

Saliva – a marker for the diabetic: a comparative study of healthy and the diabetic individuals

Kumar J

Department of Biotechnology, Hindustan College of arts & Science, Kelambakkam-603103, Tamil Nadu, India
jayanthi_vijisan@yahoo.co.in

Abstract

Saliva contains a wide range of proteins, antibodies, nucleic acids that makes it an important diagnostic fluid. The most effective non-invasive source of DNA, RNA and protein is saliva, which comes from buccal epithelial cells. The saliva from the healthy and the diabetic individuals was collected with volunteers concern. We quantified the protein content spectrophotometrically and it was found that the protein was decreased in the diabetic individual. The protein was resolved in SDS-PAGE and the protein profiles of whole saliva of healthy and diabetic were compared. Considerable variations between individuals were observed. The spectrophotometric quantification of sialic acid showed a significant increase in its levels in diabetic individuals as compared with non-diabetics.

Keywords: Saliva; Diabetics; Comparative study; Spectrophotometry; Metabolic disorder.

Introduction

Diabetic mellitus is a common chronic metabolic-disorder, which affects millions of people. The prevalence of diabetes for all age groups worldwide was estimated to reach 4.4 % by 2030. Recent World Health Organization calculations indicated that worldwide, almost 3 million deaths per year are attributed to diabetes, equivalent to 5.2% of all deaths.

Saliva is the watery and frothy substance produced from the mouth of other animals and in humans. Saliva is secreted from the salivary gland. Human saliva is composed 99.5% of water, but also includes electrolytes, mucus, antibacterial compounds and various enzymes. Enzymes present in saliva break down some of the starch and fat in the food at the molecular level. Saliva contains a wide range of proteins, antibodies and nucleic acids, which makes it an important diagnostic fluid in assessing various diseases. The collection of saliva is convenient, noninvasive and cost-effective. Saliva is an effective cleaning agent and used in conservation. Production of saliva is stimulated by the sympathetic nervous system and parasympathetic nervous system.

Human saliva is an informative body fluid containing an array of analytes (proteins, DNA and RNA) that can be used as biomarkers for translational and clinical applications. Salivary biomarkers have received special attention since they are readily accessible and easily obtained. Salivary α -amylase has been proposed has a sensitive biomarker for stress-related changes in the body that reflect the activity of sympathetic nervous system. Saliva contains biochemical system known to be involved in soft tissue repair, many antibacterial and antiviral and antifungal components including lysozyme, lactoferrin, histatins, and various antioxidants.

A major challenge in the utilization of biological analytes for clinical applications is the necessity to stabilize and maintain the integrity of informative biomarkers for clinical diagnostics. One of the values of saliva is the ease of sampling and high subject compliance for sample collection, which includes field application as well as home collection. It acts as a universal stabilizer, which provides a user friendly and easy to use processor for major salivary diagnostics (Jiang *et al.* 2009).

Recently, the use of saliva has provided a substantial addition to the diagnostic

armamentarium as an investigative tool for disease processes and disorders. In addition to its oral indications, the analysis of saliva provides important information about the functioning of various organs within the body (Chiappin *et al.* 2007).

The problems with current diagnosis of diabetes are sometimes-painful needle sticks to draw blood for tests. Some individuals avoid getting tested in fear of the discomfort. This may also discourage patients from properly monitoring their blood sugar levels. Saliva tests are completely non-invasive and provide results as quickly as the blood tests. The present study was carried out to find the biomarkers in the patients' saliva. These markers will provide the basis for new, non-invasive tests for diabetes screening, detection, and monitoring.



Fig. 1. Nanovue

Materials and methods

Sample collection

The normal and diabetic individuals were donated saliva samples. Healthy sample was obtained from the donors who had no history of diabetes mellitus. From one healthy and two diabetic individual 2 ml of the saliva was collected in the 2ml microfuge tube along with the written

instruction for collecting the sample. About 1 hour after they brushed their teeth the saliva was spitted in the microfuge tube.

Estimation of total soluble proteins

0.5 ml of saliva was collected and centrifuged at 8000 rpm for 5 minutes. The supernatant was collected and 1 ml of Bradford's reagent (100 mg Coomassie Brilliant Blue G-250 was dissolved in 50 ml 95% ethanol, and 100 ml 85% (w/v) phosphoric acid was added. It was diluted to 1 liter when the dye has completely dissolved, and filtered through Whatman #1 paper just before use) was added and read spectrophotometrically at 595 nm.

