Original Article

The Impact of Dental Environment Stress on Dentition Status, Salivary Nitric Oxide and Flow Rate

Raghad Ibrahim Kadhum Al-Moosawi, Alhan Ahmed Qasim

Department of Pedodontics
and Preventive Dentistry,
College of Dentistry,
University of Baghdad,
Baghdad, Iraq

ABSTRACT

Aim: This study aimed to assess the dental caries experience among dental students with different levels of dental environment stress in relation to salivary nitric oxide (NO) and flow rate of whole unstimulated saliva. **Materials and Methods:** The study involved 300 dental students. They were classified into three categories (mild stress, moderate stress, and severe stress) according to dental environment stress questionnaire (DESQ); clinical examination for dental caries was carried out. Unstimulated salivary samples were collected from the mild and severe stress groups for measuring the salivary flow rate. Estimation of salivary NO was carried out by using salivary NO test strips. All data were analyzed using the Statistical Package for the Social Sciences software, version 21.0 (SPSS, Chicago, Illinois). **Results:** Dental caries experience was higher among severe and moderate stress groups with nonsignificant differences ($P > 0.05$). Mean value of salivary flow rate was lower among severe stress group with nonsignificant differences ($P > 0.05$). NO was significantly higher among severe stress group ($P < 0.05$). Flow rate was weak negatively correlated with caries experience among both mild and severe stress groups except for the decay surface (DS), which was weak positive among mild stress group. NO was weak negatively correlated with DS among both mild and severe stress groups. All these correlations were statistically not significant ($P > 0.05$). **Conclusion:** Dental environment stress appears to affect oral health, shown by higher dental caries among dental students with severe dental environment stress by affecting the normal level of salivary flow rate and NO.

KEYWORDS: *Dental caries, dental environment stress, flow rate, nitric oxide, stress*

INTRODUCTION

Stress is experienced by numerous individuals in a diversity of social, academic, and work settings. Although stress can be a cause of motivation, extreme stress can be debilitating that could lead to disease.[1] The central nervous system (CNS) reacts to stress. This reaction involves different morphological and neurochemical alterations, for example, oxidative stress.[2] Oxidative stress comprises an imbalance between the amounts of reactive oxygen species (ROS) and the capability of antioxidant systems to equalize them.[3] Dentistry is known as a very stressful occupation,

and dental education is considered stressful education environment because of the dental occupation demanding interpersonal dexterity and clinical competencies as well as theoretical knowledge.[4] The main sources of stress among dental students include the education environment, apprehension of fail, hardness in dealing with patients, clinical requirements, hardness in dealing with transitions in curriculum, and

Address for correspondence: *Raghad Ibrahim kadhum*
Al-Moosawi, BDS,
MSc Student, Department of Pedodontics and Preventive Dentistry,
College of Dentistry, University of Baghdad, Baghdad, Iraq.
E-mail: iraghad823@gmail.com

How to cite this article: Al-Moosawi RI, Qasim AA. The impact of dental environment stress on dentition status, salivary nitric oxide and flow rate. J Int Soc Prevent Communit Dent 2020;10:163-70.

difficult relationships with academic staff.[5] Dental caries is a complex, chronic, and multifactorial disease that occurs due to imbalance between demineralization and remineralization of dental hard tissues.[6] When the individual exposes to stressful event, the psychological reaction to this event activates the sympathetic nervous system and the hypothalamic–pituitary–adrenocortical (HPA) axis, which causes a decrease in salivary flow, and in this manner, changes salivary capability with regard to oxidation reduction.[7] The reduction in salivary flow decreases the defensive actions created by saliva, and as a result, increases the hazard for dental caries.[8] Another study found that there is no relation between stress and salivary flow rate.[9] Nitric oxide (NO) is a free radical gas and a detrimental chemical in the atmosphere; however, it presents in little well-controlled concentrations in the body and plays a key role in several physiological and pathological processes. NO is implicated in the stress physiology and is involved in disease processes related to stress; it counteracts norepinephrine activity and sympathetic reactivity.[10] NO is strong antimicrobial chemical, and it implicates in oxidative stress-linked disturbances.[11] Results of studies on the relation between NO levels and dental caries were controversy. Therefore, the main aim of this study was to determine the dental caries experience among different stress levels of dental students and to assess an evaluation of its relationship with salivary NO and flow rate. The null hypothesis was that there is no relation between salivary NO and flow rate with the development of dental caries experience among different stress levels of dental students.

