ORIGINAL RESEARCH

Estimation of Salivary Amylase in Diabetic Subjects Exposed to COVID 19 Infection.

Premika Sri, Suganya AP, SR Thiruvanthicka, Mahalakshmi T, M.V.Bhuvaneswari, R.Sathish Muthukumar

Abstract

Introduction: Diabetes represents one of the leading causes for mortality in the world. Diabetic patients are clinically recognised as being highly susceptible to infectious diseases, possibly due to their dysregulated immune systems. Chronic insulin resistance and relative insulin deficiency in target cells cause hyperglycemia. Salivary Alpha-amylase, one of the biomarkers has been shown to increase in diabetics compared to nondiabetics. Hence, the present study is intended to evaluate post covid alterations of salivary amylase levels in diabetics, thereby assessing the metabolic functioning of these subjects.

Aim and Objectives: The aim of the current study is to evaluate any post covid alterations of salivary amylase levels in diabetics, thereby assessing the metabolic functioning of these subjects

Materials and Methods: A total of 90 participants in the varied age range of 30–60 years, were divided into three groups as Group I (Controls- non-diabetic and non-covid exposed), Group II - Diabetic subjects exposed to covid and Group III - Diabetic subjects unexposed to covid.

Results: Salivary amylase levels were found to be high in group 2 and group 3 subjects when compared to group 1 subjects. The inter-group comparison between groups 1 and group 2 and group 1 and group 3 showed a statistically significant P value of 0.000. But the comparison of amylase values between group 2 and group 3 subjects doesn't show any statistically significant value. (P value -0.487). Thus, the salivary amylase levels were found to have similar values in diabetics affected with covid and diabetics without covid infection.

Conclusion: From our study, we conclude that there were no differences in the levels of salivary amylase levels between the subjects with diabetes affected with COVID infection and diabetes who were not affected by COVID infection.

Keywords: Salivary amylase, Diabetics, COVID, Diabetes

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion, resistance to peripheral actions of insulin, or both leading to the development of disabling and life-threatening health complications, most noticeable of which are microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular complications leading to a 2-to-4-fold increased risk of cardiovascular diseases.[1] About one-third of diabetic patients grumble of dry mouth (xerostomia) which may be due to overall decreased flow of saliva ensuing from systemic dehydration and an augment in the salivary glucose level. In Diabetics, Salivary glands exhibits decreased function and increased vulnerability to oral infections such as caries or periodontitis have long been known sign and symptoms of diabetes mellitus.[2]

In accordance with the International Diabetes Federation - 2021,537 million adults (20-79 years) have diabetes, accounting for one in every ten. This figure is set to rise to 643 million by 2030 and 783 million by 2045. Over three-quarters of diabetic adults live in low- and middle-income countries. Diabetes mellitus will be responsible for 6.7 million deaths in 2021, making up for one death every five seconds. Diabetes has ultimately resulted in a 316% increase in health-

Department of Oral and Maxillofacial Pathology, Chettinad Dental College and Research Institute, Rajiv Gandhi salai, Kelambakkam, Chennai- 603103.

Corresponding Author: M.V.Bhuvaneswari, Room no.5B, Department of Oral and Maxillofacial Pathology, Chettinad Dental College and Research Institute, Rajiv Gandhi salai, Kelambakkam, Chennai - 603103.

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care expenditure over the last 15 years. 541 million adults have impaired glucose tolerance (IGT), attempting to put them at high likelihood of developing type 2 diabetes.[3]

The only conventional method for diagnosing or monitoring the disease is blood analysis in the form of random, fasting, or postprandial blood glucose levels. HbA1c, or glycosylated haemoglobin, provides an accurate estimate of glucose levels in the blood over the previous 2-3 months and aids in overcoming inconsistency in at-home monitoring records. Monitoring blood glucose levels at regular intervals, on the other hand, can cause discomfort and mental trauma in many people. As a result, the use of saliva has made it valuable in young, old, and infants due to its simple and non-invasive collection procedure.[4]

Saliva can be a convenient, non-invasive alternative to serum. It could be a useful alternative, particularly when mass screening is required and patient compliance is an issue. Leuchs described human salivary amylase (1, 4 -D glucan, 4 glucanohydrolase) in 1831. Human salivary amylase is a major component of human salivary secretions that is responsible for the hydrolysis of starch into small sugar molecules.[5] Hence the present study is done to evaluate post covid alterations of salivary amylase levels in diabetics, thereby assessing the metabolic functioning of these subjects.

Materials and Methods

In the present study (Case- Control), a total of 90 participants in the varied age range of both sexes, were divided into three groups, namely Group I- Controls (non-diabetic and non-covid exposed), Group II- Diabetic subjects exposed to covid and Group III - Diabetic subjects unexposed to covid.

