Section: Biochemistry

Original Research Article

DETERMINATION OF REFERENCE INTERVAL OF SALIVARY GLUCOSE AND ITS COMPARISON WITH SERUM GLUCOSE IN EUGLYCAEMIC INDIVIDUALS

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Abstract

Background: Salivary diagnostics offer a fast, non-invasive method for detecting and preventing oral and systemic diseases. Human saliva, an exocrine fluid, contains water, electrolytes, enzymes, immunoglobulins, albumin, and polypeptides. Unstimulated saliva is preferred in diagnostic studies due to the dilution effect in stimulated saliva. Salivary glucose, a small molecule transferable across salivary gland epithelium, has shown promise as a diagnostic tool due to its correlation with blood glucose levels. This non-invasive approach is particularly significant for diagnosing and monitoring diabetes mellitus. The study aimed to determine the reference interval of salivary glucose in euglycemic individuals and compare salivary glucose levels with serum glucose levels. **Materials and Methods:** An observational cross-sectional study was conducted on 150 healthy individuals aged 20–60 years at Govt. Medical College, Thrissur. Saliva samples were collected using plastic straws into polypropylene vials (2–3 ml), while blood samples (2.5 ml) were collected in fluoride tubes. Both samples were centrifuged, and supernatants were analyzed using the hexokinase method in an autoanalyzer. Data analysis was performed using MedCalc 18.9 software. **Result:** The majority of participants were aged 31–35 years, with 70% being female. The reference interval for salivary glucose ranged from 0.01 to 2.56 mg%, with most values between 0.01 and 0.30 mg%. Spearman's rank correlation coefficient (+0.4) indicated a moderate correlation between salivary and serum glucose levels. **Conclusion:** The study established the reference interval for salivary glucose as 0.01–2.56 mg% and confirmed a moderate correlation between salivary and serum glucose levels in euglycemic individuals.

INTRODUCTION

Salivary diagnostics plays an important role in the early detection and prevention of many oral and systemic diseases in a fast and non-invasive way. The human saliva, an exocrine fluid secretion, has high potential for screening health and diseases.[1] It consists of water, electrolytes and a variety of proteins like enzymes, immunoglobulins, albumin and different polypeptides.

Saliva is said to be the ultra-filtrate of blood. It is functionally comparable to blood in reflecting the physiological and pathological status of the body .The concentration of some components in saliva may have association with certain systemic illnesses that are reflecting the metabolic, nutritional, hormonal, immunological and neurological states of the patient.[2] Saliva collection is easier and non-invasive compared to blood collection.[2,3] These facts prompted many researchers in evaluating the possibility of using saliva as a diagnostic resource.[1] Unstimulated whole saliva is found to be used in the majority of diagnostic studies because, in stimulated saliva there is dilution causing modulation in pH.[4] Glucose is one of the blood components that are transferable across the salivary gland epithelium. Glucose is a small molecule that can diffuse easily through the membrane of the blood vessels and through gingival sulcus, to the gingival fluid and making its way to the saliva.[2] Studies that explore the diagnostic value of salivary glucose are promising due to the non-invasiveness of the procedure and its correlation with blood results.[1]

Diabetes mellitus is perhaps one of the oldest diseases studied and it was reported for the first time about 3000 years ago from Egyptian manuscript.[5,6]

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Now diabetes has become a common global health problem worldwide. It has become one of the leading reasons of death and disability.[5] It is an endocrine and/or metabolic disorder with a growing prevalence rate and worldwide incidence.[6] The prevalence in 2000 was estimated to be 2.8% and is expected to increase to 4.4% by 2030.[6] DM is represented by chronically increased glucose level assigned to the autoimmune destruction of pancreatic beta (β)-cells of the pancreas with insulin inadequacy to abnormalities that result in insulin resistance.[7] The deficient insulin action on target tissues, mainly striated muscle fibers, adipose tissue, and liver, forms the basis of disturbances of carbohydrate, fat, and protein metabolism.[8]