MATERIALS AND METHODS

STUDY DESIGN

This cross sectional study which is a type of observational descriptive epidemiological study.

STUDENT INTERVIEWING

The researcher explained the plan of study, and the students were asked if they agree to participate in this study. Three hundred students were accepted to participate in the study. The students who agreed to participate in this study and met the requirements of the study were asked to sign consent form. Then they were given the dental environment stress questionnaire (DESQ) to estimate their stress level.

All the dental students of the fourth and fifth grades were interviewed. This study was carried out in College of Dentistry, University of Baghdad, Baghdad, Iraq, from January 2019 till the end of April 2019.

SAMPLING METHODS AND CRITERIA

A total of 410 dental students of the fourth and fifth grades aged 22–23 years from College of Dentistry, University of Baghdad, were enrolled in the study. Among them, 300 students (73 male and 227 female students) met the inclusion criteria and agreed to participate in the study, 39 did not agree to participate in the study. 71 students (32 with orthodontic treatment, 33 smoker, 2 pregnant, and 4 with systemic disease) were excluded from the study. All 300 students were orally examined and classified according to DESQ into the following groups: mild stress group (83 students), moderate stress group (141 students), and severe stress group (76 students). Saliva sample was obtained from students with mild stress (55 students) and severe stress (40 students) for measuring salivary flow rate.

INCLUSION CRITERIA

The inclusion criteria of the study included all dental students (both male and female) of fourth and fifth grades from College of Dentistry, University of Baghdad.

EXCLUSION CRITERIA

The exclusion criteria of the study included students with systemic disease, students diagnosed with orthodontic treatment, smoker students, pregnant students, and students who were on hormonal supplements.

No pilot study was carried out because of the limited time for the study, but inter- and intraexaminer calibration were performed in College of Dentistry, University of Baghdad to ensure consistency of data recording. Interexaminer calibration was performed on 10 individuals of different ages; they were examined twice: the first examination was carried out by the researcher, whereas the second examination was carried out by specialist (supervisor) in the department of pedodontics and preventive dentistry. The examination included dental caries of the World Health Organization (WHO) (1987), as shown in Table 1.

Intracalibration was performed on 10 dental students; they were examined twice by researcher in which dental caries was examined with an interval period of 2 weeks between the two examinations, as shown in Table 2.

The participating students were divided according according to dental environment stress questionnaire into three groups: mild, moderate, and severe stress. after extracting the quartile rang of the DESQ, the students who have score number 81 and less it mean they are in the mild stress group, the students who have the score number (82–95) it mean they were in moderate

Table 1: Intercalibration of dental caries

Variable	Researcher observation			Supervisor observation			Paired t test	
	NO	Mean	SD	NO	Mean	SD	t test	P value
DS	10	6.200	5.138	10	6.100	5.152	1	P > 0.05
MS	10	8.500	10.512	10	8.500	10.512	*	
FS	10	8.000	6.549	10	8.000	6.549	*	
DMFS	10	22.700	16.540	10	22.600	16.507	1	P > 0.05

*The correlation and t cannot be computed because the standard error of the difference is 0

Table 2: Intracalibration of dental caries

Variable	First observation			Second observation			Paired t test	
	NO	Mean	SD	NO	Mean	SD	t test	P value
DS	10	4.50	3.171	10	4.40	2.989	1	> 0.05
MS	10	2.00	3.496	10	2.00	3.496	*	
FS	10	5.70	4.620	10	5.70	4.620	*	
DMFS	10	12.20	6.356	10	12.10	6.190	1	> 0.05
DMFT	10	7.20	2.741	10	7.10	2.644	1	> 0.05

*The correlation and t cannot be computed because the standard error of the difference is 0

stress and the students who have score number 96 and above then it mean they were in the severe stress group.

