	12.24±1.17 10.5-14	12.35±1.4 10-14	1.49	0.22
PLT: ($x10^3/mm^3$)	*Mean ± SD* *Range*	262.61±44.68 198-387	277.04±54.72 198-400	358.52±43.13[a,b] 299-414	259.13±47.02 [c] 198-400	**22.21**	**<0.001 ****
AST: (U/L)	*Mean ± SD* *Range*	22.74±6.22 14-35	20.48±3.96 15-30	23.43±6.42 14-39	20.17±4.31 14-30	2.13	0.10
ALT: (U/L)	*Mean ± SD* *Range*	21.65±4.73 15-30	25.43±5.61 15-40	23.43±5.79 15-39	25.3±5.3 15.33	2.55	0.06

D.Bilirubin: (mg/dl)	*Mean ± SD* *Range*	0.22±0.09 0.1-0.4	0.20±0.09 0.1-0.3	0.24±0.11 0.1-0.4	0.19±0.08 0.1-0.3	1.47	0.23
T.Bilirubin: (mg/dl)	*Mean ± SD* *Range*	0.78±0.10 0.6-0.9	0.74±0.11 0.6-0.9	0.75±0.10 0.6-0.9	0.73±0.11 0.6-0.9	0.45	0.72
s.albumin: (g/dl)	*Mean ± SD* *Range*	3.71±0.3 3.3-4.5	3.87±0.38 3.5-4.5	3.94±0.34 3.5-4.5	2.6±0.32[a,b,c] 2.1-3.1	**81.61**	**<0.001 ****
T.protine: (g/dl)	*Mean ± SD* *Range*	6.33±0.65 5-7.5	6.77±0.46 6-7.5	6.43±0.53 5.5-7	5.15±0.38 [a,b,c] 4.7-6	**42.69**	**<0.001 ****
INR:	*Mean ± SD* *Range*	0.89±0.18 0.5-1.2	0.84±0.20 0.5-1.2	0.86±0.20 0.6-1.2	0.84±0.20 0.6-1.2	0.28	0.84
S.creatinine: (mg/dl)	*Mean ± SD* *Range*	0.76±0.10 0.6-0.9	0.75±0.10 0.6-0.9	0.74±0.11 0.6-0.9	3.5±0.64 [a,b,c] 2.1-4.5	**396.1**	**<0.001 ****
BUN: (mg/dl)	*Mean ± SD* *Range*	13.83±3.68 8-21	13.65±3.89 8-21	13.87±3.85 8-22	58.39±11.09[a,b,c] 39.3-83.4	**274.9**	**<0.001 ****
GFR: (ml/min/1.73m^2)	*Mean ± SD* *Range*	88.79±7.27 79.3-98.8	89.66±7.47 79.3-98.8	88.62±7.71 79.3-100	47.88±6.75 [a,b,c] 40.1-62.3	**182.3**	**<0.001 ****
ACR: (mg/g)	*Mean ± SD* *Range*	19.48±3.76 12-26	20.35±4.41 12-28	20.7±5.23 13-29	683.43±155.22[a,b,c] 40.1-62.3	**418.9**	**<0.001 ****
Duration: (years)	*Mean ± SD* *Range*	------	32.61±5.72 22-43	28.65±6.74 17-40	29.96±6.53 18-41	2.32	0.11
HbA1C: (%)	*Mean ± SD* *Range*	4.48±0.62 3-5.3	7.23±0.37 [a] 6.8-8	7.89±0.69 [b] 6.9-9.2	7.91±0.63 [a,b] 7.1-10	175.5	**<0.001 ****
FBG: (mg/dl)	*Mean ± SD* *Range*	83.22±5.62 21-92	137.04±16.25[a] 114-187	162.48±22.64 [b] 119-194	158.96±19.2 [a,b] 116-198	104.6	**<0.001 ****
2HPPG: (mg/dl)	*Mean ± SD* *Range*	113.3±8.03 100-129	241.09±31.92[a] 201-301	253.09±26.92[a] 209-290	246.91±26.53[a] 203-291	164.7	**<0.001 ****
F.insulin: (uIU/ml)	*Mean ± SD* *Median* *Range*	3.55±0.74 3.5 2.5-5.3	6.52±3.48 [a] 6.1 2.3-15	9.83±4.04 [b] 11.5 2.25-15.4	8.74±3.11 [a,b] 8 5-15.2	KW 37.89	**<0.001 ****
HOMAIR:	*Mean ± SD* *Median* *Range*	0.74±0.14 0.8 0.5-1	2.28±1.38 [a] 1.9 0.9-5.3	4.1±1.94 [b] 4.9 0.8-6.8	3.52±1.6 [a,b] 2.7 1.3-6.8	55.38	**<0.001 ****
C-peptide: (ng/ml)	*Mean ± SD* *Range*	0.63±0.17 0.23-1.18	1.05±0.29 [a] 0.66-2.06	2.34±0.62 [b] 1.26-3.42	1.89±0.61 [a,b] 0.86-2.96	F 59.9	**<0.001 ****
Cholesterol: (mg/dl)	*Mean ± SD* *Range*	130 ±17.22 100-156	141.61±11.81 120-165	233.52±61.49[a,b] 140-322	186.09±51.51[c] 141-322	29.77	**<0.001 ****
TG: (mg/dl)	*Mean ± SD* *Range*	102.96±7.99 90-116	106±10.77 89-130	144.39±29.14[a,b] 100-187	116.65±20.37[c] 87-173	22.68	**<0.001 ****
LDLc: (mg/dl)	*Mean ± SD* *Range*	82.83±7.16 70-96	83.65±7.35 70-96	162.91±49.6 [a,b] 80-251	126.04±43.52 [a,b,c] 91-248	30.46	**<0.001 ****
HDLc: (mg/dl)	*Mean ± SD* *Range*	65.87±5.45 58-76	59.78±3.98 54-70	37.78±7.1 [a,b] 28-51	35.04±5.59 [a,b] 28-50	173.8	**<0.001 ****
VLDL: (mg/dl)	*Mean ± SD* *Range*	20.59±1.6 18-23.2	21.22±2.15 17.8-26	28.88±5.83 [a,b] 20-37.4	23.33±4.07 [c] 17.4-34.6	22.65	**<0.001 ****