Saliva is a complex fluid produced by the salivary glands, an essential function of which is to maintain the well-being of the mouth. It has become a helpful fundamental sampling tool for clinical conclusions and research as it can be gathered effectively and non-invasive. The presence of glucose in the salivary secretion is a settled actuality, although it was accepted beforehand that salivation does not contain glucose as a typical constituent, and the mean values did not depend on the gender.[9,10]

As indicated by past investigations, the connection between salivary glucose and serum glucose was reliable in both the diabetics and controls. Henceforth, they presumed that salivary glucose gives off an impression of being a solid pointer of serum glucose concentrations, especially in diabetic patients. The salivary glucose concentration will be higher in patients with diabetes. Prevention, well timed diagnosis and management are important in patients with diabetes. Currently blood testing remains to be the gold standard in the diagnosis but it is invasive and offer great deal of discomfort and anxiety to patients in addition to the risk of infection.[10]

So, there is an important need of non-invasive method for diagnosing and monitoring treatment of diabetes mellitus. Curiosity in saliva as a diagnostic fluid is currently developing.[5,11]

Several authors have shown that increase in the concentration of glucose in saliva is related to glycaemic status.[2] Considering the limited amount of studies that evaluate the salivary glucose level, this study is aimed to determine the reference interval of salivary glucose and comparing it with Serum Glucose Level in healthy adults.

Objective

1. To determine the reference interval of salivary glucose in euglycaemic individuals.
2. To compare salivary and serum glucose levels in euglycaemic individuals

MATERIALS AND METHODS

Study setting: Department of Biochemistry, Govt Medical College, Thrissur. Analysis of samples collected from study subjects will be done at Clinical Chemistry Section of Central Laboratory of Govt. Medical College, Thrissur.

Study design: Observational, Cross-sectional study

Study population: Apparently healthy individuals between the age group 20 – 60 years will be selected after getting informed consent, from health workers, of Govt. Medical College, Thrissur, who received 2 doses of covid vaccine and based on inclusion and exclusion criteria as shown below. Health status will be assessed by history and clinical examination. A proforma will be used for this purpose.

Inclusion criteria to determine reference interval: Apparently healthy adults between 20 to 60 years, who received 2 doses of covid vaccine.

Exclusion criteria to determine reference interval:[6]

1. Pregnant women
2. Patients diagnosed with diabetes mellitus, and those on regular medication for any other systemic diseases
3. Patients with oral pathology, those undergoing radiotherapy for head and neck cancer, oral mucosal or salivary gland disorders, corticosteroid therapy.
4. Patients who are smokers.

Sample size: The sample size for this study is set as 120 as per NCCLS (National Committee for Clinical Laboratory Standards) for finding out reference interval of a biomarker.[12] Extra 30 is also included in the study to accommodate the outliers.

Study procedure:

Study variables: Random salivary glucose level and random serum glucose level are the different variables to be tested. Apparently healthy individuals in the community will be found out by clinical examination using a proforma.

Specimen collection: The subjects were briefed about the study and a written consent is obtained for the procedure

Saliva sample collection: The subject should be made to sit comfortably at least for 5 minutes, then should wash mouth with tap water to remove food debris and rinse finally with distilled water. Each subject is instructed to discard saliva of the initial two minutes to avoid collection of diluted saliva.(70) Saliva is collected through short plastic straw and transferred it in to polypropylene vials (2-3 ml).(71) Samples will be stored at room temperature, if analysis could be carried out within 30-90 minutes of collection, or at 40c if analysis done 3-6 hours from collection, or at -200c if analysis could be done within days to months after collection(72), depending on feasibility.

Blood sample collection: 2.5 ml blood will be collected by aseptic precautions into Flouride containing blood collection tubes.

Salivary glucose and blood glucose estimation: Each saliva sample and blood sample will be centrifuged at 2000rpm for 5 minutes. Clear supernatants are used immediately for estimation of glucose. Glucose is analyzed by hexokinase method. Automatic analyzer is used for estimation.'

