RESEARCH
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PROFILING OF SALIVARY ALPHA AMYLASE AMONG YOUNG ADULTS IN HUMAN SALIVA

Running title: Salivary Alpha amylase profiling

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Abstract
Introduction: Saliva is a watery fluid produced by glands present in the oral cavity. Salivary amylase is a glucose-polymer cleavage enzyme. There are two types of amylases - AMY1 and AMY2. Salivary amylase is involved in maintaining oral health by breaking food particles and preventing dental plaque formation, antimicrobial properties, Taste Perception by releasing taste compounds from food. They increase in secretion due to Stress and mainly It acts as a diagnostic marker for various conditions like diabetes, obesity, and certain types of cancer. These provide insights into overall health status and serve as a non-invasive diagnostic tool for the future.
Aim: To find human salivary Alpha amylase production and profiling among young adults.
Materials and method: Collection of salivary samples from normal healthy men and females of 20 to 30 yrs old. Extraction of functional protein from salivary samples. Salivary alpha-amylase activity and identification of amylase protein in Native PAGE.
Result: The bands visualized as transparent bands on a dark blue background are Alpha amylase. Amylase profile pattern of Female saliva was more than male. It may be due to fluctuating Hormones, such as estrogen and progesterone, Stress response and Genetic factors. In males, M2 and M3 had Alpha amylase production. Whereas M1 and M4 had very low levels. In females, All had Alpha amylase production. Comparatively F3 had more than other females.
Conclusion: We can conclude that salivary alpha production depends on Environmental factor, Genetic factor, stress response, hormone level and health status. Salivary amylase production acts as a diagnostic tool for determining health of individuals.

Key words: Alpha Amylase, Enzymes, Native PAGE, Profiling, Saliva sample.

INTRODUCTION:

Saliva is a watery fluid produced by glands present in the oral cavity, primarily the salivary glands, like Parotid, Submandibular and Sublingual salivary gland aid in the process of digestion and to maintain oral health. It serves several important functions in the body like Moistening, Digestion, Protection, Taste, Speech and pH regulation. Moistening food, making it easier to chew and swallow(1). This moisture lubricates the oral cavity, making it more comfortable to swallow without obstruction. Saliva contains enzymes, such as amylase, which begin the process of breaking down starches and carbohydrates in food into simpler sugars. This initial digestion is a crucial and important step in the overall digestive process. It helps protect the oral cavity from infections and tooth decay by rinsing away food particles and harmful bacteria. Bacterial Presence in human mouths naturally are like Streptococcus mutans and Lactobacillus species. These bacteria are part of the oral microbiome which forms Plaque. When you consume food and beverages, especially those rich in sugars and carbohydrates, the bacteria in your mouth feed on the leftover food particles and sugars. They metabolize these sugars and produce acid as a byproduct. The acids produced by these bacteria can lower the pH level in your mouth, making it more acidic. This acidic environment weakens the enamel, which is the hard, protective outer layer of your teeth. Enamel is primarily composed of minerals like hydroxyapatite, and acid can demineralize it, leading to enamel erosion. As the enamel demineralizes, it becomes softer and more vulnerable to decay. This is the early stage of tooth decay and is often referred to as a "cavity." Over time, if the acidic environment persists, the demineralized enamel can progress to form cavities or holes in the teeth. These cavities provide a sheltered environment for bacteria to further thrive and multiply. If left untreated, the decay can extend deeper into the tooth, affecting the dentin (a softer layer beneath the enamel) and potentially reaching the inner pulp of the tooth. At this point, the decay becomes more painful and requires more extensive dental treatment, such as a filling, root canal therapy, or extraction. Preventing tooth decay primarily involves maintaining good oral hygiene practices to limit the growth of harmful bacteria and reduce acid production. These practices include regular brushing and flossing to remove plaque and food particles, limiting the consumption of sugary and acidic foods and drinks, and visiting a dentist for regular check-ups and cleanings. These Tooth decay are primarily Reduced and maintained by salivary alpha amylase(1,2).

