Article published online: 2021-11-01

ORIGINAL ARTICLE

Oral glucose tolerance test in unstimulated saliva of healthy individuals

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ABSTRACT

Objective: The aim of this study was to investigate oral glucose tolerance test (OGTT) in unstimulated whole saliva as a diagnostic specimen in clinical practice for detection of diabetes mellitus (DM). **Materials and Methods:** An interventional study was carried out in 30 apparently healthy individuals aged 24–59 years. Serum and saliva samples were obtained in fasting, 1 h and 2 h after glucose intake (75 g). Glucose concentration was determined by enzymatic colorimetric glucose oxidase-prostatic acid phosphatase assay. Statistical analysis of the repeated ANOVA (followed Bonferroni *post-hoc* test) and Pearson correlation coefficient were used. **Results:** The mean serum glucose concentration was significantly higher 1 h after glucose intake (152.32 ± 31.06) than both fasting state (106.38 ± 41.08; $P < 0.001$) and 2 h after glucose intake (125.21 ± 51.71; $P < 0.001$). Saliva glucose was also significantly higher 1 h after glucose intake (5.46 ± 2.41) than both fasting state (2.84 ± 1.46; $P < 0.001$) and 2 h after glucose intake (4.01 ± 1.91; $P < 0.001$). There were significant positive correlation between saliva and serum glucose concentration in fasting state ($r = 0.502$; $P = 0.044$), 1 h ($r = 0.756$; $P = 0.0001$), and 2 h ($r = 0.543$; $P = 0.023$) after oral glucose intake. **Conclusion:** It seems that unstimulated saliva can be used as an alternative to serum for diagnosis of DM in OGTT.

Key words
Diabetes mellitus, glucose tolerance test, saliva

INTRODUCTION

Diabetes mellitus (DM) is a chronic life-long disease caused by a carbohydrate metabolic block. The prevalence of diabetes is expected to rise to at least 300 million by 2025.[1] The conditions of diabetics are very complicated and very frequently, so it is difficult to cure. If diabetes cannot be well-controlled, the functions and metabolism of some tissues and organs will be disordered, which results in weakness, poor immunity, and complications. These complications can bring great pain to patients and even endanger their lives. Diabetes can be well-controlled by such convenient means as adjusting diet if it is diagnosed in time. Otherwise, once it reaches an advanced stage, it can result in serious diseases, such as heart diseases, renal diseases, blindness, and paraplegia.[2] However, the onset of diabetes occurs 4–7 years before clinical diagnosis.[3]

For clinical purposes, an oral glucose tolerance test (OGTT) to establish diagnostic status need only be considered if casual blood glucose values lie in the uncertain range (i.e., between the levels that establish or exclude diabetes) and fasting blood glucose levels are below those which establish the diagnosis of diabetes. If an OGTT is performed, it is sufficient to measure the blood glucose values while fasting and at 2 h after a 75 g oral glucose load. The advantage of using saliva as a biological specimen is the quick, uncomplicated, and noninvasive nature of sample collection. In addition, oral fluid sampling is safe for both the operator and the

Access this article online	
Quick Response Code:	Website: www.ejgd.org
	DOI: 10.4103/2278-9626.172736

How to cite this article: Mirzaii-Dizgah MH, Mirzaii-Dizgah I, Mirzaii-Dizgah MR. Oral glucose tolerance test in unstimulated saliva of healthy individuals. Eur J Gen Dent 2016;5:15-8.

patient and has easy and low-cost storage.[4] It is used to aid in the diagnosis of diseases and assessment of the severity of some illnesses.[5-14] The aim of the study was thus to evaluate OGTT in the saliva of apparently healthy individuals.

MATERIALS AND METHODS

Subjects

Thirty apparently healthy individuals (17 men and 13 women) were asked to participate in an interventional study, conducted at the Physiology Department, Aja University of Medical Sciences. The participants were aged 28.5 ± 9.3 years (range, 24–59 years), were not taking any medication at the time of the study, lesion(s) in their mouths and DM.

The Ethics Committee of Aja University of Medical Sciences, Iran approved the study protocol. Informed permission was obtained from all the participants.

Saliva and serum collection

An OGTT was performed with 75 g of glucose, given as an aqueous solution (300 ml) and ingested within 5 min immediately after the OGTT the subjects brushed their teeth and rinsed their mouth extensively with tap water. Venous blood and saliva were collected simultaneously before and 1 and 2 h after the glucose intake from the each participant. Samples were collected under resting conditions in a quiet room between 08.00 and 11.00 AM. Unstimulated salivary samples were obtained by expectoration in the absence of chewing movements into a plastic tube. Blood specimens were obtained by venipuncture, collected in 10-ml glass vacuum tubes without additive, and allowed to clot. The blood and saliva were then centrifuged (2000 g, 10 min) and the serum and supernatants of saliva were separated. Immediately after collection of saliva and serum, serum and saliva glucose were measured.