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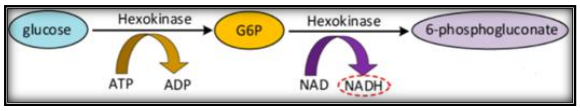
Estimation of salivary glucose:
Method: Hexokinase method
Principle: Glucose phosphorylated by Hexokinase (HK)`in the presence of ATP and magnesium ions to produce glucose – 6 – phosphate and adenosine diphosphate (ADP). Glucose – 6 – phosphate dehydrogenase (G6P – DH) specifically oxidises Glucose – 6 – phosphate to Glucose – 6 – phosphate with concurrent reduction of NAD+ to NADH. The increase in absorbance at 340nm is proportional to the glucose concentrate in the sample.



Reagent composition:
Pipes buffer (pH 7.6): 24.0 mmol/L
ATP: ≥ 2.0 mmol/L
NAD+: ≥ 1.32 mol/L
Mg2+: 2.37 mmol/L
Hexokinase: ≥ 0.59 ku/L
G6P-DH Preservative: ≥1.58 ku/L
Storage and stability: Regents are stable, unopened, up to the stated expiry date, when stored at 2 – 8 0C.
Linearity: The test is linear within a concentration range of 0.6 to 45 mmol/L. The lowest detectable level is 0.04 mmol/L.
Preparation and stability of reagent: Reagents are ready to use. Reagents are stable, unopened, up to the stated expiry date when stored at 2 – 8 0C. Opened vials stored on board the instrument are stable for 30 days.
Sample: Human Saliva and blood sample as fresh as possible and estimation done by Beckman coulter AU analyser (AU600).
Statistical analysis:

1. To find out reference interval: If reference distribution shows Gaussian distribution, reference limits can be found out by parametric methods which was then calculated as values of 2SD below and above mean (mean±2 SD). Nonparametric method can be used to find out reference interval if reference distribution does not show Gaussian distribution. Inter percentile interval of 95 % bounded by 2.5 and 97.5 percentiles will be used for reference interval determination.
2. To compare salivary glucose and serum glucose level: If the variables show normal distribution Pearson correlation coefficient will be used to find out correlation between salivary glucose and serum glucose level. If the variables do not follow normal distribution Spearman's rank order correlation coefficient was used.

Data entry and Analysis: Data analysis will be done using MedCalc free trial version 18.9.

RESULTS

In this observational cross sectional study 150 apparently healthy subjects were selected based on inclusion and exclusion criteria, and the data obtained from the subjects were entered in excel worksheet and analysed by MedCalc free trial version 18.9.
Age Distribution of Study Subjects: The age distribution of the study population showed that majority of the patients were in the age group of 31-35 years followed by 26-30 years age group. The lowest number were observed in age group of 56-60 years.

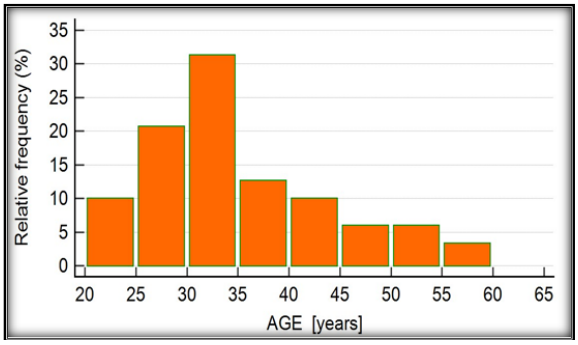


Figure 1: Age Distribution

Gender distribution: The gender distribution showed that 70% of the study subjects were females & 30% were males. [Male -1, Female -0]

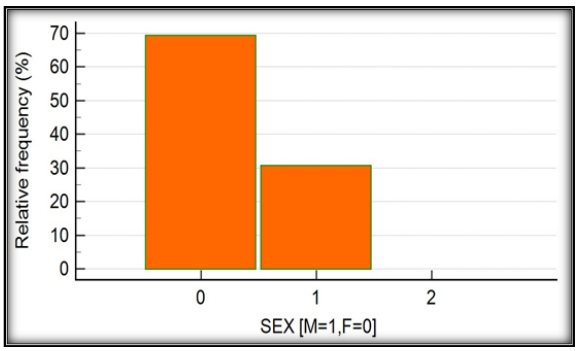


Figure 2: Gender Distribution

Reference interval of Salivary glucose: The reference interval of salivary glucose is found to be from 0.01 to 2.56 mg% based on the non-parametric percentile method.