*: Significant (P<0.05) **: highly significant (P<0.001)

Table (3): Vaspin level among the studied groups:

Variable		Group I (Control) (*n*=23)	Group II (DM) (*n*=23)	Group III (DM+Macro) (*n*=23)	Group IV (DM+Micro) (n=23)	F	P
Vaspin: (ng/ml)	*Mean ± SD*	1.54 ± 0.19	3.57 ± 0.25 [a]	0.34 ± 0.19 [a,b]	0.70 ± 0.15 [a,b,c]	**1178**	**<0.001 ****
	Range	1.22-1.79	3.11-3.99	0.06-0.59	0.43-0.92		

*: Significant (P<0.05) **: highly significant (P<0.001)

Table (4): Correlation between Vaspin level and age, duration, anthropometric measures, blood pressure and laboratory findings among Group IV:

	S.vaspin (ng/ml) (n=23)	
	r	p
age(years)	0.187	0.393
Weight (Kg)	**0.490***	**0.018**
Height (cm)	-0.440	0.056
BMI (Kg/m2)	**0.874****	**<0.001**
WC (cm)	0.041	0.852
HC (cm)	0.314	0.141
WHR	**0.516***	**0.012**
Duration (years)	**-0.617**	**0.002***
SBP (mmHg)	**0.473***	**0.016**
DBP (mmhg)	-0.170	0.437
WBCs (1000/m3)	0.260	0.231
Hb (gm/dl)	-0.164	0.455
Platelets (x1000/mm3)	0.198	0.366
AST (IU/L)	0.356	0.095
ALT (IU/L)	0.212	0.331
D bilirubin (mg/dl)	0.086	0.696
T bilirubin (mg/dl)	-0.119	0.587
Albumin (gm/dl)	0.134	0.212
T Protein (mg/dl)	-0.105	0.633
INR	0.043	0.846
Creatinine (mg/dl)	**-0.551***	**0.002**
BUN (mg/dl)	**-0.451***	**0.031**
GFR (ml/min/1.73m2)	**0.892****	**<0.001**
ACR (mg/g)	**-0.600***	**0.002**
HbA1c (%)	0.015	0.945
FBS (mg/dl)	0.262	0.228
2HPPG (mg/dl)	0.057	0.794
F.Insulin (uIU/ml)	**0.909****	**<0.001**
HOMA- IR	**0.902****	**<0.001**
c-peptide (ng/ml)	**0.721****	**<0.001**
Cholesterol (mg/dl)	0.334	0.120
TG (mg/dl)	**0.542***	**0.008**
LDL-c (mg/dl)	**0.756****	**<0.001**