Saliva is a complex fluid that contains a variety of components with important functions in the oral cavity and the digestive process. It mainly contains Water, Electrolytes, Mucus, Enzymes. Saliva is primarily composed of water, making up the majority of its volume. This watery base helps in dissolving and transporting food particles, facilitating taste, and maintaining oral hydration. As it contains electrolytes such as sodium, potassium, calcium, and bicarbonate ions, which helps to maintain the pH balance in the mouth and assist in various chemical reactions during digestion. Mucins present in saliva are proteins that give saliva its slimy or mucous consistency. Mucus helps in lubricating food, making it easier to swallow, and also provides protection for the oral mucosa. Saliva contains several enzymes, including salivary amylase (alpha-amylase) and lingual lipase. Salivary amylase helps initiate the breakdown of carbohydrates in the mouth, while lingual lipase plays a role in digesting fats. antimicrobial proteins and peptides present in saliva that helps to control bacterial growth in the mouth, contributing to oral hygiene. Immunoglobulins, such as IgA, are present in saliva and help provide immune protection to the oral cavity by neutralizing pathogens. Lysozyme is an enzyme in saliva that can help break down bacterial cell walls, contributing to its antibacterial properties. Saliva contains growth factors that can aid in tissue repair and wound healing within the oral cavity. Small amounts of hormones and metabolic waste products can be found in saliva, it a potential source of biomarkers for certain health conditions. The composition of saliva can vary among individuals and can be influenced by factors such as diet, hydration, and overall health(3,4).

Saliva contains several enzymes that play essential roles in the initial stages of digestion and oral health. Some are Salivary Alpha amylase, lingual lipase, Lysozyme, Statherin, Carbonic anhydrase, Proline-Rich Proteins (PRP), Glycosyltransferases. Salivary amylase is one of the most well-known enzymes in saliva. Its primary role is to initiate the digestion of carbohydrates. This process is important for breaking down complex carbohydrates into forms that can be further digested in the small intestine. Lingual lipase is another enzyme found in saliva, although its activity is relatively low in the mouth. Its main function is to start the digestion of fats. Lingual lipase becomes more active in the acidic environment of the stomach, where it plays a more significant role in breaking down dietary fats into fatty acids and glycerol. Lysozyme is an enzyme with antibacterial properties. It can break down the cell walls of certain bacteria, contributing to the mouth's natural defense against harmful microorganisms and maintaining oral hygiene. Salivary peroxidase (Statherin) plays a role in the antimicrobial defense of the mouth. It helps to inhibit the growth of bacteria and fungi by reactive oxygen species. Additionally, statherin can help regulate the formation of dental plaque. Carbonic anhydrase assists in the production of bicarbonate ions, which helps in maintaining the pH balance in the mouth. This enzyme helps to neutralize acidic substances, preventing damage to tooth enamel. PRPs have various functions, including binding to and clearing away potentially harmful tannins from foods and beverages. They can also help form a protective layer on tooth enamel. Glycosyltransferases enzymes are involved in the synthesis of complex carbohydrates in the glycoproteins that make up the protective mucin layer in the mouth. This layer helps lubricate the mouth and protect the oral tissues. These enzymes collectively contribute to the digestion of food in the oral cavity, maintain oral hygiene by combating harmful bacteria, and protect the oral tissues. Proper saliva production and enzyme activity are crucial for overall oral health and the efficient processing of food during the digestive process(4–6).

Salivary alpha-amylase is an enzyme plays a crucial role in the digestion of carbohydrates, particularly starches. Alpha-amylase works by breaking down long complex chains of starch molecules into simpler sugars, such as maltose and glucose. This initial step of carbohydrate digestion occurs in the mouth while food is being chewed and mixed with saliva. The enzyme's activity begins as soon as you start chewing food, as it helps to convert complex carbohydrates into more easily digestible sugar forms. This process continues in the stomach and small intestine with the help of other enzymes like pancreatic amylase, eventually leading to the complete breakdown of starches into individual sugar molecules that can be absorbed by the body for energy. Monitoring the activity of salivary alpha-amylase can be used in research and clinical settings to assess stress levels. The enzyme's production can be influenced by the body's stress response, and its levels in saliva can be measured to provide insights into an individual's physiological response to stressors. Overall, salivary alpha-amylase is an important enzyme for the