Table 1: Reference interval of salivary glucose.

Reference Interval [n=150]	90 % Confidence Interval	
	Lower limit	Upper limit
0.01 to 2.56	0.01 to 0.02	2.34 to 3.41

Frequency distribution of salivary glucose: The majority of the study subjects (38%) were in salivary glucose level 0.01 to 0.30 mg% followed by 0.31 to 0.70 mg% (18%).

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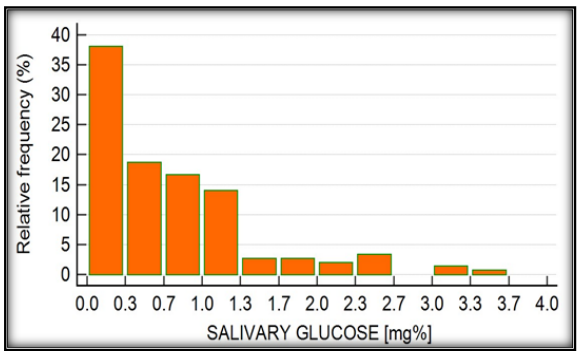

Figure 3: Frequency distribution of salivary glucose

Serum Glucose: The 2.5 & 97.5 percentile values for serum glucose levels were 77 mg% and 132mg% respectively based on nonparametric percentile method.

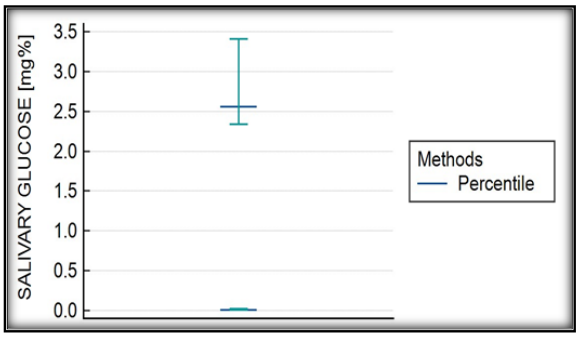

Figure 4: Nonparametric reference interval of salivary glucose.

Table 2: Serum glucose

Serum glucose level [n=150]	90 % confidence interval	
	lower limit	upper limit
77 to 132	77.00 to 78.00	130.00 to 137.00

Frequency distribution of serum glucose

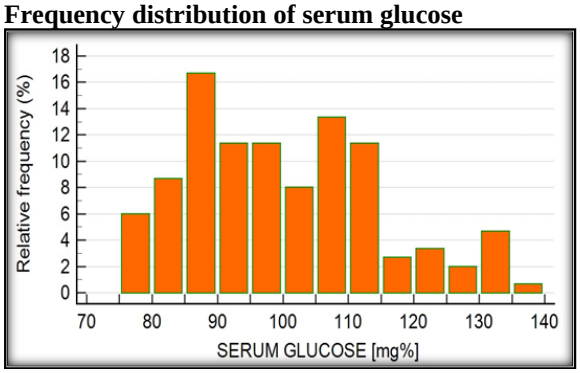

Figure 5: Frequency distribution of serum glucose

Correlation between serum and salivary glucose: The spearman's correlation coefficent was +0.4 which showed a moderate correlation between serum and salivary glucose levels, (P < 0.0001).

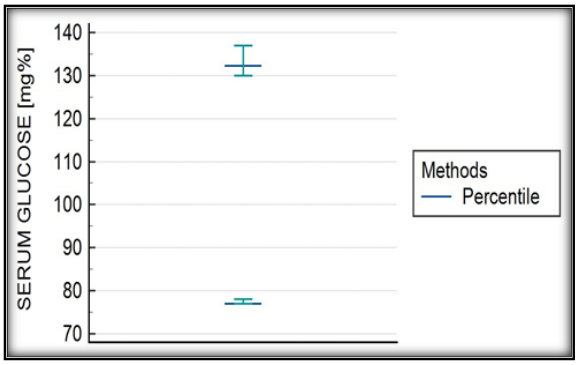

Figure 6: Percentile of serum glucose

Table 3: Correlation between serum and salivary glucose levels

Spearman's coefficient of rank correlation (rho)	0.410
Significance level	P<0.0001
95% Confidence Interval for rho	0.267 to 0.535

