RESEARCH
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COMPARATIVE ANALYSIS OF CATALASE ENZYME PRODUCTION IN NORMAL HUMAN SALIVA

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Abstract

Aim
The aim of this research is to explore and analyze the variation in catalase enzyme production within the saliva of healthy individuals and make comparisons regarding its activity levels among different subjects.

Introduction
Saliva contains a diverse array of enzymes, proteins, and other components that play important roles in maintaining oral health and aiding in the digestion process. Catalase is an enzyme that is found in various tissues and body fluids, including human saliva. Catalase acts to break down hydrogen peroxide into water (H20) and oxygen (02). This enzymatic reaction helps prevent the accumulation of hydrogen peroxide, which can be damaging to cells due to its oxidative properties.The production and activity of catalase in human saliva can vary among individuals due to genetic and environmental factors.

Methods
The salivary samples were collected by Spitting method. In this way, the patient was allowed to put it into a special sterile plastic tube (falcon). To 0.05 ml of sample 1.0 ml Citrate buffer and 0.45 ml of water is added. The Reaction is initiated by the addition of 0.5 ml of H2O2 and the reaction mixture is incubated at 37 °C for 1 min. Native gel electrophoresis was done in the same conditions as SDS-PAGE, but the polyacrylamide gel did not contain SDS and the migration was realized at 4°C.

Results
The results are interpreted based on the color change, which can be correlated with the catalase activity in the saliva sample. The higher the catalase activity, the more H2O2 is converted to O2 and H2O, resulting in a weaker color change in the final solution.Factors such as age, gender, and health conditions can influence catalase production. Environmental conditions, such as exposure to pollutants, dietary factors, and lifestyle choices, can impact catalase production. For example, smoking and alcohol consumption will decrease catalase activity.

Conclusion
The study concludes that the salivary catalase activity showed a minor variation among male and female samples. This investigation provides a new view on saliva enzymes that may be a useful tool for further investigation on the adaptive response, diet and for the evaluation of health and nutritional status of healthy and unhealthy persons.

Keywords
Human saliva, Catalase enzyme, healthy individuals, comparative analysis, oxidative stress.

INTRODUCTION

Saliva, as a readily accessible clinical specimen, has garnered significant research interest due to its rich array of enzymes and molecules, along with its unique role in diagnosing and managing a wide range of diseases. The assessment of salivary samples offers several benefits over serum-based methods. The ease of collection, cost-effectiveness, and minimal associated risks make salivary analysis particularly appealing. This approach holds promise as an economical and non-intrusive means of conducting large-scale screenings within diverse populations(1). Saliva stands as a promising medium for comprehensive biochemical analysis. Expanding research efforts, encompassing larger sample sizes, and employing heightened sensitivity devices hold the potential to enhance the precision of detecting subtle biochemical alterations within saliva. This evolution could position saliva on equal footing with the established gold standard of serum diagnostics(2). Human saliva contains a variety of detoxification enzymes that hold a significant role in preserving oral well-being. These enzymes encompass glutathione S-transferase, peroxidase, and aldehyde dehydrogenase (ALDH). Specifically, human salivary ALDH (hsALDH) assumes a protective function by participating in the detoxification process of harmful aldehydes that are naturally found in dietary sources. Moreover, hsALDH contributes to shielding the body against detrimental aldehydes stemming from sources like cigarette smoke, environmental contaminants, and diverse medications(3). Free radicals and the resultant oxidative stress play a pivotal role in the development of numerous diseases, ranging from inflammatory and allergic conditions to metabolic disorders, malignancies, and even the natural aging process. To counteract the potential detrimental effects of free radicals, the body has evolved a dedicated defense mechanism known as the antioxidant system. The effectiveness of this antioxidant system varies depending on the specific oxidative stress triggers and the affected organ. Saliva, containing a wealth of antioxidants like catalase (CAT) and glutathione peroxidase, serves as a protective shield for oral tissues, shielding them from the harmful impacts of free radicals(4). Catalase is a heme protein enzyme found in saliva, acting as a potent antioxidant. Its function involves converting hydrogen peroxide into water and oxygen through catalysis.Catalase exhibits four peroxidase activity groups, and is linked to diabetes-related oxidative effects. Enhancing catalase levels appears to mitigate diabetes complications. Individuals with type 2 diabetes have lower salivary catalase levels compared to non-diabetic individuals(5). The breakdown of hydrogen peroxide in saliva has led to the observation that the primary agents responsible for this breakdown are the enzymes catalase and peroxidase. During the oxidation of the substrate, the activity of catalase was significantly reduced. This suggests that when certain oxidizable substrates, such as free thiol groups, are present, the decomposition of hydrogen peroxide in saliva likely follows a peroxidatic reaction pathway (AH2 + H2O2 —> A + 2H2O).On the other hand, in situations where suitable hydrogen donors are not available, the decomposition of peroxide appears to be primarily driven by the catalytic reaction (2H202 —> 2H20 + O2), leading to the molecular breakdown of hydrogen peroxide(6). Smoking has been associated with alterations in salivary antioxidant concentrations, which could potentially impact the harmful effects of smoking on oral mucosal health. These changes may also suggest broader systemic implications and alterations in the levels of oxidative agents in the bloodstream (7). The aim of this study is to investigate the production of catalase enzymes in normal human saliva and compare its activity levels among individuals. To identify any significant differences in catalase activity based on age and gender. To explore any correlations between catalase activity in saliva and oral health conditions. To contribute to the understanding of the factors influencing catalase production and activity in human saliva.

