Review Article

Serum and Salivary Glucose Levels in Diabetes Mellitus: A Review

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Abstract

The salivary fluid has an old history of study, but its physiological importance has only been recognized recently. In the past 50 years, the pace of salivary research has accelerated with the advent of new techniques that illuminated the biochemical and physicochemical properties of saliva. The interest in saliva increased, further, with the finding that saliva is filled with hundreds of components that might serve to detect systemic diseases and/or act as an evidence of exposure to various harmful substances as well as provide biomarkers of health and disease. The role of saliva in the diagnosis as well as monitoring of glycemic control has, also, been attracting attention of clinical researchers in recent times although results have been conflicting. To conclude, saliva is a whole, diverse fluid, that serves various purposes discussed in detail in the literature. The recent introduction of molecular biology opens up, once again, new vistas and a new search of the role of salivary fluid as a potential diagnostic tool which has an added advantage of being noninvasive. This review presents such insight into the possible use of salivary fluid for the monitoring of serum glucose levels and in the detection of glycemic control in diabetic patients.

Keywords: Diabetes mellitus, diagnostics, saliva, systemic diseases

Introduction

Saliva is a mixture of fluids secreted mainly by three pairs of major salivary glands, viz., parotid, submandibular, and sublingual glands. A plethora of minor salivary glands distributed over the buccal mucosa, lips, and along the mucosa of the upper aerodigestive tract present from the nasal cavity to the larynx and pharynx, also, participates in this secretion. Together, they are responsible for the remaining 5% of saliva secreted in humans. It is considered that humans secrete approximately 0.5 L of saliva per day in response to stimulation of the sympathetic and parasympathetic sections of the autonomic nervous system.[1] Whole saliva is a multiglandular secretion complex consisting of gingival fluid, desquamated epithelial cells, microorganisms, and products of their metabolism, food debris, leukocytes, and mucus from the nasal cavity and the larynx and pharynx. Saliva has varied functions from tissue repair to protection, digestion, taste, antimicrobial action, in the maintenance of tooth integrity and antioxidant defense system.[1,2] The average daily volume of saliva production is 500 to 1000 mL with the submandibular gland producing around 70% of the total volume, parotid contributing for 25%, whereas the sublingual gland contributing to about 5% of the total salivary secretion. The contribution of minor salivary glands toward the total volume of saliva, although, has more or less local effects.[2]

Saliva as Diagnostic Fluid

The salivary fluid has an old history of study but its physiological importance has only been recognized recently. In the past 50 years, the pace of salivary research has accelerated with the advent of newer techniques that have illuminated the biochemical and physicochemical properties of saliva. The interest in saliva increased, further, with the finding that saliva is filled with hundreds of components that might serve to detect systemic

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diseases and/or act as an evidence of exposure to various harmful substances and provide biomarkers of health and disease.[3]

Diabetes Mellitus

Malamed[4] defined diabetes mellitus as a syndrome of disordered glucose metabolism and inappropriate hyperglycemia, resulting from an absolute deficiency in insulin secretion and/or a reduction in the biological effectiveness of insulin, and/or both.

