

ASSESSMENT OF VASPIN AND RISK FACTORS IN RELATION WITH DIABETIC MELLITUS TYPE II

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ABSTRACT

Visceral adipose tissue-derived serine protease inhibitor (Vaspin) is a novel adipocytokine. Several studies have indicated that Vaspin may exert an important role in the development of metabolic disorders. The study was carried out from February 2013 to July 2013. The age of patients and control groups were range of 35-65y. The concentration of fasting blood glucose, cholesterol, triglyceride, LDL, VLDL, HDL, Vaspin and BMI were estimated in patients and control groups. This study was conducted on randomly selected 68 Type-2 diabetic patients (27 Males and 41 Females) attending the diabetes mellitus center in Al-Sadder Teaching City in Al-Najaf province, Iraq and a group of 20 apparently healthy subjects (10 Males and 10 Females) were included as a control group. Those parameter were higher in diabetic patients than in control group ($P < 0.05$) in fasting blood glucose, cholesterol, triglyceride, LDL, VLDL and Vaspin levels in patients compared with control groups. The results revealed that Vaspin not significant difference ($p > 0.05$) in patients and control groups at different ages. The results also revealed that Vaspin level increase significantly ($P < 0.05$) in males than females in both patients and control groups. The results also revealed a significant increase ($P < 0.05$) in BMI in patients compared with control groups. The results also show that Vaspin concentration increase significantly ($P < 0.05$) with increasing BMI in males than females compared with control groups. The results have been shown significant positive correlation ($P < 0.05$) between Vaspin, FBG, cholesterol, triglyceride, LDL and VLDL in patients (males and females), while significant negative correlation ($P < 0.05$) was observed between Vaspin and HDL in patients (males and females). The present study concluded that Vaspin level was a marker for detection and diagnosis of diabetic patients Type-2, as you did not find significant difference between diabetic groups and control one, this conclusion is not justified.

Keywords: diabetes mellitus, Risk Factors and Vaspin.

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INTRODUCTION

Diabetes Mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces¹. There are mainly four types of diabetes; Type-1 diabetes is immune-mediated and requires daily administration of insulin. The other common type is Type-2 diabetes and characterized by insulin resistance or relative insulin deficiency¹⁻². Other rare types of this disease include neonatal diabetes, congenital diabetes, cystic fibrosis-related diabetes and steroid diabetes³. Type-2 diabetes is the most common form and comprises of 90% of people with diabetes around the world. The prevalence of Type-2 diabetes rates continue to increase with increasing number of patients at risk of serious diabetes-related complications. Having Type-2 diabetes increase the risk of a myocardial infarction two times and the risk of suffering a stroke two to four times. It is also a leading cause of blindness, limb amputation and kidney failure³⁻⁶. Vaspin, a member of serine protease inhibitor family, is a visceral adipose tissue-derived adipokine with potential antiprotease properties. Vaspin cDNA was isolated from visceral white adipose tissues (WATs) of rat, an animal model of abdominal obesity with Type-2 diabetes. Vaspin is seen in the diabetic obesity metabolic syndrome by sensitizing insulin action, especially in WATs. Studies on Vaspin has centered on for the identification of the potential protease substrate leading to the development of antiprotease inhibitor therapy, which could facilitate the improvement of insulin sensitivity in the metabolic syndrome⁷. The objective of the current study was the possibility of using biomarker (Vaspin) as indicators for diabetic patients Type-2. In addition to, the linear correlation between biomarkers and lipid profile.

EXPERIMENTAL

Material and Methods

The study was conducted on randomly selected 68 Type-2 diabetic patients (27 Males and 41 Females) and a group of 20 apparently control subjects (10 Males and 10 Females) were included as a healthy. Diabetes Mellitus was diagnosed by consultant doctors. The information of patients were obtained through a questionnaire consisted of the sex, age and BMI. Patients with renal dysfunction, heart diseases, who were on drugs affect oxidative stress, i.e. antioxidants, antihyperlipidemic agents were excluded from the current investigation. Five milliliters of venous blood samples were drawn using a disposable needle and plastic syringes from each patients and controls subject in jell-activated sample tubes. Blood was left at room temperature for 10 minutes for clotting, centrifuged 6000 rpm for 10 minutes, and then serum was separated and transported into new disposable tubes and kept at freezing temperature (0 to 4 degrees C). Vaspin ELISA Kit for quantitative determination of Vaspin in human serum was supplied by RayBiotech, Inc (made in china). Kit reagents were kept on ice during reagent preparation steps and equilibrated plate to room temperature before opening the sealed pouch. Vaspin ELISA Kit is an in vitro quantitative assay for detecting Vaspin peptide based on the principle of Competitive Enzyme Immunoassay.