MATERIALS AND METHODS

Sample preparation:

The salivary samples were collected by Spitting method. In this way, the patient was allowed to collect saliva in the mouth and put it into a special sterile plastic tube (falcon). Approximately 5 mL of saliva was collected using this method. This saliva should be collected from individuals following ethical guidelines. Take 5 mL of saliva and mix it with 0.1% Triton X (a detergent that helps to lyse cells and release intracellular components). The Triton X might be used to ensure efficient cell lysis and access to intracellular catalase. Store this mixture at -20°C, likely to preserve the integrity of the samples until you are ready to proceed with the assay

Centrifugation

Centrifuge the saliva-Triton X mixture at 16000 rpm for 15 minutes. This step separates the cellular debris and other solids from the liquid portion, leaving you with a clear supernatant containing soluble proteins and enzymes like catalase.

Catalase assay

Take 0.05 ml of your sample (presumably containing catalase). Add 1.0 ml of Citrate buffer. The buffer is used to maintain a stable pH in the reaction mixture. Add 0.45 ml of water to dilute the sample and bring the total volume to 1.5 ml.To initiate the reaction, add 0.5 ml of hydrogen peroxide (H2O2) to the mixture. The catalase in your sample will immediately start breaking down the H2O2 into water and oxygen.Incubate the reaction mixture at 37°C for 1 minute. During this time, catalase in your sample will catalyze the decomposition of H2O2, and oxygen gas will be produced. To stop the reaction, add 2.0 ml of dichromate-acetic acid reagent. This reagent likely contains potassium dichromate and acetic acid. It serves to halt the catalase reaction and stabilize any remaining H2O2.Prepare a set of standard H2O2 solutions with known concentrations in the range of 4-20 moles. Treat these standard solutions in the same manner as your sample, starting with the addition of Citrate buffer, water, and H2O2.After adding the dichromate-acetic acid reagent to both the sample and standard tubes, heat all the tubes in a boiling water bath for 15 minutes. This step likely serves to enhance the color development of the reaction products. After boiling, cool the tubes.

Spectrophotometric Measurement:

Measure the absorbance of the reaction mixtures at 570 nm using a spectrophotometer. The absorbance at this wavelength is proportional to the concentration of the reaction products, which can be correlated with the amount of oxygen generated.The higher the catalase activity in sample, more H2O2 will be decomposed, resulting in a lower absorbance reading at 570 nm. The change in color is indicative of the amount of unreacted H2O2 in the sample, which is proportional to catalase activity