Investigations for Diabetes Mellitus

The recognition that many patients had obvious diabetes mellitus when their glucose was measured after a test meal led to the development, by the 1960s, of at least six different recommendations for standardized oral glucose loads, ranging from 50 to 100 g, based on body size, and/or independent from this. The procedure to execute the oral glucose tolerance test was standardized by establishing a glucose load of 75 g orally. Thereafter, samples are collected at half-hourly intervals for at least 2 h and their glucose content is estimated.[5] Reinauer *et al*.[6] listed various methods for glucose estimation in urine. These include primarily qualitative paper test strips such as Diabur, Diastix, Glucostix, and others; various enzymes, including glucose oxidase and peroxidase; semiquantitative tests including visual evaluation by enclosed color charts such as Clinistix and Multistix, and others; and the various quantitative tests including hexokinase, glucose dehydrogenase, and *O*-toluidine tests. The main disadvantages of using urinary glucose in diagnosis or screening of miabetes mellitus include marked individual variations in the renal threshold for glucose, poor reflection of changing levels of hyperglycemia, and a lack of sensitivity and specificity of the various qualitative and semiquantitative procedures.[6] Reinauer, also, enumerated various methods for glucose estimation in the sera, including various chemical and enzymatic methods, including *ortho*-toluidine, neocuproine, and ferricyanide in the former while hexokinase-G6 Hexokinase/Glucose-6-phosphate dehydrogenase (PDH), glucose dehydrogenase, glucose oxidase–peroxidase, and glucose oxidase with other indicator reactants in the latter category of methods.[6] American Diabetes Mellitus Association,[7] introduced glycosylated hemoglobin (HbA1c) assay to be performed routinely in all patients with diabetes mellitus to document the degree of glycemic control at the time of initial assessment and then as a part of the continuing care. Rohlfing *et al*.,[8] correlated HbA1c levels with mean sera glucose levels in diabetic patients. Goldstein *et al*.,[9] stated that glycated sera proteins (GSPs) can be used as an index of glycemic control in cases where HbA1c cannot be measured as in cases of hemolytic anemias. GSPs are measured by means of fructosamine assay. They provide an index of glycemic status over preceding 1 to 2 weeks although HbA1c provides the glycemic status over a longer period of time, viz., 2 to 3 months.[9] Reynolds *et al*.,[10] suggested that HbA1c assay measures the amount of glucose that has been in the sera during the past 2 to 4 months by measuring the amount of glucose that binds to hemoglobin within the circulating erythrocytes and remains attached for the life cycle of the red blood cells. As red blood cells have a life span of 3 to 4 months, it is easy to assess how sera glucose levels have varied in that period and whether the patient is having a good control or not over the glycemic status. This test is advisable once in 6 months for people with HbA1c values less than 6.5%, whereas once in every 3 months for those with values more than 6.5% for indicating a change in therapy. Thus, it is the preferred test for the medical evaluation of glycemic control because it measures the sera glucose levels over a period of 8 to 12 weeks.[10] Frier and Fisher,[11] stated that venous plasma values are most reliable for diagnostic purpose than arterial or capillary blood. Also, the concentration of cholesterol and low- and high-density lipoproteins and triglycerides is another important index of the overall metabolic control in diabetic patients and should be measured regularly. Ketone bodies in urine can be identified using sodium nitroprusside reaction which is specific for acetoacetate. This test is carried out using tablets and/or dipsticks for ketones.[11] Tura *et al*.,[12] enlisted around 14 technologies and 16 devices that could be used for noninvasive glucose monitoring, including near-infra-red spectroscopy, mid-infrared spectroscopy, optical coherence tomography, temperature-modulated localized reflectance, Raman spectroscopy, polarization changes, ultrasound technology, fluorescence technology, thermal spectroscopy, ocular spectroscopy, impedance spectroscopy, electromagnetic sensing, fluid harvesting, and iontophoresis.[12]

Various Studies Confirming the Diagnostic Role of Saliva as a Diagnostic Adjunct in the Regular Monitoring of Diabetes Mellitus Correlating Serum and Salivary Glucose Levels

Forbat *et al.* conducted a study to investigate the relationship between salivary and sera glucose levels in 31 diabetics in which parotid fluid samples were obtained by cannulation of the parotid duct. Glucose concentration was determined by glucose oxidase method using Beckmann glucose analyzer. The results revealed that salivary glucose concentration was independent of sera glucose levels.[13] Borg and Birkhed conducted a similar study on 20 healthy patients to follow the secretion of free glucose in parotid saliva in various patients after a single oral intake of different carbohydrates and compared the salivary glucose concentration with concentration in the sera. Approximately 1.5 mL of citric acid-stimulated parotid saliva was collected before (at 0^{th} min) and then 15, 30, 45, 60, and 120 min after the intake. Salivary concentration of glucose was analyzed enzymatically. Most of the 0^{th} min samples showed a variation in glucose concentration from 3 to 25 mmol/L. After glucose, fructose, and sucrose intakes, the salivary glucose levels increased to about two to four times, especially in the 30^{th} min samples. The correlation between the glucose concentration in saliva and sera was found to be higher after, than, before the carbohydrate intake.[14] Darwazeh *et al.*

