International Journal of Biochemistry Research & Review

29(1): 1-9, 2020; Article no.IJBCRR.54568
ISSN: 2231-086X, NLM ID: 101654445

Correlation of Leptin and Vaspin Serum Concentration in Newly Diagnosed Type 2 Diabetes Mellitus

K. Priya[1*], S. K. Bansal[2], D. K. Sharma[3] and K. Y. Birendra[4]

[1]Department of Biochemistry, ASMCS, Ayodhya, U.P, India.
[2]Department of Biochemistry, SGT Medical College, Hospital and Research Institute, Gurugram, Haryana, India.
[3]Department of Medicine, SGT Medical College, Hospital and Research Institute, Gurugram, Haryana, India.
[4]Department of Biochemistry, Banas Medical College and Research Institute, Palanpur, Gujarat, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author KP designed the study, performed the statistical analysis, wrote the protocol and managed the literature searches. Authors SKB and DKS managed the analyses of the study and wrote the first draft of the manuscript. Author KYB helped and managed sample analysis during this work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2020/v29i130160
Editor(s):
(1) Dr. Halit Demir, Professor, Department of Chemistry, Faculty of Art and ScienceYuzuncu, Yil University, Turkey.
Reviewers:
(1) Brijesh Mukherjee, Hitech Medical College, India.
(2) Moutawakilou Gomina, University of Parakou, Benin.
Complete Peer review History: http://www.sdiarticle4.com/review-history/54568

Original Research Article

Received 25 November 2019
Accepted 30 January 2020
Published 07 February 2020

ABSTRACT

Objectives: The aim of the study was to explore the correlation of circulating leptin and vaspin levels with lipid profile, fasting blood sugar, HbA1c and anthropometric variable as inflammatory markers between diabetic patients and non-diabetic subjects.
Material and 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 Leptin, Vaspin, FBS, PPBS, HbA1c and lipid profile (TC, TG & VLDL). No significant differences were found between BMI, LDL & HDL parameters of T2DM patients compared to non-diabetic subjects. The

**Corresponding author: E-mail: priyayadav199025@gmail.com;*

results have been shown a significant positive correlation between Vaspin and Leptin in T2DM patients, (r = .755) and (*P<0.01*) as compared to controls. The body mass index was positively correlated with Vaspin in T2DM patients, (r = .50) and (*P <0.01*) and with leptin in T2DM patients, (r = .265) and (*P <0.01)*. A positive correlation had also observed between vaspin and LDL in T2DM patients, (r = .189) and (*p <0.05*). We also found that significant increased level of leptin and vaspin in females compared to males in our study group.

Conclusions: Serum leptin and vaspin level is positively associated with BMI and LDL and negatively correlated with fasting blood sugar, post-prandial glucose, HbA1c, VLDL and age.

Keywords: T2DM; leptin; vaspin and BMI.

ABBREVIATIONS

T2DM : Type 2 Diabetes Mellitus;
NDS : Non-Diabetic Subject;
BMI : Body Mass Index;
tPA :Tissue Plasminogen Activator;
PPAR : Peroxisome Proliferate-Activated Receptor Y;
NGT : Normal Glucose Tolerant;
FBS : Fasting Blood Sugar;
PPBS : Post-Prandial Blood Sugar;

1. INTRODUCTION

Some adipokines are involved in the development of insulin resistance, which is an important pathological link among various metabolic dysfunctions, including obesity, diabetes and cardiovascular diseases [1].

Leptin is the first identified endocrine product of adipose tissue and was found to regulate vascular function through local and central mechanisms [2]. There is also some evidence supporting the effects of leptin on the cardiovascular system. Leptin was shown to promote the development of atherosclerosis by inducing oxidative stress in endothelial cells, increasing platelet aggregation, hypertrophy and proliferation of vascular smooth muscle cells [3]. Additionally, it was shown that a high leptin level predicts subsequent development of T2DM [4]. Leptin decrease pre-proinsulin mRNA expression in β cells thus decrease the synthesis of insulin. It also reduces the release of insulin from human pancreatic β cells, which leads to the development of T2DM. Previous epidemiological studies investigating the association between circulating leptin levels and incident of T2 DM yielded discrepant results [5,6].

