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Correlation of Serum Vaspin with Cardiovascular Risk Factors in T2DM

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ABSTRACT

Background: Vaspin is a visceral adipose tissue-derived serine protease inhibitor. Previous data suggest that vaspin may be involved in the glucose metabolism and the development of T2DM in humans.

Objectives: This study is intended to compare the serum vaspin and atherogenic propensity by comparing fasting plasma glucose, post-prandial plasma glucose, lipid profile, atherogenic index and BMI with Ox-LDL level as a marker for cardiovascular risk between diabetic patients and non-diabetic subjects.

Material & Methods: This study was conducted with 120 newly diagnosed type 2 diabetes mellitus (T2DM) patients with age-matched 120 non-diabetic subjects as controls.

Results: We found that there is significant increase in the parameters like serum Vaspin, FPG, PPPG, lipid profile (Total Cholesterol, Triglycerides & Very low density lipoprotein), Oxidized-Low density lipoprotein level and Atherogenic index (TC/HDL). No significant differences were found between BMI, LDL & HDL parameters of T2DM patients compared to non-diabetic subjects. The results have been shown positive correlation ($P < 0.01$) between Vaspin with BMI and LDL while negative correlation ($P < 0.01$) was observed between Vaspin with FBS, PPBS, Ox-LDL & VLDL in T2DM patients.

Conclusion: Our results indicate that circulating Vaspin and Ox-LDL are potential new independent CVD risk biomarker in T2DM.

Key Words: T2DM, Risk factors, Vaspin and Ox-LDL

INTRODUCTION

Vaspin (visceral adipose tissue-derived serine protease inhibitor), a novel adipocytokine, was firstly identified in obese OLETF (Otsuka Long-Evans Tokushima Fatty) rat. Vaspin belongs to the serpin super family, clade A (Serpina 12). It is composed of 415, 412, and 414 amino acids in humans, rats, and mice, respectively [1]. In addition to adipose tissue, vaspin gene expression has been observed in human stomach, liver and pancreas and vaspin expression has also been observed in the hypothalamic of db/db and C57BL/6 mice [2-4]. It has been suggested that vaspin has potential insulin-sensitizing effects [1]. In humans, vaspin expression in terms of mRNA was detected in human visceral and subcutaneous adipose tissue [2]. Recent studies also found that vaspin gene expression in human adipose

tissue and circulating vaspin levels were positively associated with obesity-associated diseases and T2DM [5, 6]. Furthermore, it is indicated that vaspin plays a role in adipoinstular axis, and may be associated with insulin resistance in obese subjects, including patients with T2DM and polycystic ovary syndrome [7, 8]. Therefore, all these data suggest that vaspin may be involved in the glucose metabolism and the development of T2DM in human. Up to date, all studies on roles of vaspin in human metabolic diseases were cross-sectional, but it is still unclear what the real role of vaspin is in the progression of diabetes in a longitudinal process. It remains unclear whether the observed alterations in serum Vaspin and/or inflammatory parameters in T2DM are due to excess adipose tissue mass and/or directly associated with the diabetic state [9, 10].

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This study is intended to compare the serum vaspin and atherogenic propensity by comparing fasting plasma sugar, post-prandial plasma glucose, lipid profile, atherogenic index and BMI with Ox-LDL level as a marker for cardiovascular risk between diabetic patients and non-diabetic subjects. The purpose of this study was to explore the correlation of serum Vaspin with lipid profile, fasting plasma glucose, and oxidized LDL level as inflammatory markers for cardiovascular risk between diabetic and non-diabetic patients and with anthropometric variables in patients with T2DM.

MATERIALS & METHODS

Study participants

This study was conducted with 120 newly diagnosed type 2 diabetes mellitus (T2DM) patients with age-matched 120 non-diabetic subjects as controls.

Inclusion criteria were patients in the age group of 30-60 years of both males and females. Patient with newly diagnosed Type 2 DM based on fasting plasma glucose level \geq 126 mg/dl or 2-hour postprandial plasma glucose \geq 200 at two separate occasions after an overnight fast 8- 12 hours (based on American Diabetic Association) [11].

