Original Article

Assessment of serum and salivary visfatin levels in newly diagnosed patients of type-II DM

Faith R. Jerusha[1], Vandana Raghunath[2]

[1]Post Graduate Student, [2]Department of Oral and Maxillofacial Pathology and Oral Microbiology, Narayana Dental College, and Hospital, Chinthareddypalem, Nellore, Andhra Pradesh, India

Abstract

Background: Type II diabetes mellitus (T2DM) is a metabolic disorder. It is thought to be an autoinflammatory disease, as inflammatory mediators are associated with the progression of the disease. Visfatin, an adipokine, is linked to insulin resistance.

Aims: We aimed to study serum and salivary visfatin levels, at the time of diagnosis of T2DM, in middle-aged individuals, in the context of other diagnostic parameters like fasting blood sugar (FBS), postprandial blood sugar (PPBS), and glycated haemoglobin A1c (HbA1c).

Materials and Methods: Thirty newly diagnosed T2DM (Group-I, T2DM) patients and 30 healthy nondiabetic individuals (Group-II, health controls [HC]) matched for age and sex were studied. In both the groups, we assessed serum and salivary visfatin levels, and serum high-sensitivity C-reactive protein (hs-CRP) levels. We also compared serum and salivary visfatin levels and serum hs-CRP levels between Group-I and Group-II individuals.

Statistical Analysis: The correlation between the groups was tested using Pearson's correlation. A *P*- value < 0.001 was considered to be statistically significant. The data was tabulated using software MS Excel and analysed using IBM Statistical Package for the Social Sciences (SPSS) Version 22.0.

Results: A positive correlation with a value of 0.8836 and a *P* value of 0.001 was noted between serum and salivary visfatin of Group-I.

Conclusion: This is the first study in the Indian scenario to study the serum and salivary visfatin in newly diagnosed T2DM individuals. Serum visfatin and hs-CRP levels increased in T2DM, thus defining the link between visfatin, inflammation and T2DM, but we failed to notice a positive correlation.

Keywords: Diabetes mellitus, hs-CRP, T2DM, visfatin

Address for correspondence: Dr. Vandana Raghunath, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Narayana Dental College and Hospital, Chinthareddypalem, Nellore – 524 003, Andhra Pradesh, India.
E-mail: vandanaraghunath@hotmail.com

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INTRODUCTION

Type II Diabetes mellitus (T2DM) is a Chronic metabolic disorder with clinical signs and symptoms of increased serum glucose levels, insulin resistance (IR), and relative deficiency of insulin.[1] Micro and macrovascular complications of diabetes increase the risk of cardiovascular mortality and morbidity. Insulin resistance is associated with hyperglycemia, abnormal lipid profiles, and alterations in

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inflammatory mediators.[2] Inflammatory mediators like interleukin-1β (IL-1β) and C-reactive protein (CRP) have been found to be indicators T2DM and are involved in the progression of T2DM. Thus, T2DM is said to be an auto-inflammatory disease, leading to β-cell dysfunction.[3] A novel group, 'adipocytokines,' secreted by adipocytes, are now linked to IR, thus relating to obesity and T2DM.

Fukuhara *et al.*[4] isolated an adipokine – Visfatin/Nampt in 2005, produced from the visceral fat of humans and mice. Later, it was found that visfatin was both synthesised and released from adipocytes and inflammatory cells. Visfatin/Nampt is involved in the progression of atherosclerosis and is a marker of endothelial dysfunction.[5] It binds to the insulin receptor and activates it, to exert several insulin-mimetic actions.[4,6] Visfatin is implicated to have a vital role in glucose homeostasis.[6-8] Conditions such as sepsis, acute lung injury, rheumatoid arthritis and inflammatory bowel disease due to an active state of inflammation are noted to have elevated levels of visfatin.[6]

We aimed to investigate serum and salivary visfatin levels, at the time of diagnosis of T2DM, in middle-aged individuals, in the context of other diagnostic parameters like like fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycated hemoglobin (HbA1c). As inflammation is the common tie between T2DM, obesity, and visfatin, high-sensitivity CRP (Hs-CRP) is an inflammatory marker (increased T2DM and obesity), and visfatin being a pro-inflammatory cytokine; their correlation in T2DM patients was also explored.