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 superfamily, clade A (Serpina 12). It is composed of 415, 412, and 414 amino acids in humans, rats, and mice, respectively [7]. It has been suggested that vaspin has potential insulin-sensitizing effects [7]. In humans, vaspin expression in terms of mRNA was detected in human visceral and subcutaneous adipose tissue [8]. 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 [9,10]. Therefore, all these data suggest that vaspin may be involved in the glucose metabolism and the development of T2DM in human. A significant correlation between serum vaspin and leptin concentrations supports previous human studies that serum vaspin concentration reflects body fat mass in human [11]. 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 adipokines and/or inflammatory parameters in T2DM are due to excess adipose tissue mass and/or directly associated with the diabetic state [12,13]. In this study, we examined the alteration in serum adipokines (leptin & vaspin) levels in newly diagnosed T2DM patients to explore the relationship between serum leptin & vaspin level with age, gender, body mass index (BMI), glucose metabolism and lipid metabolism as inflammatory markers between diabetic patients and non-diabetic subjects.

2. MATERIALS AND METHODS

2.1 Study Participants

The present study was conducted in the Department of Biochemistry, SGT Medical College, Hospital & Research Institute, Gurugram, Haryana. One hundred & twenty (58 female & 62 male) newly diagnosed T2DM

patients attending medicine OPD of SGT hospital and one hundred & twenty (60 female & 60 male) non-diabetic subjects as controls were enrolled in the study.

Inclusion criteria for cases: Patients in the age group of 30-60 years of both males and females and Patient with newly diagnosed Type 2 DM based on fasting blood sugar level ≥ 126 mg/dL or 2-hour postprandial plasma glucose ≥ 200 mg/dL or HbA1c ≥ 6.5% at two separate occasions after an overnight fast 8-12 hours (based on American Diabetic Association) [14] were included in this study.

Exclusion criteria for cases: The patients age < 30 years or > 60 years, Known cases of Type 1 diabetes mellitus, Patient on medications such as hypolipidemic drugs, hypoglycemic drugs, hormone replacement therapy, tissue plasminogen activator (tPA), anticoagulant therapy (heparin), steroid, Pregnant and lactating women were excluded from this study.

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 = weight (kg)/height $(m)^2$ [15]. 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.

2.2 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 blood sugar (FBS), Post-prandial plasma glucose [16,17], total cholesterol (TC) [18,19], triglycerides (TG) [20], high density lipoprotein (HDL) [21-23] and low density lipoprotein (LDL) [24-26] were assayed on fully automated analyzer (EM-200). HbA1c was assayed on fully automated analyzer (BS-300) [27,28].

Assay of circulating Leptin and Vaspin in serum: The assay was performed after bringing all reagents, diluted standards and samples to the room temperature. Leptin was assayed using leptin ELISA kit CAN-L-4260 (Diagnostic Biochem Canada Inc, DBC) with limit of detection is 0.50 ng/mL and sensitivity of less than 5%, with no cross-reactivity [29,30]. 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 [31].

2.3 Statistical Analysis

Analysis of data was performed using SPSS version 25.0 software. Data were expressed as mean ± standard deviation (SD) for continuous variables. Normally distributed data were compared using Student's t-test for two groups. The correlations between serum Leptin and Vaspin 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.

3. RESULTS

The characteristics of the patients and the controls are shown in Table 1. Correlation analysis between adipokines and other variables in T2DM patients are shown in Tables 2 & 3. A significant positive correlation between vaspin & leptin in T2DM patients are shown in Figure 1. Serum leptin & vaspin level in females were significantly higher than that in males are shown in Figs. 3 & 4.