Native PAGE

A polyacrylamide gel is prepared, similar to SDS-PAGE conditions, but without the addition of sodium dodecyl sulfate (SDS). The gel is kept cold at 4°C during the electrophoresis

process. This is typically done for native gels to maintain the native conformation and activity of proteins without denaturation. Sample mixtures containing proteins or biomolecules are loaded into wells at the top of the gel.An electrical current is applied across the gel, causing the molecules to migrate through the gel based on their charge, size, and shape. The gel acts as a molecular sieve, separating the molecules according to their properties.After electrophoresis, the gel is removed from the apparatus and placed in a container or tray.It is then incubated in a 10% hydrogen peroxide (H2O2) solution for 10 minutes. This step is often used to enhance the visualization of specific biomolecules. H2O2 can react with certain compounds, creating detectable products. After the incubation with H2O2, the gel is rinsed in distilled water for 2 minutes. This step is crucial to remove excess H2O2 and prevent unwanted reactions during the subsequent staining step. The gel is submerged in a solution containing 1% potassium ferricyanide and ferric chloride. This staining solution reacts with specific biomolecules to form colored or precipitated products. The greenish band you mentioned likely indicates the presence of specific proteins or biomolecules that reacted with the staining solution. After sufficient staining time, the gel is carefully examined to visualize the greenish band(s). This band corresponds to the presence of particular proteins or other biomolecules within the gel. The intensity and position of the band(s) can provide information about the abundance and migration of these molecules during electrophoresis.
This staining method is often used when you want to detect specific molecules while preserving their native conformation and activity. The reaction of H2O2 with certain compounds can generate visible products that allow for visualizing specific bands in the gel. The specific biomolecules that react with the staining solution can vary depending on the experimental setup and the staining reagents used.

STATISTICAL ANALYSIS

Principal Component Analysis was carried out in origin 8.5 software.

RESULTS

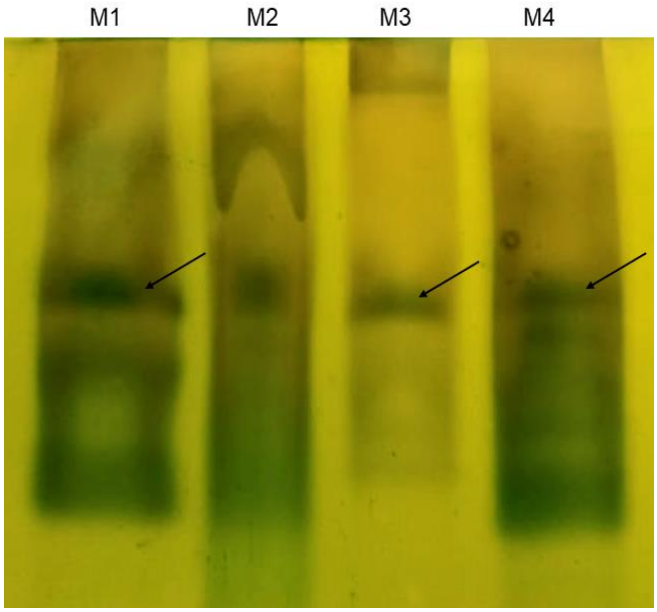

Figure1: Catalase profile pattern of Male saliva samples in non-denaturing-Polyacrylamide Gel Electrophoresis.

In male saliva samples (M1, M2, M3, and M4):
M1 and M4 exhibit higher catalase production compared to M2 and M3. This suggests that M1 and M4 individuals have a relatively higher level of catalase enzyme activity in their saliva. The catalase profile in males appears to vary among individuals, with some showing higher catalase production than others.

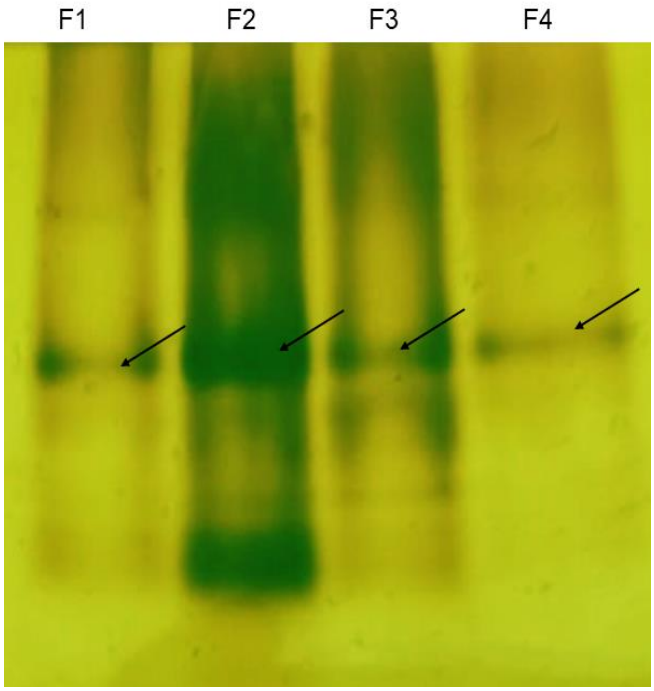

Figure2: Catalase profile pattern of Female saliva samples in non-denaturing-Polyacrylamide Gel Electrophoresis.

