Original Article

A Comparison Between Catalase and Salivary Alpha Amylase Level in Patients with Diabetic and Non Diabetic

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ABSTRACT

Objective: To compare the salivary catalase and alpha amylase level in patients with diabetes and non-diabetes.
Study Design: Analytical descriptive study
Place and Duration of Study: This study was conducted at the Physiology department of Bakhtawar Amin Medical & Dental College**,** Multan from February 2022 to January 2023.
Materials and Methods: A total of 112 patients were enrolled in study. Saliva of 56 diabetic individuals 56 non diabetic individuals was gathered and sent to laboratory. Level of salivary alpha amylase and catalase was determined separately. Level of enzymes was compared between diabetic and non-diabetic groups. Mean (standard deviation and frequency (percentages) were calculated by using SPSS version 23 for data analysis.
Results: The mean salivary catalase enzyme in diabetes and non-diabetes patients was 497.21±52.81 KU/I and 292.52±39.08 KU/I, respectively. In diabetes patients, the average level of salivary catalase enzyme was greater than the non-diabetes patients. This difference was statistically significant, ($p<0.000$). The mean alpha-amylase level in diabetes and non-diabetes patients was 160060.71±166.15IU/ml and 82922.75±175.36IU/ml, respectively. In diabetes patients, the average alpha-amylase level was greater than the non-diabetes patients.
Conclusion: In diabetes type 1 patient's level of salivary alpha amylase and catalase is higher in diabetic patients as compared to diabetic healthy subjects.

Key Words: Salivary enzyme, Alpha amylase, Catalase, Diabetic patients, Non diabetic individuals.

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INTRODUCTION

In the context of diabetes, several factors contribute to the increased production of free radicals and the impaired antioxidant defense system. Chronic hyperglycemia (high blood sugar levels) is one of the key factors that promote oxidative stress in diabetes[1]. High glucose levels can lead to the overproduction of ROS through various mechanisms, such as increased glucose autoxidation, activation of protein kinase C (PKC), and increased mitochondrial production of superoxide radicals[2].

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The excess production of free radicals and the insufficient antioxidant defense system can lead to tissue damage and contribute to the mechanism of its development and progression of diabetes-related complications[3]. For instance, oxidative stress has been implicated in the mechanism of pathogenesis of diabetic neuropathy, nephropathy, retinopathy, and cardiovascular complications. Free radicals can damage cellular components, including DNA, proteins, lipids, and leading to inflammation, impaired cellular function, and tissue injury[4].

Catalase is an enzyme that helps convert hydrogen peroxide into water and oxygen. It is found in nearly all living organisms and is particularly abundant in the liver, red blood cells, and cells that are exposed to high levels of oxygen, such as those in the lungs[5]. Catalase helps protect cells from oxidative damage caused by reactive oxygen species (ROS), which are natural byproducts of metabolism[6]. In individuals with type 1 diabetes, oxidative stress can be increased due to chronic hyperglycemia and other factors related to the disease. It is possible that catalase activity or levels may be affected in these individuals[7].

Salivary alpha-amylase is an enzyme produced by the salivary glands and is involved in the breakdown of starch into smaller sugar molecules, such as maltose and glucose[8]. Its activity can be influenced by various

factors, including stress and sympathetic nervous system activation. Studies have shown that salivary alpha-amylase levels can be increased in response to stressors such as physical or psychological stress[9]. It is worth noting that stress can affect glucose metabolism and glycemic control, and individuals with type 1 diabetes may experience additional stressors related to their condition[10].

MATERIALS AND METHODS

This study was conducted at physiology department of Bakhtawar Amin Medical & Dental College**,** Multan from February 2022 to January 2023 in one year. Hospital bored of ethics approved the study protocol and consent Performa. Sample size was calculated by using openepi.com an online sample size calculator with 80% power of study, 955 confidence interval, 0.5% margin of error and mean level of salivary catalase enzyme 447.9 ± 143 KU/I in diabetic participants 283.7 ± 229.7 KU/I in non-diabetic participants.

