

## **Salivary Alpha-Amylase and Calcium levels Evaluation as a Biomarker in Stress Patients Undergoing Surgical Wisdom Tooth Extraction**

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**Abstract:** *The salivary enzyme alpha-amylase has been proposed as a biomarker for stress-induced activity of the sympathetic nervous system. In the growing field of amylase research, recent studies have underscored the usefulness of salivary alpha-amylase in this regard. Calcium is the major component of bones and teeth, and it is not surprising that disturbances in calcium metabolism have been implicated in most of the major chronic diseases, including renal disease, osteoporosis; and periodontal tissues.*

*Twenty healthy Iraqi male undergo wisdom tooth extraction from undergraduates dental students were considered in this study with age mean of 22 years old, 2 ml samples of stimulated saliva were collected prior ten mints of surgical wisdom tooth removal, and recollected after one week from surgery from*

*these students, the samples were analyzed to assess changes in  $\alpha$ -amylase and calcium ion concentration under controlled conditions.*

*The collected data predicted elevation of amylase level from ((7652.965  $\pm$ 34.0410) to (7674.710  $\pm$  68.8958) IU/L) with a significant change. And also increase in the calcium ion concentration level from (0.1465  $\pm$  0.048) to reach level (0.1975 $\pm$  0.100) mmol/L. Positive correlation were recorded between alpha amylase and calcium concentration when plotted the correlations values of stress and no stress individual's.*

*In a conclusion. Acute stress caused by wisdom tooth removal will elevate level of amylase in saliva significantly, however significant increasing in calcium concentration was observed in the stimulated saliva in comparison with the non-stimulated saliva with significant mean changes. However, our result predicted elevated levels of calcium concentration of the stimulated saliva than the non-stimulated (after extraction).*

***Key word: saliva  $\alpha$ -amylase, saliva calcium, acute stress, wisdom tooth extraction.***

## Introduction

Saliva is the familiar fluid present in the mouths of humans and some animals, which serves to moisten and lubricate the mouth. In addition, it contains enzymes that begin the process of digesting food, it aids our sense of taste, and it helps cleanse and protect the teeth, gums, and other tissues inside the mouth [1-4].

The value of salivary biomarkers for diagnostic and prognostic assessments has become increasingly well established in medicine, pharmacology, and dentistry. Certain salivary components mirror the neuro-endocrine status of the organism. Other saliva products are protein in nature, and can serve to reflect immune surveillance processes. The autonomic nervous system regulates the process of salivation, and the concentration of yet other salivary components, such as  $\alpha$ -amylase, which provide a reliable outcome measure of the sympathetic response [5].

stress is the body's reaction to a change that requires a physical, mental or emotional adjustment or response. Stress can come from any situation or thought that makes you feel frustrated, angry, nervous, or anxious. Stress is caused by an existing stress-causing factor or "stressor.". Stress reaction is a biological reaction caused by stress-induced alterations in the autonomic nervous system and endo-crinological and immunological functioning; the complexity of this relationship has not been fully elucidated [6-7]. Acute stress has an immediate onset, which forces the body into an immediate reaction. Examples of acute stressors might include a minor traffic accident, examinations, an argument, or an impending deadline one is straining to meet. Risks to health and should be taken seriously. Stress depletes important minerals, vitamins and nutrients from the body. Stress reduction strategies imbalances are created and these important systems begin to malfunction leading to illness and disease [8-9].

The  $\alpha$ -amylases are calcium metallo-enzymes, completely unable to do function in the absence of calcium. By acting at random locations along the starch chain,  $\alpha$ -amylase breaks down

long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and limit dextrin from amylopectin [10].

The autonomic nervous system regulates the process of salivation, and the concentration of certain salivary components (e.g., S.  $\alpha$ -amylase) mirrors the sympathetic response. Other salivary constituents (e.g., cortisol; cytokines) reflect physiologic endocrine-immune regulation [11-12].

Calcium is a potent intracellular signaling agent which has been implicated in the regulation of a wide variety of cellular activities . Despite the huge volume of studies of the effect of calcium on cell growth and differentiation, little is known about the molecular mechanism by which fluctuations of intracellular calcium stores contribute to specific induction of gene expression [13-14].