4. DISCUSSION

In this study, we investigated the adipokines (leptin & vaspin) levels in newly diagnosed T2DM patients and it was correlated with age, gender, BMI, diabetic profile and lipid profile as inflammatory markers between diabetic patients and non-diabetic subjects. In this study, it has been found that serum leptin levels in type 2 DM patients with (mean ± S.D 20.79 ± 35.01 ng/mL) were significantly (*P* < *0.05*) increased compared to healthy controls with (mean ± S.D 11.51 ± 6.31 ng/mL) and serum vaspin level in T2DM patients with (mean ± S.D 18.18 ± 14.98 ng/mL) was also significantly (*P* < *0.001*) increased compared to non-diabetic subjects with (mean ± S.D 12.53 ± 6.58 ng/mL) (Table 1).

The comparison between FBS of T2DM patients with (mean ± S.D 173.7 ± 63.95 mg/dL), PPBS

with (mean ± S.D 271.558 ± 84.04 mg/dL) & HbA1c with (mean S.D 8.974 ± 3.49%) was found to be significantly higher (*P < 0.001*) than FBS of non-diabetic subjects with (mean ±S.D 101.133 ± 10.19 mg/dL), PPBS with (mean ± S.D 144.167 ± 15.34 mg/dL) & HbA1c with (mean ± S.D 3.798 ± 0.764%)(Table 1).

The comparison between serum TC of T2DM patients with (mean ± S.D 183.404 ± 49.94 mg/dL), serum TG (mean ± S.D 238.483 ± 111.86 mg/dL) and serum VLDL (mean ± S.D 43.75 ± 17.99 mg/ dL) was found to be significantly higher than serum TC of non diabetic subjects with (mean ± S.D 162.9 ± 46.45 mg/dL), serum TG (mean ± S.D 164.0967 ± 83.73 mg/dL) and serum VLDL (mean ± S.D 33.42 ± 17.25 mg/dL) (P < 0.001).

There was no significant difference (*P>0.05*) found between serum LDL of T2DM patients with (mean ± S.D 100.46 ± 37.43 mg/dL), serum HDL (mean ± S.D 46.528 ± 14.93 mg/dL) and BMI (mean ± S.D 26.019 ± 5.523 kg/m^2) compared to non-diabetic subjects of serum LDL with (mean ± S.D 92.16 ± 38.52 mg/dL), serum HDL (mean ± S.D 46.051 ± 11.17 mg/dL) and BMI (mean ± S.D 25.667 ± 4.962 kg/m^2).

The waist-hip ratio of T2DM patients with (mean ± S.D 0.9926 ± .0917) was significantly (*P < 0.001*) increased compared to non-diabetic subjects with (mean ± S.D 0.93 ± .135) in this study (Table 1). We also found that BMI and waist-hip ratio was positively correlated (r = .310) and (*P <0.01*) in T2DM patients compared to non-diabetic subjects (Table 3).

In our study, a positive correlation had observed between leptin & BMI (r = .265) and (*P <0.01)*, Vaspin & BMI in T2DM patients, (r = .50) and (*P <0.01*) (Table 2) and a positive correlation had also observed between Vaspin and LDL in T2DM patients, (r = .189) and (*P <0.05*) (Table 2). A progressive increase in serum Vaspin, Leptin and LDL concentration was observed with an increase in BMI in present study. Our findings are supported with the results from previous studies [8,32-37].

As per Brennan et al. the elevated plasma levels of Leptin are associated with adipocyte dysfunction in the presence of risk factors (increased BMI, CRP, LDL-c and TG) [32].

As per Stefanovic et al. a positive correlation between oxidative stress status parameters and leptin in obese patients was observed which suggests that increased oxidative stress and increased leptin levels, both consequences of obesity, may play a role in type 2 diabetes mellitus development [33].

Krasnodebski et al. studied 58 patients with CAD and detected high plasma leptin levels in diabetic patients [34].

The study conducted by Paul et al. found that strong association between leptin levels and body mass index. Increased levels of serum leptin in obese subjects without type 2 diabetes mellitus were reported in this study [35].

Kazmi et al. reported that higher levels of leptin in female [36].