examined mixed salivary glucose concentration in 41 diabetics and 34 healthy controls with a mean age of 52 ± 16 and 52 ± 18 years, respectively. Salivary glucose levels were analyzed by modified enzymatic ultraviolet detection method. Glucose concentration in saliva of diabetics was found to be significantly higher than in the controls and was directly related to the sera glucose levels.[15] Belazi *et al.* conducted a study to examine the flow rate and composition of unstimulated whole saliva and serum in children with newly diagnosed insulin-dependent diabetes mellitus (IDDM) and compared the values derived with the values obtained for a group of healthy controls. They observed that no significant difference was seen in the salivary flow rates between the two groups, whereas significantly higher concentrations of glucose were seen in the saliva and serum in children with IDDM. Salivary IgA concentration was, also, found to be higher in the test group as was serum IgG.[16] Amer *et al.* suggested that salivary samples of the nondiabetic control patients did not show the presence of glucose even in the slightest concentrations, whereas the samples obtained from the type 2 diabetics (non-IDDM) showed significant concentration of glucose in the saliva. The conclusion was that salivary glucose concentrations seemed to correlate with the serum glucose concentrations exclusively in patients with diabetes mellitus and that it could be used for monitoring changes in the sera glucose levels in only such patients.[17] Lopez[18] studied 20 diabetic children (3–15-years old) and 21 controls in the same age group (5–12-years old) and demonstrated that total sugars, glucose, urea, and total proteins were greater in diabetic patients than in the controls, whereas calcium values were found to be decreased. Aydin examined salivary glucose levels in unstimulated saliva samples collected from 20 obese and 20 nonobese type 2 diabetes mellitus patients in an age range of 41 to 66 years and 20 healthy controls. The results showed significantly higher salivary glucose levels in diabetic patients when compared to the controls.[19] Jurysta *et al.* conducted a study to evaluate salivary glucose concentration in unstimulated and mechanically stimulated saliva of normal and diabetic patients. Sera glucose levels were measured by glucose oxidase method, whereas salivary glucose levels were measured by hexokinase method. They observed higher glucose concentration in the saliva of diabetic patients than in the controls. This was applicable for both unstimulated and stimulated saliva. No significant correlation, however, could be observed between glycemia and glucose concentration in both unstimulated and stimulated saliva of diabetic patients.[20] Soares *et al.*[21] performed a study to determine salivary glucose levels in healthy adult controls and stated that the concentration of salivary glucose is not dependent on capillary glycemia and that the concentration of salivary glucose does not present significant differences between the measurements for males and females. Panchbhai *et al*.[22] studied salivary glucose levels in 120 age- and sex-matched individuals who were divided into three groups of 40 each, uncontrolled and controlled diabetics and healthy controls and observed significantly elevated mean salivary glucose levels in both uncontrolled and controlled diabetic patients when compared with the healthy controls. Sashi Kumar and Kannan assessed salivary glucose concentration and oral candidal carriage in type 2 diabetic patients. The study included 150 adults, 100 with type 2 diabetes mellitus, and 50 controls aged in between 40 and 60 years. Diabetic status was determined by assessment of random, non-fasting sera glucose levels and HbA1c levels. Salivary glucose levels were measured in the unstimulated and stimulated salivary samples by glucose oxidase method. The findings of the study revealed higher salivary glucose levels in the diabetics than in the nondiabetic patients. A significant positive correlation was, also, observed between salivary and sera glucose levels. They concluded that salivary glucose concentration is a potentially useful tool to monitor the glycemic control. Increased salivary glucose is, also, associated with increased prevalence of oral candida in such patients.[23] Vasconcelos *et al.* conducted a study to evaluate the concentrations of sera and salivary glucose levels in 40 type 2 diabetics with a mean age of 57.7 ± 8.9 years and 40 healthy controls with a mean age of 50.2 ± 12.3 years. Saliva collected was stored frozen until use in the glucose assay. The absorbance values of salivary glucose assay were read on a spectrophotometer at wavelength of 500 nm. Salivary glucose concentration was found to be significantly higher in type 2 diabetics although they could not observe a significant positive correlation between salivary and sera glucose levels in diabetic patients. They concluded that diabetes mellitus influences the concentration of salivary glucose and as salivary glucose is not directly influenced by glycemia, salivary assessment of glucose cannot be used to monitor glycemic control in diabetics.[24] Vaziri *et al.* examined salivary glucose levels in 40 type 1 diabetic patients in the age range of 9 to 61 years, 40 type 2 diabetic patients in the age range of 39 to 82 years, whereas 40 healthy controls in the age range of 39 to 67 years and observed no significant difference in the glucose concentrations between type 1 and type 2 diabetic patients and their matched controls and concluded that as alterations in the oral cavity might have some role in the development and severity of oral changes, determination and monitoring of salivary constituents may be useful in description and management of oral findings in diabetic patients.[25] Nagalaxmi and Priyanka studied 50 type 1 diabetes mellitus patients with 50 age- and sex-matched healthy controls and obtained a significant correlation between salivary and serum glucose levels in type 1 diabetic patients and in the controls. The levels of salivary glucose did not vary with age and gender of the patient in type 1 diabetes mellitus patients.[26] Lasisi and Fasanmade conducted a study to determine the effects of type 2 diabetes mellitus and periodontal disease on salivary flow rates and biochemical composition including salivary glucose and potassium levels and found significantly higher values (P value = 0.002 and 0.04, respectively) in the diabetic patients regardless of the periodontal disease status (mean = 100.7 ± 9.33, 111.5 ± 32.85 and 23.79 ± 5.19, 22.9 ± 6.25 mg/dL, respectively) compared with the nondiabetic patients (mean = 80.5 ± 30.85, 62.5 ± 31.89 and 19.23 ± 5.04, 17.74 ± 4.68 mg/dL, respectively).[27] Abikshyeet *et al.* conducted a study to substantiate the role of saliva as a

