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Original Research Article

Low Circulating Vaspin Level is not associated with Insulinemic Status in Impaired Glucose Tolerant Subjects

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ABSTRACT

Background: Vaspin is a potential insulin-sensitizing adipokine and its low levels may be linked to onset and progression of type 2 diabetes mellitus (DM). However, the exact mechanisms in relation to DM are not completely understood and conflicting.

Aim of the study: To compare serum vaspin levels in subjects with impaired glucose tolerance (IGT) with apparently healthy controls to explore its relationship with insulinemic status or impaired glucose tolerance.

Methods: This study included 47 subjects with IGT and age-sex matched 30 apparently healthy controls. Fasting serum insulin and vaspin levels were measured by enzyme linked immunosorbent assay. Insulin secretory capacity (HOMA %B), insulin sensitivity (HOMA %S) and insulin resistance (HOMA IR) were assessed from fasting glucose and insulin using HOMA2 calculator.

Results: Fasting serum vaspin levels were 1.24 ± 0.85 ng/ml in IGT and 2.17 ± 1.22 ng/ml, p = 0.0006. Vaspin levels showed no significant relationship HOMA %B, HOMA %S or HOMA IR in IGT or controls. However, vaspin showed negative trend with HOMA %B, HOMA IR and positive trend with HOMA %S in the total subjects. Multiple regression analysis showed that β value was not significant for insulin (p = 0.383), HOMA % B (p = 0.763), HOMA %S (p = 0.441) and HOMA IR (p = 0.381) on adjusting age, BMI and glycemic group.

Conclusion: It may be concluded that low circulating vaspin level is not associated with insulinemic status in impaired glucose tolerant subjects.

Keywords: Vaspin, Impaired glucose tolerance, Beta cell function, Insulin sensitivity, Insulin resistance.

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INTRODUCTION

The adipose tissue secretes several bioactive molecules known as adipokines or adipocytokines in addition to energy storage. These adipokines play an important role in reducing the progression of atherosclerosis through several mechanisms involving inhibition of insulin resistance, monocyte attachement, foam cell formation, endothelial cell activation, oxidative stress and vascular inflammation or stimulation of plaque stabilization.[1] The visceral adipose tissue-derived serine protease inhibitor (vaspin) is a novel adipokine that was identified recently in obese OLETF rats and found to have potential insulin-sensitizing effects.[2,3] It improves glucose tolerance and affects the candidate genes for insulin resistance[2] and acutely reduces food intake in obese mice.[4] In addition to adipose tissue,[5] vaspin gene expression has been observed in human stomach,[4] liver and pancreas.[6] Vaspin gene expression in adipocytes and circulating vaspin levels are found to be positively associated with obesity, obesity-associated diseases and type 2 diabetes mellitus.[5,7-9] A number of studies[10,11] reported a higher serum vaspin level in type 2 diabetic subjects than normoglycemic controls whereas opposite results are also available.[12-14] One recent study[15] found that low circulating vaspin is a risk factor for the development and progression of type 2 DM which indicated that lower vaspin level may be linked to intermediate hyperglycemia. In prediabetes stage, some studies[13,14] reported a higher serum vaspin level that were in agreement with its diabetic counter parts. However, the exact mechanism by which vaspin is linked to the impairment of glucose homeostasis, insulin sensitivity or developing type 2 DM are not clearly understood. Since low circulating vaspin has found to be associated with type 2 diabetes and decreased insulin sensitivity in subjects with type 2 DM of Bangladeshi origin[12] in contrast to other studies,[16] in this study, we aimed to determine serum vaspin and to explore the relationship of serum vaspin with β-cell function or insulin sensitivity in subjects with impaired glucose tolerance (IGT).