Estimation of sialic acid

0.5 ml of saliva was collected and centrifuged at 8000 rpm for 5 minutes. The supernatant was collected and 1 ml of Ninhydrin reagent was added and read spectrophotometrically at 570 nm.

Concentration of DNA and RNA

The concentration of DNA and RNA were estimated using NanoVue uV visible spectrophotometer (Fig. 1).

SDS-PAGE analysis of proteins

It is performed by Laemmli method.

Sample loading

To the 100 µl of healthy salivary sample (H) and to 100 µl of diabetic



Fig. 2. DNA concentration of healthy and diabetic salivary sample: A. healthy, B. Diabetic 1, C. Diabetic 2

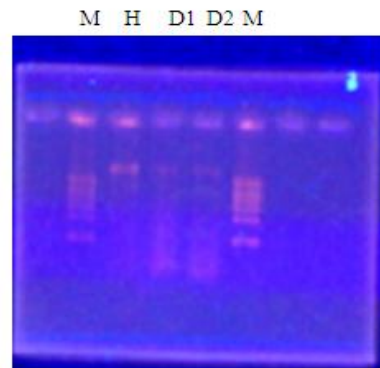


Fig. 3. gDNA of healthy (H) and diabetic Samples (D1, D2) were run along with the marker

salivary sample (D1, D2) 10 µl of sample buffer was added and heated for a minute and kept in ice. 40 µl of healthy sample (H) was loaded in lane 6 and lane 8 respectively and 40 µl of D1 and D2 sample was loaded in lane 7 and 5 respectively (Fig. 7).

Isolation of DNA



Fig. 4. RNA isolation from healthy sample

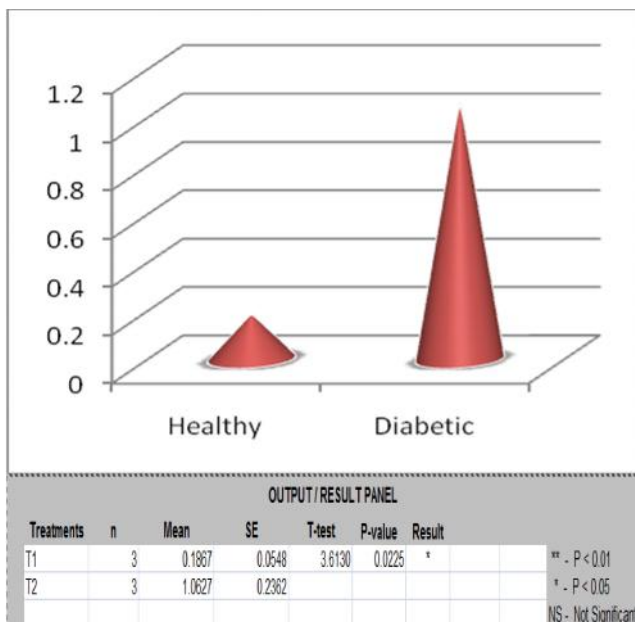


Fig. 5. Statistical analysis of sialic acid content in healthy and diabetic samples

2 ml of the saliva sample was collected and centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded and the pellet was collected. In order to maximize the pellet size again 2 ml of saliva was collected and centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded and the pellet was collected. The pellet was washed with TE buffer by centrifuging it at

5000 rpm for 5 minutes. Then 1 ml of lysis buffer was added and dissolved the pellet by vigorous vortexing. Then add 10 µl of 10 mg/ml proteinase-K and incubate it at 58°C for two hours. Then add equal volume of phenol and chloroform and centrifuge at 10,000 rpm for 5 minutes. Then the top aqueous layer was transferred to a new tube. Then add 1/10 volume of 3 M sodium acetate and incubate at -20°C for two hours. Then centrifuge at 10,000 rpm for 5-8 minutes. Then pour off the supernatant. Wash the invisible pellet with 95% ethanol. Then discard the supernatant. Then re-suspend the DNA with 20 µl of TE buffer. The concentrations of DNA of both healthy and diabetic sample were identified in Nanovue (Fig. 2). The isolated DNA was run in 0.8% agarose gel electrophoresis (Fig. 3).

