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# Investigation the Influence of Stress on Salivary Features, Oral Hygiene and Gingival Health Condition among a Group of Adolescents Male Students

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### Abstract

Background: Stress is a condition that causes pressure and is caused by several factors in teens, such as unplanned events. Subjects and Method: This observational comparative study included 260 male students divided into three groups: low stress, moderate stress, and high stress who were provided according to the Adolescent Stress Questionnaire Scale (ASQ-S). Oral hygiene examination, including plaque index, was done according to Silness and Loe, 1964 index, and calculus examination was done according to Ramfjord, 1959 index, while gingival health examination was performed according to Loe and Silness, 1967 index. Salivary pH, flow rate, salivary Vitamin  $B_{12}$ , and Folic acid were measured in unstimulated saliva samples from the low and high-stress groups. **Results:** It has been shown that the higher means of plaque and gingival indices among high-stress level students was statistically non-significant (p>0.05). The high-stress group has a reduced salivary flow rate, with significant differences (P < 0.05). Vitamin  $B_{12}$  and Folic acid levels were more critical in the high-stress group (P > 0.05). Among both low and highstress groups, there was a negative, non-significant correlation between pH and flow rate with plaque and gingival indices. In high-stress students, the correlation of Vitamin  $B_{12}$  and Folic acid with plaque and gingival index was damaging. Conclusion: Augmentation of stress is linked with disturbance of psychological well-being, which tends to influence oral hygiene and gingival health by lowering the rate of saliva flow and impacting the levels of Vitamin  $B_{12}$  and Folic acid in saliva. Keywords: Folic acid, Gingival health, psychological well-being, Stress, Vitamin B<sub>12</sub>.

Article Information

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# **INTRUDUCTION**

Stress can refer to a physical, mental, or emotional issue that generates tension in the body or mind. Adolescence is a crucial time of life because of the rapid physical, social, cognitive, and emotional development that occurs then (1). These changes substantially affect one's health and well-being in later years (2). Stress leads to an increase in glucocorticoids and catecholamines, as well as an alteration in the immune response, which in turn influences health-related behaviors. It may lead to infections and periodontal tissue loss. The activation of these hormones decreases the activity of natural killer cells and the number of lymphocytes, among other immunological processes.(3)

Saliva is an ideal and non-intrusive biological specimen for the quantitative and qualitative evaluation of chemical and physiological

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mediators linked to many conditions, including stress-related disorders<sup>(4)</sup>. The sympathetic and parasympathetic nervous systems control the volume and composition of saliva during stress, which can reduce the rate at which saliva is secreted from the salivary glands, a condition frequently accompanied by dry mouth (5). A nutritional deficiency might result from a lack of vitamin intake, which substantially impacts dental and general health. Vitamins have a considerable impact oral health<sup>(6)</sup>. on Micronutrients such as vitamins C, B9, and E, calcium, zinc, copper, iron, and antiinflammatory and antioxidant properties play a role in development. A lack of certain micronutrients contributes significantly to developing periodontitis and caries <sup>(7)</sup>. Vitamin B12 (cobalamin) and B9 (folic acid) are B complex vitamins required for cell metabolism <sup>(8)</sup>. Vitamin B<sub>12</sub> is a vital micronutrient for brain development and function. It is essential for a child's early development because it affects memory, reasoning, attention, metabolism, the formation of red blood cells, and oral health (910)

This study was conducted because the Iraqi school system currently lacks a mental health philosophy and the Iraqi environment is undergoing several stressful life events. To avoid parental control, the presence of complete permanent dentition was ensured to minimize the effect of gender variation. Therefore, this study aims to determine the impact of stress on salivary variables, oral hygiene, and gingival health in a sample of 15-year-old male students. The null hypothesis proposes no correlation between salivary state and oral hygiene and gingival health about stress.

# PATIENTS AND METHODS Study Design

This descriptive cross-sectional study was done in Al Najaf City Center/Iraq, from 16th February 2023 until the end of May 2023. The 15-year male students were randomly selected from a third-grade intermediate of ten Iraqi schools. The researcher initially explained the purpose of the study to the students. A total of 260 students accepted to participate and those who fulfilled the study's conditions and agreed to participate were asked to sign a consent form. Any student with systemic disease that might interfere with oral health conditions like diabetes or disorders of salivary glands, a student on vitamin B12 and folic acid supplements therapy, taking medications, on orthodontic treatment, a smoker, and a student who refuses to participate in the research were excluded.