Table 1. Comparative analysis of adipokines, other biochemical measurements and anthropometric variables as inflammatory markers between diabetic and non-diabetic subjects using student's t test

Parameters	Diabetic patients (n=120)	Non- diabetic (n=120)	t-value	*P*-value
BMI (Kg/m^2)	26.019 ± 5.523	25.667 ± 4.962	0.519	0.604*
Fasting blood sugar (mg/dL)	173.7 ± 63.95	101.133 ± 10.19	12.28	0.000***
Post-prandial glucose (mg/dL)	271.558 ± 84.04	144.167 ± 15.34	16.34	0.000***
HbA1c (%)	8.974 ± 3.49	3.798 ± 0.764	15.88	0.000***
TC (mg/dL)	183.404 ± 49.94	162.9 ± 46.45	3.293	0.001***
TG (mg/dL)	238.483 ±111.86	164.0967 ± 83.73	5.832	0.000***
LDLc (mg/dL)	100.46 ± 37.43	92.16 ± 38.52	1.689	0.093*
HDLc (mg/dL)	46.528 ± 14.93	46.051 ± 11.17	0.281	0.779*
VLDLc (mg/dL)	43.75 ± 17.99	33.42 ± 17.25	4.465	0.000***
Leptin (ng/mL)	20.79 ± 35.01	11.51 ± 6.31	2.858	0.005**
Vaspin (ng/mL)	18.18 ± 14.98	12.53 ± 6.58	3.783	0.000***
Waist-hip ratio	0.9926 ± .0917	0.93 ± .135	4.112	0.000***

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

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

Variable	T2DM (n = 120) r – value	
	Leptin	Vaspin
BMI (kg/m^2)	+0.265**	+0.502**
Fasting blood sugar (mg/dL)	-0.034	-0.009
Post-prandial glucose (mg/dL)	+0.014	-0.006
HbA1c (%)	-0.03	-0.04
TC (mg/dL)	+0.073	+0.157
TG (mg/dL)	+0.013	+0.045
LDLc (mg/dL)	+0.131	+.189*
HDLc (mg/dL)	-0.04	+0.02
VLDLc (mg/dL)	+0.013	-0.02
Vaspin (ng/mL)	+0.75**	1
Age	-0.113	-0.071

*Correlation is significant at the 0.01 level (2-tailed) **; Correlation is significant at the 0.05 level (2-tailed) **

Table 3. Correlation of BMI & waist-hip ratio among Non-diabetic subjects (NDS) & T2DM patients

Parameters	BMI (NDS)	Waist-hip ratio (NDS)
BMI (NDS)	1	0.064
BMI (T2DM)	BMI (T2DM)	Waist-hip ratio (T2DM)
	1	.310**