HDL-c (mg/dl)	0.159	0.468
VLDL (mg/dl)	0.398	0.060

*: Significant (P<0.05) **: highly significant (P<0.001)

Figure (1): Correlation between Vaspin level and ACR among Group IV (DM+Micro).

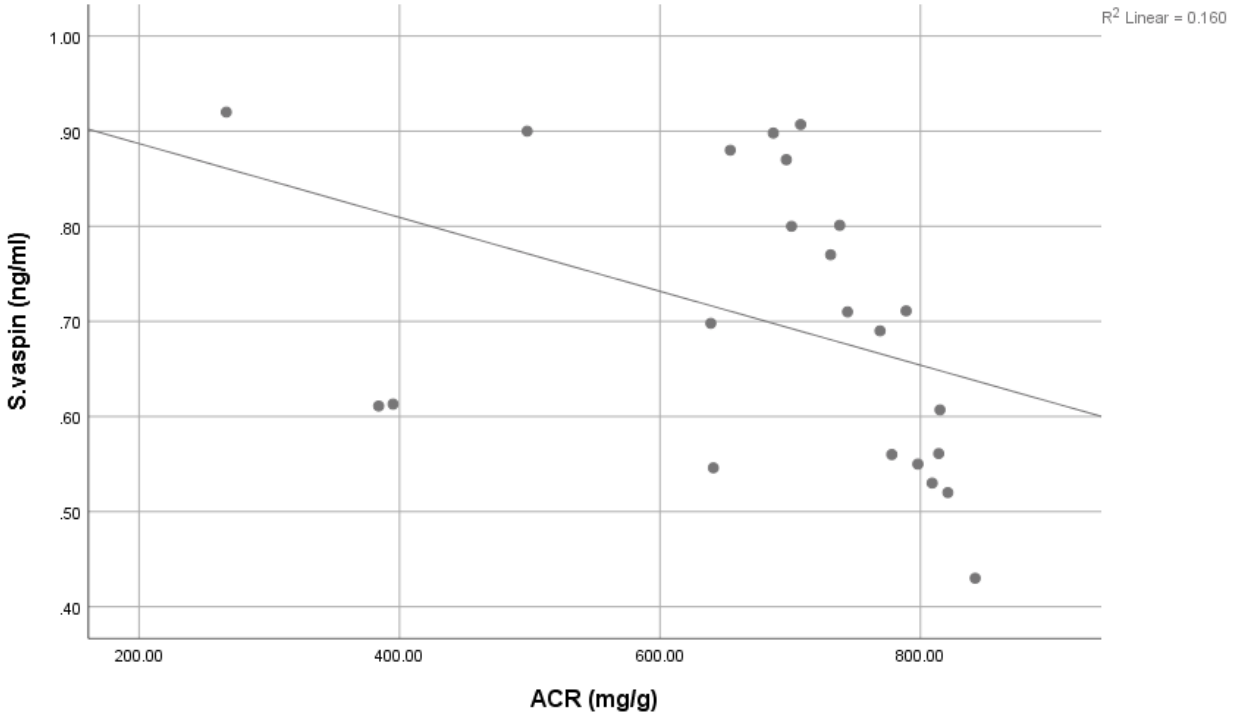

Table (5): Validity of Vaspin in differentiate between Macro and Micro complication among the complicated DM cases:

Cut off	AUC (95%CI)	Sensitivity	Specificity	PPV	NPV	Accuracy	P
>1.62	0.92 (0.84-0.99)	87%	78.3%	80%	85.7%	82.6%	**<0.001 ** **

Table (6): Multivariate linear regression analysis for significant predictors of Vaspin level among the studied groups:

	Standardized Coefficients Beta	SE	P
Age(years)	0.135	0.01	0.32
Weight: (Kg)	**0.421**	**0.02**	**<0.001****
Height (cm)	0.205	0.004	0.12
BMI: (Kg/m^2)	**0.514**	**0.10**	**<0.001****
WC (cm)	0.162	0.05	0.29
HC (cm)	0.263	0.01	0.09
WHR	0.024	0.005	0.89
Duration (years)	**-0.331**	**0.02**	**0.01***
SBP (mmhg)	0.301	0.04	0.07
DBP (mmhg)	0.066	0.12	0.85

WBCs (1000/m3)	0.293	0.085	0.09
Hb (gm/dl)	0.048	0.008	0.80
Platelets (1000/m3)	0.113	0.013	0.46
AST (IU/L)	0.096	0.003	0.93
ALT (IU/L)	0.331	0.011	0.11
D bilirubin (mg/dl)	0.048	0.087	0.87
T bilirubin (mg/dl)	0.014	0.027	0.97
Albumin (gm/dl)	0.283	0.161	0.16
T Protein (gm/dl)	0.194	0.014	0.14
INR	0.086	0.06	0.56
Creatinine	0.351	0.03	0.06
BUN (mg/dl)	0.247	0.012	0.21
ACR (mg/g)	0.120	0.017	0.17
GFR (ml/min/1.73m2)	0.173	0.009	0.19
HbA1c (%)	**0.466**	**0.034**	**0.003***
FBS (mg/dl)	**0.457**	**0.004**	**0.004***
2HPPG (mg/dl)	0.188	0.001	0.29
Fasting insulin (uLU/ml)	0.214	0.13	0.16
HOMA-IR	0.013	0.04	0.90
C-peptide (ng/ml)	0.239	0.06	0.08
Cholesterol(mg/dl)	**0.521**	**0.02**	**0.001***
TG(mg/dl)	**0.496**	**0.001**	**<0.001****
LDL-c(mg/dl)	0.217	0.06	0.21
VLDL (mg/dl)	0.145	0.014	0.14
HDL-c (mg/dl)	**-0.481**	**0.007**	**<0.001****

*: Significant (P<0.05) **: highly significant (P<0.001)

Discussion

Our research evaluated serum vaspin levels as related to the macrovascular and microvascular complications in elderly patients with T2DM compared with healthy group. This study is a case control study and includes a total sum of 92 elderly patientd (above 65 years old), divided into four groups.

The current study revealed markedly significant elevation of serum vaspin levels in diabetic patients without complications compared to healthy in the controls (3.57 ± 0.25 ng/ml versus 1.54 ± 0.19 ng/ml, p < 0.001). Additionally, there was a, logistic regression analysis that indicated that serum vaspin levels serve as an independent predictor of diabetic macroangiopathy. Compared to individuals with T2DM without complications (Group II), patients with diabetic macrovascular complications (Group III) exhibited a significantly, higher levels of HbA1c and FPG, fasting insulin, HOMA IR and C-peptide among diabetic cases with macrovascular complications (Group III). 7.89±0.69 for HBA1C, 162.48±22.64 mg/dl for FPG, 253.09±26.92 mg/dlFor PPPG, 9.83±4.04 uIU/ml for fasting insulin, 4.1±1.94for HOMA-IR , 2.34±0.69 ng/ml for C-peptide compared to T2DM patients without complications (groups II) 7.23±0.37 for HBA1C, 137.04±16.25 mg/dlfor FPG,

241.09±31.92 mg/dlFor PPPG, 6.52±3.48 uIU/ml for fasting insulin, 2.28±1.38for HOMA-IR.