Exclusion criteria were age $<$ 30 and $>$ 60 years, conditions that could potentially alter vaspin concentrations such as prolonged fasting, advanced chronic diseases (such as chronic liver disease, chronic kidney disease, congestive heart failure, thyroid disease, etc.), Patient on medications such as hypolipidemic drugs, hypoglycemic drugs, hormone replacement therapy, tissue plasminogen activator (tPA), anticoagulant therapy (heparin) and steroid, Known cases of Type 1 diabetes mellitus, Pregnant and lactating women.

Diagnosis of diabetes was based on the criteria of American Diabetes Association [11]. The information of patients were obtained through a questionnaire consisted of the sex, age, height, weight and BMI. BMI was calculated using the following formula: $BMI = \text{weight (kg)}/\text{height (m)}^2$ [12]. Informed consent was obtained from all participants and the

study protocol was approved by the Ethics Committee of the SGT Medical College, Gurugram, Haryana and India.

METHODS

Assay of biochemical markers

Five milliliters of venous blood samples were collected from each patients and controls subject after 12 h of overnight fast in serum separator tubes. After clot formation, samples were centrifuged at 1000 x g for 20 minutes, and then serum was separated and transported into new disposable tubes and kept at -20°C for one month. Fasting plasma glucose (FPG), Post-prandial plasma glucose [13, 14], total cholesterol (TC) [15, 16], triglycerides (TG) [17], high density lipoprotein (HDL) [18-20] and low density lipoprotein (LDL) [21-23] were assayed on fully automated analyzer (EM-200).

Assay of circulating Vaspin and Ox-LDL in serum

The assay was performed after bringing all reagents, diluted standards and samples to the room temperature. Vaspin was assayed using Human vaspin ELISA Kit SEA706Hu (Cloud-Clone Corp, Inc., USA) with detection range from 0.156 ng/ml to 10 ng/ml and sensitivity of 0.056 ng/ml, with no significant cross-reactivity or interference with other analogues [24]. Ox-LDL was assayed using Human Oxidized Low Density Lipoprotein (Ox LDL) ELISA Kit SEA527Hu (Cloud-Clone Corp, Inc., USA). The detection range is from 15.6 pg/ml to 1000 pg/ml with an estimated sensitivity of less than 6.2 pg/ml. Intra-assay and inter-assay precision coefficients of variation (CV%) were $<$ 10% and $<$ 12%, respectively [25].

Statistical analysis

Analysis of data was performed using SPSS version 25.0 software. Data were expressed as mean \pm standard deviation (SD) for continuous variables. Normally distributed data were compared using Student's t test for two groups. The correlations between serum vaspin and Ox-LDL with other variables were tested using Pearson correlation coefficient (r) and correlations were analyzed using Spearman's correlation coefficient. P value $<$ 0.05 was considered significant.

Results:

Table 1: Comparative analysis of adipokines with cardiovascular risk factors between diabetic and non-diabetic patients using Student's t test

Parameters	Diabetic patients (n=120) Mean \pm S.D	Non- diabetic (n=120) Mean \pm S.D	t-value	P-value
BMI (Kg/m ²)	26.019 \pm 5.523	25.667 \pm 4.962	0.519	0.604*
Fasting plasma glucose (mg/dl)	173.7 \pm 63.95	101.133 \pm 10.19	12.28	0.000***