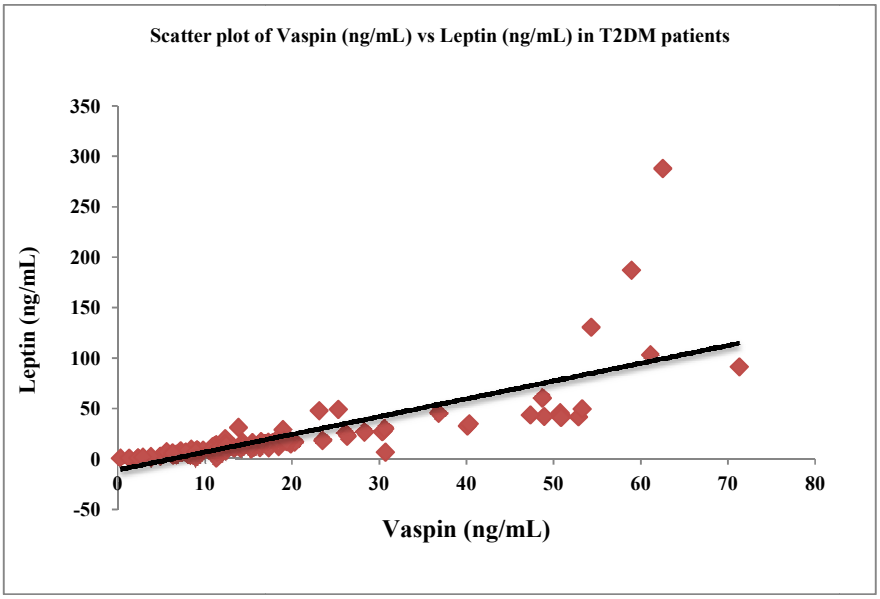

Fig. 1. Correlation between Vaspin & Leptin in T2DM patients

Das P et al. showed that there is no correlation between leptin and insulin resistance in an indian population. This study concluded that, obesity mainly of central or abdominal type might be responsible for the insulin resistance in type 2 diabetes mellitus whereas leptin, a potential marker for obesity was probably not involved. This perhaps points towards the multifactorial causation of insulin resistance in type 2 diabetes mellitus [37,38].

As per Najam et al. leptin levels are high in obese subjects irrespective of the diabetes status [39].

Ahmed et al. reported that the plasma Leptin level was positively correlated to fasting blood glucose level contrary to our results showing at no significant correlation found in the level of fasting blood glucose, Post-prandial plasma glucose and HbA1c in T2DM patients [40].

In present study, the higher levels of Leptin had observed in females comparison to males.

The present study also demonstrated that the serum vaspin level of the newly diagnosed T2DM subjects was significantly (*P < 0.001*) higher than that of the controls. Our findings are supported with the results from previous studies [7, 41-46].

As per Hida et al. 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 [7].

Youn et al. 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 [41].

G. Sun et al. reported that vaspin was associated with lipid profile and up-regulate peroxisome proliferate-activated receptor ϒ (PPAR) activity and play an important role in development of atherosclerosis in diabetic patients [42].

As per Jian et al. the serum Vaspin was significantly correlated with BMI, waist-hip ratio and HOMA of insulin resistance in T2DM patients [45].

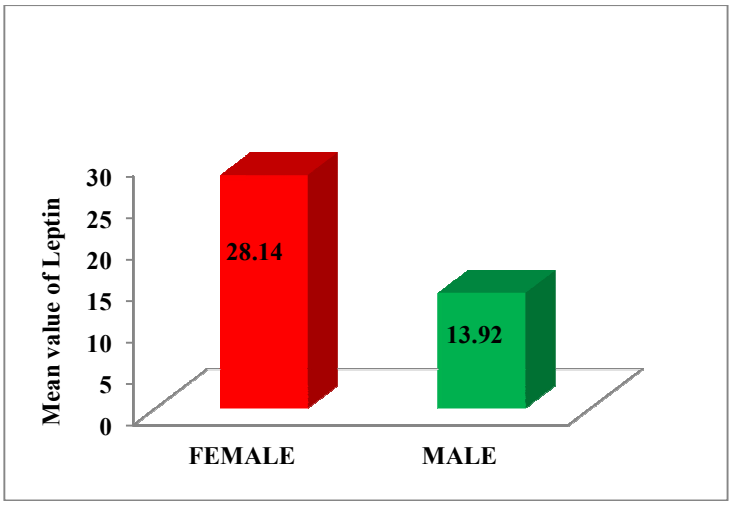

Fig. 2. Distribution of leptin among male and female

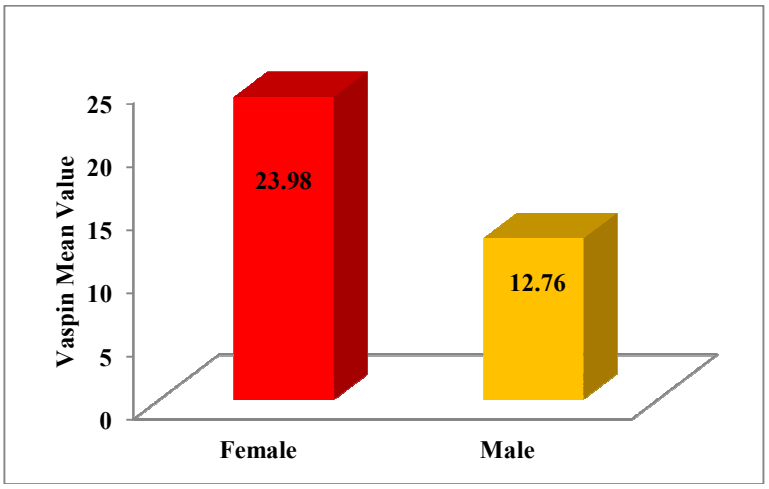

Fig. 3. Distribution of vaspin among male and female

Dai et al. found that serum vaspin concentrations were elevated in T2DMpatients and that serum vaspin concentrations were higher in obese T2DM patients than in lean T2DM patients and non-diabetic obese subjects [46].