The adolescents' stress level was estimated using the Adolescent Stress Questionnaire Scale (ASQ-S) <sup>(11)</sup>. An oral examination included measuring the plaque index according to Silness and Loe,1964 index criteria <sup>(12)</sup> as shown in (Fig 1,2,3) calculus index according to Ramfjord 1959<sup>(13)</sup> for oral hygiene assessment as shown in (Fig 4), and gingival index according to Loe and Silness,1967 index criteria <sup>(14)</sup> for gingival health assessment, the level of gingival inflammation shows in (Fig.4,2).

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Figure 1: Moderate accumulation of plaque within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye.



Figure 2: Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin, with mild inflammation, slight redness, edema, no bleeding on probing.



Figure 3: A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen only after the application of the disclosing solution or by using the probe on the tooth surface. No calculus presented. No inflammation of gingiva



Figure 4: Moderate lingual supra gingival calculus with gingivitis

# **Data Collection Method**

The students' stress levels were measured by using the ASQ-S, a self-recording questionnaire taken from a previous study (11). There were fifty-four items in the initial version of the questionnaire; after introducing the scale to experts, eighteen items had been dropped out; the adjusted questionnaire consisted of 36 items related to stress resulting from daily lives; adjustments were made considering the Iraqi culture. The stress of becoming an adult, dealing with peer pressure, home life, taking on adult responsibilities, attending school regularly, balancing work and play, and performing well in school, and the stress of financial pressure are among the 36 self-rated items included in the ASQ-S. The answer for each item was scored as: Not stressful to me= 1, moderately stressful to me=2, severely stressful to me=3, very severely stressful to me=4; and I had not passed=0.

The validity of the ASQ-S was checked to establish the correlation between each item's score and the scale's total scores (Table 1).

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Pearson correlation was employed to determine the correlation between each item's score and the sample's total score, which consisted of 260 questionnaires; the correlation coefficient ranged from (0.85 - 0.36),and results demonstrated that each relationship was statistically significant when compared to the essential Pearson correlation value (0.12) at the P-value (0.05). The Cronbach  $\alpha$  method was employed to assess a scale's reliability using this approach, giving the results of the reliability coefficient, which was 0.90.

# Table 1. Items validity of ASQ scale using themethod of correlation of item score and totalscore of the scale

No	Pearson correlation	No	Pearson correlation	No	Pearson correlation
1	0.40	13	0.53	25	0.48
2	0.40	14	0.42	26	0.43
3	0.51	15	0.42	27	0.42
4	0.54	16	0.54	28	0.43
5	0.39	17	0.47	29	0.53
6	0.44	18	0.46	30	0.36
7	0.40	19	0.57	31	0.40
8	0.36	20	0.41	32	0.58
9	0.37	21	0.53	33	0.49
10	0.39	22	0.47	34	0.49
11	0.47	23	0.43	35	0.51
12	0.45	24	0.55	36	0.57

Saliva Collection Method

The collection of unstimulated salivary samples was conducted within a specially designated area at the school during the morning hours from 9 to 11 AM. The samples were obtained by the saliva collection procedures established by the University of Southern California School of Dentistry <sup>(15)</sup>. The students were instructed to take their seats and rinse their mouths with distilled water, allowing themselves to rest for a minimum of five minutes. They were requested to minimize their physical activity and to position their forehead above and the test cup below. Subsequently, they were directed to keep their lips open to facilitate the collection of saliva into the cup for five minutes. Upon the conclusion of the designated collecting period, the students were instructed to expel any residual saliva from their oral cavity expeditiously by expectorating it into the provided cup. Every student's cup was assigned a distinct code, denoted by a serial number on their case sheet. The measurement of salivary flow rate was conducted using the equation SFR = volume  $\div$  time (ml/min) <sup>(16)</sup>. The pH measurement was conducted by using a pH meter manufactured by Hanna Instruments, a company based in the United States. The saliva samples were centrifugated at 3000 rpm for 15 minutes in a researcher's laboratory at the Medical College, University of Kufa. Following centrifugation, the resulting clear supernatant was carefully separated using a micropipette. The supernatant was then divided into two sections, each placed in separate Eppendorf tubes, for subsequent analysis of Vitamin B12 and Folic acid.