General procedure

The general procedure is as follows, step wise-

1. Kit reagents were kept on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. 100 µl anti-Vaspin antibody was added to each well and incubated for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec) and also incubated overnight at 4°C.
3. The solution was discarded and washed wells 4 times with 1x Wash Buffer (200-300 µl each), Washing may be done with a multichannel pipette or an automated plate washer. Removal of liquid was completed at each step is essential to good assay performance. After the last wash, removed any remaining Wash Buffer by aspirating or decanting and invert the plate and blotted it against clean paper towels.
4. 100 µl of each standard was added, positive control and sample into appropriate wells that included a blank well (Assay Diluent only). Wells were covered and incubated for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4°C.
5. The solution was discarded and washed 4 times.
6. 100 µl of prepared HRP-Streptavidin solution was added to each well and incubated for 45 minutes with gentle shaking at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes.
7. The solution was discarded and washed 4 times.
8. 100 µl of TMB One-Step Substrate reagent was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).
9. 50 µl of stop solution (8ml) was added to each well and read absorbance at 450 nm immediately.

Statistical analysis

The results were expressed as (mean $\pm$ standard deviation)⁸. ANOVA test has been used for the comparison between the patients and control groups while Pooled t- test has been used for the comparison among subdivided groups in the measured parameters. Pearson's correlation coefficients (r) were calculated to estimate the correlation between parameters.

The difference between groups is considered as statistically different when (p<0.05). All statistical analysis were performed using SPSS Statistics version 19, while the figures constructed using EXCEL program of Microsoft Office 2007.

RESULTS AND DISCUSSION

Fasting blood glucose and serum Lipid profile level

The results of Table-1 indicate a significant increase (P<0.05) in fasting blood glucose (FBG) level in diabetic patients (274.29 $\pm$ 59.20 mg/dl) in comparing with control group (102.05 $\pm$ 9.66 mg/dl). Also the

results show that there is a significant increase ($P < 0.05$) in serum Cholesterol, Triglycerides, LDL-C and VLDL-C level in diabetic patients (5.46 ± 0.12 , 3.06 ± 0.06 , 3.17 ± 0.04 and 1.37 ± 0.11 mmol/L) respectively comparing with control group (4.12 ± 0.06 , 1.65 ± 0.11 , 2.36 ± 0.06 and 0.79 ± 0.07 mmol/L) respectively, and a significant decrease ($P < 0.05$) in HDL-C level in diabetic patients (0.86 ± 0.08 mmol/L) in comparing with control group (1.28 ± 0.14 mmol/L).

Table-1: Serum level of FBG and lipid profile components in patients and control groups

Parameters	Mean $\pm$ S.D	
	Control	Patients
	n = 20	n = 68
FBG (mg/dl)	102.05 $\pm$ 9.66	274.29 $\pm$ 59.20 *
Cholesterol (mmol/L)	4.12 $\pm$ 0.06	5.46 $\pm$ 0.12 *
Triglyceride (mmol/L)	1.65 $\pm$ 0.11	3.06 $\pm$ 0.06 *
LDL-C (mmol/L)	2.36 $\pm$ 0.06	3.17 $\pm$ 0.04 *
VLDL-C (mmol/L)	0.79 $\pm$ 0.07	1.37 $\pm$ 0.11 *
HDL-C (mmol/L)	1.28 $\pm$ 0.14	0.86 $\pm$ 0.08 *

* means significant difference at $P < 0.05$.

Vaspin level

The results in figure-1 show a significant increase ($P \leq 0.05$) in Vaspin level in diabetic patients (559.97 ± 72.02 pg/ml) in comparing with control groups (199.64 ± 25.82 pg/ml).