MATERIALS AND METHODS

We hypothesised serum visfatin to be increased in middle-aged newly diagnosed T2DM patients, as it is responsible for IR. Saliva is an ultra-filtrate of plasma, and Hs-CRP, an inflammatory marker, should reflect levels of visfatin. Our study population comprised 30 newly diagnosed T2DM (Group-I, T2DM) patients and 30 healthy nondiabetic individuals (Group-II, HC-healthy controls) matched for age and sex. Group-I patients were drawn from the Out-patient Department of General Medicine, Narayana Super Specialty Hospital, Nellore, Andhra Pradesh, and the healthy individuals were the patients and patient attendees attending the Oral Medicine Department of Narayana Dental College and Hospital, Nellore (A.P). For Group-I, we included patients diagnosed with FBS (>126 mg/dl), PPBS (>140 mg/dl) and HbA1c levels (>6.5) and as per criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1998), aged 30–40 years and having a body mass index (BMI) of 18.5–24.9. The control group included healthy nondiabetic patients matched for age and sex, with no symptoms of diabetes mellitus with FBS (<126 mg/dl), PPBS (<140 mg/dl) and HbA1c levels (<6.5). Subjects in both groups belonged to the same race, ethnicity, socio-economic status and living standards. Subjects with severe diabetic complications (retinopathy, peripheral neuropathy and nephropathy), blood pressure (>130/90), systemic complications/severe periodontitis/oral lesions other than dental caries, kidney/liver dysfunction (elevated serum glutamic oxaloacetic transaminase [SGOT]/Serum glutamic pyruvic transaminase [SGPT]/creatinine levels), endocrine malignancy/any other chronic inflammatory diseases/severe systemic illnesses or any recent weight change, other disorders altering immune system and smokers were excluded from the study. The objective of our study was to assess serum FBS, serum PPBS, and serum HbA1c assay in only Group-II. We assessed serum and salivary visfatin levels, and serum hs-CRP levels in both groups. We also compared serum and salivary visfatin levels and serum hs-CRP levels between Group-I and Group-II individuals. We also planned to correlate serum visfatin with salivary visfatin and serum visfatin with FBS, PPBS, HbA1c, and hs-CRP in Group-I individuals.

Ethics

Ethical approval was obtained from the Institutional Ethical Committee, Narayana Dental College and Hospital. The study procedure was explained in detail, and oral and written consent was obtained from all participants. All samples were analysed at GREEN PATH ELISA DIAGNOSTIC LAB, KURNOOL. Both serum and salivary samples were then assayed for visfatin levels using a Human Visfatin ELISA kit obtained from Chongqing Biospes Co., Ltd, China (Biospes Catalog No.: BYEK1137) using enzyme-linked immunosorbent assay method. With the remaining serum, hs-CRP levels were assessed using the Turbilatex Turbidimetry Test Kit, obtained from Lab Care Diagnostics Pvt., Ltd, India turbidimetric immunoassay method.

Statistical Analysis

Numbers and percentages were used to represent categorical variables, mean and standard deviation were used for continuous variables. The mean differences were assessed using the Student's *t*-test. Pearson's correlation was used to test the correlation between the groups. A *P*- value of <0.001 was considered to be statistically significant. The data was tabulated using software MS Excel and analysed using IBM Statistical Package for the Social Sciences (SPSS) Version 22.0.