The aim of this research is the evaluation of  $\alpha$ -Amylase and calcium ion levels in saliva during acute stress before surgical wisdom tooth extraction and evaluates the mean concentration changes of amylase level in 25, 50 and 90 percent of the investigated patients. Investigate the correlation between the stress and non-stress patients. We select the period of examination which considered the more available and control condition. study of acute stress with the same room temperature & with stress free condition. Up to our knowledge, No similar research were reported to the time of writing this research.

## **Material and method:**

### **Chemical reagents:**

All chemicals and reagents used in this research were with high purity for analytical purposes. The  $\alpha$ -Amylase kit estimation were used in this research have supplied by Quanti Chrom™ (DAMY-

100) plus - USA. The  $\alpha$ -Amylase detection in this kit was based on colorimetric method of assay. The spectrophotometer used in this work was Scinco 2021, 200-700 nm double beam spectrophotometer, Korea.

### **Calcium reagents:**

Calcium reacts with Cresolphthalein complex in 8-hydroxyquinolinem to form a colored complex (purple color) that absorbs at 570 nm (550 – 580 nm). The intensity of the color is proportional to the calcium concentration. Color intensifiers and a stabilizer are present to minimize interference by other metallic ions. The analysis has been performed by using optima spectrometer – Japan with spectrum.

### **Saliva samples :**

Saliva specimens were collected into sterile graduated disposable plastic test tubes in conditions without stimulation, in period time between 9:00 and 10:00 hrs, after the last meal. Before collection of the test specimens of the saliva the subjects were rinsed their mouths with water. Saliva then was collected into plastic graduated test tubes with the volume scale accuracy to 0.5 ml. The volumes of the collected saliva were recorded after 5 min, then collecting of saliva was continued to reach the volume of 10 ml. During saliva collection test tubes were placed in a container with ice. Then the collected material was centrifuged at 5,000 rpm for 15 min, frozen at a temperature of  $-2^{\circ}\text{C}$  and stored in such conditions until biochemical tests were performed with period not more than 10 hours.

### **Subjects:**

Twenty Iraq male students were submitted for wisdom tooth extraction from Al-Mustansiria University /college of dentistry during their wisdom tooth extraction (1 hour before dental extraction) as stressed group. Stressor age mean were of  $22\pm 0.4$  years old clinically healthy students. We retest the same 20 students with the same room condition were considered as control,

without presence of stress condition one week after wisdom surgery.

### **Results:**

All collected data were statistically calculated by using SPSS Ver. 19.0 and Microsoft Excel -2010. In this study, the saliva of dental students was assessed on two occasions, before and after wisdom tooth extraction (one week) the results showed significant increase in alpha-amylase concentrations from  $(7587.6917 \pm 688.958)$  to reach level  $(7640,967 \pm 340.410)$ , respectively) when compared with the non-stress full sample (Table 1). While salivary calcium concentration levels were increased from  $(0.1465 \mp 0.487)$  to reach level  $(0.1975 \mp 0.10036)$  (Table 2).

All these results were recorded significant changes in between stress and changes in level of alpha amylase and calcium level concentration with a positive correlation according to the ANOVA test.

The mean concentration changes of alpha \_amylase are plotted in Figure 1, while patient's values of alpha-amylase are listed in Figure 2. The concentration changes of calcium between the stress and non-stress were plot in Figure 3.

The calcium evaluation calibration curve was plotted in Figure 4, while individual's values of calcium ion changes are listed in Figure 5. The correlation between stress and non-stress condition for the same students are plotted in Figure 6.

### **Discussion:**

Salivary glands not only act as an amplifiers of a low level of nor epinephrine, but also respond more quickly and sensitively to psychological stress [15].

Our result presents that acute stress lead to significant changes in amylase and calcium levels. Many researchers were find that

many mental illnesses are traced to trauma and serious changes, whose damage surfaces in times of stress and change. The evaluated statistical data had evidence that stress - in ourselves or in someone about whom we care – should be taken in our concern and treatment seriously [16-17].

