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Biochemistry Section

Association of Salivary Osteopontin Levels with Glycaemic Status and Microalbuminuria - in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Introduction: The monitoring of glycaemic status in patients with T2DM is mainly through blood tests (Fasting plasma glucose and HbA1c), which are invasive and involves painful pricks. This leads to poor patient compliance and soon could lead to various micro and macro vascular complications, which hamper the quality of life. There are no sensitive and specific markers to predict these complications at the earliest. Sialochemistry has recently gained attention for monitoring chronic diseases. Osteopontin is a phospho-glycoprotein molecule, elevated in many inflammatory conditions.

Aim: The aim of the study was to evaluate the role of serum and salivary osteopontin in Type 2 Diabetes mellitus (T2DM).

Materials and Methods: In this case-control study, we recruited 33 cases of T2DM and 31 age and gender matched healthy controls. Body Mass Index (BMI), Waist/Hip Ratio (WHR), Waist Circumference (WC) and blood pressure was recorded. Fasting Plasma Glucose (FPG), salivary glucose, HbA1c, microalbuminuria, systolic BP, serum and salivary osteopontin levels were estimated.

Results: FPG, salivary glucose, HbA1c, microalbuminuria, systolic BP, BMI, waist / hip ratio serum and salivary osteopontin levels were significantly high in T2DM cases compared to control subjects. Serum and salivary osteopontin levels were significantly correlated with HbA1c and microalbuminuria in T2DM cases.

Conclusion: Serum and salivary osteopontin levels are significantly elevated in subjects with T2DM and are associated with glycaemic control and microalbuminuria.

Keywords: Diabetic nephropathy, Fasting Plasma Glucose, HbA1c

INTRODUCTION

Prevalence of Type 2 Diabetes mellitus (T2DM) is rapidly increasing, especially in India [1]. The incidence of diabetes mellitus may rise up to 500 million by the year of 2030 worldwide [2]. T2DM is a chronic "polymetabolic disorder" characterised by Insulin Resistance (IR), cellular glucopenia with high glucose levels in the extracellular fluids [3]. At present, the diagnosis of T2DM is mainly based on the Fasting Plasma Glucose (FPG) and random blood glucose levels correlating with signs and symptoms. Recently, World Health Organization has recommended HbA1c ≥6.5% is also a diagnostic criterion for T2DM [4].

The patients is usually monitored by the glycaemic status through self-examination by capillary blood glucose estimation with glucometers. These investigations for emergency, screening and monitoring purposes, mostly involve painful pricks, which causes inconvenience to the patients, also chances to get infected. Few researchers now are looking at saliva as a potential sample for screening and monitoring the glycaemic status [3]. The concentration of many analytes in saliva, including glucose reflects its respective concentrations in circulation [5]. Moreover, collection of saliva is convenient. Processing saliva as a sample is cost effective, easy to store and transport. For some analytes it is the preferred sample with a greater sensitivity and specificity [6].

T2DM patients with poor glycaemic status are prone to develop Diabetic Nephropathy (DN) at the early stages [7,8]. DN is characterized by microalbuminuria, hypertension and decline in glomerular filtration rate in the beginning. Later if not treated, patient may land up in end stage renal disease [9,10]. Estimated glomerular filtration rate (eGFR) and microalbuminuria are currently available tools to monitor, but not before the onset of pathology [11]. Thoughts are now needed to come up with a marker which could alarm us or predict DN, before the onset of glomerular changes.

Osteopontin is a phospho-glycoprotein with a molecular weight of 32kDa [12]. It is expressed in glomerular cells, mesangial cells, podocytes, endothelial cells, osteoblasts, macrophages, activated T cells and also vascular smooth muscle cells [13]. Osteopontin plays a prominent role in inflammation, promoting macrophage infiltration and retention in tissues [14]. Osteopontin recently has come to light as a potential marker which is found associated with many metabolic and inflammatory diseases including obesity, T2DM and cardiovascular diseases. Few evidences document the association of osteopontin levels with DN [15].

The aim of the study was to evaluate the role of serum and salivary osteopontin in T2DM.