These findings are along with other studies. According to **Kumari et al**., a considerable difference ($p < 0.001$) in serum vaspin levels were found between type 2 diabetic and non-diabetic controls **[10]**. **El-Lebedy et al.** also found that, compared to healthy volunteers, diabetics had higher serum vaspin concentrations **[11]**. Additionally, **Yang et al.** proved markedly elevated serum vaspin levels in T2DM group as compared to the healthy control group **[12]**.

Conversely, **Baig et al.** found that level of serum vaspin are depress in type 2 diabetes group in comparison to a healthy control group, which contradicts our findings. Their study included 75 patients with type 2 diabetes and 75 age-, gender-, and BMI-matched healthy controls. Similarly, **Taşdemir et al.** observed lower plasma vaspin levels in diabetic rats when comparing to control animals. Their study, conducted on Wistar albino rats, consisted of seven rats in the control group, seven in the diabetes group, and only one in the therapy group **[13,14]**.

Our findings demonstrated a significantly lower serum vaspin levels in diabetic patients with macrovascular complications than in healthy individuals (0.34 ± 0.19 ng/mL vs. 1.54 ± 0.19 ng/mL, $P < 0.001$). This aligns with the study by **Montaser et al. (2021)**, which found reduced serum vaspin levels across patients with coronary artery disease (CAD) compared to healthy controls. Their study included 20 individuals with normal coronary arteries as the control group (Group I) and 68 CAD patients divided into three subgroups (Groups II, III, IV) based on disease severity **[15]**.

However, our results contradict the research of **Rashad et al. (2020)**, who reported significantly elevated serum vaspin levels in T2DM patients who suffer from ischemic stroke compared to the control group [16].

Additionally, our study revealed a notable positive correlation between vaspin levels and triglycerides (TG) as well as LDL-C . In contrast, a significant negative correlation was obtained between vaspin levels and HDL-C among Group III (diabetes with macrovascular complications).

Montasera et al., (2021) reported thatpositive significant correlation of circulating vaspin level was found with HDL-C levels and significant negative correlation of serum Vaspin levels with TC, TG, and LDL-C in CAD patients' groups. **[15]**

Our study demonstrated a statistical significance rise in HbA1c and FPG, HOMA IR, PPPG, fasting insulin, and C-peptide among diabetic cases with microvascular complications (Group IV) 7.91±0.63 for HBA1C, 158.96±19.2 for FPG, 246.91±26.53For PPPG, 8.74±3.11 for fasting insulin, 3.52±1.6 for HOMA-IR , 1.89±0.54 ng/ml for C-peptide compared to all diabetic cases without complications (group II). 7.23±0.37 for HBA1C, 137.04±16.25for FPG, 241.09±31.92For PPPG, 6.52±3.48 for fasting insulin, 2.28±1.38for HOMA-IR, 1.02±0.43 ng/ml for C-peptide .

Tony et al. found that the type 2 diabetic Patients with deminished renal function had lower blood sugar and hemoglobin A1c levels than patients with type 2 diabetes who did not have impaired renal function (171.65± 69.09 vs. 209.96±79.70 and 7.02 ±1.34vs. 8.93±2.01mg/dl, respectively, P<0.001 for each). Our study demonstrated a statistical significance higher concentration levels of TC, TG, LDL-C and decrease in HDL-C among diabetic cases with microvascular complications (GroupIV) 186.09±51.51 mg/dl for TC, 116.65±20.37 mg/dl for TG, 126.04±43.52 mg/dl for LDL-C, 35.04±5.59 mg/dl for HDL-C (P<0.05). compared to all diabetic cases without complications (groups II) 141.61±11.81 mg/dl for TC, 106±10.77 mg/dl for TG, 83.65±7.35 mg/dl for LDL-C, 59.78±3.98 mg/dl for HDL-C (P<0.05). **[17]**

There was a statistical significance increase in WHR within T2DM patients with diabetic complications (macrovascular or microvascular) compared to other groups (I, II) (1.12±0.18 VS. 0.90±0.08, 0.92±0.08 and 0.88±0.07) respectively. Otherwise, no statistically significance change was perceived between the studied groups as regards weight or height.