Our result predicted that the changes in concentration could be attributed to some individuals are more sensitive to some stress factor than others; so, the person's characteristics and behavior patterns must be looked at to determine their importance and their vulnerability to stress [18-19].

Elevation of salivary amylase and calcium levels can be the result of both physiological and psychological stress as was previously mentioned.

In this research, we have no changes in the stress responses of patients prior to surgery, but on the day of surgery there was a highly significant increase demonstrated in patients having wisdom tooth extraction under local anesthesia. However, although this trend is notable in the group as a whole, the error bars indicate that the test would not be suitable for measuring the stress response of all individuals.

The overall results seem initially surprising but they perhaps reflect two factors. The first of these is that patients were only selected on the basis of their own preference for anesthesia and its known by the general population to carry a higher risk of morbidity and mortality. The elevation of calcium in saliva predicted calcium dissolution from teeth matrix and develop gingivitis and periodontitis, as well as dry socket.

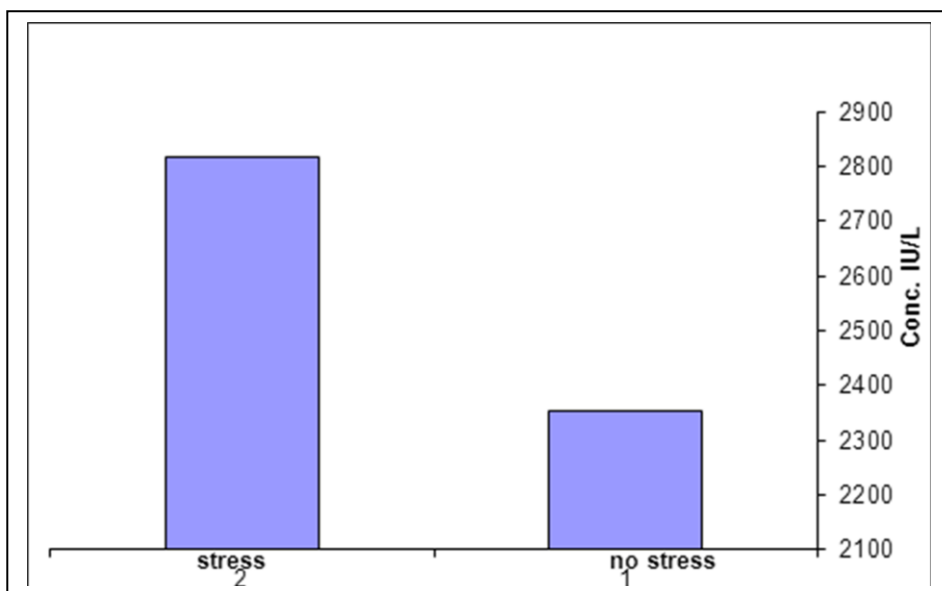
Our clinical observation recorded poor soft tissue and mall bone union and increase the possibilities for post extraction infection.no similar result were reported by any researcher reported significant changes of alpha amylase level changes with stressed patients. This could be attributed to the suitable selected intervals for monitoring the amylase (one month after extraction). This changes improved by the positive correlation between the expected and observed

result according to the statistical evaluation. The positive correlation provided a strong evidence of effect of stress on healing condition and play an important role on efficiency of extraction and possibility of failure procedure. However, we found that alpha amylase required a long period for elevation in comparison with other biochemical molecules. We highly recommend that alpha amylase changes must be evaluated kinetically and recorded changes with time to select the suitable period of analysis.

<i>Function</i>	<i>Before extraction</i>		<i>After extraction</i>	
<i>N</i>	20		20	
<i>Mean ±SD</i>	7640.9670		7587.6917	
<i>Median</i>	7652.9650		7674.7100	
<i>Variance</i>	115879.329		474663.996	
<i>Range</i>	1565.78		3202.74	
<i>Confidence 95 % intervals</i>	<i>lower</i>	6326.40	<i>lower</i>	4705.226
	<i>upper</i>	7892.18	<i>Upper</i>	7908.00
<i>Results In percentages</i>	25	7631.000	25	7631.000
	50	7652.9650	50	7674.7100
	90	7868.4609	90	7904.0450
<i>Sig &lt;0.05</i>	0.01		0.01	