QUESTIONNAIRE METHOD

Dental environment stress was recorded for all dental students by means of self-recorded questionnaire using DESQ, which was designed for this purpose and was adopted from other study.[12] The first copy of the questionnaire consisted of 41 items, 17 items were dropped out after introduction to a group of experts, 24 items were retained. The researcher asked the students to answer the questionnaire items on a five-point Likert scale (not stressful, slightly stressful, moderately stressful, severely stressful, very severely stressful) as previously mentioned to the students.

To facilitate the understanding of the DESQ the researcher used the Arabic version that used by several Arabic country among them Iraq in Al-Qadisiyah University.

The validity of DESQ was checked by statistician by finding the correlation between the score of each item with total scores of scale, to achieve this, Pearson correlation was used to find the correlation between score of each item of scale and the total score of sample of research which was (300) questionnaire, correlation coefficient ranged between (0.316 and 0.575), when the t test used to find the difference between these correlation coefficient the result showed that all correlation was statistically significant when compared with table value of Pearson correlation (0,11) at $P = 0.05$ and degree of freedom (298) as in Table 3.

Cronbach α is the coefficient of reliability used commonly for measurement of the internal consistency or reliability of a scale. The Cronbach α formula was used to find the reliability of the DESQ, suggesting that the reliability obtained using this method was 0.85.

Oral examination was carried out for dental caries following the criteria of decay, missed, and filled surfaces (DMFS) and decay, missed, and filled teeth (DMFT) of WHO 1987 index.

SALIVA COLLECTION METHOD

Saliva sample collection was carried out in the morning between 9:30 and 11:30 AM. The collection of unstimulated saliva was performed under standard condition according to the University of Southern California School of Dentistry guidelines for saliva collection.[13] After asking the students to sit on the dental chair of the clinic of educational hospital of College of Dentistry, University of Baghdad, the students were given a cup of distilled water to rinse his mouth. Then relax for 5 minutes and minimize movement, after that start to collect the unstimulated saliva sample by inclining the head forward with mouth retained opened slightly to permit saliva to drool into a graduated sterilized jar which is coded with same identification number written on the case sheet for each students, which was serial numbers, at the end of the period of saliva collection, the students asked to gather any remaining saliva in the mouth and expectorate it quickly into the sterilized jar quickly. The salivary flow rate was measured by the researcher in the clinic of educational hospital of College of Dentistry, University of Baghdad by dividing the volume of collected saliva in milliliter (mL) by the time required for collection in minute (min). Flow rate (mL/min) = volume (mL)/time (min).[14] The estimation of salivary NO was carried out by the researcher by using the salivary NO test strips (Berkeley test, L2G North America, Chicago,

Table 3: Statistical analysis of the items of dental environment stress scale using method of correlation between item score and total score of scale

Number of items	Pearson correlation	Number of items	Pearson correlation	Number of items	Pearson correlation
1	0.316	9	0.530	17	0.351
2	0.468	10	0.463	18	0.416
3	0.339	11	0.487	19	0.575
4	0.529	12	0.548	20	0.407
5	0.394	13	0.561	21	0.523
6	0.521	14	0.496	22	0.520
7	0.443	15	0.471	23	0.573
8	0.394	16	0.532	24	0.483

USA), after the researcher estimated salivary nitric oxide by using NO test strips. The students followed the manufacture instructions as shown in Figure 1. The test can be done any time during the day but if food or drink (except water) was consumed 10 min must pass before testing. The researcher compare the color appeared on NO test strips with NO scale, then these levels were recorded on students case sheets. Therefore, the researcher does not need to send the salivary specimen to an institution for laboratory analysis.