In female saliva samples(M1,M2, M3, M4):
F2 displays a notably higher level of catalase production compared to other female samples, indicating a relatively elevated catalase enzyme activity in the saliva of F2. In this case, F2 stands out as having the highest catalase content among the female participants.

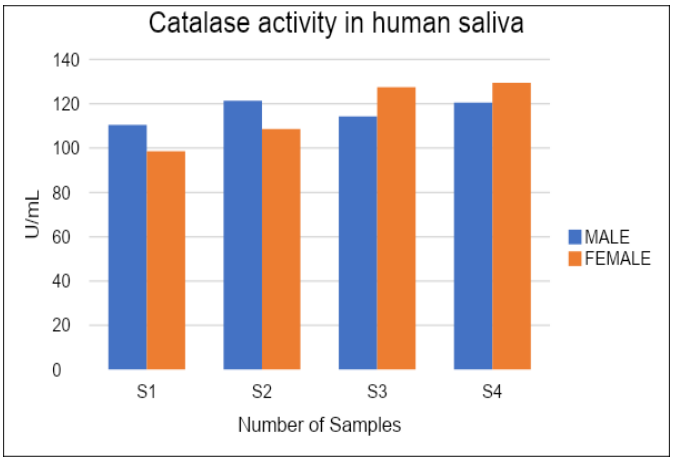

Figure 3: Catalase content in human saliva samples.

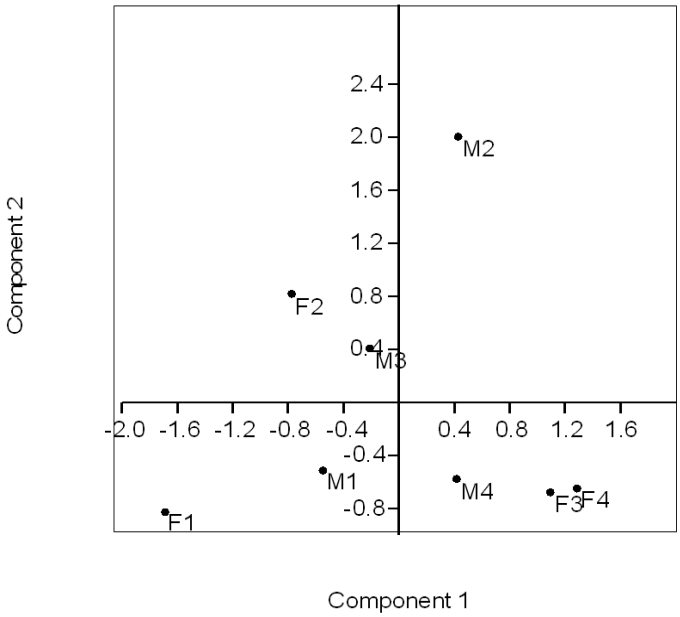

Figure 4: Principal Coordinate analysis of Catalase content among male and female saliva samples.

