Original Article

Effect of 2 h of room temperature storage on salivary glucose concentration

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Abstract

Background of the Study: Although many studies are available validating the role of saliva as an alternative diagnostic tool, no reliable data are available on the duration of time, a salivary sample can be reliably stored at room temperature for estimation. It varies from one analyte to another and has to be researched.

Aim: The aim of the study was to determine the effect of 2 h of room temperature storage on salivary glucose concentration.

Materials and Methods: Saliva samples obtained by spitting method from thirty healthy volunteers were centrifuged and glucose concentration determined in the supernatant obtained. The test was repeated 2 h later following room temperature storage of the supernatant.

Results: The data obtained were analyzed using wilcoxson signed rank test. No significant difference between was observed between the two values.

Conclusion: Salivary glucose can reliably estimate on centrifuged samples following 2 h of room temperature storage.

Keywords: Glucose, room temperature, saliva, storage

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INTRODUCTION

Saliva is a composite fluid secreted by the salivary glands and plays an important role in maintaining oral health. The fact that many components in blood reaches saliva through transcellular or paracellular pathways have propelled many researches and proved that saliva could reflect the physiological and pathological state of the body. Whole saliva is the most sought after diagnostic medium as it is easy to collect, requires minimal technical skill, easy to repeat, no privacy issues as with urine collection, no risk of needle prick injuries as with blood collection and is acceptable for all age groups.[1]

Although many studies are available validating the role of saliva as an alternative diagnostic tool, no reliable data are available on the duration of time, a salivary sample can be reliably stored at room temperature for estimation. The general storage protocol states that, up to 90 min, the sample can be stored at room temperature, but it could vary

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from one analyte to another depending on factors such as physical and chemical property, stability, and reactivity of the analyte in question, thus warranting more researches to benchmark the reliable storage criteria for each analyte.[2,3]

As studies are lacking in this regard, this pilot study was planned as a small initiative (with slightly extended time from the recommended protocol) to determine the effect of 2 h of room temperature storage on salivary glucose concentration. Glucose was considered as it is one of the most frequently estimated analytes due to increasing incidence and prevalence of diabetes worldwide[4] and many studies have shown a good correlation between serum and salivary glucose.[5,6]

Aims and objectives

The aim of the study was to estimate the glucose concentration in the salivary sample immediately after collection to obtain a baseline value and repeat the test on the same sample after 2 h of room temperature storage and compare the values thus obtained.

MATERIALS AND METHODS

The study population comprised thirty healthy volunteers aged above 18 years. Individuals suffering from any systemic disease or on any medications that could affect salivary characteristics were excluded from the study.

After obtaining informed consent, 2 ml of unstimulated whole saliva was collected by spitting method. The participants were refrained from eating or drinking for 1 h and asked to rinse their mouth with distilled water before sample collection. They were asked to sit in a comfortable position with eyes open and head tilted slightly forward. They were instructed to pool the saliva in the floor of the mouth and spit every 60 s or whenever there is an urge to swallow. The samples were centrifuged at 3000 RPM for 10 min and supernatant separated.[7]

Glucose concentration of the supernatant was estimated using oxidase-peroxidase method (Glucose-EGD, M/S Excel Diagnostics Pvt. Ltd) in automatic analyser (BIOTRA-K 3000). The method works on the principle: glucose is oxidized by the enzyme glucose oxidase to give D-Gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of enzyme peroxidase oxidises phenol which combines with 4-aminoantipyrine to produce a red-colored quinoneimine dye. The intensity of color developed is proportional to glucose concentration in the sample.[8]

The test was again repeated after 2 h, and the values obtained were tabulated and subjected to statistical analysis.

RESULTS

The mean value of glucose concentration immediately assayed was found to be 0.651 mg/dl (standard deviation [SD] 0.546) and that estimated 2 h later was 0.652 mg/dl (SD 0.502). The means were compared using Wilcoxson signed rank test and found no significant difference between them [Table 1]. This shows that room temperature storage of 2 h does not affect the glucose concentration in saliva, and thus it can be reliably estimated from the centrifuged sample even after 2 h.

DISCUSSION

This study is first of its kind and no literature is available as yet with regard to stability of glucose in saliva. As blood is considered as the gold standard for majority of the diagnostic tests, literature was searched with regard to the fate of glucose in blood. It was surprising to find that, in collected samples of blood, despite the addition of sodium fluoride, there is a 5%–7% loss of glucose per hour through glycolysis.[9] This is because the sodium fluoride inhibits enolase rather late in the glycolysis by which time the glucose has already phosphorylated with available adenosine triphosphate.[10] Increased white blood count and ambient temperature have also been found to contribute to further loss of glucose in collected samples.

On the other hand, the results of our study in saliva suggest that there is no significant alteration in the salivary glucose concentration even after 2 h of sample collection. Glucose is an aldohexose, a simple monosaccharide which is soluble in water. The cyclic form of glucose is more stable due to the pyranose ring formed by the five carbon atoms and the location of all the hydroxy group (except the one on anomeric carbon) in equatorial position.[11] In our study, the salivary samples were centrifuged immediately and the supernatant separated which removed all the cellular components that could potentially utilize glucose through glycolysis. This could be the reason why we could achieve a reliable result even after 2 h of room temperature storage. A similar finding was observed by Turchiano *et al*.[12] in their study where centrifugation of the collected blood samples yielded a better result. The results of our study show that there is not even any requirement to add inhibitors of

Table 1: The comparison of the salivary glucose values obtained at an interval of 2 h following sample collection using Wilcoxon signed rank test

Glucose estimation	Mean	*n*	SD	*P*
Immediately (mg/dl)	0.651	30	0.546	0.797#
After 2 h (mg/dl)	0.652	30	0.502	

#Denotes not statistically significant. SD: Standard deviation

glycolysis such as fluoride and just a mere centrifugation following saliva sample collection will yield us a reliable result, thus unfolding another distinct advantage of saliva as a diagnostic medium.

This study shows that the diagnostic services could be established with minimal technical requirements. This would be a boon to developing and underdeveloped countries where accessibility to diagnostic laboratories is still difficult and providing health care to every individual in need remains a great challenge.[13] The extended storage time will enable many camps to be conducted in rural areas where large sample collections for mass screening for diabetes, etc. could be performed and transported for testing with ease, as the expensive storage equipment requiring continuous power supply may no longer be necessary.

This study is first of its kind and has to be extended to a large sample involving other analytes to validate and benchmark the sample stability and diagnostic ability of saliva.

CONCLUSION

Our study highlights another distinct and unexplored advantage of saliva that is minimal storage requirements, making it more feasible and sought after diagnostic medium. From this study, we conclude that reliable salivary glucose concentration can be determined even after 2 h of room temperature storage following sample collection. The study may be in an initial stage, but it is definitely a promising one as it is establishing many avenues for research to advocate saliva as the routinely used diagnostic medium.

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Conflicts of interest

There are no conflicts of interest.

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