initial stages of carbohydrate digestion, and its measurement can also be useful in understanding stress-related responses(7–10).
Salivary alpha-amylase is primarily classified into two types based on their genetic origin and tissue-specific expression such as AMY1 and AMY2. Salivary Alpha-Amylase 1 (AMY1) is primarily produced in the parotid glands, one of the major salivary glands located near the ears. It is responsible for breaking down starches and complex carbohydrates in the mouth during the initial stages of digestion. Humans can have different numbers of copies of the AMY1 gene, and this genetic variation can influence an individual's ability to efficiently digest starches. Whereas Salivary Alpha-Amylase 2 (AMY2) is another type of salivary alpha-amylase, and it is produced in the salivary glands, particularly in the submandibular and sublingual glands. AMY2 also plays a role in carbohydrate digestion, working in conjunction with AMY1 to break down starches into simpler sugars. Both types of salivary alpha-amylase enzymes have similar functions, but they may have different properties and variations in their expression levels depending on genetics and individual factors. The genetic diversity in the AMY1 gene, in particular, can lead to differences in the efficiency of carbohydrate digestion among individuals, which is an area of research interest in fields such as nutrition and genetics(3).
Several factors can reduce the activity of salivary alpha-amylase like Stress, which triggers the body's "fight or flight" response, leading to a decrease in salivary flow and a reduction in alpha-amylase activity. This is part of the body's natural response to divert resources away from digestion during stressful situations. Some medications, particularly anticholinergic drugs, can inhibit the production of saliva and, consequently, reduce the levels of salivary alpha-amylase. Inadequate hydration can lead to reduced saliva production, which in turn can lower alpha-amylase levels. As people age, salivary gland function may decline, leading to decreased salivary alpha-amylase activity. In Certain medical conditions it affects the salivary glands, such as Sjögren's syndrome or salivary gland tumors, can reduce both saliva production and alpha-amylase levels. Smoking has been shown to reduce saliva production and may also impact the activity of salivary alpha-amylase. Excessive alcohol intake can lead to dehydration and decrease saliva production, which can subsequently reduce alpha-amylase activity. Conditions such as periodontal disease or infections in the mouth can affect the salivary glands and reduce salivary alpha-amylase levels. A diet high in processed foods or low in starches may result in lower salivary alpha-amylase activity because the enzyme's production is influenced by the presence of starches in the mouth. Hormonal fluctuations, such as those that occur during pregnancy or menopause, can impact salivary gland function and potentially reduce alpha-amylase activity. It's important to note that some of these factors are temporary, while others may have long-term effects on salivary alpha-amylase levels. Additionally, the specific mechanisms and extent of these effects can vary from person to person. If you suspect a problem with your salivary alpha-amylase levels or overall oral health, it's advisable to consult a healthcare professional for a proper evaluation and guidance(3).
In recent years, Salivary Alpha amylase has gained attention as a potential biomarker for stress and other physiological and psychological conditions. Profiling salivary alpha-amylase can provide insights into an individual's stress response and overall health. profiling of salivary alpha-amylase works in Stress Research, Psychological Studies and Health Assessment. Profiling salivary alpha-amylase is commonly used in stress research to understand how individuals respond to stressors. It can help identify differences in stress responses among individuals. Salivary alpha-amylase profiling has been used in studies related to emotions, anxiety, and other psychological factors. In some cases, salivary alpha-amylase levels have been explored as potential indicators of overall health or as biomarkers for certain health conditions(11).
Profiling salivary alpha-amylase can provide valuable information in various research and clinical contexts, especially in the fields of stress physiology, psychology, genetics, digestive health, Prediction of Diabetes, Cancer, Oral cancer, Oral disease, Smoking related disease, Respiratory Disease and GIT diseases. Our study is To find human salivary Alpha amylase production and profiling among young adults.

MATERIALS AND METHOD:

The current study was done in the Department of Forensic Odontology in Saveetha Dental College Chennai.

