

A COMPARATIVE STUDY OF SERUM AND SALIVARY BIOCHEMICAL MARKERS IN DIABETES MELLITUS TYPE II WITH HEALTHY INDIVIDUALS

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Article Info: Received 02 June 2020; Accepted 23 June 2020

DOI: <https://doi.org/10.32553/ijmbs.v4i6.1230>

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Conflict of interest: No conflict of interest.

Abstract

Introduction: Blood is the gold standard body fluid for diagnosis of Diabetes Mellitus (DM) but saliva can offers an alternative to serum as a biological fluid for diagnostic purposes because it contains serum constituents. People wants that medical science should promote non invasive method for diagnosis of disease.

Aim: The study was conducted to estimate and compare serum and salivary glucose, total protein, calcium and phosphorus levels in DM type II and healthy subjects and to evaluate whether saliva can be used as a diagnostic fluid in DM type II patients. The target population of this study was individuals who have Type-2 DM for different period of time and resident INDORE.

Materials & Methods: Study consisted of 50 subjects from OPD/IPD Index Medical College & Research Center, INDORE, MADHYA PRADESH, India. The study groups were divided into Group I-25 DM patients (TypeII) and Group II-25 healthy subjects. The saliva and serum samples were collected from each subject and levels of different biochemical parameters were estimated.

Result: were noted. On comparing values in saliva and serum, among two groups, a significant difference ($p < 0.005$) was found between few of them.

Conclusion: Values regarding blood and salivary biochemical parameters were distinctly different between two groups suggesting salivary parameters can be used as a diagnostic alternative to blood parameters for diabetes mellitus.

Introduction

Type II Diabetes mellitus is an endocrine disease characterized by a short fall in the production of insulin with consequent alteration of process of assimilation, metabolism and balance of blood glucose concentration which when left untreated can lead to serious complications(1). Early diagnosis of diabetes plays a significant role in successful clinical treatment. Monitoring individuals with diabetes involve repeated evaluation of glucose levels. Regular pricking becomes quite cumbersome and painful which reduces the compliance. Thus there is a splurge of interest in non-invasive diagnostic method which necessitates the need for other bodily fluids (2)

Noninvasive methods for frequent measurements of biomarkers are increasingly being investigated. Application of salivary diagnostics has gained importance with the establishment of significant similarities between the salivary and serum proteomes.(3) Saliva is an organic bio-fluid, with complex composition and specific roles for monitoring health and disease states of an individual(4). Saliva is an accessible clinical sample that has been in the spotlight of the researchers' attention due to its

possession of different enzymes, molecules and distinctive function in the diagnosis and treatment of various diseases.(5) Saliva has been put forth as a potential diagnostic tool for surveillance of disease due to its several advantages. It clearly offers an inexpensive, simple and easy to use screening methods.(6)

Monitoring of serological parameters of diabetes typically involves invasive techniques with associated pain and distress. Hence, the development of noninvasive methods for frequent monitoring of biomarkers is a growing area of research. Some of the alternative methods evaluated include assessing skin auto-fluorescence for accumulation of advanced glycation end products and measuring the analytes in exhaled breath, urine, or saliva.(7) .

Due to hyperglycemia in DM patients, the glucose metabolic products cause changes in the microvasculature and basal membrane of salivary glands and other oral mucosal tissue 8)

B Among all salivary parameters, glucose, amylase, and total proteins have been found to be closely related to the oral environment in patients with T2D.(9)

In last few years, more specific and sensitive techniques for detection of total salivary proteome have been used. Analysis and identification of albumin and globulin is important not only for understanding of oral pathophysiology, but it may be useful as a potential biomarker &

Changes in serum electrolyte concentrations can affect systemic disease. Saliva is saturated with calcium ions that play important role in enamel remineralization. The DM patients have demonstrated an increase in calcium concentration in saliva compared to the control group.(8)

Limited studies have attempted to investigate association of salivary and serum correlation of glucose, amylase, proteins, calcium and phosphorus DM [10]. This study aimed to evaluate salivary and serum glucose, total protein calcium & phosphorus levels in adult patients with T2D compared with healthy controls and to determine the correlation of salivary biomarkers with blood biomarkers in both diabetic patients and healthy controls.

Material & Method

This study consisted of 25 cases of selected type II DM (Group I) and 25 healthy individuals (Group II) with the age range between 30 – 65 years inclusive of both the genders who reported to the Department of Medicine in IMCHRC INDORE.

Inclusion criteria

1. Patients who were diagnosed as Type II Diabetes mellitus with no other systemic disease. 2. Healthy subjects with blood glucose within normal level for controls.

Exclusion criteria

1. Pregnancy.
2. Patients with salivary gland disorders and on treatment for salivary gland diseases.
3. Patients who had undergone surgery of the salivary glands.
4. Patients who had been exposed to chemotherapy/radiation for head and neck.

Sample Collection - Serum: Fasting blood sample was withdrawn from both the patients suffering from DM and healthy volunteers.

Saliva: The patients were detailed about collection of saliva. They were advised to avoid food and fluid intake 2 hours prior to collection of saliva. The saliva was collected in resting position after rinsing the mouth with distilled water between 9.00am and 11.00am. The patient was instructed to spit in a sterile plastic container over a period

of 5 minutes. The 2ml of unstimulated whole salivary sample was collected from both control and study groups, stored at 20°C temperatures and sent to laboratory immediately

Estimation of serum and salivary glucose: The serum and salivary glucose estimation was performed using glucose oxidase-peroxidase (GOD-POD) end-point method. Then serum and saliva sample by automatic analyzer.

Estimation of serum and salivary total protein: The estimation of serum and salivary total protein was performed by modified Biuret, end point method.