Extraction of RNA

To the sample of 450µl RLT and 5µl β-mercaptoethanol and vortex vigorously and incubate at 56°C for three minutes. Transfer it to the lilac column and centrifuge at 13,000rpm for two minutes. Then add 250µl ethanol to it. Then transfer to pink column and centrifuge at 10,000 rpm for 15 seconds. Discard the flow throw. In pink column RNA retained. Then add 500 µl of RWI to it and centrifuge at 10,000 rpm for 30 seconds. Then discard the flow throw. Add 450 µl of RPE at 10,000rpm for 30 seconds. Then discard the flow throw. Then transfer the content from the pink column to 2ml collection tube. Then add 450µl RPE and centrifuge at 10,000rpm, for one minute. Then transfer the pink column to 1.5ml Eppendorf tube. Then add 30µl RNase free water to it. The concentrations of RNA of healthy were identified in Nanovue (Fig 4).

Results & discussion

Diabetic mellitus is a chronic disease that may influence personal behavior and socio economic status. Dialectologists all over the world are trying to find immunological, molecular biological and biochemical markers for rapid and cost-effective diagnosis. Most of the investigations have

concentrated on plasma serum for analyses; we selected saliva as diagnostic samples for its availability, easiness and non-invasive collection.

Serum or plasma sialic acid is considered as one of the biochemical markers for cardiovascular risk factors. The sialic acid concentration of healthy and diabetic saliva was measured spectroscopically. It was found that the sialic acid content in diabetic salivary sample was considerably increased (Fig.5). Thus, a positive correlation between sialic acid levels and diabetes was observed. Sialic acid is a constituent of many salivary glycoproteins. Studies have been reported elevated levels of sialic acid in pregnancy and diabetic mellitus. Abdella *et al.* (2000), Hallikeri (2008) performed the experiment on the serum sialic acid analysis on Kuwaiti Type-2-diabetics people and Kuwait non-diabetic people they found that there was a significant elevation in serum total concentrations in the diabetic individual than the healthy individuals.

Quantitative comparison of the protein concentration of healthy and diabetic saliva was made spectrophotometrically. The protein content was statistically lower in diabetic sample when compared to the healthy sample (Fig.6). In experimental diabetes, Ghitescu *et al.* (2001) observed diabetes stimulated changes in the levels of soluble proteins namely Actin and Annexins I and II. Reduction in total soluble proteins can be further analyzed with proteomic approaches.

The saliva sample of both healthy and diabetic was run in SDS-PAGE, a thick peptide band was seen in healthy sample whereas no such thick band was observed in the diabetic sample (Fig.7). Hoe *et al.* (1997) and Huang (2004) found that the saliva from diabetic patients showed more pink-violet bands in the molecular weight region below 56KDa. The saliva from one healthy subject showed that the pink-violet band at the molecular weight of more than 97.4KDa. The saliva from another healthy subject did not showed that band. The pink-violet band is because of meta-chromatic effect. They found considerable variation between individual to individual. Similar to this result, we also observed enhanced levels of polypeptides