### Immunological Assay and Statistical Analysis

Both main B<sub>12</sub> and Folic acid were analyzed by kit using enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Vitamin B<sub>12</sub> and Folic acid (YLbiont, Human). The data description, analysis, and presentation were performed by using Statistical Package for

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Social Science (SPSS version -22, Chicago, Illinois, USA), for frequency and percentage for qualitative variables and mean and standard error (SE) for the quantitative variables. Pearson correlation is a parametric test for the linear correlation between two quantitative variables while Analysis of Variance (ANOVA) is used for testing the difference between independent groups using a Games-Howell post hoc test. The level of significance is P≤0.05.

## **RESULTS**

The total number of students is classified into three groups with different stress levels. The sample distribution was done according to the stress level scale, as shown in Table 2. About two-thirds (65.38%) of students had moderate stress levels, whereas 16.92% and 17.69% showed low and high stress levels respectively. The mean values of plaque, calculus, and gingival indices (1.590, 0.100,1.306), respectively, were higher in the high-stress level group with a statistically non-significant difference (P>0.05) (Table 3)

Table 2. The distribution of the sample according to stress level.

Stress levels	N	%
Low"<=40"	44	16.92
Mod."40-60"	170	65.38
High ">=60"	46	17.69

<b>Fable 3. Plaque and</b>	gingival index	according to stress levels	s.
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Vars.	Stress levels								To	otal
	Low"<=40" Mod."40-60"		High ">=60"							
	Mean	±SE	Mean	±SE	Mean	±SE	F	P value	Mean	±SE
PLI	1.449	0.081	1.440	0.038	1.590	0.075	1.620	0.200	1.468	0.032
CalI	0.071	0.022	0.083	0.016	0.100	0.067	0.140	0.869	0.084	0.016
GI	1.047	0.138	1.000	0.060	1.306	0.100	2.712	0.068	1.062	0.050

In Table 4, the mean pH value was lower in the group experiencing low-stress levels. However, the observed difference did not reach a statistical significance (P>0.05). In contrast, the group subjected to high levels of stress had a lower mean flow rate value, and this difference was shown to be statistically significant (P<0.05); the means of both vitamin  $B_{12}$  and Folic acid were high in the high-stress level student group with a statistically non-significant difference (P>0.05)

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Vars.		S	tress	Statistical tests		
	Low"	"<=40" H		h ">=60"		
	Mean ±SE		Mean	±SE	Т	P value
PH	6.923	0.162	7.113	0.036	1.169	0.246
SFR	0.980	0.043	0.735	0.032	4.595	<0.001
B12	117.656	1.381	118.111	1.576	0.216	0.829
Folic acid	1.705	0.052	1.759	0.089	0.511	0.610

 Table 4. Salivary physical and chemical constituents among students with low and high stress

 levels with statistical difference

The pH and flow rate of the low and high-stress groups had negative relationships with plaque, calculus, and gingival indices among the low and high-stress students, except the calculus index, had a positive connection (P>0.05) for both low and high-stress levels. This is in Table 5. Table 6 shows that vitamin B12 correlated positively with plaque index and negatively with calculus and gingival indices in low-stress groups. Folic acid negatively correlated with plaque, calculus, and gingival index. B12 and Folic acid correlated negatively with plaque and gingival indices and positively with calculus index in high-stress individuals. None of these associations were significant.

Table5. Correlations of salivary physical and chemical constituents with oral hygiene and gingival health condition according to stress level.

Stress level		pН		SFR	
		r	р	r	Р
	PLI	-0.077	0.620	-0.115	0.456
Low"<=40"	CalI	-0.006	0.969	0.067	0.666
	GI	-0.043	0.780	-0.046	0.766
	PLI	-0.100	0.507	-0.238	0.111
High ">=60"	CalI	0.150	0.319	0.165	0.272
	GI	-0.049	0.745	-0.010	0.948

Table 6. Correlations of salivary	chemical constituents	with oral	cleanliness	and	gingival	health
condition according to stress level	l.					