MATERIALS AND METHODS

This cross-sectional observational study was conducted during the period of February – December 2015 and included 47 subjects with impaired glucose tolerance (31 male and 16 female) aged between 25 to 56 years and 30 age-sex matched healthy subjects (20 male and 10 female). IGT was defined according to WHO criteria.[17] Subjects with diabetes mellitus (according to WHO definition),[17] subjects with a previous or current histories of gestational diabetes mellitus (GDM), hypertension, patients with serious comorbid diseases (infection, stroke, myocardial infarction, major surgery), subjects using drugs that significantly affect glucose metabolism (anti-hyperglycemic agents, glucocorticoids, thiazide diuretics etc.) and pregnant or lactating mother were excluded.

Blood samples were collected after an overnight fast and plasma samples were kept at -70 °C for subsequent assay. Plasma glucose was measured by hexokinase method by the automated chemistry analyzer, Dimension RxL Max (Siemens Healthcare Diagnostics Inc., USA). Glycated hemoglobin levels (%HbA_{1c}) were estimated by a dedicated high-performance liquid chromatography using Variant® Turbo Hemoglobin A_{1c} Program (Bio–Rad Laboratories, Inc., USA). Serum total cholesterol, triacylglycerol, high-density lipoprotein cholesterol, creatinine and alanine amino transferase were measured spectrophotometrically using Dimension RxL Max chemistry analyzer.Low-density lipoprotein cholesterol was calculated by Friedewald formula.[18] Serum vaspin and insulin concentrations were determined using enzyme-linked immunosorbent assay (Cloud-Clone Corp., USA).

Data were analyzed by Student t test, Spearman rank correlation and multiple linear regression using MedCalc 11.4 or STATISTICA 8 and A two-tailed p value of <0.05 was considered statistically significant.

RESULTS

In this cross-sectional observational study, 77 adults subjects were recruited according to inclusion-exclusion criteria, among them 47 subjects had impaired glucose tolerance (IGT) and the rest 30 were age-sex matched apparently healthy subjects. The study was conducted to explore the association of circulating vaspin levels with serum insulin, insulin secretory capacity (HOMA %B) and insulin sensitivity (HOMA %S) or insulin resistance (HOMA IR) in IGT subjects of Bangladeshi origin.

Characteristics of the study subjects

Characteristics of the study subjects and controls are presented in table 1. IGT group and controls were matched for age (p = 0.650) with a central tendency that represent middle age (Table 1). The age ranges for IGT and control groups were 25 – 56 years and 30 – 56 years respectively. In IGT and control groups, 25% and 75% percentile value of ages were 39%, 48% years and 37%, 48% years respectively. In IGT group, 66% were male, in control, 67% were male. Body mass index (BMI) in subjects with IGT was higher than that of controls (p = 0.048) and 35% of IGT and 20% of control had BMI ≥ 27.5 Kg/m^2. The waist-hip ratios (WHR) were similar in both groups (P = 0.218). Systolic and diastolic blood pressures were higher in IGT group compared to control group but it was significant only for diastolic blood pressure (p = 0.045). The IGT group had significantly higher fasting and postprandial plasma glucose levels compared to control group (Table 1). Compared to the mean of HbA_{1c} in the control group, the mean of HbA_{1c} values was significantly higher in IGT group (p < 0.0001) and mean was within the range of prediabetes according to ADA diagnostic criteria based on HbA_{1c}. Among the lipid parameters studied, fasting serum total cholesterol, fasting serum triacylglycerol and low-density lipoprotein cholesterol were higher and fasting HDL-cholesterol was lower in IGT groups compared to control but not statistically significant (Table 1). Serum creatinine and ALT levels were similar in IGT and control subjects.

Insulinemic status of the study subjects

Insulinemic status of control group and of IGT group is presented in Table 2. The mean value of fasting serum insulin differed significantly between control and IGT (p < 0.0001) and it was 59% higher in IGT group compared to control. Compared to control, insulin secretory capacity as assessed by HOMA %B was 46% higher in IGT group and it was statistically significant (p < 0.0001). Insulin sensitivity as assessed by HOMA %S in IGT group was significantly reduced compared to control (32%, p < 0.001) and insulin resistance as assessed by HOMA IR was significantly higher in IGT group (53%, p < 0.0001).