The estimation of Then total protein concentration was measured in both serum and saliva sample by automatic analyzer.

Estimation of serum and salivary calcium: It was done using calcium test kit (Arsenazo III), end point method. Then calcium concentration was measured in both serum and saliva sample by automatic analyzer.

Estimation of serum and salivary phosphorus:

The estimation of serum and salivary inorganic phosphorus was done by using end point method. Then inorganic phosphorus concentrations were measured in both serum and saliva sample by automatic analyzer.

STATISTICAL ANALYSIS -Statistical analysis was done with the help of ANOVA TEST. The values thus obtained were tabulated and subjected to statistical analysis. The paired and unpaired t-test and Pearson Coefficient correlation (p-value) was determined between salivary and serum biomarkers.

Result

In the present study, 50 subjects were investigated out of which 25 were DM subjects (20 males and 8 females) and 25 were non-diabetic subjects (16 males and 9 females). The age ranges from 36-65 years for both groups with mean age (48±9.0 years) for Group I and (43.16±6.9 years) for Group II subjects. The significant correlation was found between both genders and two study groups (p<0.005).

Table 1: Age and gender distribution between Group I (T2DM) and Group II (NON DIABETIC)

Variable	Group 1	Group 2	P value
Males	20	16	0
Females	5	9	
Age +SD years	48±9	43.16±6.9	

(NS: p>0.05; Not Significant; *p<0.05; Significant; **p<0.001; Highly Significant; r=Pearson Correlation Coefficient)

Table 2: Distribution and comparison of salivary & serum components between Group I (DM TYPE II)

Parameters	Serum	Saliva	P value
Glucose	132 ± 5(mg/dl)	11±1.5(mg/dl)	0
Total protein	6.8±.86(gm/dl)	1.5± .22(mg/dl)	0
Calcium	9.1±1.7(mg/dl)	10.2±1.8(mg/dl)	.2857
Phosphorus	4.3 ±.40(mg/dl)	10.5±2.1(mg/dl)	0

(NS: $p>0.05$; Not Significant; * $p<0.05$; Significant; ** $p<0.001$; Highly Significant; r =Pearson Correlation Coefficient)

The above table shows that there was significant correlation in between all marker except calcium .

Table 3: Distribution and comparison of salivary components between Group I & II.

Parameters	Group I	Group II	P value
Glucose	11 ±1.5	1.5 ±.64(mg/dl)	0
Total Protein	1.5 ±.22	1.3±.50	0.03
Calcium	10.2 ±1.8	7.2 ±1.1	0
Phosphorus	10.5±2.1	8.1 ±.56	0

(NS: $p>0.05$; Not Significant; * $p<0.05$; Significant; ** $p<0.001$; Highly Significant; r =Pearson Correlation Coefficient)

The present study shows the significant correlation of salivary marker in between T2DM patients and healthy individual. **Discussion:**

The alterations in salivary component could impact the incidence, signs, and severity of oral changes in diabetic patients[11] and metabolic syndrome as a metabolic abnormality can increase risks of T2DM.[12] This study estimated salivary glucose, total protein, calcium and phosphorus levels in T2DM patients compared with the healthy controls as well as the correlation of salivary glucose level with serum glucose in the patients and controls.

This study revealed that the salivary glucose level of T2D patients was significantly higher than that of the healthy controls, as most of the studies reported similar results. S Amer et.al found a significant correlation between salivary and serum glucose levels in diabetic patients and healthy controls.[13].Agrawal *et al.*[14] reported similar result association between fasting saliva and fasting plasma glucose levels of diabetic patients and healthy controls.

This study indicated that salivary total protein level was slightly significantly higher in T2DM patients than the healthy controls. A study conducted by Chorzewski M et.al confirmed the results significantly higher total protein in T2DM patients.[15] The increased salivary total protein in diabetics could be attributed to the increase in basement

membrane permeability, allowing easy and increased passage of serum proteins into the whole saliva through salivary gland.[16] The observed differences may have different reasons. Total protein level is influenced by the saliva collection method, determination method, diet fluctuations of protein concentration, and even the speed and time of centrifugation.[17]

In the present study, the salivary calcium levels were found the salivary calcium levels were found significantly higher ($p<0.001$) in diabetics than non-diabetics, the results were similar as Lopez ME et al. whereas found in present study non significant relation were found in serum and salivary T2DM and healthy individuals. Shirzaiy M et al., found no significant difference of salivary calcium levels between two groups [18]. The serum calcium is decreased because of decreased insulin leading to its stimulatory action on proliferation of osteoblast and calcium homeostasis impairment. The reason of increase in salivary calcium in DM patients is due to reduction in salivary flow rate, or with an increase of the concentration of specific proteins which make special bonds with calcium phosphate

This study demonstrated a significantly high concentration of salivary phosphorus in DM patients compared to controls. Similar results were seen by Sultan E et al., studies [19]. This might be due to reduction in salivary flow and release of binded phosphorus into the saliva from the degraded periodontal proteins in DM patients.

Limitation:

The main limitation of the study was the small sample size, which definitely calls for a more extensive and large sample size research to substantiate the usefulness of saliva as diagnostic marker in DM..

Conclusion:

The highly significant correlation was found between serum and salivary levels of glucose, total protein and phosphorus in DM patients while on intergroup comparison, significant correlation was found between diabetics and non-diabetics for salivary glucose, calcium and phosphorus. Hence, present study will add new dimensions and lay the foundation for further research on large populations in making use of salivary biochemical parameters (glucose, total protein calcium & phosphorus) for screening, diagnosis and monitoring of DM to blood.

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