diagnostic tool in the monitoring of diabetes mellitus. The study included 106 patients, newly diagnosed with type 2 diabetes mellitus, with 15 healthy controls in the age group of 36 to 65 years. The salivary and sera samples were collected from the patients early in the morning after an 8-h fasting period. The study found increased fasting salivary glucose levels in patients with diabetes mellitus with a significant positive correlation that was observed between salivary and serum glucose levels in the diabetic as well as control patients. Based on their results, they concluded that fasting salivary glucose levels can be used as a noninvasive diagnostic and monitoring tool to assess the glycemic status in diabetic patients.[28] Panchbhai conducted another study on 80 diabetics who were divided into two groups of 40 each, a group with uncontrolled diabetes mellitus and the other group with controlled diabetes mellitus with a group 3 that was composed of age- and sex-matched healthy, nondiabetic controls. The second set of sample consisted of 150 study patients in groups of 50 each as classified in the earlier sample. A significant positive correlation (P value < 0.05) of salivary glucose levels and fasting sera glucose levels was observed in patients with uncontrolled diabetes mellitus in both sets of the samples.[29] Agrawal *et al*.[30] performed a study on 40 diabetic and 40 nondiabetic patients and found a correlation coefficient for nondiabetic and diabetic patients to be + 0.58 and + 0.40, respectively, proving the correlation between fasting salivary and sera glucose levels statistically significant. Prathibha *et al*.[31] conducted a study on 30 nondiabetic and diabetic patients and stated that significant variations were observed in the salivary physical and biochemical parameters between the diabetic and nondiabetic patients. Jha *et al.* conducted a study on 90 patients and their diabetic status was determined by estimation of random, nonfasting sera glucose levels and HbA1c levels. Salivary glucose levels were found to be significantly higher in the diabetic than in the nondiabetic patients. Also mean unstimulated salivary glucose level was found to be 1.15 mg/dL in the controls, whereas 2.04 mg/dL in the controlled diabetic and 3.99 mg/dL in the uncontrolled diabetic patients, suggesting a significant positive correlation between salivary and sera glucose levels.[32]