Subjects attending the outpatient ward of Chettinad Dental College and Research Institute were included in the study. An Institutional Ethics Committee approval and a written Informed consent were obtained before commencing the study. Demographic details of the patient were recorded along with their COVID history. The procedure was carried out with precautionary and proper COVID 19 protocols. Healthy volunteers, diabetic subjects with history of covid before three months and diabetic subjects without covid infections were included in this study. Covid positive subjects and subjects with other systemic illness were excluded from the study.

Saliva samples was collected from subjects for estimation of salivary amylase level. The salivary samples were be collected after oral rinse. The patient was made to sit straight in the chair, and after spitting any residual saliva in the mouth, he or she was asked to spit in a sterile container every minute for 10 minutes to collect unstimulated whole saliva. Each sample of 5 ml was centrifuged at speed of 5000 rpm for 5 min to remove any debris thereof. Thereafter, salivary amylase levels of each sample were determined using semiautomatic analyzer with direct substrate method (kinetic enzyme assay).

Results

The results obtained; levels of salivary amylase were tabulated after analysis. The statistical analysis was done, along with inter group comparison using standard T- Test of our case control study. Salivary amylase levels were found to be high in group 2 and group 3 subjects when compared to group 1 subjects. The inter group comparison between group 1 and group 2 and group 1 and group 3showed statistically significant P value of 0.000. But the comparison of amylase values between group 2 and group 3 subjects doesn't show any statistically significant value. (P value -0.487). Thus, the salivary amylase levels were found to have similar values in diabetes affected with covid and diabetic without covid infection. (Table-1, Graph-1)

Discussion

Diabetes mellitus ((DM) is a metabolic disorder associated with significant immune system dysfunction. Many aspects of the systemic immune response, such as polymorphonuclear leukocyte function (leukocyte adherence, chemotaxis, and phagocytosis), bactericidal activity, and antigen challenge response gets altered.[5]

Asia is a major area of the rapidly emerging Type 2 Diabetes Mellitus global epidemic, with China and India the top two epicentres. Genetic predisposition determines individual susceptibility to Type- 2 Diabetes Mellitus (T2DM), an unhealthy diet and a sedentary lifestyle are important drivers of the current global epidemic; early developmental factors (such as intrauterine exposures) also have a role in susceptibility to T2DM later in life.[6] Many cases of T2DM could be prevented with lifestyle changes, including maintaining a healthy body weight, consuming a healthy diet, physically active, non-smoking and drinking alcohol in moderation. Most patients with T2DM have at least one complication, and cardiovascular complications are the leading cause of morbidity and mortality in these patients.[7]

The main oral complication attributed by diabetes is periodontal disease (PD), considered to be the sixth complication of DM, higher prevalence of mucosal disorders possibly associated are chronic immunosuppression, delayed healing, and/or salivary hypofunction.[8] Impaired immune system along with salivary glandular dysfunction and hyperglycemic state leads to oral fungal infections such as oral candidiasis. Vascular dysfunction and reduced immune responses results in delayed wound healing and higher chances of acquiring infection post-surgically. High quality systematic review by F. D'Aiuto, et al (2017) suggests that there is an elevated risk of precancerous and cancerous condition.[9]

The prevailing technique for determining blood glucose levels necessitates venepuncture, which can be incredibly

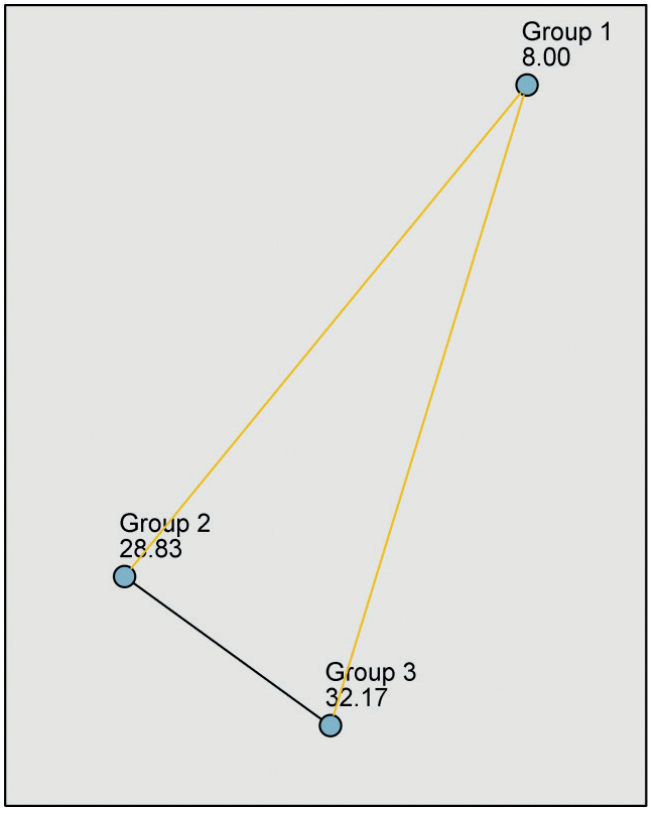
Table 1: Statistical analysis done in the subjects of group 1,2, 3 with levels of salivary amylase