Medical history suggest the diabetes type and patients were interviewed for duration of diabetes, smoking status, HbA1c level and other medical conditions. All patients were insulin dependent as their disease requirement. Individuals who have active periodontal inflammation or other oral conditions such as oral infections, ulcers, or lesions, systemic disease that affects salivary glands, smokers or alcohol users were excluded from study.

The patient is provided with special sterile plastic tubes, typically Falcon tubes, for collecting saliva. These tubes are used to maintain the sterility of the samples. The patient is instructed to collect saliva in their mouth over a specified period of time, typically every 60 seconds, for a duration of 15-5 minutes. During this time, the subject should avoid eating, drinking, or any oral stimulation to obtain an unstimulated sample. Approximately 5 mL of saliva is collected from each subject using this method. The specific volume may vary depending on the requirements of the study or analysis being conducted. The saliva collection is performed between 8 to 9 a.m., in a fasting state, to avoid potential circadian changes that can affect the composition of saliva. Once collected, the saliva samples are immediately placed on ice to maintain their integrity during transportation to the laboratory. Keeping them on ice helps preserve the biochemical properties of the samples. Upon reaching the laboratory, the saliva samples are centrifuged at 4 °C for 10 minutes at 800 g. This centrifugation step helps separate squamous cells and cellular debris from the saliva, allowing for a cleaner sample for analysis.

The samples were frozen at a temperature of -80 °C. After the samples were collected and prepared, necessary tests were conducted. The nature of these tests is not specified in the given statement, but it mentions the use of prepared kits and atomic absorption spectrophotometry. Data analysis for mean (SD) and frequency (percentages) were calculated by using SPSS version 23.

RESULTS

Overall, 112 patients were included in our study, in which 56 (50.0%) patients were suffered from type-I diabetes and 56 (50.0%) patients were non-diabetes patients.

Table No. 1: Distribution of salivary catalase enzyme and alpha-amylase level in diabetes and non-diabetes patients

Variable	Type-I Diabetes		Test of significance
	Yes, 56 (50.0%)	No, 56 (50.0%)	
Salivary catalase enzyme	497.21±52.81	292.52±39.08	t=23.32, p<0.001
Alpha-amylase level	160060.71±166.15	82922.75±175.36	t=2389.56, p<0.001

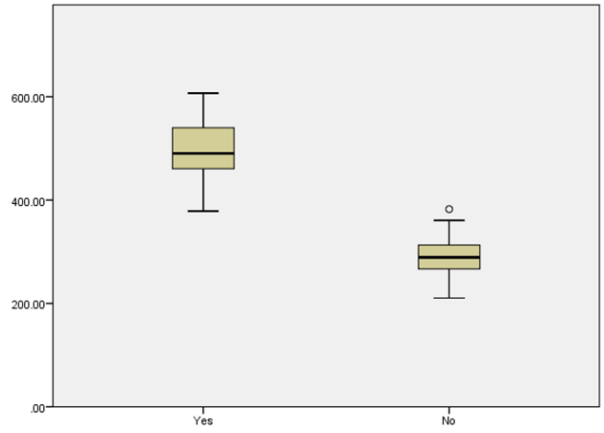

Figure No. 1: Comparison of salivary catalase Level in type-I diabetic and non-diabetic patients.

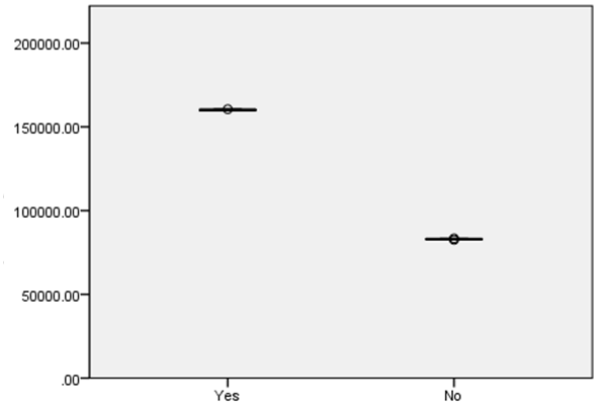

Figure No. 02: Comparison of Alpha-amylase level in type-I diabetic and non-diabetic patients.