Post-prandial glucose (mg/dl)	271.558 ± 84.04	144.167 ± 15.34	16.34	0.000***
TC (mg/dl)	183.404 ± 49.94	162.9 ± 46.45	3.293	0.001***
TG (mg/dl)	238.4837 ± 111.86	164.0967 ± 83.73	5.832	0.000***
LDL (mg/dl)	100.46 ± 37.43	92.16 ± 38.52	1.689	0.093*
HDL (mg/dl)	46.528 ± 14.93	46.051 ± 11.17	0.281	0.779*
VLDL (mg/dl)	43.75 ± 17.99	33.42 ± 17.25	4.465	0.000***
Vaspin (ng/ml)	18.18 ± 14.98	12.53 ± 6.58	3.783	0.000***
Oxidized LDL (pg/ml)	29.80 ± 18.68	10.54 ± 6.06	10.741	0.000***
Atherogenic index (TC/HDLc)	4.16 ± 1.355	3.66 ± 1.04	3.181	0.002**

*Not significant ($p > 0.05$), **Significant ($p < 0.05$), *** Highly significant ($p < 0.001$)

Table 2. Correlation analysis between serum vaspin and other variables in T2DM patients.

Variable	T2DM (n = 120) r value
BMI	+0.502**
Fasting plasma glucose (mg/dl)	-0.009
Post-prandial glucose (mg/dl)	-0.006
TC (mg/dl)	+0.157
TG (mg/dl)	+0.045
LDL (mg/dl)	+0.189*
HDL (mg/dl)	+0.02
VLDL (mg/dl)	-0.02
Oxidized LDL (pg/ml)	-0.00
Atherogenic index (TC/HDLc)	+0.107

Correlation is significant at the 0.01 level (2-tailed) **

Correlation is significant at the 0.05 level (2-tailed) *

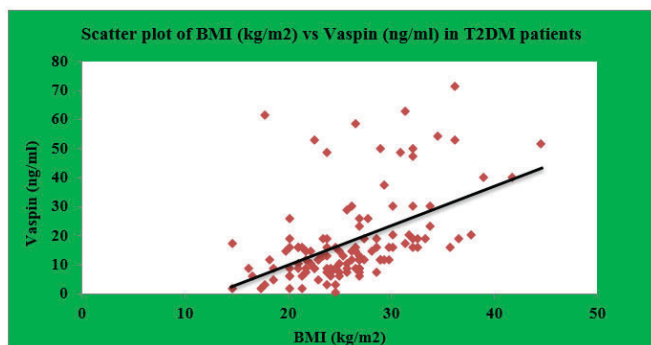


Figure 1: Correlation between BMI & Vaspin in T2DM patients.

The above figure shows significant positive correlation between BMI & Vaspin in T2DM patients with $r = .502$ & $P < 0.01$

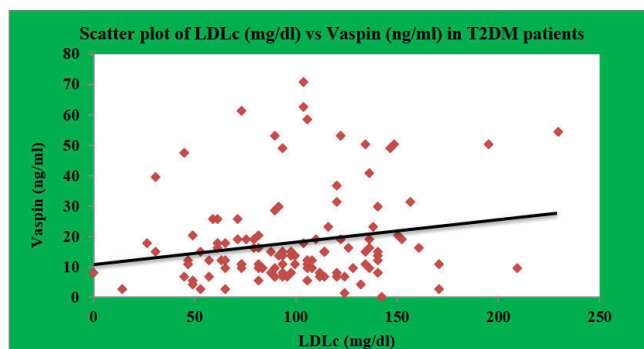


Figure 2: Correlation between LDL & Vaspin in T2DM patients.

The above figure shows significant positive correlation between LDL & Vaspin in T2DM patients with $r = 0.189$ & $P < 0.05$

DISCUSSION

In this study, we investigated the serum vaspin and atherogenic propensity by comparing fasting plasma glucose, post-prandial plasma glucose, lipid profile, atherogenic index and BMI with Ox-LDL level and their correlation as a marker for cardiovascular risk between T2DM patients and non-diabetic subjects.

We demonstrated significantly higher serum vaspin levels ($P < 0.001$) in patients with T2DM compared to non-diabetic subjects.

The comparison between FPG of T2DM patients and PPPG was found to be significantly higher ($P < 0.001$) than non-diabetic subjects.