MATERIALS AND METHODS

In this case-control study, we recruited 33 cases of T2DM and 31 age and gender matched healthy controls. This study was conducted in Department of Biochemistry in collaboration with Department of General Medicine, Mahatma Gandhi Medical College and Research Institute, SBV, Pillayarkuppam, Puducherry, India. The project was initiated after obtaining the permission from institutional research review board and institute human ethical committee, during the period May 2015 to February 2016. The T2DM cases were recruited from the diabetic clinic; controls samples were healthy volunteer staff working at SBV and those who visited our hospital for master health check-up.

Inclusion criteria: Male and female subjects with T2DM between the ages 30 to 60 years with no established complications were included in the study, after obtaining a written informed consent.

The subjects were instructed not to consume alcohol/tobacco for a period of 12 hours prior to the collection of saliva sample.

Exclusion criteria: Patients with other specific types of diabetes mellitus, known organ dysfunction, smokers and chronic alcoholics, those who consume smokeless tobaccos, with oral/dental infections/other pathology were excluded from this study. Three ml of fasting venous blood sample was collected from subjects of both the groups to estimate the biochemical parameters. Un-stimulated saliva sample was collected to estimate the salivary osteopontin levels.

Collection of saliva

Procedure was explained to the subjects of both the groups and salivary samples were collected in fasting state after obtaining consent. The subjects were asked to rinse their mouth with normal saline and made to sit comfortably and then un-stimulated salivary samples were collected in sterile graduated container by spit technique [16]

Study parameters

Body Mass Index (BMI), waist circumference, waist/hip ratio, blood pressure, eGFR, Fasting Plasma Glucose (FPG), salivary glucose, HbA1c, microalbuminuria, serum and salivary osteopontin levels.

- HbA1c levels were estimated by using ion-exchange High-Performance Liquid Chromatography (HPLC) method.

- Serum and salivary osteopontin levels were estimated by using Solid Phase Sandwich human ELISA kit (ab100618) abcam.

- Estimated GFR was calculated using the 4-variable Modification of Diet in Renal Disease study (MDRD) equation [17]: eGFR (ml/ min 1.73 m2) = 186×(plasma creatinine{µmol/l} divided by 88.4)–1.154×(age)–0.203×(0.742 if female).

- Glucose levels in serum and saliva were estimated by GOD-POD method.

STATISTICAL ANALYSIS

All data are expressed as mean ± standard deviation. Unpaired Student t-test was used to compare the data between the two groups. Pearson's correlation analysis was used to find the association and degree of relationship between serum and salivary osteopontin levels with glycaemic status and microalbuminuria in subjects with T2DM. SPSS version 16.0 was used for analyzing the data. A p-value <0.05 was considered to statistically significant.

RESULTS

The results were expressed as mean and Standard Deviation (SD). All anthropometric measurements and biochemical variables of both T2DM cases and healthy controls were tabulated and compared by unpaired student's t-test.

All anthropometric measurements were significantly high in cases compared to healthy controls except the waist circumference. We observed significantly high salivary glucose, FPG, HbA1c, serum and salivary osteopontin in cases compared to controls [Table/Fig-1]. The difference in mean eGFR was not statistically significant. The association of serum and salivary osteopontin levels with microalbuminuria and glycaemic control was drawn by Pearson's correlation analysis. Serum and salivary osteopontin levels were significantly associated with microalbuminuria and glycaemic control [Table/Fig-2].

DISCUSSION

This study aimed to evaluate the serum and salivary osteopontin levels in T2DM cases and their association with the glycaemic control. Both serum and salivary osteopontin levels were found significantly elevated in subjects with T2DM compared to healthy controls [Table/Fig-1]. This could add to the existing literature of