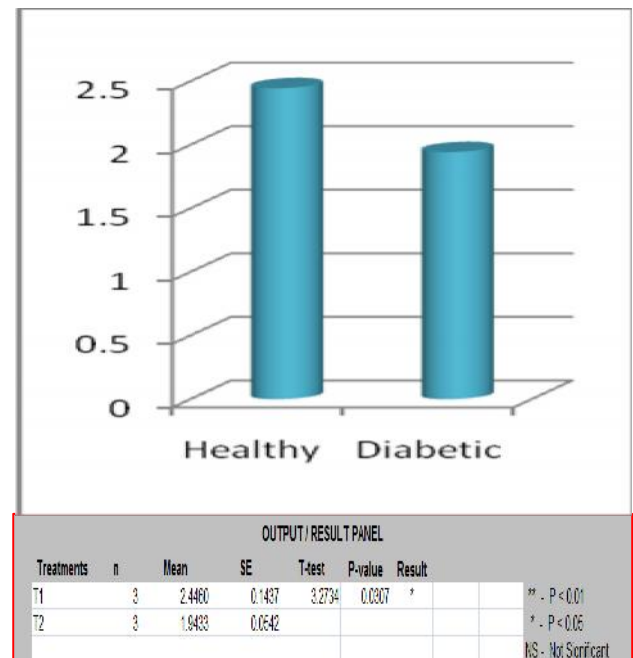


Fig. 6. Statistical analysis of protein content in healthy and diabetic samples

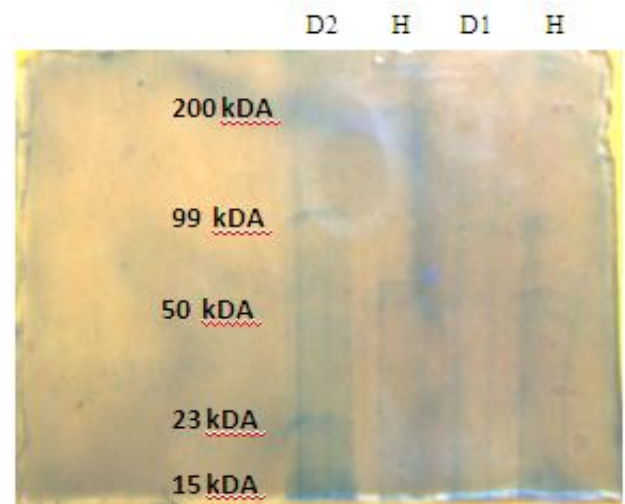


Fig. 7. SDS-PAGE profile of healthy and diabetic sample around 50 kDa. Proteomic approaches of this specific band will provide the insight of molecular nature and functional role in diabetic patients.

The gDNA of the diabetic individual was found to be reduced when compared to the healthy. Kanto *et al.* (2005), Daniel *et al.* (2006) and Sachwark (2006) were also found that saliva can be used as a marker for some diseases.

Conclusion

In the present study an attempt was made to evaluate the differences of sialic acid contents, levels of total soluble proteins and the nucleic acid. Results revealed that an increase in sialic acid is a precautionary signal for cardiovascular complications in the diabetic volunteers. Reduction in soluble proteins & nucleic acid indicates the impairment of protein synthetic machinery and this recommends more intakes of vegetables in the daily dietary menu of diabetic patients.

References

1. Abdella N, Akanji AO, Mojiminiyi OA, Assoussi AA and Moussa M (2000) Relation of serum total sialic acid concentrations with diabetic complications and cardiovascular risk factors in Kuwaiti Type 2 diabetic patients. *Diabetes Research and Clinical Practice* 50, 65-72.
2. Chiappen S, Antonelli G, Gatti R and Palo EF (2007) Saliva specimen: a new laboratory tool diagnostic and basic investigation. *Clin chim Acta*, 38, 30-40.
3. Daniel PK, David K, Serena Choo and Chia KS (2006) Saliva as a viable alternative source of human genomic DNA in genetic epidemiology. *Clinical Chimica Acta*, 367, 81-85.
4. Hallikeri K, Sanjay PR and Shivashankara AR (2008) Evaluation of salivary sialic-acid, total protein, and total sugar in oral cancer: A preliminary report. *Indian J Dent Res*, 19, 288-91.
5. Hoe AZ, Pendek R, Lam SK and Rahim ZHA (1997) Preliminary study on from diabetic and non-diabetic individuals by polyacrylamide gel electrophoresis. *Annals Dent* 4, 7-8.
6. Huang CM (2004) Comparative proteomic analysis of human whole saliva. *Archives of Oral Biology* 49, 951-962.
7. Jiang Jiang, Noh Jin Park, Shen Hu and David T Wong (2009) A universal pre-analytic solution for concurrent stabilization of salivary proteins, RNA and DNA at ambient temperature. *Oral biology*, 54, 268-273.
8. Kanto EA, Hirata MH, Hirata RD, Wunes FD, Hattenhoff RF and Oliveira MR (2005) DNA extraction from human saliva deposited on skin and its use in forensic identification procedures. *Oral biology*, 56, 119-128.
9. Ghitescu DL, Gugliucci A and Dumas F (2001) Actin and Annexins I and II Are among the Main Endothelial Plasmalemma-Associated Proteins Forming early Glucose Adducts in Experimental Diabetes. *Journal of diabetics*, 50, 1666-1674.
10. Sachwark NV, Malysz V, Fremdt H, Koch C, Simeoni E and Schwark T (2006) Fast and simple DNA extraction from saliva and sperm cells obtained from skin or isolated from swabs. *Legal Medicine* 8, 177-181.