Collection of Saliva and Preparation of Sample Pools:

Stimulated saliva samples were collected from Eight healthy, ranging in age from 20-30 years (Four male and Four females). All volunteers exhibited good oral health and overall good systemic conditions. The collection of saliva was done in the morning before breakfast, to reduce the effects of the circadian cycle. Saliva was collected until 5 mL of falcon tube was reached. Centrifugation at 16000 ×g for 15 min at 4∘C was used to separate pellet and the supernatant. Only Saliva supernatants were pooled together. Pellets were discarded. Each pool was made with 5 mL of supernatant from each volunteer. Saliva collected was used fresh for all experiments and was kept to refrigerate from collection to the preparation of aliquots. No protease inhibitors were added to the saliva samples.

Separation of Amylase Complex from Saliva supernatant:

Amylase assay was performed according to Hassanabatar et al. (2013). Rice starch in 0.1 M Phosphate buffer of pH 6.8 was used as a 0.5 ml of substrate and 0.2 ml of 1% NaCI were incubated at 37 degree for 10 min. Then 0.3 ml of saliva samples were added and incubated at 37 degrees for 15 minutes. The reaction was stopped by adding 1 ml of dinitro salicylic reagent and kept in a boiling water bath for 10 min. The solution was cooled and 10 ml of distilled water was added.
The color developed was measured at 520 nm(9).

Native PAGE

Native gel electrophoresis was done in same conditions as SDS-PAGE, but the polyacrylamide gel did not contain SDS and the migration was realized at 4 °C. After electrophoresis the gel was incubated at 55 °C in soluble starch solution (1%) prepared in 0.1 M sodium acetate buffer (pH = 6.0). Subsequently the gel was stained with iodine reagent (KI-I2 solution). The amylase bands were visualized as transparent bands on a dark blue background(7).

STATISTICAL ANALYSIS:

Principal Component Analysis was carried out in the original 8.5 software.
ETHICAL APPROVAL:
There is no ethical approval needed for this study.

RESULTS:

The bands visualized as transparent bands on a dark blue background is Alpha amylase production seen in Human Saliva.

Amylase production was compared between Male and females of the age group 20-30 years. Amylase production pattern was observed. Eight samples were taken where 4 females(F1, F2, F3, F4) and 4 males(M1, M2, M3, M4). On comparing Male and Female, (Figure 1 and 2) Amylase profile pattern of Female saliva was more than male. It may be due to fluctuating Hormones, such as estrogen and progesterone, Stress response and Genetic factors. In males, M2 and M3 only had Alpha amylase production. Whereas M1 and M4 had in very low or no production. In females, All had Alpha amylase production. Comparatively F3 had more than other females.

Figure 1:

