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## Alpha – amylase enzyme evaluation In Saliva of acutely stressed student

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### Abstract

Alpha - amylase is one of the major protein components of saliva. The main function of salivary alpha-amylase is the enzymatic digestion of carbohydrates, but it is also important for mucosal immunity in the oral cavity. The salivary enzyme alpha-amylase has been proposed as a marker 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.

Fifteen Iraqi male students were considered in this study with mean age of 19.0 years old during examination period as stress students, another fifteen students without examination were considered as .control.  $\alpha$ -Amylase assay were achieved by using colorimetric photometry method.

The collected data predicted elevation of amylase level from  $2354.260 \pm 379.757$  to  $2817.977 \pm 125.375$  IU/L with a significant change. Positive correlation was recorded when plotted the correlations values of stress and no stress individual's.

In Conclusion we found that acute stress caused by examination associated with elevation of amylase in saliva significantly, however one of the main causes of academic stress is test anxiety for Undergraduate students seem to be more emotionally vulnerable due to examinations.

**Key word: saliva  $\alpha$ -amylase, stress, acute stress on amylase level.**

### Introduction

Amylase is an enzyme that catalyses the breakdown of starch into sugars. Amylase is present in human saliva, where it begins the chemical process of digestion. Among other proteins, alpha-amylase is synthesized and secreted by acinar cells, which make up more than eighty percent of the cells in the major salivary gland<sup>(1)</sup>. The  $\alpha$ -amylases are calcium metalloenzymes, completely unable to 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<sup>(2)</sup>.

Stress reaction is a biological reaction caused by stress-induced alterations in the autonomic nervous system and endocrinological and immunological functioning; the complexity of this relationship has not been fully elucidated<sup>(3,4)</sup>. 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<sup>(5,6)</sup>. Stress reduction strategies imbalances are created and these important systems begin to malfunction leading to illness and disease<sup>(7)</sup>.

The aim of this research is the evaluation of  $\alpha$ -Amylase changes in saliva during acute stress and evaluates the mean concentration of amylase level in 25, 50 and 75 percent of the collected result and evaluate the correlation between the stress and non-stress student. We select the period of examination which considered the more available and control study case of acute stress. Up to our knowledge No similar research was reported to evaluate the  $\alpha$ -Amylase level in acute stress condition from saliva until the time of writing this research.

## Material and method

### Material

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

### Procedure

Samples are assayed freshly. However When stored frozen,  $\alpha$ -amylase was stable for one month.

1. *Reaction.* In appropriately labeled 1.5-mL Eppendorf tubes, transfer 10  $\mu$ L saliva. Then add 190  $\mu$ L Substrate, vortex briefly to mix and incubate for 5 min.
2. A blank control, 10  $\mu$ L water plus 190  $\mu$ L Substrate and 80  $\mu$ L Stop

Reagent. Alternatively a blank control can be prepared in the order: 10  $\mu$ L sample, 80  $\mu$ L Stop Reagent and 190  $\mu$ L Substrate. This would be necessary if the sample has a background color that is visibly blue.

3. Add 80  $\mu$ L Stop Reagent to each sample tube to terminate the reaction. Vortex to mix and centrifuge for 5 min at 6000 rpm., followed by carefully transfer 200 $\mu$ L supernatant into wells of a clear bottom 96-well plate. In separate wells, transfer 200  $\mu$ L water and 200  $\mu$ L Calibrator.
4. The optical density was recorded at 595nm<sup>(8)</sup>.

### Subjects

Fifteen Iraq male students were used in this study from Al Rafidain University college –dentistry department during examination of biochemistry ( about 1 hour before examination ) as stressed group. Stressor mean age was of 19.0 year old clinically healthy students. Another fifteen students with the same age period without examination stress or other stress factors were considered as control.

### Results

All collected data were statistically calculated by using SPSS Ver. 15.0 and Microsoft Axel -2007.