For salivary analysis, 95 students were selected using simple random sample methodology only from the mild stress group (55 students: 27 male and 28 female) and from the severe stress group (40 students: 11 male and 29 female) to compare the lowest and highest levels of stress, and to see if there is correlation between the stress and the salivary flow rate and NO. Ninety-five students were selected because the NO test strips were only for 50 tests, and the researcher purchased only two containers because it was expensive, so it will be enough for 100 persons. Caries experience was diagnosed according to DMFS/DMFT of WHO 1987 index. The unstimulated saliva was collected under standard condition according to the University of Southern California School of Dentistry guidelines for saliva collection.[13]

The collected unstimulated saliva sample was poured directly after collecting in a graduated test tube to determine the volume collected through the 5-min period.[14] The amount of unstimulated salivary flow rate collected was 0.2–1.1 mL/min.

To avoid the confounding factors the researchers follow the exclusions criteria as mentioned previously as it may affect the stress level and saliva. There was no bias in selection of samples.

Regarding the age and gender, they were adjusted by using chi-square test between them and stress levels. There was no significant association between age, gender, and stress levels so they were no confounding factors.

OBSERVATIONAL PARAMETERS

According to the direction of use, the students were asked to (1) place the NO test strip with saliva here side on the tongue for 5 s and then remove and (2) fold the strip over and gently press the two sides together for 10 s.

Then the researcher simply separated and compared color on test pad to the NO scale, which was categorized into five levels (depleted, low, threshold, target, and high). The level recorded on the case sheet.

STATISTICAL ANALYSIS

Data analysis, description, and presentation were performed by using the Statistical Package for the Social Sciences software, version 21.0 (SPSS, Chicago, Illinois).

All the variables were normally distributed according to Kolmogorov–Smirnov test. Multivariant analysis cannot be performed because some assumptions couldn't be met like correlation between variables so splitting ANOVA is the best.

The statistical analysis was grouped into two classes:

Descriptive analysis:
- The mean, standard error and standard deviation was used for quantitative variable.

Inferential statistics:

- One-way ANOVA for analysis of variance: It was used for parametric test determination and to find the difference between independent samples. No post hoc used because no significancy after ANOVA. Significance was accepted at $P < 0.05$.
- Independent sample t test: It was carried out to find the mean differences between two samples.
- Pearson correlation was used to find the linear correlation between two variables.

RESULTS

About the missing data, the moderate stress group was not included in the analysis of saliva sample because

the aim of this study was to compare between the lowest level of stress and the highest level of stress to see how the stress affects the level of NO and salivary flow rate.

Chi-square test was used to verify whether there is a significant association between stress groups and gender and between the stress groups and age or grade. The result was not significant so these variables (gender and grade) were excluded. Confidence interval was 95%.

Table 4 shows the caries experience (DMFT, DMFS and its components decay surface [DS], missed surface [MS], and filled surface [FS]) among dental students according to dental environment stress levels for the total sample. The mean value of DMFT and DS fraction was higher among severe stress level with statistically nonsignificant difference ($P > 0.05$), whereas DMFS, FS, and MS fractions were higher among moderate stress level with statistically nonsignificant difference ($P > 0.05$).

Table 5 shows the mean value of flow rate and NO. For salivary flow rate, it was lower among students with severe dental environment; however, the difference was not statistically significant ($P > 0.05$), whereas the mean value of NO was higher among students with severe dental environment stress with statistically significant difference ($P < 0.05$).

Table 6 shows the correlation between salivary physicochemical characteristics and dental caries. The caries experience (DMFT, DMFS and its components DS, MS, and FS) were weak and negative correlated with flow rate among students with mild dental environment stress except for the DS was weak and positive correlated with flow rate; while among the

student with sever dental environment stress there were weak and negative correlation between the caries experience and flow rate. The correlation of caries experience with NO was weak negative among students with mild dental environment stress, whereas among students with severe dental environment stress it was weak positive except for DS for which it was weak negatively correlated with NO. All these correlations were found to be statistically not significant ($P > 0.05$).