Table 1: Clinical and biochemical characteristics of the study subjects

Parameters	IGT (n=47)	Control (n=30)	p value
Age (yrs)	43.3 ± 7.7	42.5 ± 7.5	0.65
Sex (male/female)	20/10	31/16	-
BMI (kg/m^2)	26.6 ± 3.7	25.0 ± 3.2	0.048
WHR	1.00 ± 0.06	0.98 ± 0.08	0.218
SBP (mmHg)	118 ± 13	112 ± 12	0.061
DBP (mmHg)	79 ± 10	75 ± 10	0.045
FPG (mmol/L)	5.7 ± 0.6	5.1 ± 0.4	<0.0001
PPG†† (mmol/L)	9.7 ± 0.9	5.9 ± 1.0	<0.0001
HbA_{1c} (%)	6.3 ± 0.4	5.4 ± 0.4	<0.0001
Fasting TG (mg/dl)	177 ± 80	156 ± 78	0.279
Fasting Chol (mg/dl)	189 ± 73	169 ± 58	0.206
Fasting HDLC (mg/dl)	38.2 ± 8	39.8 ± 11	0.636
Fasting LDLC (mg/dl)	92 ± 56	89 ± 45	0.904
S Creatinine (mg/dl)	0.92 ± 0.43	0.86 ± 0.32	0.514
ALT (U/L)	30 ± 15	27 ± 13	0.371

Results are expressed as mean (SD) and compared by Unpaired t test, BMI = Body Mass Index, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Presure, FPG =Fasting plasma glucose, PPG†† =Postprandial Plasma Glucose 2 hours after 75g oral glucose load, HbA1c = Glycated hemoglobin, TG = Serum Triacylglycerols, Chol = Serum Total Cholesterol, HDLC = High- Density Lipoprotein Cholesterol, LDLC = Low-Density Lipoprotein Cholesterol, ALT = Serum alanine amiono transferase.

Table 2: Comparison of serum insulin, insulin secretory capacity, insulin sensitivity, insulin resistance between control and IGT.

Parameters	IGT (*n*=47)	Control (*n*=30)	p value
Fasting Insulin (μIU/ml)	19.1 ± 5.5	12.0 ± 2.8	**<0.0001**
HOMA %B	152.6 ± 38.2	104.8 ± 38.1	**<0.0001**
HIOMA %S	38.2 ± 13.2	56.4 ± 13.5	**<0.0001**
HOMA IR	2.9 ± 0.8	1.9 ± 0.4	**<0.0001**

Two-tailed un-paired t test between case and control; HOMA %B, insulin secretory capacity; HOMA %S, insulin sensitivity; HOMA IR, insulin resistance.

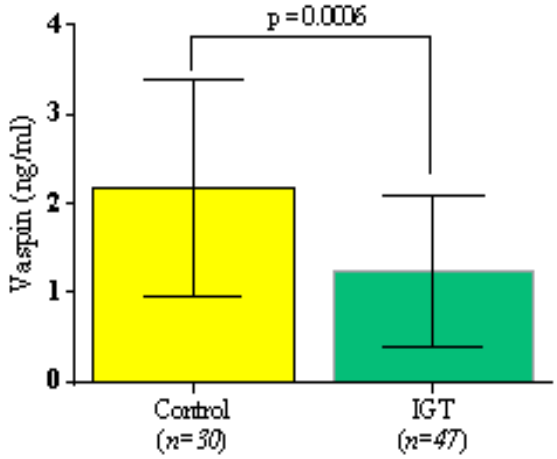

Fig 1: Comparison of serum vaspin (ng/ml) between control and IGT.