<b>Table 2 the statistical evaluation of saliva calcium before and after</b>				
<i>Function</i>	<i>Before extraction</i>		<i>After extraction</i>	
<i>N</i>	20		20	
<i>Mean ±SD</i>	0.1975		0.1465	
<i>Median</i>	0.1500		0.1257	
<i>Variance</i>	0.010		0.002	
<i>Range</i>	0.33		0.15	
<i>Confidence 95 % intervals</i>	<i>lower</i>	0.11	<i>lower</i>	0.10
	<i>upper</i>	0.44	<i>Upper</i>	0.25
<i>Results In percentages</i>	25	0.1288	25	0.1091
	50	0.1500	50	0.1257
	90	0.3667	90	0.2250
<i>Sig &lt;0.05</i>	0.01		0.01	



**Figure 1. The Mean Concentration Changes Of Saliva Alpha Amylase Before And After Extraction,**

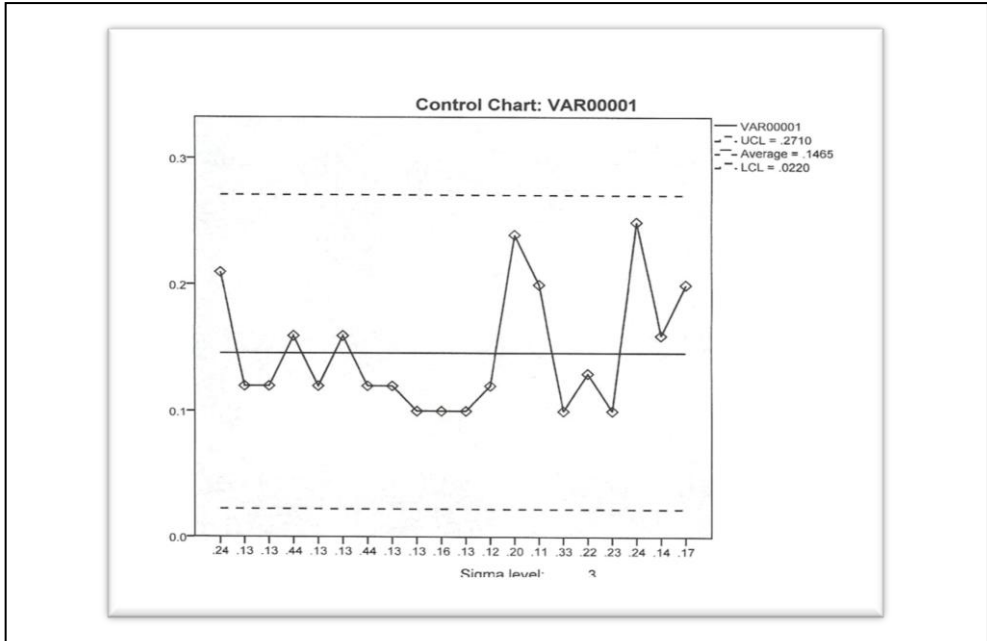


Figure 2. Salivary  $\alpha$ - Amylase of Investigated Patients.