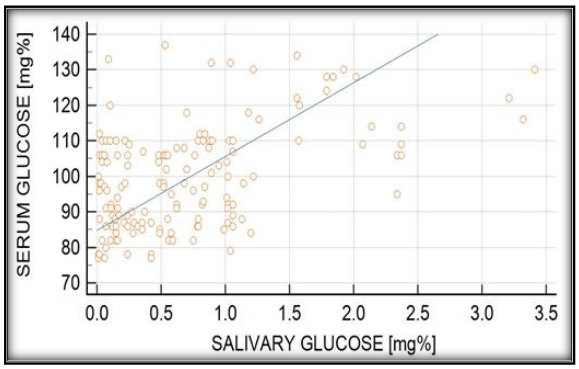

Figure 7: Correlation between serum and salivary glucose levels

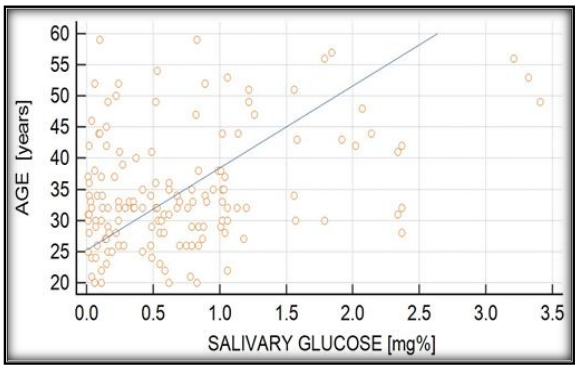

Figure 8: Correlation between age and salivary glucose levels

Correlation between age and salivary glucose
The spearman's rank correlation coefficent was + 0.2 which showed a low correlation between age and salivary glucose levels (P= 0.0061).

Correlation between age and Serum glucose
The spearman's rank correlation coefficent was + 0.4 which showed a moderate correlation between age and serum glucose levels (P < 0.0001).

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Table 4: Correlation between age and salivary glucose levels

Spearman's coefficient of rank correlation (rho)	0.223
Significance level	P= 0.0061
95% Confidence Interval for rho	0.0649 to 0.370

Table 5: Correlation between age and serum glucose levels

Spearman's coefficient of rank correlation (rho)	0.414
Significance level	P < 0.0001
95% Confidence Interval for rho	0.271 to 0.538

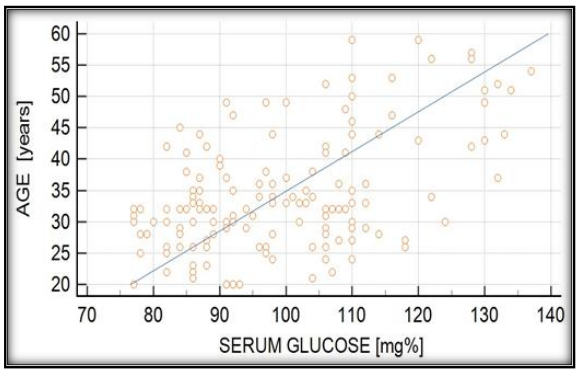

Figure 9: Correlation between age and serum glucose levels

DISCUSSION

In our study the age distribution of the study population showed that majority of the study subjects were in the age group of 31 to 35 years (32%), followed by 21% in 26 to 30 years age group. The lowest number were observed in age group 56 to 60 years [Figure 1]. The gender distribution showed that 70% of study subjects were females, and 30% were males [Figure 2]. This was similar to the reviewed studies.

In this study the reference interval of salivary glucose was found to be from 0.01 to 2.56 mg%, based on non-parametric percentile method [Table 1]. In them majority of the subjects were in salivary glucose level 0.01 to 0.30 mg% (38%), followed by 0.31 to 0.70 mg% in nearly 18% [Figure 3]. Harshada Ragunathan et al,[4] showed that, out of 20 patients with diabetes mellitus, 9 of them had the serum glucose level of 150–200 mg/dL, 7 had a level of 200–250 mg/dL, and 4 with a level of 300–400 mg/dl and in control group out of 20 patients 13 patients had the serum glucose level below 100 mg/dl and 7 of them with the level of 100–110 mg/dl. A distinct difference was observed in the salivary glucose between the control and diabetic group. The salivary glucose level for control was between 0.1 and 0.7 mg/dl and for diabetic, the level was between 1.0 and 9.6 mg/dl.