Parameters	Controls (n=31)	Cases (n=33)	p-value
Gender M/F (%)	51/49	52/48	NS
Age (years)	47 ± 2.24	48±3.21	NS
Salivary glucose (mg/dl)	5.6 ±1.27	6.41±2.13	0.003*
Fasting Plasma glucose (mg/dl)	101.17±17.58	138.97±29.42	0.001*
HbA1c (%)	6.01±0.75	7.59±1.37	0.001*
Serum osteopontin (ng/ml)	26±1.3	33±1.1	0.001*
Salivary osteopontin (µg/L)	2.4±0.4	3.7±0.6	0.003*
eGFR (ml/min/1.73 m^2)	93.30 ± 20.11	92.30 ± 28.89	0.1
Microalbuminuria (mg/24hrs)	24±1.5	44±1.3	<0.001*
Systolic blood pressure (mmHg)	117.53±7.08	127.60±14.60	<0.001*
Diastolic blood pressure (mmHg)	77±7.72	79.83±9.24	0.1
Waist circumference (inches)	37.13±2.70	36.67±3.36	0.2
Waist / Hip ratio	0.91±0.04	0.88±0.05	0.01*
BMI	28.14±5.79	24.91±2.86	0.004*

[Table/Fig-1]: Comparison of anthropometric and the biochemical variables between the cases and the controls.
Comparison was done by unpaired student t-test * indicates statistically significant p-value. NS: Not Significant

Osteopontin levels	HbA1c (%)		Microalbuminuria (mgs/24 h)	
Serum (ng/ml)	r=0.8	p=<0.001*	r=0.9	p=<0.001*
Saliva (µg/L)	r=0.7	p=<0.001*	r=0.8	p=<0.001*

[Table/Fig-2]: Association of serum and salivary osteopontin levels with microalbuminuria and glycaemic control (HbA1c).
Association was seen by using Pearson's correlation analysis, * indicates statistically significant p-value for respective r values

elevated osteopontin in chronic inflammatory conditions [18] more significant in T2DM.

T2DM is proved to be associated with systemic inflammation [19] and if untreated could lead to micro and macro vascular complications, which hamper the quality of life [20]. Monitoring the glycaemic control is the key, but fails to predict these complications in subjects with uncontrolled T2DM. It is really challenging and we need a specific and sensitive biomarker to predict complications in T2DM, as some develops these complications early and some at the later ages. Sialochemistry for various analytes (e.g., cortisol, glucose etc.,) is recently gaining attention, especially in chronic diseases like T2DM as it improves the patient compliance in monitoring the response to treatment. In addition if properly used, salivary analytes correlates with that of plasma [21].

Osteopontin plays an important role as an inflammatory protein as it is expressed in macrophages, activated T lymphocytes and vascular smooth muscle cells [13]. Osteopontin also promotes macrophage infiltration and retention in tissues [14]. However, further studies with larger samples are needed to evaluate this protein as a marker to predict micro and macro vascular complications and especially DN, as it is abundantly expressed in glomerular cells, mesangial cells, podocytes [22]. Studies on the individual complications are necessary to come to any conclusion, as osteopontin levels are elevated in many conditions with systemic inflammations, chronic infection and carcinomas [23].

There exists a significant association of osteopontin (both in serum and saliva) with glycaemic control [Table/Fig-2], throwing new lights on osteopontin and its potential as a useful adjuvant tool for monitoring glycaemic control. There is evidence for osteopontin levels which was significantly correlated with the degree of kidney function compromise in DN [24-26]. Elevated osteopontin levels were detected in urinary diabetic albuminuric patients and nephrotic syndrome too. This study shows an association of osteopontin (serum+salivary) with microalbuminuria [Table/Fig-2].

Hence, osteopontin is a potential tool for predicting the complications of T2DM. As the study excluded the cases with complications, there was no significant association of osteopontin with eGFR. Future studies could concentrate specifically on

individual complications and their association with osteopontin levels. There was no significant association of osteopontin with anthropometric measures and blood pressure.

LIMITATION

This study excluded the T2DM cases with complications. The micro and macro vascular complications could not be done, to see the correlation between osteopontin (serum+salivary) and the respective complications of T2DM. Also, the comparison of existing markers of systemic inflammation like, hsCRP, adipokines with osteopontin was not done.

CONCLUSION

Osteopontin levels were elevated in subjects with T2DM and were associated with poor glycaemic control. This shows the significance of osteopontin, as a potential early indicator of micro and macro vascular complications. Elevated osteopontin indicates systemic inflammation and oxidative stress. Hence, low doses of anti-inflammatory agents and anti-oxidants could help in achieving a good glycaemic control in subjects with T2DM.

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