The mean salivary catalase enzyme in diabetes and non-diabetes patients was 497.21±52.81 KU/I and 292.52±39.08 KU/I, respectively. In diabetes patients,

mean salivary catalase level was greater than the non-diabetes patients. This difference was statistically significant, ($p<0.000$). (Figure-1). The mean alpha-amylase level in diabetes and non-diabetes patients was 160060.71±166.15IU/ml and 82922.75±175.36IU/ml, respectively. In diabetes patients, the average alpha-amylase level was greater than the non-diabetes patients. This difference was statistically significant, $p<0.000$. Figure-2, Table-1.

DISCUSSION

In this study saliva samples of 56 diabetic patients were compared with saliva of 56 non diabetic patients regarding alpha amylase level and catalase level and salivary amylase level was found much higher in diabetic patients. A study was conducted by Panchbhai et al[11] in 2010 and compared insulin dependent diabetic patients with non-insulin dependent diabetic patients in terms of salivary amylase and catalase level. It was reported that salivary alpha amylase level is much lower in patients with controlled diabetics.

Another study was conducted by Maleki et al[12] and reported that salivary alpha amylase and catalase level were always remain higher in diabetic patients as compared to non-diabetic patients. Level of catalase in diabetic patients was 447.9 ± 143 KU/I and in non-diabetic patients it was 283.7 ± 229.7 KU/I. Similarly, level of salivary alpha amylase enzyme was 150075 ± 158356.8 IU/m and 81825 ± 66742.2 IU/ml in diabetic and non-diabetic patients respectively. Aydin's study indicated an increase in the concentration of salivary alpha amylase in diabetic patients. This finding suggests that diabetic patients may have higher levels of this enzyme in their saliva compared to non-diabetic individuals[13].

Recent studies have found a correlation between rise in basal membrane permeability in salivary glands and diabetes. This increased permeability allows salivary proteins such as amylase to penetrate the membrane. Additionally, it was found that there is an increase in the expression levels of amylase receptors in diabetic patients[14]. In a study by Piras et al[15] showed that factors may contribute to the elevation of salivary amylase levels in individuals with diabetes which is not associated with non-diabetic subjects.

In another study by Reznick et al[16] diabetic and non-diabetic patients were compared in terms of salivary catalase and alpha amylase level. Twenty patients of age 13-19 years were enrolled. It was concluded that antioxidantability of salivary enzyme was increased in diabetic subjects and in non-diabetic healthy subjects remains unchanged. The studies conducted by Ibuki et al[17] and Leite et al[18] likely aimed to explore the relationship between diabetes-induced oxidative stress and salivary catalase levels and reported that level of catalase activity can be indicative of oxidative stress, which is often associated with diabetes.

In 2009 Gumus et al[19] conducted a study on 60 patients and compared diabetic and non-diabetic subjects in terms of antioxidant activity level of saliva and reported that it was much lower in diabetics than normal healthy subjects. In another study Carda et al determined the level of alpha amylase and catalase in diabetic patients and compared with non-diabetics and found no significant change among both groups[20].

CONCLUSION

In diabetes type-1 patient's level of salivary alpha amylase and catalase is higher in diabetic patients as compared to diabetic healthy subjects.

Suggestions: It suggests that more research is needed in order to develop guidelines for early diagnosis and treatment in diabetic individuals. Specifically, it is recommended to conduct further studies with larger populations and take into account confounding factors that may influence the outcomes.

Author's Contribution:

Concept & Design of Study:	Amna Riaz
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Revisiting Critically:	Amna Riaz, Romana Mehwish
Final Approval of version:	Amna Riaz

Conflict of Interest: The study has no conflict of interest to declare by any author.

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