In this study the spearman's rank correlation coefficent was +0.4, which showed moderate correlation between serum and salivary glucose levels [Table 3]. The other spearman's rank correlation coefficent obtained were + 0.2 which showed low correlation between age and salivary glucose levels [Table 4], +0.4 which showed moderate correlation between age and serum glucose levels [Table 5]. Similarily, the study by Sreedevi, et al,[16] A highly significant correlation was found between salivary glucose and serum glucose before the treatment and also after the control of diabetes. The correlation between salivary glucose and serum glucose was also highly significant [0.7] in controls. it has an added advantage of being non-invasive procedure with no need of special equipment. Avanindra Kumar et al,[17] showed the presence of glucose was detected in both groups; however, the levels were raised in people with diabetes compared to healthy individuals. The study indicated a substantial increase in salivary and serum glucose levels in diabetic patients compared to healthy controls. The concentration of glucose in saliva increases with the increase in serum glucose concentration.

Preethi Balan et al,[18] study assessed glucose levels using the glucose oxidase method in blood and unstimulated saliva in 90 subjects who were divided into 3 equal groups of controlled type 2 diabetes, uncontrolled type 2 diabetes and those without diabetes. Salivary glucose levels were significantly higher in patients with diabetes than controls. There was a significant positive correlation between salivary and plasma glucose levels in patients with diabetes and concluded that glucose concentration in saliva is higher in diabetics but hyperglycaemia does not influence salivary glucose levels. . Shruti Gupta, et al,[19] the study revealed a significant correlation between salivary and serum glucose levels in both diabetic and nondiabetic subjects. No significant relationship was observed between salivary glucose levels and gender or age in both diabetics and non-diabetics and between salivary glucose levels and duration of diabetes in diabetics. Mean salivary glucose levels (19.48 ± 5.511) in diabetic subjects were found to be significantly higher than the levels in non-diabetic subjects (7.82 ± 2.423) (P < .001). On the basis of the findings, it was concluded that salivary glucose levels could serve as a potentially non-invasive adjunct to monitor glycaemic control in diabetic patients.

Limitations of the Study: Age specific and gender specific reference intervals of salivary glucose were not found in this study

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Table 6: Comparison of present study with other studies

Author	Present study	Sreedevi et al,[16]	Abikshyeet et al,[11]	Balan et al,[18]	Shruti Gupta et al,[19]	Nadaf et al,[6]	Raghunathan et al,[4]	Avanindra Kumar et al,[17]
Year	2020	2008	2012	2014	2015	2017	2019	2019
Study design	Obs. CS	CC	CC	CC	CC	CC	CC	CC
Sub(n)	150	C-120 N-120	C-106 N-15	90	C-100 N-100	C-30 N-30	C-20 N-20	C-150 N-50
Age	20-60	<35 to >55	35 -65	30 - 60	≤40 to >60	20 -75	-	<45 &>45
Method	HK	GOD-POD	GOD-POD	GOD	GOD	HK	GOD-POD	GOD-POD
Salivary Glucose level (mg%)	0.01-2.56	0.7 to 1.3	0.51-2.32		4.3-12.9		0.1-0.7	
Mean		1.0 ± 0.1	1.23 ±0.52	1.18 ± 0.675	7.82 ± 2.42	0.72 ± 0.08		6.36 ± 0.693
r between Saliva &Serum Glucose	Rho - +0.4	r-0.74	r-0.52	r-0.68		r-0.79		
Degree of r	Mod.			Good			High	
Significance	YES							

CONCLUSION

The reference interval of salivary glucose was found to be from 0.01 to 2.56 mg%, with the majority of the salivary glucose were distributed in 0.01 to 0.30 mg% range.

The spearman's rank correlation coefficent was +0.4 which showed moderate correlation between serum and salivary glucose levels.

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