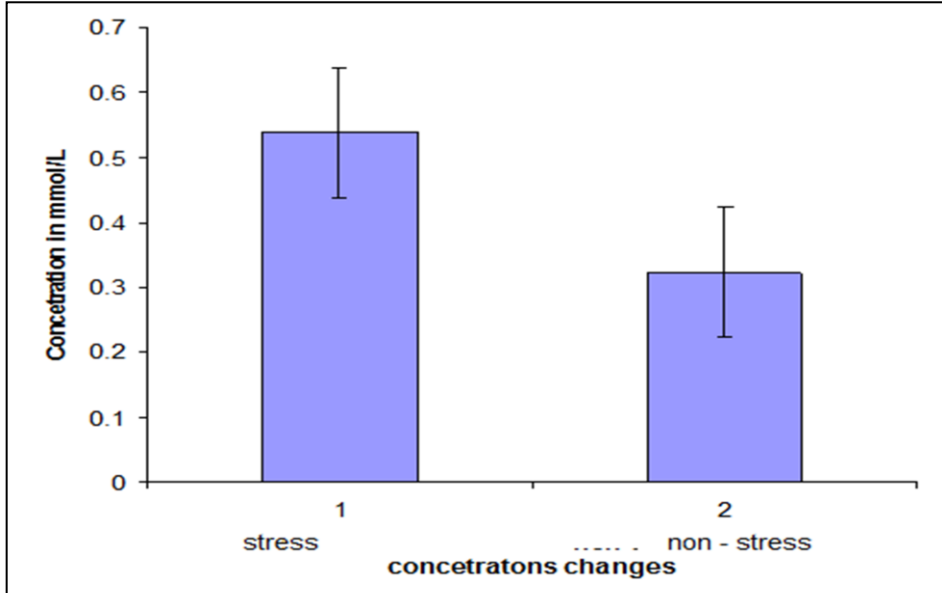
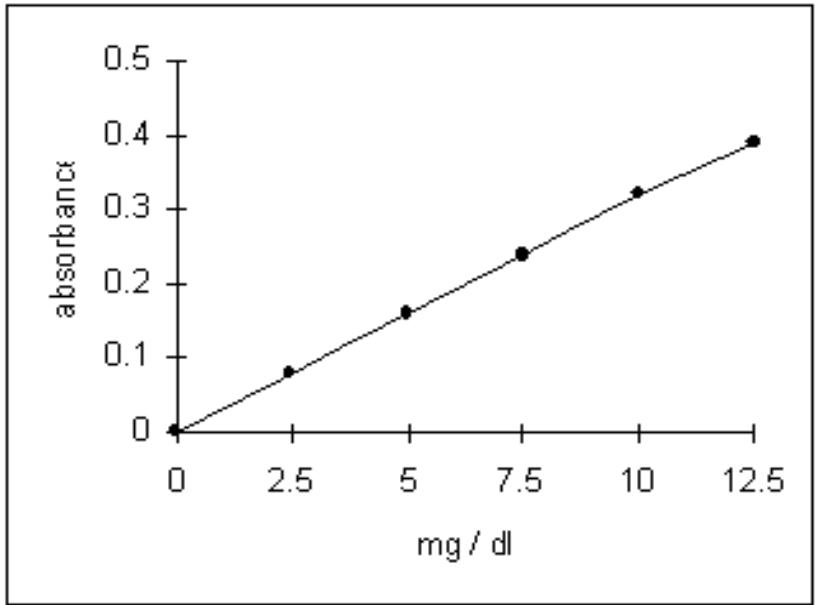
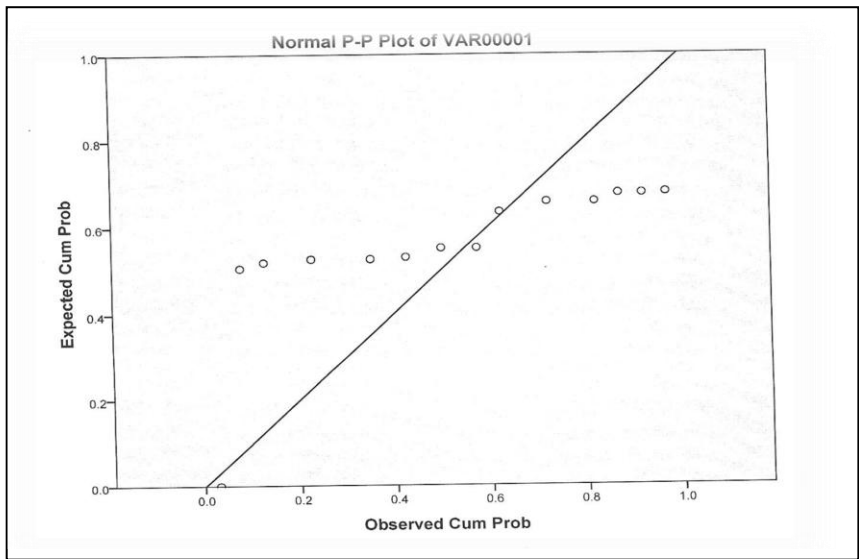