Sample 1-Sample 2	Test Statistic	Std.Error	Std.Test Statistic	Sig	Adj.Sig
Group 1 -Group 2	-`20.833	4.795	-4.344	.000	.000
Group 1 -Group3	-24.167	4.795	-5.040	.000	.000
Group 2 – Group 3	-3.333	4.795	-.695	.487	1.000

distressing for patients, particularly children. Along with the physical trauma, the procedure provokes mental trauma and anxiety in the patients, further negatively impacting them. It is of foremost importance to assess the severity of this problem in order to make blood glucose assessment more appropriate to patients while not meddling with their regular visits to diabetic clinics. As a consequence, it is necessary to devise a method for measuring BGL that is non-invasive.[10]

Saliva aids in the maintenance of the oral cavity and gastrointestinal tract by lubricating the mouth, impeding potentially harmful microbes, and facilitating oral tissue healing. Amylase is a digestive enzyme produced by the salivary glands and pancreas that cleaves the glycosidic linkages in starch molecules, resulting in smaller saccharides such as maltotriose, maltose, and trace amounts of glucose. There has been increasing interest in salivary biomarkers in recent years. The main justification for their use is their ability to monitor the disease initiation and its progression, and to observe the outcome of treatment. Specific biomarkers associated with the state of health or disease, can be detected and monitored in a non-invasive way.[11]

Salivary amylase plays a vital role in the oral cavity which is responsible for metabolizing both complex and simple carbohydrates into glucose. Diabetes alters the volume of saliva, the composition of amylase levels, and other salivary biomarkers pertaining to catecholamine and other substances such as cortisol due to its association with the autonomic system.[12] Salivary amylase activates starch hydrolysis in the mouth, and this process accounts for no more than 30% of total starch hydrolysis due to its inactivity in the stomach due to an acidic pH. Sympathetic and parasympathetic nerves innervate the acinar cells, which produce salivary amylase. Sympathetic nervous system activation increases amylase synthesis, which increases amylase concentration in saliva, and parasympathetic nervous system activation increases saliva flow. Salivary amylase is related to the autonomic system and it is involved in in glycemic digestion, so it could be a good biomarker for assessment and follow-up in Diabetes Mellitus.[13]



Graph 1: Graphical values of salivary amylase levels among group 1, 2 and 3

The COVID-19 pandemic is creating major difficulties for world health Organization as the virus is constantly changing its structural properties. The mutation process associated with the COVID-19 virus is creating major difficulties for doctors and researchers in finding the necessary cure for the disease.[14] Saliva specimen collection has the advantages of being more acceptable for patients and more secure for healthcare workers in covid diagnosis as it's a non-invasive method of collection and estimation. To date, three methods for collecting saliva have been reported: coughing, saliva swabs, and directly from the salivary gland duct.[15] The present study is intended to evaluate post covid alterations of salivary amylase levels in diabetics, thereby assessing the metabolic functioning of these subjects.

The study done by Kheirmand Parizi Marjan et al (2019) demonstrated that salivary IgA and amylase levels are associated with diabetes. Moreover, s-IgA levels are associated with T2DM in patients with oral candidiasis, white plaque, abscesses, or xerostomia manifestations, while s-amylase levels are associated with T2DM in patients with oral candidiasis or erythematous candidiasis manifestations.[16] A systematic review done by Rozita Naseri et al (2018) suggests that salivary amylase level was lower in patients with T2D than the healthy controls, which was in agreement with the results of Yavuzyilmaz et al., Panchbhai et al., KMP et al., and Indira et al., but was higher in two studies which could be attributed due to the hormonal and metabolic alternations in diabetic patients.[17]

From the results of our study, we found a statistically significant value difference between Controls (non-diabetic and non-covid exposed) with Diabetic subjects exposed to covid and also the difference between Controls (non-diabetic and non-covid exposed) with Diabetic subjects unexposed to covid. But, there was no statistical difference between Group B and Group C.

Conclusion

From our study, we conclude that there were no differences in the levels of salivary amylase levels between the subjects of diabetes affected with COVID infection and diabetes who was not affected with COVID infection. The number of included subjects among the groups may be one of the limitations of our study and also comparison with HBA1c would give appropriate results. But from our inference, we recommend estimation of salivary amylase can be used as a diagnostic means of monitoring diabetic levels in situation of pandemics.

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