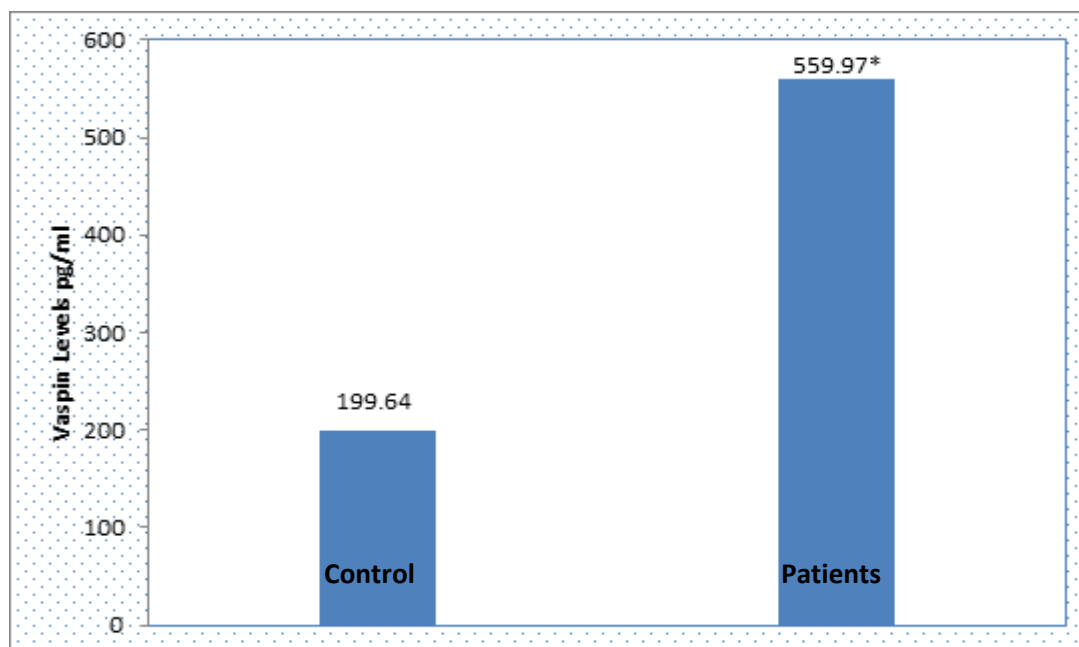


Fig.-1: Vaspin level in diabetic patients and control groups

* means significant difference at ($P < 0.05$)

Comparison serum Vaspin level between diabetes and control groups according to age

The results of Table-2 show a significant increase ($p < 0.05$) in serum Vaspin level in patients at different ages, comparing with control groups. Furthermore the results show that there is no significant difference ($p > 0.05$) in serum Vaspin in patients at different ages.

Comparison serum Vaspin level between diabetes and control groups according to gender

The results of Table-3 reveal a significant increase ($p < 0.05$) in serum Vaspin level in males than females in both patients and control groups, while serum Vaspin level are highly significant increase ($p < 0.05$) in both males and females in patients comparing with control groups.

Table-2: Serum Vaspin level of patients and control groups in different age

Age	Mean $\pm$ S.D	
	Control	Patients
	n = 20	n = 68
35 -45	197.31 $\pm$ 27.00a	518.13 $\pm$ 115.65b
46 - 55	238.22 $\pm$ 65.87 a	553.40 $\pm$ 113.15b
56 - 63	199.22 $\pm$ 17.89a	556.03 $\pm$ 128.28b

Where, a and b means significant difference at ($P < 0.05$), between patients and control groups

Comparison Vaspin level between diabetes and control groups according to BMI

The figure-2 shows a significant increase ($P < 0.05$) in Vaspin level in all groups normal weight, over weight and obese weight in comparing with control groups. Also the same figure show a significant increase ($P < 0.05$) in Vaspin level in all groups normal weight, over weight and obese weight in males (551.76, 615.79, 568.68 pg/ml) in comparing with females (410.93, 585.72, 488.29 pg/ml).

Relationship between Vaspin and fasting blood glucose levels

The results of correlation and linear regression between Vaspin and FBG in male patients are indicated that there is no a significant correlation ($P > 0.05$) between Vaspin levels (pg/ml) and FBG levels (mg/dl) of Diabetic Mellitus patients ($r = 0.06$).

The results of correlation and linear regression between Vaspin and FBG in female patients are indicated that, there is no a significant correlation ($P > 0.05$) between Vaspin levels (pg/ml) and FBG levels (mg/dl) of Diabetic Mellitus patients ($r = 0.035$).