Serum vaspin levels in control and IGT groups

In control group, the mean (SD) of serum vaspin concentration was 2.17 ± 1.22 ng/ml. It was 1.24 ± 0.85 ng/ml in IGT group. Figure 1 represent the comparison of serum vaspin levels between control and IGT group. The mean difference was statistically significant (p = 0.0006). Serum vaspin concentrations were higher in control for both male and female but It was significant only for male (Table 3). Further, no significant difference was observed between male and female within each group (Table 3). In obese group (BMI ≥ 27.5 Kg/m^2), circulating level of serum vaspin was higher in control compared to IGT (p = 0.261) whereas it was significantly higher for BMI < 27.5 kg/m^2 in control compared to IGT (Table 3). No significant difference was found between subjects with BMI ≥ 27.5 Kg/m^2 and BMI<27.5 kg/m^2 within IGT or control (Table 3).

Relationship of serum vaspin with HOMA %B, HOMA %S, HOMA IR and other variables

Table 4 shows the relationship of serum vaspin with HOMA %B, HOMA%S, HOMA IR, age, BMI, FPG, PPG, HbA_{1c}, fasting insulin in control and IGT groups. No parmeters showed statistically significant relationship with serum vaspin levels in control or IGT group. However, in the total subjects, FPG, PPG and HbA_{1c} showed statistically significant negative correlation with serum vaspin (Table 4).

Multiple regression analysis

In multiple linear regression analysis considering insulin, HOMA %B, HOMA %S and HOMA IR as dependent variable and serum vaspin, age, BMI as independent continuous variables and gender, glycemic groups as categorical independent variables showed no significant association serum vaspin (Table 5).

Table 3: Comparison of serum vaspin

Parameters	IGT (*n*=47)	Control (*n*=30)	p value
Male	1.20 ± 0.80	1.99 ± 1.06	**0.014**
Female	1.32 ± 0.99	2.49 ± 1.49	**0.059**
p value	*0.700*	*0.387*	
BMI ≥ 27.5 Kg/m^2	1.43 ± 0.92	2.27 ± 1.57	0.261
BMI < 27.5 Kg/m^2	1.16 ±	2.13 ± 1.14	**0.003**
p value	*0.410*	*0.842*	

Table 4: Correlation of serum vaspin with different parameters

Parameters	All Subjects (*n*=77)	Control (*n*=30)	IGT (*n*=47)
	r(*p*)	r (*p*)	r (*p*)
Age	0.050 (*0.692*)	-0.021 (0.919)	0.132 (0.424)
BMI	0.064 (*0.617*)	-0.021 (0.919)	0.306 (0.061)
WHR	0.003 (0.983)	0.043 (0.837)	0.157 (0.399)
FPG	**-0.308 (*0.013*)**	-0.382 (0.054)	0.030 (0.857)
PPG	**-0.284 (*0.022*)**	0.021 (0.918)	**0.387 (0.015)**
HbA_{1c}	**-0.428 (*0.0009*)**	-0.197 (0.336)	-0.197 (0.288)
Fasting Insulin	-0.159 (*0.205*)	0.131 (0.523)	0.149 (0.364)
HOMA %B	-0.185 (*0.140*)	0.016 *(0.938)*	0.071 *(0.670)*
HOMA %S	0.134 (*0.289*)	0.155 (0.449)	-0.112 (0.496)
HOMA IR	-0.146 (*0.247*)	0.136 (0.507)	0.151 (0.361)

Table 5: Association of serum vaspin with insulinemic variables.

Independent variable	Insulin	HOMA %B	HOMA %S	HOMA IR
Vaspin	β = 0.098	β = 0.035	β = -0.089	β = 0.101
	p = 0.383	p = 0.763	p = 0.441	p = 0.381
Group (IGT)	β = 0.619	β = 0.539	β = -0.546	β = 0.595
	p < 0.001	p <0.001	p <0.001	p < 0.001
Age	β = -0.113	β = -0.219	β = 0.115	β = -0.095
	p = 0.290	p = 0.053	p = 0.298	p = 0.389
BMI	β = 0.235	β = 0.323	β = -0.278	β = 0.222
	p = 0.040	p = 0.007	p = 0.019	p = 0.059