Arshad Noori Al-Dujaili et al. reported that there were no significant correlation (*P>0.05*) between vaspin levels (pg/mL) and FBG levels (mg/dl) in Type 2 diabetes mellitus patients (r = 0.227) [47].

But, the finding of present study was in contradictory to Ye et al. [48,49] and Li et al. [49] who attributed a positive correlation between Vaspin and post–prandial plasma glucose levels. In our study, negative correlation had observed between vaspin with FBS (r = -0.009), PPBS (r = -0.006), HbA1c (r = -0.04) and VLDL (r = -0.02) level in T2DM patients. In this study, negative correlation had also observed between leptin with age (r = -0.113) and vaspin with age (r = -0.071) in T2DM patients.

In present study, vaspin and leptin levels of T2DM patients was found to be significantly higher than vaspin and leptin levels of non-diabetic subjects. A significant positive correlation observed between Vaspin and Leptin in T2DM patients as compared to control groups. A positive correlation had also observed between Vaspin and LDL in T2DM patients. We also found that Vaspin and leptin are most directly correlated with BMI parameters. So, BMI and waist-hip ratio measured easily predicted rates of these biochemical parameters involved in T2DM and maintaining a BMI and waist-hip ratio “ideal” may prevent the increase levels of vaspin, leptin, LDL and disturbances that follow.

This study showed that the serum vaspin level in females were significantly higher than that in males. Our results were consistent with the results of Seeger et al. [50]. The reasons for the gender difference remain unclear. Some studies have shown that leptin levels are affected by gender and that androgen may suppress the expression of adipokines [51,52]. High estrogen levels result in increased leptin level, which may explain the increased vaspin levels as well.

5. CONCLUSION

Thus, it can be concluded that there is an association between increased serum adipokines (leptin & vaspin) level and type 2 DM patients. Higher leptin and vaspin levels may consider as an additional risk factor in patients of type 2 DM with high BMI and waist-hip ratio (obesity) and dyslipidemia. Vaspin and Leptin level could be a marker for detection and diagnosis of T2DM patients. Vaspin and Leptin might be a predictor of poor glucose control and insulin resistance of T2DM.

CONSENT AND ETHICAL APPROVAL

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kershaw EE, Flier JS, et al. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89:2548-56.
2. Bakker W, Eringa EC, et al. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. Cell Tissue Res. 2009; 335:165-89.
3. Kowalska et al. Role of adipose tissue in the development of vascular complications in type 2 diabetes mellitus. Diabetes Res Clin Pract. 2007;78:14-22.
4. Yanagawa T, Taniguchi, et al. Leptin, triglycerides and interleukin 6 are independently associated with C-reactive protein in Japanese type 2 diabetic patients. Diabetes Res Clin Pract. 2007; 75:2-6.
5. Ley SH, Harris et al. Adipokines and incident type 2 diabetes in an aboriginal Canadian [corrected] population: The Sandy Lake health and diabetes project. Diabetes Care. 2008;31:1410–5.
6. Schmidt MI, Duncan et al: Leptin and incident type2 diabetes: Risk or Protection. Diabetologia. 2006;49:2086–96.
7. Hida K, Wada J, Eguchi J, Zhang H, Baba M, et al. Visceral adipose tissue-derived serine protease inhibitor: A unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci USA. 2005;102:10610–5.
8. Kloting N, Berndt J, Kralisch S, Kovacs P, Fasshauer M, et al. Vaspin gene expression in human adipose tissue: Association with obesity and type 2 diabetes. Biochem Biophys Res Commun. 2006;339:430–6.