Table-3: Vaspin levels in both gender of patients and control groups

Marker	Mean $\pm$ S.D			
	Control		Patients	
	n = 20		n = 68	
	Male	Female	Male	Female
	n = 10	n = 10	n = 27	n = 41
Vaspin pg/ml	207.22 $\pm$ 25.70*	192.06 $\pm$ 25.94	561.37 $\pm$ 16.83*#	538.58 $\pm$ 127.21#

* means significant difference at ($P < 0.05$), different gender at the same group

means significant difference at ($P < 0.05$), at the same gender in different group

Relationship between Vaspin and lipid profile levels**A. Cholesterol**

The results of correlation and linear regression between Vaspin and cholesterol in male patients are indicated that, there is no a significant correlation ($P > 0.05$) between Vaspin levels (pg/ml) and cholesterol levels (mmol/L) of Diabetic Mellitus patients ($r = 0.039$).

The results of correlation and linear regression between Vaspin and cholesterol in female patients are indicated that, the presence of a significant positive correlation ($P \leq 0.05$) between Vaspin levels (pg/ml) and cholesterol levels (mmol/L) of Diabetic Mellitus patients ($r = 0.227$).

B. Triglycerides

The results of correlation and linear regression between Vaspin and triglycerides in male patients are indicated that, there is no a significant correlation ($P > 0.05$) between Vaspin levels (pg/ml) and triglycerides levels (mmol/L) of Diabetic Mellitus patients ($r = 0.028$).

The results of correlation and linear regression between Vaspin and triglycerides in female patients are indicated that, the presence of a significant positive correlation ($P \leq 0.05$) between Vaspin levels (pg/ml) and triglycerides levels (mmol/L) of Diabetic Mellitus patients ($r = 0.107$).

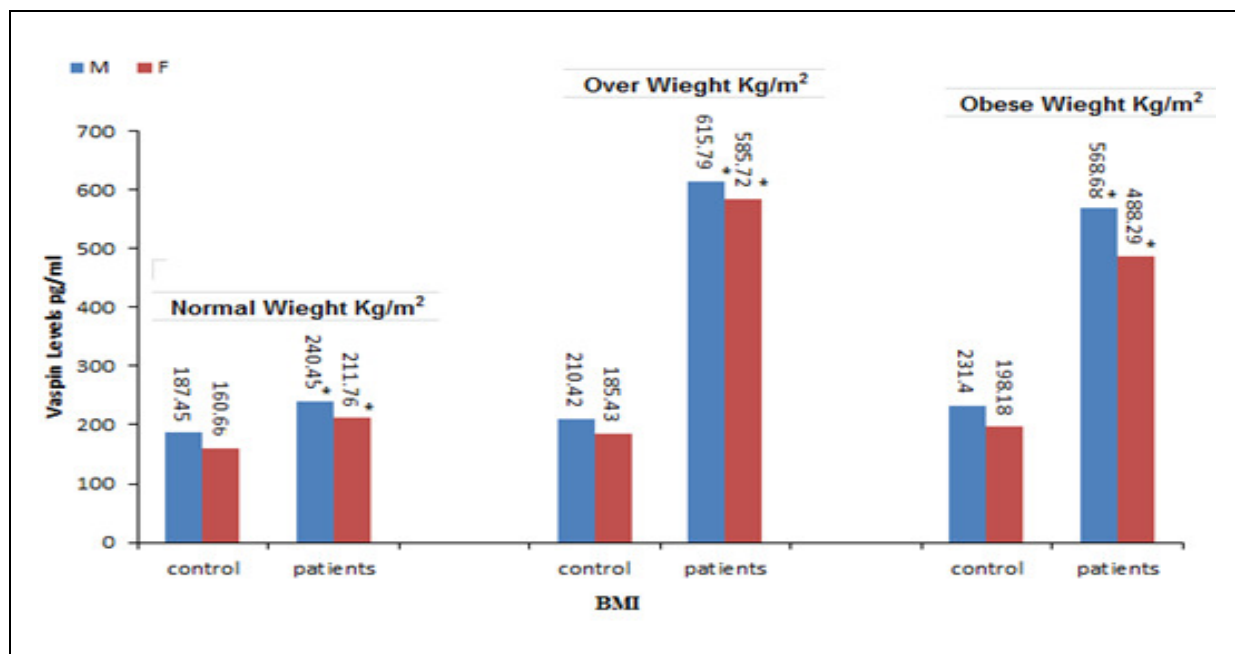


Fig.-2: Vaspin levels of patients and control groups according to BMI

* means significant difference at ($P < 0.05$)

C. LDL-C

The results of correlation and linear regression between Vaspin and LDL in male patients are indicated that, there is no a significant correlation ($P > 0.05$) between Vaspin levels (pg/ml) and LDL levels (mmol/L) of Diabetic Mellitus patients ($r = 0.064$).