DISCUSSION

In this study, the mean value of fasting serum insulin, insulin secretory capacity (HOMA %B) and insulin resistance as assessed by HOMA IR were significantly higher with reduced insulin sensitivity (HOMA %S) in IGT group compared to control that represented the characteristic feature of intermediate hyperglycemia. These results are inaccordance with previous studies done on Bangladeshi population.[12,19]

The mean (SD) of serum vaspin was 2.17 ± 1.22 ng/ml in the control group (consisting 30 apparently healthy adults) and it was higher than the previous study done on Bangladeshi adult healthy subjects (0.83 ± 0.28 ng/ml).[12] Considerable differences in demographic variables and insulinemic status and ELISA kit from different sources may be responsible for this variation in these studies (Current study and Tasnim et al[12]). Exisisting data indicated that mean serum vaspin level in adult healthy control were 0.065 – 1.55 ng/ml.[9,14,15,20-23] In subjects with IGT, compared to control significantly lower serum vaspin level was observed with a mean (SD) value of 1.24 ± 0.85 ng/ml in this study. This finding is inconsistent with other studies by level and trend. Tönjes et al[23] reported a higher serum vaspin level in IGT compared to control and it was higher than the mean value in IGT in the present study (Tönjes et al vs current study: 1.89 ± 1.76 vs 1.24 ± 0.85 ng/ml). In another study Atya et al[14] found a significantly higher serum vaspin level in prediabetes (0.34 ± 0.08 ng/ml) compared to control (0.07 ± 0.02 ng/ml). This inconsistency has also been observed in previous study done in Bangladeshi type 2 diabetic subjects that found significant lower level of serum vaspin in type 2 diabetes compared to control and it was opposite to that obtained by Atya el al[14] and Tönjes et al[23]. However, current finding (this study) and previous reports[12] in this population are in the line of recent cohort study which concluded that low serum vaspin level is a risk factor for the pregression of type 2 diabetes.[15]

Several studies indicated that circulating level of serum vaspin is related to age, gender, BMI, WHR but these are not common. In this study, no significant difference was observed between male and female or normal and obese subjects in control or IGT group. Unlike the previous study,[12] we observed positive trend (r= 0.306, p=0.061) of circulating vaspin with BMI in IGT and is consistent with reports of Feng et al[13]. Furthermore, we observed significant positive relationship of circulating vaspin with post-glucose load plasma glucose levels in IGT and inverse relationship with fasting and post-glucose load plasma glucose and glycated hemoglobin levels in the total subjects.

The relationship of circulating vaspin with insuliniemia status in different glycemic stages are conflicting.[24] Though study on IGT subjects is rare, some studies found a positive or no relationship with insulin sensitivity in control or type 2 DM and negative or no correlation with insulin secretion and insulin resistance.[14,23] This study revealed no relationship of circulating vaspin with fasting plasma glucose, HbA1c, insulin sensitivity as assessed by HOMA %S, insulin secretion (HOMA %B) or insulin resistance (HOMA IR) in IGT or control groups. The flat curve of vaspin with these parameters may be due to small sample size, narrow range of plasma glucose and serum vaspin. In this study, serum vaspin showed no significant association with insulinemic status as assessed by multiple linear regression analysis. However, we observed a negative trend of circulating vaspin with insulin hypersecretion or insulin resistance and positive trend with insulin sensitivity in the total subjects indicating a protective role to the progression of type 2 diabetes mellitus.

The role of circulating vaspin related to type 2 diabetes is thought to be protective. In a study on Chinese type 2 diabetic subjects showed lower vaspin level in type 2 diabetic subjects with carotid plaque compared to those without carotid plaque.[21] In another Chinese 2-year cohort study showed that low circulating vaspin is a risk factor for the new onset and progression of type 2 DM. Since type 2 diabetes is a polygenic disorder, more studies in differents subsets of hyperglycemia required to be carried out to investigate to role of vaspin on development and progression of type 2 diabetes mellitus in our population.

It may be concluded from this study that fasting serum vaspin is lower in IGT than healthy subjects and low circulating vaspin level is not associated with insulinemic status in impaired glucose tolerant subjects.

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