RESULTS

The baseline characteristics of the study population are enumerated in Table 1. Serum FBS levels ranged from 178–129 mg/dl in Group-I, while in Group-II individuals, it ranged from 112–75 mg/dl. Mean Fasting blood sugar (FBS) values were higher in Group-I (T2DM) (149.2333 ± 14.9958) compared to Group-II (98.200 ± 10.9431) individuals. It was statistically significant with a *P*- value <0.001. [Table 2]. Serum PPBS levels ranged from 210–162 mg/dl in Group-I, while in Group-II individuals, it ranged from 135–96 mg/dl. Mean PPBS values were higher in Group-I (T2DM) (183.2667 ± 15.3980) compared to Group-II (117.200 ± 11.9175) individuals. This, again, was statistically significant with a *P*- value <0.001. [Table 2] Serum HbA1C levels ranged from 12.9–6.1% in Group-I, while in Group-II individuals, it ranged from 9.2–4.1%. Mean HbA1C values were higher in Group-I (T2DM) (8.4067 ± 1.9483) compared to Group-II (6.1033 ± 1.3984) individuals. [Table 2]. It was statistically significant with a *P*- value of <0.001. Serum hs-CRP levels ranged from 6.2–3.2 mg/l in Group-I, 2 while in Group-II individuals, it ranged from 4.6–0.02 mg/l. Higher mean hs-CRP values were seen in Group-I (T2DM) (4.2633 ± 1.0337) compared to Group-II (1.8073 ± 1.1517) individuals. It was statistically highly significant, with a *P*- value of <0.001. [Table 2] The mean serum visfatin levels were higher in Group-I (14.9233 ± 2.116) compared to Group-II (6.6133 ± 1.948); this was also statistically significant with a *P*- value of <0.001. [Table 3] On comparing the mean salivary visfatin values, a higher mean value was noted in Group-I (12.5567 ± 1.654) compared to Group-II (3.9167 ± 1.491), which was statistically highly significant with a *P*- value of <0.001 [Table 3]. Among Group-I individuals, a positive correlation was noted between serum and salivary visfatin levels with a correlation value of 0.8836. It was statistically significant, with a *P*- value of 0.001 [Figure 1].

Table 1: Baseline characteristics of the study population

Baseline data	T2DM Cases (*n*=30)		HC Control (*n*=30)	
	Mean	SD	Mean	SD
Age	37.76666667	2.284631312	37.3	2.3946
	Frequency		Frequency	
FBS				
≥126 mg/dl	30		0	
<126 mg/dl	0		30	
PPBS				
≥140 mg/dl	30		0	
<140 mg/dl	0		30	
HbA1c				
≥6.5	27		11	
<6.5	3		19	
	No.	%	No.	%
Gender				
Female	15	50%	15	50%
Male	15	50%	15	50%

FBS=Fasting blood sugar, HC=Healthy control, HbA1c=Glycated Hemoglobin, PPBS=Postprandial blood sugar, T2DM=TypeII

FBS=Fasting blood sugar, HC=Healthy control, HbA1c=Glycated Hemoglobin, PPBS=Postprandial blood sugar, T2DM=Type-II diabetes mellitus, SD=Standard deviation

DISCUSSION

Our study had participants in the age range of 30–40 years, with diabetes predominantly diagnosed within this age range.[9] A proportionately equal number of males and females were recruited to prevent bias due to gender. The baseline characteristics in both groups were similar in the study. A higher mean value of 14.9 ± 2.11 could be noted in Group-I than in Group-II (6.61 ± 1.94) individuals. It was also statistically significant with a '*P*- value' of <0.00.1.

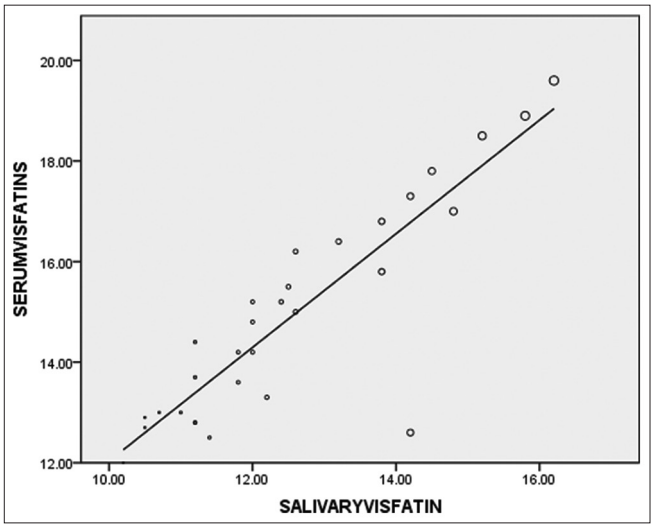

Figure 1: Showing positive correlation between serum and salivary visfatin levels