The level of total cholesterol (TC), triglycerides (TG) and VLDL was significantly increased ($P < 0.001$) compared to non-diabetic subjects. There was no significant difference ($P > 0.05$) found between the level of LDL, HDL and BMI of T2DM patients compared to non-diabetic subjects.

In our study, a positive correlation had observed between Vaspin and LDL in T2DM patients, ($r = .189$) and ($P < 0.05$) and BMI with Vaspin in T2DM patients, ($r = .50$) and ($P < 0.01$). Our findings are supported with the results from previous studies [1, 26-32].

As per Hida et al. (2005) the levels of serum Vaspin may change with the progression of diabetes. Vaspin may increase at the beginning and decrease with worsening of diabetes in human [1].

Youn et al. (2008) have observed that serum vaspin levels are associated with the presence of obesity and impaired insulin sensitivity in subjects with NGT but not in subjects with T2DM [26].

G. Sun et al (2009) reported that vaspin was associated with lipid profile and up-regulate peroxisome proliferate-activated receptor γ (PPAR) activity and play an important role in development of atherosclerosis in diabetic patients [33].

As per Jian et al. (2014) the serum Vaspin was significantly correlated with BMI, waist-hip ratio and HOMA of insulin resistance in T2DM patients [30].

Dai et al. (2016) found that serum vaspin concentrations were elevated in T2DM patients and that serum vaspin concentrations were higher in obese T2DM patients than in lean T2DM patients and non-diabetic obese subjects [31].

Arshad Noori Al-Dujaili et al. (2016) reported that there were no significant correlation ($P > 0.05$) between vaspin levels (pg/ml) and FBG levels (mg/dl) in Type2 diabetes mellitus patients ($r = 0.227$) [32].

But, the finding of present study was in contradictory to Ye et al. (2009) [34, 35], Li et al. (2011) [35] who attrib-

uted a positive correlation between Vaspin and post-prandial plasma glucose levels. In our study, negative correlation had observed between vaspin with FPG, PPPG, VLDL and Ox-LDL level in T2DM patients.

Relationship between vaspin and atherosclerosis has been examined in several previous studies. Vaspin was detected in foam cells of atherosclerotic lesions [36] and elevated serum vaspin level was associated with the severity of coronary artery disease [37] suggesting that vaspin may play a role in atherosclerosis and cardiovascular events.

In this study, it has been also found that, cardiac risk ratio was significantly ($P < 0.05$) elevated in diabetic patients, as compared to non-diabetic subjects and the level of Ox-LDL in T2DM patients was significantly increased ($P < 0.001$) compared to non-diabetic subjects which indicates higher risk of adverse cardiac events in diabetic patients. These parameters may thus be used for analyzing the risk of atherosclerosis in diabetic patients.

CONCLUSION

Thus, it can be concluded that serum Vaspin, lipid profile, atherogenic index and Ox-LDL are known risk factors for atherosclerosis in the general population. The changes in the level of vaspin secretion and increased Ox-LDL in type 2 diabetes mellitus patients which have been associated in the development of atherosclerosis and with insulin resistance, appears to be higher risk for cardiovascular disease. Patients with T2DM should be strengthened to reduce their adipose mass, through physical exercise habit, healthy food habits, or, ultimately, drug intervention, in order to diminish the risk for cardiovascular disease. The present study also showed that vaspin level could be a marker for detection and diagnosis of T2DM patients. Vaspin might be a predictor of poor glucose control and insulin resistance of T2DM.

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List of abbreviations

T2DM Type 2 diabetes mellitus

Ox-LDL	Oxidized low density lipoprotein
BMI	Body mass index
tPA	Tissue plasminogen activator
CVD	Cardiovascular disease
PPAR	Peroxisome proliferate-activated receptor γ
NGT	Normal glucose tolerant
FPG	Fasting plasma glucose
PPPG	Post-prandial plasma glucose
TC	Total cholesterol
TG	Triglycerides
LDL	Low density lipoprotein
HDL	High density lipoprotein
VLDL	Very low density lipoprotein

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