Laboratory assays

An enzymatic colorimetric glucose oxidase-prostatic acid phosphatase assay was applied to measure the serum and saliva concentrations of glucose using affiliated kits (Parsazemoon, Karaj, Iran) according to the manufacturers' instruction.

Statistical analysis

The repeated ANOVA analysis test (followed Bonferroni *post-hoc* test) was used to compare the salivary glucose among fasten, 1 and 2 h after glucose intake. The Pearson correlation test was applied to determine the association between serum and salivary concentration of glucose. Results were considered statistically significant if $P < 0.05$. Analyses were performed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA).

RESULTS

The study sample consisted of 13 women and 17 men with a mean age of 28.5 ± 9.3 years (range, 24–59 years).

According to WHO protocol,[15] 23, 4, 2, and 1 of the participants were normal, impaired fasting glycemia (IFG), impaired glucose tolerance (IGT), and DM, respectively.

The mean of serum and unstimulated saliva concentrations of glucose, 1 h after glucose intake was significantly higher than fasten and 2 h after glucose intake. In addition, it is significantly higher in 2 h after glucose intake than fasten [Table 1].

Statistical evaluation of the data using Pearson analysis indicated a correlation between the unstimulated salivary concentration of glucose and its serum concentration in fasten status ($r = 0.502$; $P = 0.044$); 1 h ($r = 0.756$; $P = 0.000$), 2 h after glucose intake ($r = 0.543$; $P = 0.023$), and area under curve ($r = 0.666$; $P = 0.007$) [Table 2].

DISCUSSION

Early detection of disease plays a crucial role in successful therapy. In most cases, the earlier disease is diagnosed, the more likely it is to be successfully cured or well-controlled. Managing a disease, especially in the early stage, may dramatically reduce the severity of its impact on the patient's life, or prevent and/or delay subsequent complications. Self-measurement of blood

Table 1: The mean values and SD of serum and unstimulated whole saliva glucose concentrations in oral GTT of apparently healthy participants

	Fasten	1 h	2 h
Serum (mg/dL)	106.38±41.08	152.32±31.06*	125.21±51.71*,#
Saliva (mg/dL)	2.84±1.46	5.46±2.41*	4.01±1.91*,#

Data are expressed as mean±SD. *Different from fasten, #Different from 1 h after intake of oral glucose, $P<0.05$. SD – Standard deviation, GTT – Glucose tolerance test

Table 2: Correlation between serum and saliva glucose level in GTT

Saliva (mg/dL)	Serum (mg/dL)			
	Fasten	1 h	2 h	AUC
Fasten	r=0.502 P=0.044			
1 h		r=0.756 P=0.000		
2 h			r=0.543 P=0.023	
AUC				r=0.666 P=0.007

GTT – Glucose tolerance test, AUC – Area under curve

glucose level is a very important aspect in monitoring the health quality of diabetic patients. Furthermore, three blood samples are required for OGTT. Often, this blood collecting causes physical and mental stress to the patient. For this reason, it is desirable to establish a noninvasive bloodless procedure to monitor the blood glucose level. Saliva, a multi-constituent oral fluid, has a high potential for the surveillance of general health and disease.[16] In this study, OGTT in unstimulated whole saliva was investigated, and we found that during the glucose tolerance test, the salivary glucose level increased within 60 min and decreased in the healthy participants after 2 h and was high in diabetic patients as were observed in serum. Hence, results of OGTT in saliva seem to be similar to serum results. To the best of our knowledge, this is the first study to assess OGTT in unstimulated saliva.

Results of the study showed that glucose exists and is detectable in saliva which is in agreement with results of several studies,[17-22] but the contrast with others.[23] It is interesting to know that in the majority of studies, more recent findings have emphasized on the existence of glucose in saliva.

Our study has demonstrated that there is significant correlation between saliva and serum glucose concentration. Our findings are in agreement with several previous reports.[17,18,22,24,25] As a modest positive correlation between serum and saliva glucose concentration was found, and salivary glucose level was lower than that detected on the serum, it is suggested that glucose may be passively entered to saliva.

For ethical limitation, we did not evaluate OGTT in the saliva of diabetic patients. However, 4, 2, and 1 of participants were IFG, IGT, and DM, respectively. Results of OGTT in the saliva of these patients were similar to results in the serum. The present study confirms the previous findings that showed elevated glucose concentration in stimulated parotid saliva at least 2 h after glucose intake in individuals with both IGT and manifest DM.[17]

CONCLUSION

It can be concluded that unstimulated saliva can be used as an alternative to serum for diagnosis of DM in OGTT.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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