Our results are in harmony with the study of **Hao et al.,** who revealed that diabetic complications (macrovascular or microvascular) were correlated positively with increase of BMI and WHR. **[18].**

Our findings regarding BMI differ from those stated by **Tony et al. [17]**. Whilst our study identified a statistically substantial difference in BMI between individuals with T2DM and the control group, no notable difference was detected between T2DM patients with and without complications.

Additionally, our results revealed a statistically significant variation in diabetes duration among the studied groups. All patients in the T2DM group with microvascular complications exhibited retinopathy, neuropathy, and nephropathy, with a statistically significant difference compared to other case groups.

Furthermore, diabetic patients with microvascular complications (Group IV) exposed a significant increase in creatinine, BUN, and ACR levels, along with a significant decline in GFR, in contrast to diabetic patients who had macrovascular complications (Group III), diabetic patients without complications (Group II), and healthy controls (Group I) ($p < 0.001$).

Our findings support the conclusions of **Khode et al. [19]**, who informed a significantly higher prevalence of comorbidities, comprising chronic kidney disease and coronary heart disease , in Type 2 DM patients with microvascular and macrovascular complications compared to the control group .

Our findings discovered a positive relationship between Vaspin levels and GFR ($r = 0.892$, $p < 0.001$), while a negative correlation between Vaspin levels and ACR, creatinine, and BUN ($r = -0.600$, $p < 0.001$), ($r = -0.551$, $p < 0.001$), and ($r = -0.451$, $p < 0.001$) in Group IV (T2DM with microvascular complications) were observed.

These findings align with those of **Inoue et al. (2012)**, who found an inverse relation between creatinine levels and serum Vaspin levels, while a positive correlation was observed with eGFR in DNP patients **[20]**.
Conversely, **Hiammohammedsalih et al. (2021)** reported no significant correlation between Vaspin levels and uACR ($r = -0.30$, $p = 0.01$) in DNP groups **[21]**.
Our results from multivariate linear regression analysis identified significant predictors of Vaspin levels among the groups studied. The current study found that weight, BMI, HbA1c, FBS, cholesterol, and TG were significant positive predictors of Vaspin, while disease duration and HDL were significant negative predictors.
Baig et al. (2021) conducted binary logistic regression analysis and demonstrated a substantially negative predictive connection amongst Vaspin and T2DM **[13]**.

In another study by **Kahraman et al. (2016)**, statistically significant distinctions were detected between the studied groups regarding elevated serum creatinine, BUN, and ACR, as well as decreased GFR in T2DM patients with microvascular complications parallelled to those without complications. However, no statistically significant differences were observed between the groups in terms of mean values of serum urea or creatinine clearance **[22]**.

Karadaga et al. [23] investigated Vaspin levels in 106 adult T2DM patients characterized based on their estimated glomerular filtration rate (eGFR). They found significantly lower Vaspin levels in patients with an eGFR above 60 mL/min/1.73m² compared to those with an eGFR below this threshold ($p = 0.03$).

Our results indicated no correlation between age and sex with Vaspin levels across all groups. This finding agree with **Yan et al.** and **Mohammed et al.**, who reported no correlation between age and Vaspin levels **[24,25]**.

Conclusions

- In conclusion, Serum Vaspin had a significant validity in prediction of complications of DM (microvasular and macrovascular) with sensitivity, specificity and accuracy all 100%. So, serum vaspin might provide as a novel marker of unrecognized symptoms of diabetes complications.
- the significant correlation noticed between serum vaspin and lipid profile may suggest a role of vaspin in lipid metabolism. Also, Vaspin correlated with anthropometric measures suggesting a role in adiposity. All these correlations urge a future study to unravel its mechanisms.
- Vaspin level is correlated negatively with ACR, Creatinine and BUN and correlated positively with GFR in elderly T2DM patients with microvascular complications

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