Controversy and Future Research Directions

Thus, on reviewing the literature so far, it could be inferred that saliva can be used as a potentially useful, noninvasive tool in the regular monitoring of diabetic patients. Numerous studies have shown a significant positive correlation between salivary and sera glucose levels; however, the specificity of the salivary glucose assay to assess the exact sera glucose levels, still, remains a big question. There is a controversy regarding the relationship between the concentration of glucose in the sera and the salivary fluid. Several factors might account for the poor correlation between sera and salivary glucose levels prevailing in diabetic patients, including oral retention of alimentary carbohydrates, glucose utilization by bacteria, and release of carbohydrates from salivary glycoproteins and contamination of the saliva by a large outflow of gingival crevicular fluid in patients with poor gingival status. Abikshyeet *et al.* formulated equations to predict fasting sera glucose levels and HbA1c percentage when fasting salivary glucose levels were known. However, accurate sera glucose levels could not be assessed by such equations in all the patients.[28] Based on the presently available data, there is an obvious need for further, extensive studies, to obtain an answer to this query, to accurately assess sera glucose levels from the obtained salivary glucose levels, and utilizing the diagnostic benefits of saliva in the clinical practice for the exact estimation of sera glucose levels.

Limitations of the Model for Using Saliva in Diagnostics

Apart from the abovementioned limitations, the potential use of saliva in diagnosis as well as in the regular monitoring of diabetic patients suffers from another possible constraint wherein in certain situations, including numerous autoimmune and/or inflammatory conditions like Sjogren syndrome and primary biliary cirrhosis, graft-versus-host disease, IG-G4-related sclerosing disease, degenerative diseases like amyloidosis, granulomatous conditions including sarcoidosis, infections including HIV/AIDS, hepatitis C, malignant conditions like lymphomas and salivary gland agenesis or aplasia apart from drug-induced xerostomia caused due to drugs including anticholinergics, antihistaminics, antihypertensives, and neurotropic drugs, including sedatives and anxiolytics, antidepressants and antipsychotics, to name a few, either a decreased salivary output/xerostomia or a possible change in salivary composition is seen and the total solids in the saliva change to the extent of not being reliable for diagnostics as well as in the regular monitoring of the patients. Patients with salivary gland changes after exposure to radiation in the head and neck area for treatment of malignancies, also, pose such challenges. Similar challenges are faced even in situations where the glucose threshold is either exceeded as in hyperglycemic crisis like diabetic ketoacidosis because of xerostomia or in cases of severe hypoglycemia because serum glucose levels have to cross a minimum threshold to appear in saliva.

Conclusion

To conclude, saliva is a whole, diverse fluid that serves various purposes discussed in detail in the literature. Also there has been sufficient literature that assays the role of saliva as a potential diagnostic tool in the monitoring of glycemic status in known and being treated diabetes mellitus patients. Further studies is, however, warranted to establish the role of saliva in the diagnosis of various conditions as well as its suitability and usage in the diagnosis as well as routine monitoring of glycemic status in persons with diabetes mellitus.

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Conflicts of interest

There are no conflicts of interest.

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