



Original Article

Assessment of Salivary Alpha-Amylase, Cortisol, and Prevalence of Temporomandibular Disorder in Patients with Major Depressive Disorder

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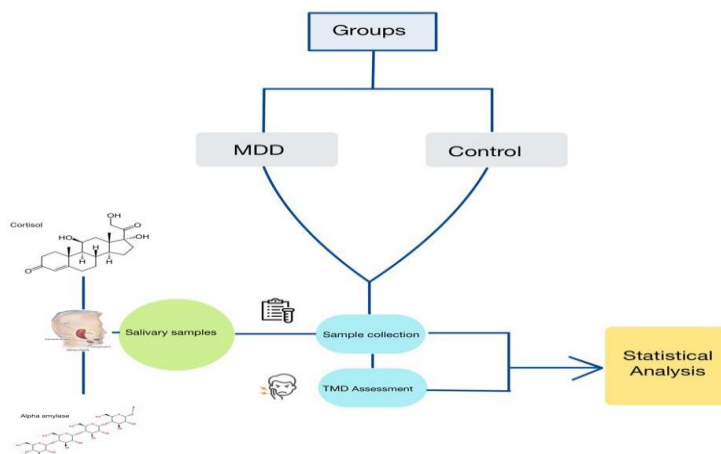
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ABSTRACT

There has been a general uptick in the number of people diagnosed with major depressive disorder (MDD) worldwide, which has led to a commensurate rise in the need for oral medicine in developed nations. Oral medicine bridges the gap between dentistry and medicine, and many illnesses and conditions that cause orofacial discomfort have traditionally been related to psychiatric conditions and disorders. Participants in the study were required to have a diagnosis of major depressive disorder and to have been getting therapy for at least two weeks before starting the study. There were 49 patients in this group. The participants in the control group were all in good health and showed no indications or symptoms of having a systemic disease. There were 34 people in this group. To conclude a diagnosis for each individual, the most recent edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) was used (DSM5). The results of t-test indicated no significant difference in the levels of salivary cortisol between the study group and control group ($p > 0.05$); this was the conclusion reached by the test. On the other hand, the salivary alpha-amylase levels were significantly greater ($p > 0.05$) in MDD patients compared to control subjects. There was a highly significant difference ($p < 0.001$) between MDD patients and control participants. MDD patients had significantly higher scores on FAI and a much higher prevalence of TMD than healthy controls. In conclusion, it was shown that patients diagnosed with MDD had higher alpha-amylase levels in their saliva.

GRAPHICAL ABSTRACT



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Introduction

Major depressive disorder (MDD), the most frequent cause of disability worldwide, can manifest itself in various ways and affect one out of every five persons [1]. There is a correlation between the MDD symptoms with structural and neurochemical impairments in corticolimbic areas of the brain [2]. The clinical manifestations of depression in individuals from Baghdad are not dissimilar to those observed in people from other parts of the world [3]. There is a significant incidence of depression in Iraq [4-6].

Depression was revealed to be a key missed criterion in a recent study carried out in Iraq, and it was found that this criterion may help in the early diagnosis of people with Behcet's disease [7].

Alpha-amylase is the main digestive enzyme in the mouth. In addition to its role in digestion, it has an immunological role by protecting the mouth from microbial invaders. Alpha-amylase serves several functions and benefits, including its role in the digestive process, which begins in mouth, and its ability to bind to oral bacteria and teeth [8].

Furthermore, it has been demonstrated that salivary alpha-amylase is a marker responsive to stimuli that stimulate the sympathetic nervous system (such as adrenaline) [9].

As salivary alpha-amylase (sAA) is secreted in response to neurotransmitter stimulation and the salivary glands are innervated by both sympathetic and parasympathetic nerves, salivary alpha-amylase is considered to be a valuable biomarker for measuring autonomic activity [10].

The parotid gland secretes salivary -amylase (sAA) in response to adrenergic activity suppressed by -blockade [11, 12]. Amylase has become a new biomarker for sympathetic nervous system reactions to psychosocial stress [13, 14].