The statistical evaluation of concentration for the stress and non and stress students were listed in table 1. The mean concentration change are plot in figure 1, while individuals values of amylase are listed in figure 3. The correlation between the stress and non stress students are plotted in figure 4.

## Discussion

Salivary glands not only act as amplifiers of a low level of norepinephrine, but also respond more quickly and sensitively to psychological stress<sup>(9)</sup>.

For many young adults, college is the best time of life. These critical years of adjustment can also be undermined by acute or chronic stress. Many researchers are found that many mental illnesses were traced to trauma, whose damage surfaces in times of stress and change, such as the college years. The evaluated statistical data are evidence that stress - in ourselves or in subjects under study should be considered and treated seriously.<sup>(10-13)</sup>

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<sup>(14,15)</sup>

Significant elevation of amylase enzyme was recorded after stress condition, However, one of the main causes to academic stress is test anxiety. Most undergraduate students seem to be more emotionally vulnerable due to examinations. Increased anxiety from tests has a debilitating effect on their performance.<sup>(16)</sup>

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Table-1 The statistical evaluation of concentration for the stress and non-stress students.

Test		No stress students	Stress students
N		15	15
Mean ±Std. dev.		2354.260 ± 379.757	2817.977 ± 125.375
Std. error of mean		120.089	323.715
Median		2350.310	2754.297
Range		1149.120	5810.240
Percentage	25 %	2689.659	3436.587
	50 %	2350.3095	2754.297
	75 %	2076.496	2129.463
Pair t –test		6.179	
Sig. ( p <0.05 )		0.0001	
95 % confidence interval of differences		Lower	1844.899
		Upper	3975.763

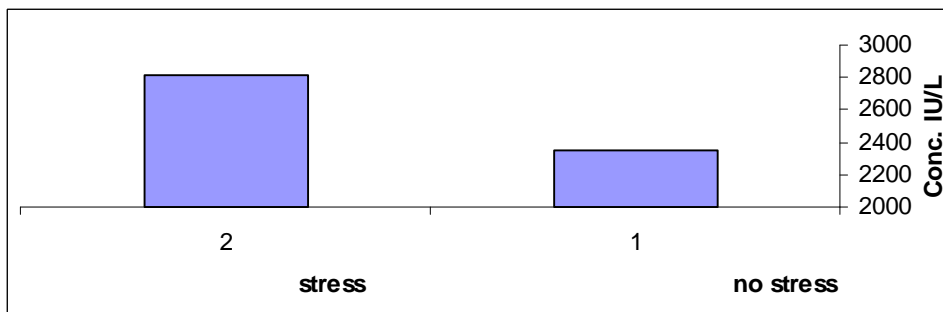


Figure 1. The mean changes of concentration of  $\alpha$ - amylase .

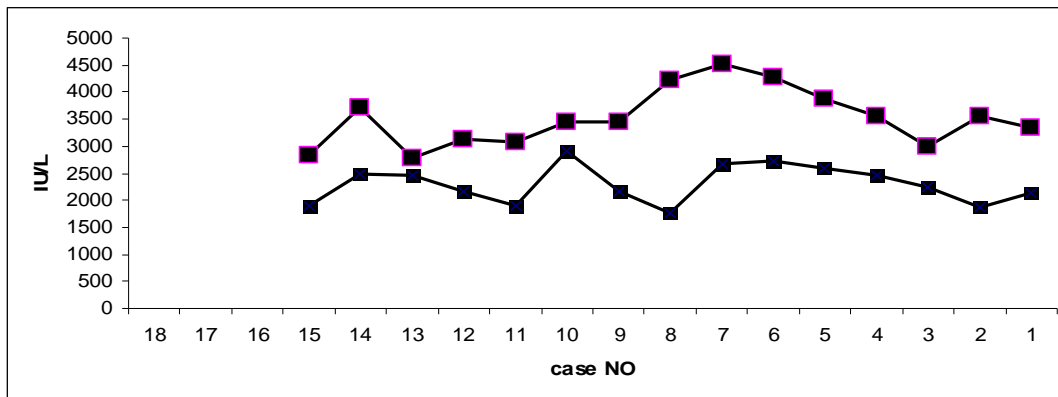


Figure -2 The concentration changes of  $\alpha$ - amylase concentration for the students without stress and with stress effect.