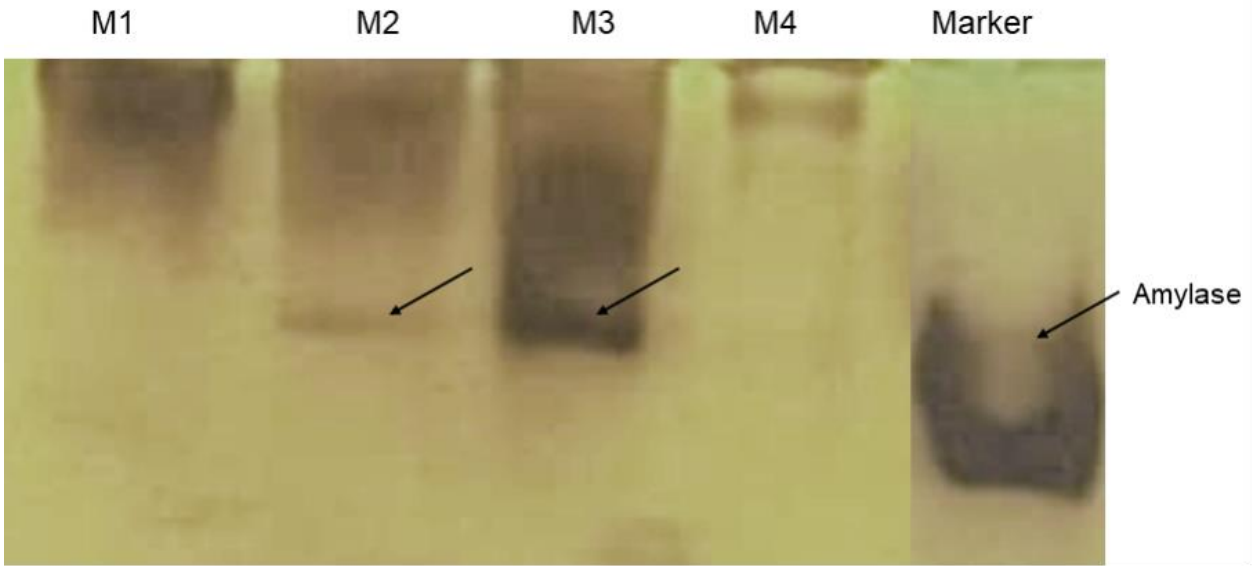
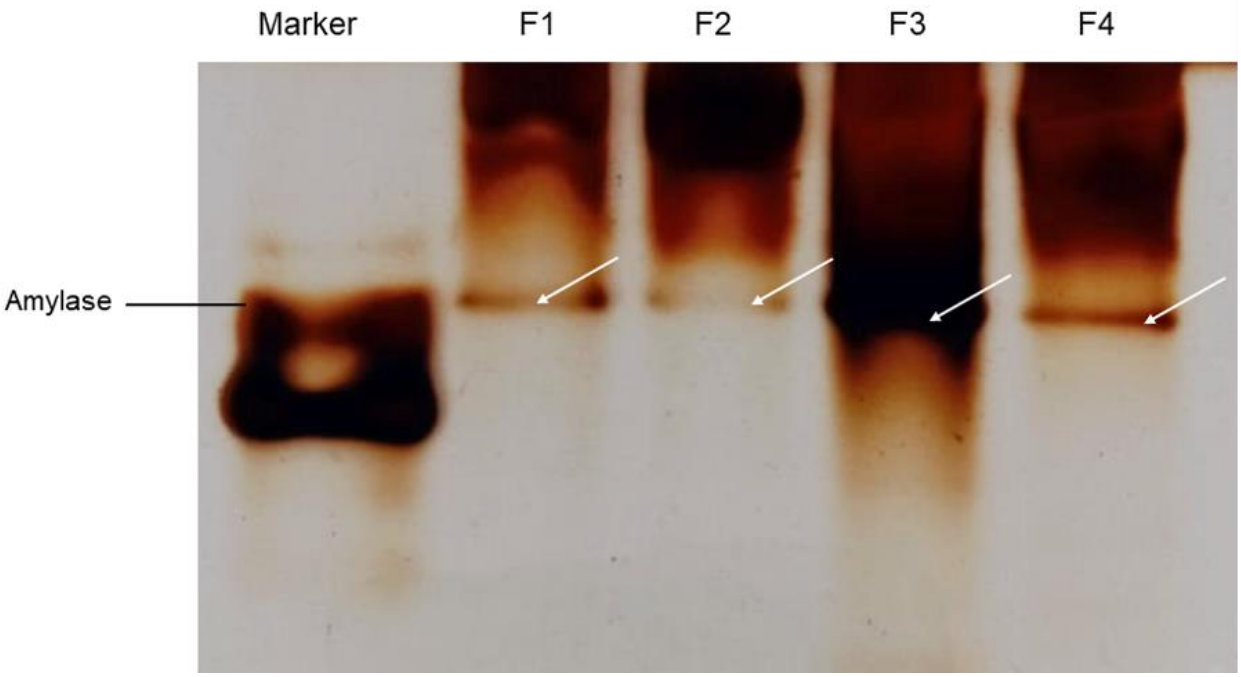
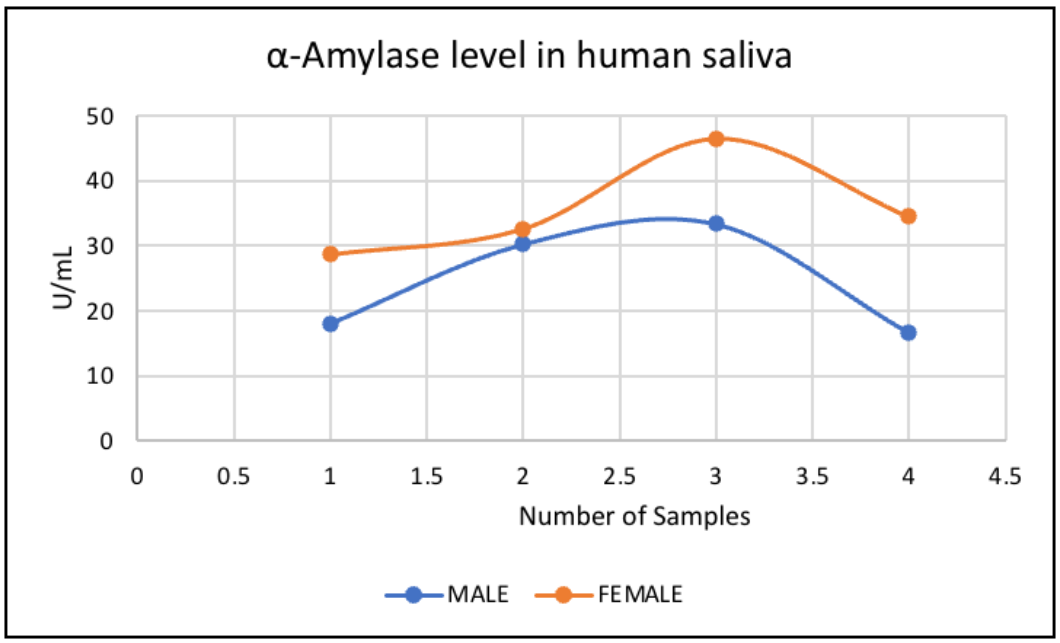