Table 2: Mean values of FBS, PPBS, HBA1C and HS-CRP of both the groups (I & II)

Parameters	Cases (*n*=30) T2DM		Control (*n*=30) HC		Mean Difference	95% Confidence Interval of the Difference		*P*
	Mean	SD	Mean	SD		Lower	Upper	
FBS	149.2333	14.9958	98.200	10.9431	51.0333	41.9801	60.0867	0.00*
PPBS	183.2667	15.3980	117.200	11.9175	66.0667	57.0134	75.1199	0.00*
HbA1C	8.4067	1.9483	6.1033	1.3984	2.3033	1.3428	3.2638	0.00*
Hs-CRP	4.2633	1.0337	1.8073	1.1517	2.4560	1.4955	3.4164	0.00*

T2DM - Type-II Diabetes Mellitus; HC - Healthy control. *statistically significant result (p-value < 0.001). PPBS: Postprandial blood sugar, Hs-CRP: High-sensitivity C-reactive protein, HbA1C: Glycated hemoglobin

Table 3: Serum and salivary visfatins in Group-I and Group-II

Visfatins	Cases (*n*=30) T2DM		Control (*n*=30) HC		Mean Difference	95% Confidence interval of the difference		*P*
	Mean	SD	Mean	SD		Lower	Upper	
Serum visfatin	14.9233	2.11 6	6.6133 6.6133	1.948	8.310	7.086 0	9.533 9	0.00*
Salivary visfatin	12.5567	1.65 4	3.9167	1.491	8.640	7.416 0	9.863 9	0.00*

HC=Healthy control, T2DM=Type-II diabetes mellitus. *Statistically significant result ($P<0.001$)

Similar to our study, Legakis *et al.*[10] studied patients with type 2 diabetes, serum visfatin levels (4.968 ± 2.13 ng/ml) were significantly higher in the fasting state compared to the control group (2.891 ± 0.61 ng/ml) with a *P*- value <0.0001. Study done by Alireza Esteghamati *et al.*[11]: The serum visfatin levels (5.49 ± 2.4 ng/ml) were higher in T2DM patients compared with controls (3.58 ± 2.2 ng/ml) with a *P*- value <0.01.

While a study conducted in 2012 by Samad Akbarzadeh[12] recorded lower levels of serum visfatin levels 1.71 ± 0.93 ng/ml) than the controls (2.69 ± 2.02 ng/m), *P* = 0.0001). Two other studies also showed lower visfatin levels or similar levels in controls and T2DM groups.[13,14] Thus, based on the above facts, though our observations cannot be compared on similar grounds, only the fact that serum visfatin levels were increased in T2DM individuals compared to healthy controls is in accordance with Legakis *et al.*'s[10] and Alireza Esteghamati *et al.*'s (2011)[11] and Sandeep *et al.*'s (2007)[15] observations and not in agreement with Samad Akbarzadeh's (2012)[12] observation.

A hallmark of T2DM is the development of pancreatic beta-cell failure, which results in insulinopenia and hyperglycemia.[16] Increasing visfatin levels with progressive pancreatic beta-cell dysfunction, suggests that an increase in visfatin is a compensatory mechanism which develops in long-standing T2DM patients. An association between insulin resistance and subclinical inflammation involving cytokines derived from adipose tissue or adipocytokines has been found[17]. Recently, there has been increasing evidence about adipocyte-derived factors to be the major regulators of insulin resistance.[18,19] Thus, all the above indicates, a major role of visfatin in the pathophysiology of insulin resistance, T2DM, and obesity.