DISCUSSION

The defensive elements of saliva and the relationship between oral diseases and psychological components were taken into consideration. It is surprising how minimal psychological stress can adjust the safeguard element in saliva. In this study, the dental caries experience was higher among severe dental environment stress category than other categories that were not statistically significant. The increase of dental caries prevalence with increased stressful situation was agreed with other studies.[9,15] This may be due to the stress has an effect on the immunity and can weaken the host defense to cariogenic bacteria,[16] by unhealthy eating habits like frequent snacking and increase the intake of sugar rich diet,[15] poor oral health behavior (flossing teeth, brushing teeth) making great condition for microbes,[17] by diminishing salivary flow prompting diminished removal of cariogenic bacteria, subjective oral dryness and unstimulated salivary flow were connected with stress so decrease the protective functions of saliva increasing the vulnerability to dental caries.[18] Although a lower unstimulated salivary flow rate was found in the severe stress group, this difference

Table 4: Caries experience (DMFS-DMFT) according to categories of dental environment stress levels						
Variable	Stress level	N	Mean	SE	F	P value
DS	Mild	83	3.33	0.390	0.748	0.474
	Moderate	141	2.89	0.253		
	Severe	76	3.38	0.411		
MS	Mild	83	1.02	0.268	1.079	0.341
	Moderate	141	1.24	0.226		
	Severe	76	0.72	0.243		
FS	Mild	83	5.02	0.685	0.308	0.735
	Moderate	141	5.72	0.626		
	Severe	76	5.63	0.612		
DMFS	Mild	83	9.37	0.899	0.087	0.916
	Moderate	141	9.84	0.753		
	Severe	76	9.72	0.756		
DMFT	Mild	83	5.30	0.438	0.461	0.631
	Moderate	141	5.46	0.347		
	Severe	76	5.88	0.412		

N = number, SE = standard error, F = Fisher's exact test
$P \leq 0.01$ (highly significant), $P \leq 0.05$ (significant), and $P > 0.05$ (not significant)

Table 5: Salivary physicochemical characteristics among dental students of mild and severe dental environment stress levels

| Variable | Stress level | | | | | | Statistical test | | |
| | Mild | | | Severe | | | | | |
	N	Mean	SD	N	Mean	SD	T-test	df	P-value
Fr	55	0.804	0.358	40	0.715	0.282	1.299	93	0.197
NO	55	71.273	66.835	40	105.000	70.347	2.375	93	0.020

Fr =flow rate, NO = nitric oxide, SD = standard deviation, df = degree of freedom

Table 6: Correlation coefficient of caries experience (DMFT, DMFS and its components decay surface, missed surface, and filled surface) with salivary physicochemical characteristics according to mild and severe dental environment stress levels

| | | | Correlations | | | | |
Stress level			DS	MS	FS	DMFS	DMFT
Mild	Fr	r	0.002	–0.179	–0.056	–0.110	–0.076
		P	0.989	0.191	0.684	0.424	0.583
	NO	r	–0.089	–0.250	–0.124	–0.204	–0.075
		P	0.519	0.065	0.367	0.136	0.210
Severe	Fr	r	–0.049	–0.074	–0.011	–0.003	–0.018
		P	0.764	0.650	0.948	0.983	0.913
	NO	r	–0.013	0.157	0.095	0.129	0.111
		P	0.935	0.335	0.558	0.426	0.497

Fr = flow rate

Figure 1: Steps of using the nitric oxide saliva test strips (Berkeley test, L2G North America, Chicago, USA)

did not reach statistical significance; same result was found by other studies.[19] This is because anxiety and fear may influence salivary flow through pathways in the amygdala, the hypothalamus, and the brain stem.[20]

The salivary flow rate was negatively correlated with caries experience among students with severe stress level. The value of $P > 0.05$ was not statistically significant, and this agreed with other studies.[9] This