Stress le	evel	B	12	Folic acid		
		r	Р	r	Р	
	PLI	0.105	0.499	-0.166	0.280	
Low"<=40"	CalI	-0.189	0.218	-0.242	0.114	
	GI	-0.187	0.223	-0.153	0.321	
High	PLI	-0.055	0.715	-0.261	0.080	
">=60"	CalI	0.057	0.708	0.059	0.697	
	GI	-0.048	0.750	-0.262	0.079	

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### DISCUSSION

Psychology-related factors may influence the levels of plaque and the state of the gingiva in adolescent students. The current study has shown that the percentage of students with moderate and high-stress categories was high; this agreed with studies<sup>(17,18,19,20,29)</sup>. The results of the study showed that means of plaque, calculus, and gingival indices were higher in levels high-stress with statistically no significance; this coincided with (19:20:22) and may be connected to the fact that stressful conditions activate the neuroendocrine system, which in turn causes the continuous release of pro-inflammatory mediators and an alteration in the normal microbiota, which ultimately leads to periodontal inflammatory disorders (22). Another study revealed a significant stress-related gingival index mean difference; results may vary due to age range, periodontal variable studied, study design, and stress measures employed<sup>(18)</sup>. According to this study's results, saliva's flow rate was significantly (P<0.05) lower in the high-stress group, as seen in previous studies<sup>(20,22)</sup>; this phenomenon can be explained by the fact that the autonomic nervous system mainly controls the secretion of saliva. Therefore, when individuals are exposed to stressful conditions, they cause a gradual accumulation of stress on various systems involved in maintaining stability in the body, such as the central nervous system, the autonomic nervous system (ANS), and the hypothalamus-pituitary-adrenal axis (HPA). Consequently, the ANS and HPA axes become dysregulated over an extended period .(23)

Salivary pH was not significantly lower in the low-stress group compared to the high-stress group; this finding agrees with <sup>(25)</sup> but contrasts the results of <sup>(20)</sup>, which indicated that both flow rate and pH decreased as stress increased. Although the sympathetic and parasympathetic systems influence salivary nervous pН regulation, the current study does not demonstrate a significant decrease in salivary pH levels under high stress. This discrepancy may be attributed to variations in saliva sampling methods, the study population, and the parameters utilized<sup>(33)</sup>. By <sup>(18 \cdot 20 \cdot 26)</sup>, salivary flow rate and pH negatively correlated with gingival and plaque indices in both high and low stress levels. This finding opposes the conclusion drawn by <sup>(19)</sup>; the plaque accumulation in the high-stress group of this study may have been caused by a reduction in flow rate. Furthermore, low pH conditions promoted the proliferation of various pathogenic bacteria responsible for the induction and advancement of periodontal disease.<sup>(18)</sup>

The results of the current study show a positive correlation of pH and SFR with calculus formation, which agrees with<sup>(34)</sup>. The results of this research revealed that the values of B<sub>12</sub> and Folic acid were statistically non-significantly higher in high-stress students, although there was disagreement with <sup>(28)</sup>.

However, a cross-sectional study by (29) found no connection between psychiatric symptoms and vitamin B<sub>12</sub> and Folic acid. It is important to note that the subjects' poor intake of these nutrients, as well as variations in eating patterns, lifestyle, nutritional evaluation techniques, classifications of psychiatric symptoms, and other confounding variables, may have influenced the findings. The correlation between vitamin B<sub>12</sub> and Folic acid was negative and non-significant, with plaque and gingival indices among the high-stress level group. This agrees with (30,31,32). This may be related to the existence of high levels of vitamins, which are crucial for the maturation process of the oral mucosal epithelium and the enhancement of hematopoietic factors that contribute to the prevention and treatment of infection in oral mucosa.<sup>(32)</sup>

The limitation of the study was that some students were shy, which led to difficulty in oral examination and salivary samples. The researcher had to take samples from students in the intermediated third grade early in the

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morning, which conflicted with the school and teachers' classes.

### CONCLUSIONS

Depending on the findings, an increased stress condition has considerably reduced salivary flow, which negatively influences oral hygiene and gingival health. Vitamins B<sub>12</sub> and Folic acid, two members of the B complex vitamins, have been linked to better periodontal health.

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