There is some evidence to suggest that the alpha-amylase levels in the saliva can serve as a potential salivary stress marker in those who are hypersensitive to the impacts of adverse social events [15].

Major depressive disorder (MDD) and excessive production of cortisol hormone in response to stress are frequently linked [16]. In recent years, there has been a rise in the prevalence of using free cortisol levels in saliva as a simple and non-invasive indicator of free cortisol levels. Nonetheless, there was no evidence to suggest that individuals suffering from depression had higher levels of salivary cortisol compared to the healthy controls [17].

However, the salivary cortisol levels alone are insufficient to differentiate between subjects who have depression and those who do not. This is demonstrated by meta-analyses that failed to find evidence that definitively demonstrated a difference in salivary cortisol levels between depressed patients and healthy controls [17]. Some have suggested that cortisol hypersecretion may only occur in severely depressed patients due to the complexity of cortisol levels in most moderately depressed patients [18].

Temporomandibular disorders (TMDs) are a subset of craniofacial pain problems. The temporomandibular joint, the muscles that move the jaw, and the other head and neck musculoskeletal structures are involved [19].

The TMD is believed to be caused by multiple factors [20, 21]. Biological, behavioral, environmental, social, emotional, and cognitive factors have a role [22]. However, the primary cause of TMD development still needs to be fully understood [19]. Psychological disorders are a risk factor for TMD pain, but their importance is still under research [23].

This study evaluates salivary alpha-amylase and oral findings and compares that with healthy control subjects.

Materials and Methods

The Ethical Committee of the College of Dentistry at Baghdad University approved this study. It was a cross-sectional study (Project No.:458722). Participants in the study included 49 people who had been diagnosed with major depressive disorder and were receiving treatment for an extended period (at least two weeks).

In the control group, 34 people were otherwise healthy and had no indications or symptoms of

systemic disease. Psychiatric experts from Najaf City's Al-Hakim hospital provided the study group with diagnoses derived from The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM5). The collection of samples took place between January 30th, 2021 and April 20th, 2022. The laboratory work was carried out in Najaf City at the Al-Rawan specialized laboratory.

To participate in this study, patients must be at least 18 years old and have received a clinical diagnosis of depression from a psychiatrist. Patients who consult for emergencies, pregnant women, subjects on corticosteroid treatment, and subjects with a history of radiotherapy or chemotherapy were all disqualified from participation in the study because they did not meet the exclusion criteria.

All patients were given a thorough oral exam to look for any signs of oral disease. Some oral symptoms investigated were bleeding during brushing and burning mouth syndrome (BMS). Dry mouth (xerostomia), altered taste, and halitosis are the symptoms of this condition.

TMD was examined using the Arabic version of the Fonseca Anamnestic Index (FAI).

This index is composed of ten questions, each of which can be answered in one of three ways: "yes" (worth ten points), "sometimes" (worth five points), or "no" (0 points). The score is produced from the accumulation of points collected throughout all of the questions, and using it makes it possible to allocate points to the following categories [24, 25]: A score between 0 and 15 points shows that there are no signs and symptoms of TMD; a score between 20 and 45 points suggests that there is mild TMD; a score between 50 and 65 points indicates that there is moderate TMD; and a score between 70 and 100 points indicates that there is severe TMD. The Arabic edition of the FAI was successful in all of its examinations after passing through the processes of standardized translation and cross-cultural adjustment. It was discovered that validity and reliability of the FAI (FAI-A)'s Arabic version were adequate and reasonable [26].

To conduct an immunological investigation for the objectives, it was determined that a Human

AMY1 (Amylase Alpha 1, Salivary) ELISA Kit with Catalog No. E-EL-H0320 was the appropriate instrument for measuring alpha-amylase in the saliva.

In addition, a Human Cortisol ELISA Kit was utilized to determine the amounts of cortisol present in the collected samples of saliva.