DISCUSSION

The results indicate distinct catalase profiles in male and female saliva samples analyzed through non-denaturing polyacrylamide gel electrophoresis.Overall, these findings suggest gender-related differences in catalase production within the saliva samples analyzed. Males, specifically M1 and M4, show varying catalase levels compared to females, with F2 exhibiting the highest catalase content among the female participants. These variations in catalase profiles may have implications for antioxidant defense mechanisms and potential differences in response to oxidative stress between genders. Certain health conditions, such as diabetes, liver disease, and chronic inflammation, can alter catalase production. Catalase activity has been reported to decrease with age, which may impact the enzyme production in saliva. Further research could explore the reasons behind these observed differences and their potential significance. The findings demonstrated that within saliva, Glutathione (GSH) emerges as a prominent diagnostic biomarker for Oral Squamous Cell Carcinoma (OSCC). Following closely are SuperOxide Dismutase (SOD), sialic acid, and β-2 microglobulin. Saliva proves to be an equally dependable biomarker source when compared to blood(8).The findings of this study suggest that the anomalous rise in biochemical markers observed in the saliva of OSCC patients is likely a result of the liberation of these markers from cells that have undergone pathological changes. This phenomenon appears to be driven by cellular release, rather than an upsurge in their synthesis originating from interstitial fluids leaking through compromised oral mucosa and gingival sulcus(9). Superoxide dismutase, catalase and glutathione peroxidase serve as the backbone of cellular antioxidant defense mechanisms. Lowered activities of these enzymes have been reported in various pathological conditions including oral carcinogenesis. The levels of vitamin E and reduced glutathione were significantly decreased in oral cancer patients as compared to healthy subjects. The activities of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase were also significantly decreased in oral cancer patients as compared to healthy subjects and gradually decreased from stage Il to stage IV of oral cancer patients (10). Exercise can lead to oxidative stress, prompting a growing interest in using antioxidant supplements to counter its perceived negative effects. Among these supplements, vitamin C is widely popular in the context of sports and exercise. A potential approach to assess exercise-induced oxidative stress is by examining biomarkers such as vitamin C and malondialdehyde levels in saliva(11). The findings indicate that glutathione (GSH) levels in leukocytes, erythrocytes, and saliva rank highest as potential diagnostic biomarkers for breast cancer. Following closely is the Oxygen Radical Absorbance Capacity (ORAC) in serum, which demonstrates promise as a sensitive indicator of breast cancer. Moreover, saliva emerges as an equally dependable sample source compared to blood, and hematological parameters, GSH, malondialdehyde (MDA), and catalase could be employed as cost-effective diagnostic biomarkers for various diseases, with a particular emphasis on cancer(12). The antioxidant defense system in biological systems, whether comprising enzymes or non-enzymatic components, can become compromised. This deficiency may occur as a result of either excessive consumption of antioxidants or an overwhelming presence of oxidant species. These circumstances contribute to alterations in oxidative stress levels, which in turn, play a pivotal role in the observed inflammatory reactions associated with recurrent aphthous stomatitis. In simpler terms, when the body's natural defense mechanisms against oxidative stress are weakened due to an excess of oxidants or a depletion of antioxidants, it can trigger inflammatory responses seen in conditions like recurrent aphthous stomatitis(13).The study demonstrated that the self-polymerization of dopamine (DA), which is sensitive to oxygen levels and inhibited by hydrogen peroxide (H2O2), can serve as an indicator of catalase (CAT) or CAT-like nanozyme activity. This indicator allows us to monitor the production of oxygen (O2) catalyzed by these enzymes, particularly in low-oxygen (hypoxic) environments. The research successfully optimized the conditions for a typical catalase assay. This assay proved to be versatile and applicable to a wide range of samples, including nanozymes, animal tissues, and human saliva. When compared to common methods, it exhibited advantages in terms of sensitivity, specificity, and adaptability, making it a valuable tool for establishing measurement standards and potentially standardized assays for evaluating CAT (or CAT-like nanozyme) activity(14).Findings illustrate that oxidative stress in diabetes mellitus (DM) can be expedited through multiple mechanisms. This acceleration is not solely attributable to the heightened production of reactive oxygen species (ROS) resulting from elevated blood sugar levels (hyperglycemia). It is also influenced by the diminished capacity of the antioxidant defense system, which can be attributed, at least in part, to the presence of single nucleotide polymorphisms (SNPs) in certain scavenger enzymes(15).

CONCLUSION

Our study has revealed intriguing insights into the catalase profiles within saliva samples, particularly in the context of gender differences.These findings highlight the existence of gender-related differences in salivary catalase content, suggesting potential variations in antioxidant defense mechanisms. The color change observed in the assay is directly related to catalase activity in the saliva samples. Higher catalase activity leads to more efficient conversion of hydrogen peroxide (H2O2) into water (H2O) and oxygen (O2), resulting in a less intense color change. This color-based assessment serves as a reliable indicator of catalase enzyme activity in the samples.

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.

CONFLICT OF INTEREST

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

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ETHICAL APPROVAL

There is no ethical approval needed for this study.

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