may be because of the important action of salivary flow rate as washing action and the defensive components of saliva that increase with increasing salivary flow rate,[8] whereas among mild stress category, the flow rate was positively correlated with DS, which was statistically not significant ($P > 0.05$); this agreed with other study.[9] This may be related to fact that salivary flow rate flow rate may have little effect on the activity of caries at a specific point in time and determination of salivary flow rate at one-time may not be thorough estimation of salivary flow actions.[21] In this study, the salivary NO was significantly higher among students with severe dental environment stress than those with mild dental environment stress. This finding was in good agreement with that obtained by Gammoh et al.,[22] who found that the salivary NO was high among Iraqi refugees with anxiety and stress in Jordan, indicating an oxidative stress condition and attributed this increase in salivary NO to an upregulation in NO synthases, which are responsible for synthesizing NO. This finding was in good agreement with that obtained from a study by Sundar et al.,[23] who also found increase in salivary NO with increased stress level among patients with periodontitis, whereas Kroll et al.[24] found low levels of exhaled NO in reaction to stress and depression. The correlations of dental caries with NO were negative among students with mild and severe dental environment stress with nonsignificant difference. This result was in good agreement with that of other study.[25] This may be due to the antimicrobial action of NO against caries microorganisms such as Streptococcus mutans.[26] The interactions with other reactive species, such as superoxide, and creation of a highly reactive molecule, peroxynitrite, which can have greater cytotoxic potential than NO.[27] When salivary nitrite came in touch with acid medium (acid generating bacteria like: S. mutans) round the teeth, antimicrobial mass like NO will be created leading to antimicrobial actions. Accordingly, when the nitrite exists in the oral cavity in high concentration, the development and probable existence of acidogenic bacteria is restricted. Therefore, salivary nitrate is a reservoir from which various nitrogen oxides are produced, especially NO.[28,29] In evaluating caries risk, the determination of single salivary antibacterial component is not significant as dental caries is multifactorial. Therefore, further experimental and clinical studies are required to estimate the effect of stress on NO and the anticaries activity of NO.

Conclusion

The results of this study support that the salivary flow rate and NO may serve as a biomarker of stress. Also dental environment stress affect the variable of oral health represented by higher dental caries experience among sever stress group.

Author contributions

Raghad Ibrahim Kadhum Al-Moosawi and Alhan Ahmed Qasim we both approve the final version.

Ethical policy and institutional review board statement

Ethical clearance (approval number: 46, date of approval: 9/1/2019, clinical trial registry number: 813640) was obtained before the start of the study by the scientific committee of College of Dentistry, University of Baghdad (board name: Dr. Akram Alhuwaizi), Baghdad, Iraq. All the procedures have been performed as per the ethical guidelines laid down by the Declaration of Baghdad 2019.

Patient declaration of consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Data availability statement

Data set available for request please contact the following person Raghad Ibrahim. Email: iraghad823@gmail.com

References

1. Babar MG, Hasan SS, Ooi YJ, Ahmed SI, Wong PS, Ahmad SF, et al. Perceived sources of stress among Malaysian dental students. Int J Med Educ 2015;6:56-61.
2. Sorce S, Krause KH. NOX enzymes in the central nervous system: From signaling to disease. Antioxid Redox Signal 2009;11:2481-504.
3. Sies H, Berndt C, Jones DP. Oxidative stress. Annu Rev Biochem 2017;86:715-48.
4. Alhajj MN, Khader Y, Murad AH, Celebic A, Halboub E, Márquez JR, et al. Perceived sources of stress amongst dental students: A multicountry study. Eur J Dent Educ 2018;22:258-71.
5. Gordon NA, Rayner CA, Wilson VJ, Crombie K, Shaikh AB, Yasin-Harnekar S. Perceived stressors of oral hygiene students in the dental environment. Afr J Health Professions Educ 2016;8:20-4.

6. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, *et al*. Dental caries. Nat Rev Dis Primers 2017;3:17030.

7. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: Implications for immunoprotection and immunopathology. Neuroimmunomodulation 2009;16:300-17.

8. Gao X, Jiang S, Koh D, Hsu CY. Salivary biomarkers for dental caries. Periodontol 2000 2016;70:128-41.

9. Abdul-Ameer AK, Radhi NJ, Abdul-Ghani HJ. Stressful life events in relation to dental caries and selected salivary constituents among secondary school students in Baghdad city. J Baghdad College Dent 2017;29:139-47.

10. Mobarak EH, Abdallah DM. Saliva nitric oxide levels in relation to caries experience and oral hygiene. J Adv Res 2011;2:357-62.

11. Chen HJ, Spiers JG, Sernia C, Lavidis NA. Response of the nitrergic system to activation of the neuroendocrine stress axis. Front Neurosci 2015;9:3.