Catalogue No.: E-EL0157

Data analysis based on statistics

During the data insertion and analysis procedures, in addition to Microsoft Excel, the Statistical Package for the Social Sciences (SPSS) version 23 was applied. Because the data comprised both descriptive and quantitative information, initially it is needed to test variables distribution in the study. After that, the χ^2 test and t-test were carried out to determine whether or not the variables that were a part of the study exhibited any correlation with one another.

During our investigation, the Kolmogorov-Smirnov test and correlation were the two critical techniques to ascertain whether the quantitative data adhered to a normal distribution.

Results and Discussion

The study outcomes showed that the age range of patients suffering from MDD was 23 to 66 years old, while the age range of participants in the control group was 20 to 57.

Patients determined to have major depressive disorder had an average age of 44.3 years old, with a standard deviation of 10.19 years old.

There was no statistically significant difference in the ages of the persons in control group. According to the table, the mean age of participants in control group was 41.26 years old, and the standard deviation was 10.98 years old (1).

The research results showed that the group of patients diagnosed with MDD included 26 males (53.1%) and 23 females (46.9%). In comparison, the group of healthy participants (the control group) included 19 males (55.9%) and 15 females (44.1%), with no significant difference between both groups, as listed in [Table 1](#).

Patients who participated in the research had salivary alpha-amylase levels that ranged from

0.5-2.25 ng/mL, with a standard deviation of 0.35 ng/mL and an average of 1.37 ng/mL. Patients in the control group had salivary alpha-amylase levels that ranged from 0.76-1.83 ng/mL, with a standard deviation of 0.2 ng/mL and an average of 1.19 ng/mL.

The results of this study's t-test showed a statistically significant difference in salivary alpha-amylase levels between the study group and control group ($p = 0.009$). This finding was based on the comparison of both groups. The results of this inquiry are summarized in [Table 2](#), which can be found below.

The outcomes of this study showed that the mean salivary cortisol was 206.42 ng/mL and a standard deviation of 130.01 ng/mL for patients who were a part of the study group, with a range of 28-604 ng/mL. In comparison, the mean of salivary cortisol for the control group was 289.44 ng/mL and a standard deviation of 442.37 ng/mL, with a range of 28-515 ng/mL.

In this experiment, a t-test was performed, and the results showed no statistically significant difference in the levels of salivary cortisol between the study group and the control group ($p > 0.05$). This information is presented in [Table 2](#). Patients diagnosed with major depressive disorder exhibited salivary alpha-amylase levels substantially higher than those in the control group ($p < 0.05$).

According to the findings of this research, which utilized the Fonseca amnesic scale, the study group consisted of (23) patients who had a (moderate) level of TMD, which represented (46.9%), (15) patients who had a (mild) level of TMD, which represented (30.6%), (9) patients who had a (severe) level of TMD, which represented (18.4%), and (2) patients who did not have TMD, which represented (4.1%).

There were 24 people in control group who received the score "none," which accounts for 70.6% of the total, there were 5 people who received the score "mild," which accounts for 14.7% of the total, there were 4 people who received the score "moderate"; accounting for 11.8%, and there was only one individual who received the score "severe", which accounts for 2.9% of the total, as provided in [Table 3](#).

According to the study's findings, there was a very significant difference ($p = 0.001$) between the patients diagnosed with MDD and the control subjects, with higher scores on FAI in patients diagnosed with MDD compared to the healthy controls. The results of this investigation are summarized in [Table 4](#).

According to the findings of this study, the amount of salivary alpha-amylase was significantly higher in patients diagnosed with MDD. This was shown to be the case compared to the study's healthy controls.

Previous studies [26, 27] have produced findings that align with this. It was postulated that alpha-amylase might be used as a valuable indicator of the activity of the sympathoadrenal medullary (SAM) system [26]. Patients diagnosed with MDD could have excessively high levels of sAA. In addition, the use of medications has the potential to assist in the improvement of depressive symptoms and the sAA reduction. On the other hand, some studies have led researchers to believe that the sAA liberation can be furtehrcaused by stimulation of the parasympathetic nervous system [28].