9. Tan BK, Heutling D, Chen J, Farhatullah S, Adya R, et al. Metformin decreases the adipokine vaspin in overweight women with polycystic ovary syndrome concomitant with improvement in insulin sensitivity and a decrease in insulin resistance. Diabetes. 2008; 57:1501–7.
10. Aktas B, Yilmaz Y, Eren F, Yonal O, Kurt R, et al. Serum levels of vaspin, obestatin, and apelin-36 in patients with nonalcoholic fatty liver disease. Metabolism. 2011; 60:544–9.
11. Aust G, Richter O, Rohm S, Kerner C, Hauss J, et al. Vaspin serum concentrations in patients with carotid stenosis. Atherosclerosis. 2009;204:262–8.
12. Abdelgadir M, Elbagir M, et al. Reduced leptin concentrations in subjects with type 2 diabetes mellitus in Sudan. Metabolism. 2002;51:304–6.
13. Bahceci M, et al. The correlation between adiposity and adiponectin, tumor necrosis factor alpha, interleukin-6 and high-sensitivity C - reactive protein levels. Is adipocyte size associated with inflammation in adults? J Endocrinol Invest. 2007;30:210–4.
14. Alberti KG and Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic Medicine. 1998; 15(7):539-53.
15. Ahmed A E, Mohamed A M, et al. Correlation between plasma leptin hormone in diabetic patients and the extent of coronary artery disease. Menoufia Medical Journal. 2018;31:607–12.
16. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor.Annals of clinical Biochemistry.1969;6(1):24-7.
17. Sacks DB. Carbohydrates; determination of glucose in body fluids: Tietz textbook of clinical chemistry and molecular diagnostics, 5th ed. Elsevier, USA. 2012; 718-21.
18. Allain CC, Poon LS, Chan CS, Richmond WF, Fu PC. Enzymatic determination of total serum cholesterol. Clinical Chemistry.1974;20(4):470-5.
19. National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III): final report. Circulation. 2002;106:3143-421.
20. Remaley AT, Rifai N, Warnick GR. Lipids, Lipoproteins, Apolipoproteins and Other cardiovascular risk factors: Tietz textbook of clinical chemistry and molecular diagnostics, 5th Edit. Elsevier, USA. 2012; 776.
21. Castelli WP, et al. HDL cholesterol and other lipids in coronary heart disease. Circulation. 1977;55:767.
22. Williams P, et al. High density lipoprotein and coronary risk factor. Lancet. 1979; 1:72.
23. Kannel WB, Castelli WP, et al. Cholesterol in the prediction of atherosclerotic disease; new perspectives based on the Framingham study. Ann. Intern. Med. 1979;90:85.
24. Badimon JJ, Badimon L, Fuester V, et al. Regression of atherosclerotic lesions by high-density lipoprotein plasma fraction in the cholesterol-fed rabbit. Journal of Clinical Investigation. 1990;85(6):1234-41.
25. Gordon T, et al. High density lipoprotein as a protective factor against coronary heart disease. Am .J. Med. 1977;62:707.
26. Crouse JR, et al. Studies of low density lipoprotein molecular weight in human beings with coronary artery disease. J Lipid Res. 1985;26:566.
27. Trivelli LA, Ranney HM, et al. Hemoglobin components in patients with diabetes mellitus. New England Journal of Medicine.1971;284(7):353-7.
28. Nathan DM, Singer DE, et al. The clinical information value of the glycosylated hemoglobin assay.New England Journal of Medicine.1983;29:466-9.
29. Dagogo-Jack S, Fanelli C, Paramore D, Brothers J, Landt M. Plasma leptin and insulin relationships in obese and nonobese humans. Diabetes. 1996;45(5): 695-8.
30. Chessler SD, Fujimoto WY, Shofer JB, Boyko EJ, Weigle DS. Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. Diabetes. 1998;47(2):239-43.
31. Fernhurst Dr. Enzyme-linked immunosorbentb assay kit for visceral adipose tissue derived serine protease inhibitor (Vaspin). Instruction manual. 12th ed. Cloud-Clone Corp. 2017;1-5.