The results of correlation and linear regression between Vaspin and LDL in female patients are indicated that, the presence of a significant positive correlation ($P \leq 0.05$) between Vaspin levels (pg/ml) and LDL levels (mmol/L) of Diabetic Mellitus patients ($r = 0.601$).

The study revealed a significant increase in fasting blood glucose in patients comparing with control group as presented in Table-1. These results are expected due to the fact that the main characteristic feature of DM is hyperglycemia. Blood glucose is tightly controlled by two key processes: insulin secretion by pancreatic β -cells in response to a nutrient and insulin action on major target organs, i.e., skeletal muscle, liver and adipose tissue. Type-2 diabetes mellitus (T2DM) is often associated with obesity and results from insufficient insulin production/secretion and insulin resistance (IR)⁹. Previous study showed that plasma Vaspin levels were elevated in patients with T2DM¹⁰. Another study by Tan et al demonstrated that Vaspin protein level elevated in adipocytes in a dose-dependent manner of glucose¹¹. Study of Zhang et al¹² observed that elevated Vaspin level are positively associated with fasting insulin and was an independent factor associated with Vaspin level and suggested that Vaspin may be indication of insulin sensitivity and play an important compensatory role in response to decrease insulin sensitivity. Free fatty acid is an important cause of obesity associated with insulin resistance, some studies indicated that plasma Vaspin was positively related to free fatty acid in patients with T2DM¹²⁻¹³. Recent studies confirmed the association between Vaspin and fasting insulin implying that the reduction of Vaspin was attributed to improve insulin sensitivity¹⁴⁻¹⁶. Another point of view associated Vaspin more with glycemic status rather than with insulin levels, in this respect study of Kloting *et al.*¹⁷ showed that elevated Vaspin

level lowering blood glucose. Vaspin as an adipokine was indicated as possessing interesting insulin sensitivity¹⁸. El-Mesallamy *et al*¹⁹ confirmed that T2DM is associated with increased level of Vaspin and visfatin and the results showed that Vaspin is affected by both factors T2DM and obesity. Study by Kloting *et al*.¹⁷ found that Vaspin mRNA expression is to be absent in normal glucose tolerant individual and more frequently detected in T2DM patients. Vaspin induced by hyperglycemia and significant correlation between glucose which found to cause increase Vaspin net protein production and secretion from mental adipose tissue¹⁰. Study by August *et al*²⁰ reported that Vaspin level are not increase in obese women as a result of estradiol that reduce expression of Vaspin into adipocytes. Previous study in rats showed that visceral Vaspin mRNA expression significantly correlate with percentage of body fat and BMI Human showed Vaspin concentrations in obese subject to be increased compared with lean subject^{21,22}.

Most recent studies showed a relationship of Vaspin with submarkers of lipid metabolism and these correlation are positive with cholesterol and triglycerides and lesser extent with LDL-C which indicates that Vaspin play an important role in lipid metabolism or might be indicated dyslipidemia especially because Vaspin is adipokine secreted by adipocytes¹⁹. Van *et al*. using simple linear regression analysis revealed that serum Vaspin positively correlated with BMI, cholesterol, triglycerides, LDL-C and IL-6²³. Previous study demonstrated increase risk of cardiovascular disease which positively associated with elevation in Vaspin level and insulin resistance in diabetic¹⁴⁻¹⁵. It has been reported that abnormality of Vaspin level and gene expression related to BMI and markers of lipid metabolism such as cholesterol and triglycerides in metabolic syndrome²². Vaspin reported to be associated with lipid profile and up-regulate peroxisome proliferate-activated receptor γ activity and play an important role in development of atherosclerosis in diabetic patients²⁴.

CONCLUSION

In conclusion, the present study show that Vaspin level could be a marker for detection and diagnosis of diabetic patients Type-2. Also, Vaspin might be a predictor of poor glucose control and insulin resistance of T2DM.

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