Increased salivary visfatin levels were noted in Group-I individuals (16.2 to 10.2 μg/ml) compared to Group-II individuals (7.2 μg/ml to 1.2 μg/ml). A higher mean value of (12.55 ± 1.65) could be observed in Group-I compared to Group-II (3.91 ± 1.49) individuals were statistically significant with a *P*- value of <0.001. Our study is the first study on salivary visfatin levels in Indian T2DM individuals to the best of our knowledge. The observation of increased salivary visfatin levels confirms its role in the diabetic state of T2DM. However, more studies in this regard need to be done to draw an inference. In our study, we noted increased hs-CRP (4.26 ± 1.03) mean levels in Group-I individuals (T2DM) as compared to healthy individuals in Group-II (HC) (1.80 ± 1.15), respectively. It was statistically significant with a *P*- value of <0.001. Several studies[20-22] reported an association of CRP levels and T2DM. TNF, IL-6 and CRP were significantly associated with the risk of developing T2DM. Among the above three inflammatory markers, CRP plays a major role in diabetes.[23] Many studies have noted the significance of the above parameters in T2DM individuals. Hence, they are considered diagnostic tests for T2DM.[24]

hs-CRP is a very important inflammatory marker that gets increased, as observed in our study, signifying the underlying subclinical inflammatory processes inherent to T2DM. In the present study in Group-I T2DM individuals, the visfatin levels ranged from 19.6 to 12.0 μg/ml in serum and in saliva, it ranged from 16.2 to 10.2 μg/ml, with a mean serum visfatin value of 14.9 ± 2.11 and salivary visfatin value of 12.55 ± 1.65. Thus, the serum levels were more compared to salivary levels. Both serum and salivary visfatin levels positively correlated with a correlation value of 0.8836 and a *P*- value of <0.001, which was statistically significant. Thus, this is the first study correlating serum and salivary visfatin levels in T2DM to the best of our knowledge. A negative correlation was noted between serum visfatin, FBS, PPBS, HbA1c, and hs-CRP levels, but none of these correlations were statistically significant. Moreover, no studies have correlated serum visfatin with FBS, PPB and HbA1c in T2DM till date.

Increased FBS, PPBS, and HbA1c levels are proven diagnostic tests for T2DM. Hence, to test the effectiveness of serum visfatin as a diagnostic marker for T2DM, we correlated with FBS, PPBS and HbA1c levels. Our study is the first study to correlate PPBS, HbA1c, hs-CRP and serum visfatin to the best of our knowledge. As only a few studies exist, our observations need to be supported by more such studies done amongst the Indian population in the future, adopting standardised criteria/methodology. Since our study is a preliminary study done with a smaller

sample size (30 individuals/group), other parameters were not given importance.

CONCLUSION

This is the first study in the Indian scenario to assess the serum and salivary visfatin in newly diagnosed T2DM individuals. The finding of a statistically significant increase in serum and salivary visfatin levels in newly diagnosed T2DM further confirmed the role of visfatin in T2DM. It also reflects the potential of using saliva as a diagnostic fluid. The significant positive correlation between serum and salivary visfatin, however, needs to be explored further. Though serum visfatin, FBS PPBS and HbA1C levels were significantly increased in T2DM, the finding of negative correlation amongst them could not aid in labelling visfatin as a diagnostic marker in T2DM. Serum visfatin and hs-CRP levels increased in T2DM, thus defining the link 50 between visfatin, inflammation and T2DM, but we failed to notice a positive correlation. More studies in the Indian population would help in assigning a confirmatory and diagnostic role of visfatin in T2DM. Not considering the parameters of insulin resistance and obesity-related anthropometric measurements and not assessing oral hygiene status were some of the drawbacks of our study.

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Conflicts of interest

There are no conflicts of interest.

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