Figure 2:

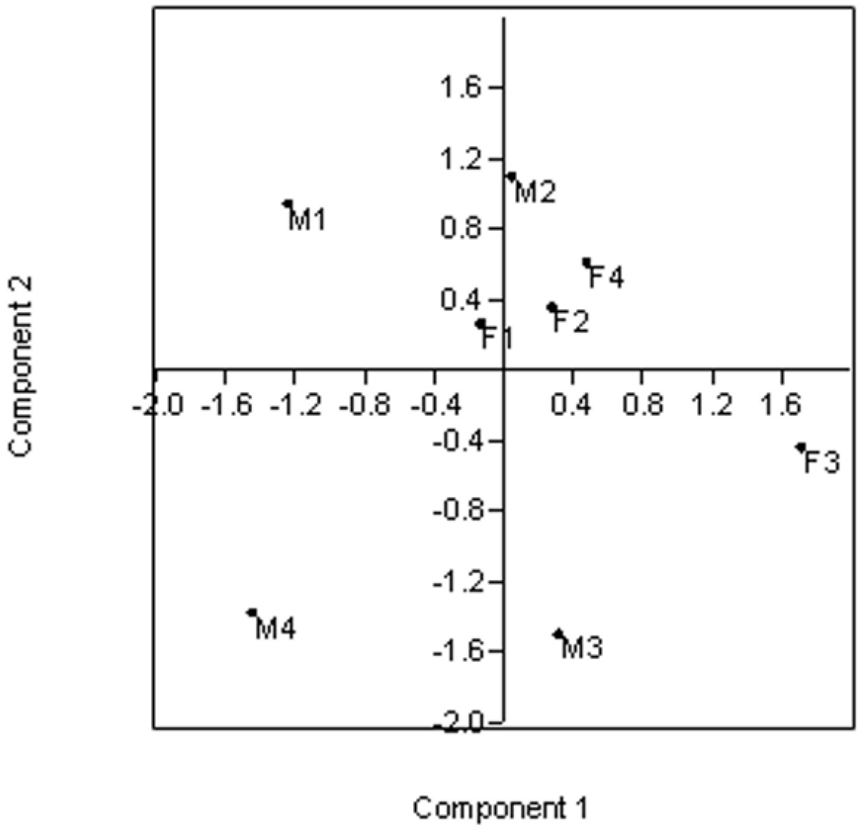


In Figure 3, Quantitative Graph comparing Male and Female shows Male having lessAlpha amylase level in saliva comparing female. The curve of Male, M3 had significant production where as in Female curve, F3 had significant production.