32. Brennan A M, et al. Circulating leptin levels are not associated with cardiovascular morbidity and mortality in women with diabetes: A prospective cohort study. Diabetologia. 2007;50:1178–85.
33. Youn BS, Klo¨ting N, et al. Serum vaspin concentrations in human obesity and type 2 diabetes. Diabetes. 2008;57:372–7.
34. Krasnodebski P, et al. Leptin in coronary artery disease and period of convalescence in patients with type 2 diabetes mellitus. Kardiol Pol. 2010;68: 648–53.
35. Paul RF, Hassan M, et al. Effect of body mass index on serum leptin levels. J Ayub Med Coll Abbottabad. 2011;23:40-3.
36. Kazmi A, et al. Association of leptin with type diabetes in non-obese subjects. J Ayub Med Coll Abbottabad. 2012;24:186-9.
37. Das P, Bhattacharjee D, et al. Association of obesity and leptin with insulin resistance in type diabetes mellitus in Indian population. Indian J Physiol Pharmacol. 2013;57:45-50.
38. Feng R-N, Wang C, et al. Vaspin in newly and previously diagnosed Chinese type 2 diabetic females: a case-control study. Asian Biomedicine. 2011;5(4):525–9.
39. Najam SS, Awan FR, et al. Leptin correlation with obesity, diabetes and gender in a population from Faisalabad, Pakistan. Arch Med. 2016;8:5-9.
40. Ahmed AE, et al. Correlation between plasma leptin hormone in diabetic patients and the extent of coronary artery disease. Menoufia Medical Journal. 2018;31:60.
41. Youn BS, Klo¨ting N, et al. Serum vaspin concentrations in human obesity and type 2 diabetes. Diabetes. 2008;57:372–7.
42. Sun G, Li L, Yang M, Liu H, Nowicki M, Zong H, Yang G. Ann. Med. 2009;41: 311.
43. Li Z, Ma C, Li L, et al. Vaspin serum concentration in patients with type 2 diabetes and carotid plaque. The Journal of International Medical Research. 2012; 40(5):1670–6.
44. Atya HB, Hassan ZA, et al. Vaspin concentration in obesity, impaired glucose tolerance and type 2 diabetes in Egypt. Advanced Research in Biological Sciences. 2013;1(1):6–13.
45. Jian W, Peng W, Xiao S, Li H, Jin J, Qin L, et al. Role of serum vaspin in progression of type 2 diabetes: A 2-Year Cohort Study. PLOS ONE. 2014;9(4):e94763.
46. Dai R, Dong Z, et al. Obese type 2 diabetes mellitus patients have higher serum vaspin concentrations. Journal of Diabetes. 2016;8(3):445–7.
47. Al-Dujaili AN, Hassan AB, et al. Assesment of vaspin and risk factors in relation with diabetes mellitus type2. Rasayan J. Chem. 2016;9(4):566-72.
48. Ye Y, Hou XH, et al. Serum vaspin level in relation to postprandial plasma glucose concentration in subjects with diabetes. Chinese Medical Journal. 2009;122(21): 2530–3.
49. Li K, Li L, Yang M, et al. Short-termcontinuous subcutaneous insulin infusion decreases the plasma vaspin levels in patients with type 2 diabetes mellitus concomitant with improvement in insulin sensitivity. European Journal of Endocrinology. 2011;164(6):905–10.
50. Seeger J, Ziegelmeier M, Bachmann A, Lössner U, et al. Serum levels of the adipokine Vaspin in relation to metabolic and renal parameters. J. Clin. Endocrinol. Metab. 2008;93:247-51.
51. Horn R, Geldszus R, Pötter E, von zur Mühlen A, et al. Radioimmunoassay for the detection of leptin in human serum. Exp. Clin. Endocrinol. Diabetes. 1996; 104:454-58.
52. Ma Z, Gingerich RL, Santiago JV, Klein S, et al. Radioimmunoassay of leptin in human plasma. Clin. Chem. 1996;42:942-6.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/54568