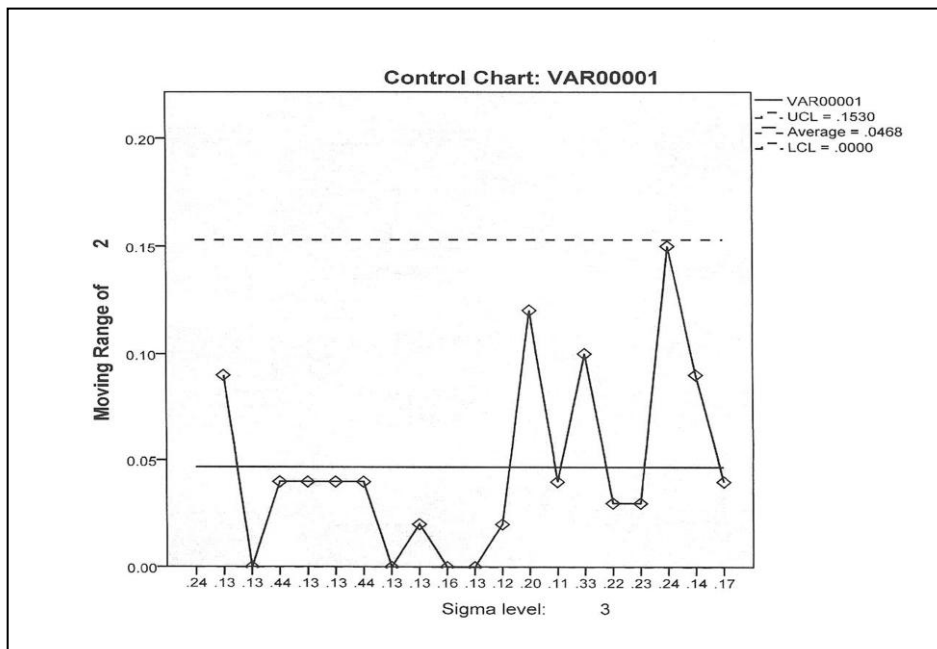
Figure 3. Calcium Concentration Change Before And After Extraction



**Figure 4. The Calibration Curve of Calcium**



**Figure 5: Individual's Calcium Changes**



**Figure 6: Calcium Level between the Observed and Expected Values**

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## قياس إنزيم الألفا- اميليز وايون الكالسيوم في اللعاب كدالة حيوية عند الشد العصبي للمرضى الخاضعين لقلع ضرس العقل جراحيا

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### المستخلص

استخدم إنزيم الاميليز اللعابي كمؤشر بيولوجي لفعالية الجهاز العصبي التلقائي ومحفز للشد العصبي. ويتطلب الوصول للفعالية القصوى للإنزيم وجود ايون الكالسيوم. يعد الكالسيوم من العناصر المتوفرة في العظام والأسنان وهو في حركة متواصلة بين النسيج وسوائل الجسم المختلفة. يتأثر تركيز الكالسيوم بالعديد من الأمراض مثل ترقق العظام، الكلية وأمراض اللثة وغيرها.

تم شمول عشرون طالب من الذكور بمعدل عمر يبلغ اثنان وعشرون سنة. تم جمع 2 مل من اللعاب قبل عشرة دقائق من قلع سن العقل جراحيا ومرة أخرى بعد اسبوع من إجراء عملية إزالة سن العقل. وتم إجراء حساب مستوى الاميليز الكالسيوم لبيان تأثير الشد العصبي. النتائج التي تم الحصول عليها تبين ارتفاع مستوى الاميليز اللعابي من  $(7652.965 \pm 34.0410)$  إلى  $(7674.710 \pm 68.8958)$  IU/L. كذلك تبين ارتفاع مستوى الكالسيوم من  $(0.1465 \pm 0.048)$  إلى  $(0.1975 \pm 0.100)$  ملي مول / لتر وبترباط إحصائي موجب.

من خلال النتائج تبين تأثير الشد العصب على رفع مستوى إنزيم الاميليز مقرون بارتفاع ايون الكالسيوم مقارنة بنفس الأشخاص ولكن في غياب تأثير الشد النفسي.