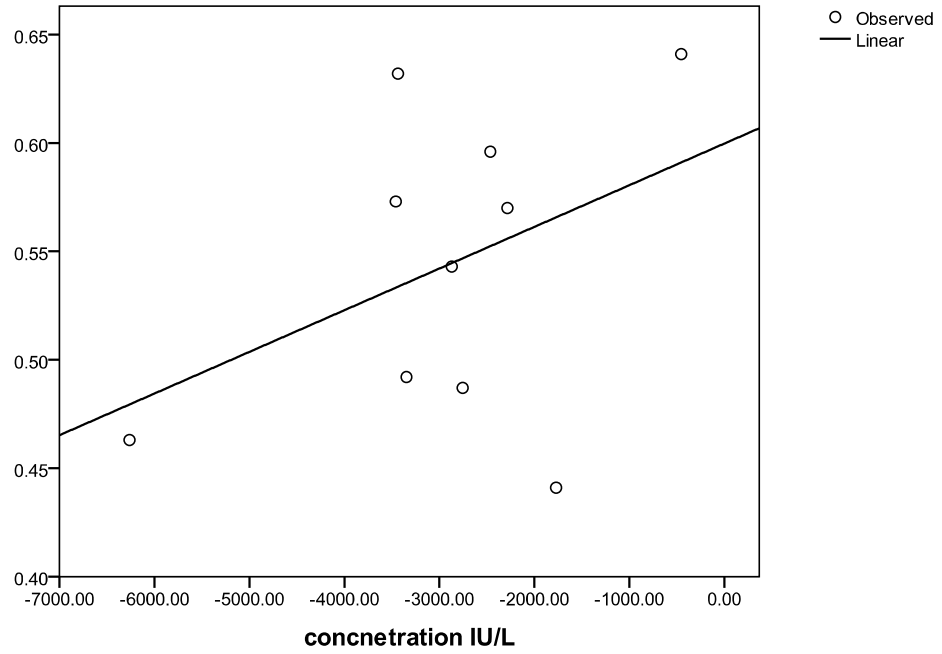


Figure 3 The correlation between the stress and non stress students.

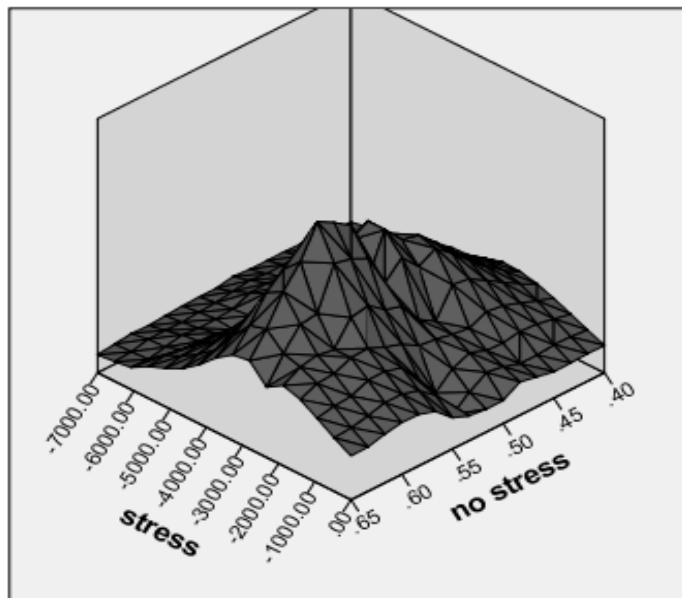


Figure -4The changes of concentration between the stress , non stress students and correlation ( 3D plot) .