Our research results showed that salivary cortisol levels did not differ significantly between healthy controls and patients diagnosed with major depressive disorder (MDD). Other studies [29, 30] found that depressed people did not have significantly greater salivary cortisol levels compared to healthy controls, which agrees with this study's results.

In depression, monitoring awake salivary cortisol may be a more sensitive method of identifying HPA axis dysfunction, as mentioned by Bhagwagar *et al.* [31].

On the other hand, the findings of a previous meta-analysis [32] indicated an association between depressive symptoms and plasma cortisol levels in response to psychological stresses.

According to the findings of the study, the TMD incidence was significantly greater in patients suffering from MDD compared to healthy controls. This was the conclusion drawn from a comparison of the two groups.

Table 1: Age and gender profile of major depressive disorder (MDD) patients compared to healthy controls: A demographic analysis

Covariates	MDD patients		Control		P-value
Age (years old)	23-66		20-57		0.133
Range					Ns
Mean±SDD	44.30±10.19		41.26±10.98		
Gender	No.	%	No.	%	0.8
Male	26	53.1	19	55.9	Ns
Female	23	46.9	15	44.1	
Total	49		34		

*Ns: Non-significant ($p > 0.05$).

Table 2: Mean, standard deviation, and range of salivary alpha-amylase and cortisol levels in patients with major depressive disorder and healthy controls

Biomarker	Groups	No.	Mean (ng/ml)	SDD	Range	P-value
Alpha-amylase	MDD	49	1.37	0.35	0.5-2.25	0.009 S
	Control	34	1.19	0.20	0.76-183	
Cortisol	MDD	49	206.41	130.01	28-604	0.218 Ns
	Control	34	289.44	442.37	28-515	

*S: Significant ($p < 0.05$).

*Ns: Non-significant ($p > 0.05$).

Table 3: The number of patients with MDD who had TMD as measured by the (Fonseca anamnestic index) and the percentage of control individuals who had TMD

TMD	Study Group		Control Group		p-value
	No.	%	No.	%	
None	2	4.1	24	70.6	0.000 [HS]
Mild	15	30.6	5	14.7	
Moderate	23	46.9	4	11.8	
Severe	9	18.4	1	2.9	
Total	49	100	34	100	

*HS=Highly significant ($p < 0.001$).

Table 4: Mean and standard deviation of patients diagnosed with the major depressive disorder as well as control subjects' Fonseca Anamnestic Index scores

Groups	No.	Mean	Std.	P-value
MDD patients	49	51.51	19.34	0.000 [Hs]
Control subjects	34	15.67	20.63	

*HS=Highly significant ($p < 0.001$).

Studies [33-35] conducted in the past that investigated TMD using the same instrument generated results consistent with our findings.

According to the findings of Liao *et al.* [36], the TMD incidence was discovered to be 2.65 times higher in individuals who suffered from depression in comparison to those who did not suffer from depression.

TMD has a tangible link with both anxiety and depression, according to several further studies [37-39] that were conducted. People under much

stress have a greater risk of developing dysfunctional habits and experiencing muscle tension [40], both of which can lead to temporomandibular joint disorder (TMD). People who suffer from TMD have parafunctional elements in their bodies, notably those that induce increased muscle tension and changes in emotional states, which are strong indications of jaw pain. This hints that anxiety and depression are etiological variables in TMD [41], which is

supported by the fact that these factors generate greater muscular tension.

Conclusion

In conclusion, it was found that MDD group had a significantly greater TMD incidence. Dentists need to evaluate the role of psychological factors in temporomandibular joint disorder (TMD). Salivary alpha-amylase levels were higher in patients with MDD, suggesting that this is a reliable tool for evaluating MDD. Salivary cortisol showed no significant difference between both groups.

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No potential conflict of interest was reported by the authors.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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