12. Al-Sowygh ZH, Alfadley AA, Al-Saif MI, Al-Wadei SH. Perceived causes of stress among Saudi dental students. King Saud Univ J Dent Sci 2013;4:7-15.

13. Navazesh M, Kumar SK; University of Southern California School of Dentistry. Measuring salivary flow: Challenges and opportunities. J Am Dent Assoc 2008;139:35S-40S.

14. Rantonen P. Salivary flow and composition in healthy and diseased adults [disseration on the internet]. University of Helsinki; 2003 [cited 2019 Sept 22]. Available from: https://helda.helsinki.fi/bitstream/handle/10138/20291/salivary.pdf?sequence=2.

15. Faizal GG, Azizan NI, Ibrahim K, Abllah Z. Diet intake and caries status among final year students in IIUM Kuantan campus. Mater Today Proc 2019;16:2245-52.

16. McEwen BS, Gianaros PJ. Stress- and allostasis-induced brain plasticity. Ann Rev Med 2011;62:431-45.

17. Ghulam O, Tola AW, Al-Saedi MA. Oral hygiene practice and its relation with stress among the adult patient visiting the National Guard Hospital and Military Hospital in Madinah. J Dent Oral Health 2018;5:1-7.

18. Hosseini-Yekani A, Nadjarzadeh A, Vossoughi M, Reza JZ, Golkari A. Relationship between physicochemical properties of saliva and dental caries and periodontal status among female teachers living in central Iran. J Int Soc Prev Community Dent 2018;8:48-55.

19. Bulthuis MS, Jan Jager DH, Brand HS. Relationship among perceived stress, xerostomia, and salivary flow rate in patients visiting a saliva clinic. Clin Oral Investig 2018;22:3121-7.

20. Proctor GB. The physiology of salivary secretion. Periodontol 2000 2016;70:11-25.

21. Thaweboon S, Thaweboon B, Nakornchai S, Jitmaitree S. Salivary secretory IgA, pH, flow rates, mutans streptococci and Candida in children with rampant caries. Southeast Asian J Trop Med Public Health 2008;39:893-9.

22. Gammoh OS, Al-Smadi A, Al-Awaida W, Badr MM, Qinna NA. Increased salivary nitric oxide and G6PD activity in refugees with anxiety and stress. Stress Health 2016;32:435-40.

23. Sundar NM, Julius A, Valiathan M, Raj K, Paddmanabhan P. Correlation of salivary nitric oxide and stress levels in patients with chronic periodontitis—A biochemical study. Drug Invent Today 2019;12:141-147.

24. Kroll JL, Werchan CA, Reeves AG, Bruemmer KJ, Lippert AR, Ritz T. Sensitivity of salivary hydrogen sulfide to psychological stress and its association with exhaled nitric oxide and affect. Physiol Behav 2017;179:99-104.

25. Scoffield J, Michalek S, Harber G, Eipers P, Morrow C, Wu H. Dietary nitrite drives disease outcomes in oral polymicrobial infections. J Dent Res 2019;98:1020-6.

26. Nambu T, Wang D, Mashimo C, Maruyama H, Kashiwagi K, Yoshikawa K, *et al*. Nitric oxide donor modulates a multispecies oral bacterial community—An *in vitro* study. Microorganisms 2019;7:353.

27. Hardingham N, Dachtler J, Fox K. The role of nitric oxide in pre-synaptic plasticity and homeostasis. Front Cell Neurosci 2013;7:190.

28. Hohensinn B, Haselgrübler R, Müller U, Stadlbauer V, Lanzerstorfer P, Lirk G, *et al*. Sustaining elevated levels of nitrite in the oral cavity through consumption of nitrate-rich beetroot juice in young healthy adults reduces salivary pH. Nitric Oxide 2016;60:10-5.

29. Yamamoto Y, Washio J, Shimizu K, Igarashi K, Takahashi N. Inhibitory effects of nitrite on acid production in dental plaque in children. Oral Health Prev Dent 2017;15:153-6.