Figure 3:



In Figure 4, on comparing components 1 and 2, F1, F2 and F4 had similar production. Whereas F3 had significantly higher production than others. In M1 and M4 there was no Alpha amylase production and M2 and M3 had significant production. Figure 4:



DISCUSSION:

In the past three decades, discoveries that enabled the noninvasive measurement of the psychobiology of stress (in saliva) have added new dimensions to the study of health and human development. Several new studies have generated renewed interest in salivary α-amylase (sAA) as a surrogate marker of the autonomic/sympathetic nervous system component of the psychobiology of stress. This article reviews salivary alpha amylase properties and functions; presents illustrative findings relating stress and the physiology of stress, behavior, cognitive function, and health; and provides practical information regarding specimen collection and assay. In our study, some females and Male had less alpha amylase production. It may be factors like stress, behavior and internal diseases and hormone fluctuations(9).

In this Study, The ability of various oral streptococci to bind with salivary α-amylase to their cell surfaces was investigated. Samples of cells were mixed with whole cleared saliva and the α-amylase remaining after removal of the cells was assayed by radial diffusion in starch-containing agarose. Seventy-five per

cent of *Streptococcus sanguis* strains bound the enzyme but strains of *Streptococcus mutans* and *Streptococcus mitior* did not. SDS-polyacrylamide gel electrophoretic analysis of *Strep. sanguis* cells which had been mixed with saliva showed that α-amylase is bound to the surface of the cells and can be recovered from them intact. In our study, amylase production was profiled and found it reduced oral pathogens. So there is reduced tooth decay found(12).

The prevalence of diabetes and its complications encouraged the researchers to find effective ways with minimal side effects in order to diagnose and cure diabetes. The alpha-amylase and catalase are salivary enzymes which could be effective in this area. The salivary alpha-amylase inhibitors reduce the absorption of complex carbohydrates and so are effective in the diagnosis of diabetes by controlling blood sugar and measurement of the concentration level of salivary alpha-amylase. Due to lack of studies and conflicting results regarding this topic, the present study aims to compare the level of catalase and salivary alpha- amylase in patients with diabetes type I with non-diabetic people. In the present descriptive analytic study, The level of salivary alpha-amylase and catalase in people affected by type 1 diabetes was higher than the non-diabetic people. In our study we only Production of Salivary alpha amylase

, in future research we can find the Problems like cancer, diabetes, disease with the help of salivary alpha amylase production(3).

Saliva and its defense systems such as antioxidants and minerals are very important in the pathogenesis of different diseases. Cigarette smoking has many destructive effects. Oxidative stresses play an important role in the side effects of smoking. This study assessed the effect of cigarette smoking on salivary levels of catalase, vitamin C, and a-amylase. This retrospective cohort study was carried out in Hamadan between 259 smokers and non-smokers. The salivary catalase levels were lower and a-amylase levels were higher in smokers, but the differences were not statistically signif- icant (P = 0.416 and P = 0.265, respectively). Smokers were younger than non-smokers. This shows that Salivary alpha amylase production varies according to external conditions like smoking(13).

CONCLUSION:

Overall, profiling using salivary alpha-amylase can provide valuable insights into an individual's stress response and physiological state, making it a useful tool in various fields of research and potentially in clinical practice. However, its interpretation should be done in the context of other relevant factors and with ethical considerations in mind. Salivary Alpha amylase production and release of salivary alpha-amylase are regulated by the autonomic nervous system and are not typically under conscious control. Food Intake, Stress, Exercise, Hormonal changes, Psychological Factors, Circadian Rhythms, Emotions and psychological states, Medications and Health Conditions, Age, Genetics, External Factor and adverse Habits. It's important to note that the relationship between these factors and salivary alpha-amylase production can be complex and may vary from person to person. Researchers continue to study the various influences on this enzyme's activity to better understand its role in digestion and stress response.

SCOPE OF FUTURE RESEARCH:

Conduct studies to understand diurnal variations in catalase activity in saliva. This would involve collecting saliva samples at multiple time points throughout the day and analyzing the catalase levels to determine if there are any consistent patterns or fluctuations. Investigate in more detail the various factors that can influence catalase activity in saliva. This could include exploring the factors such as diet, smoking, and alcohol consumption, oral hygiene practices, and the presence of specific systemic or oral health conditions

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CONFLICT OF INTEREST:

All the authors declare that there was no conflict of interest in the present study.

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