

# The Relationship Between Stress, Salivary Biomarkers, and Dental Caries Severity in Dental Students: A Clinical and Laboratory Analysis

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## ABSTRACT

**Background:** Dental education is recognized as a significant source of stress, which may influence the oral health of students. This study aimed to evaluate the relationship between stress levels and salivary parameters, including pH, flow rate, and mineral content (calcium, phosphate, and magnesium), and their potential association with dental caries severity among dental students. **Methods:** A cross-sectional study was conducted on dental students categorized into three groups based on their stress levels: mild, moderate, and severe. Saliva samples were collected and analyzed for pH, flow rate, and mineral concentrations. Dental caries severity was assessed using standardized caries indices. Statistical analyses were performed to determine correlations between stress, salivary parameters, and caries severity. **Results:** A significant decrease in salivary pH was observed with increasing stress levels ( $P=0.032$ ). However, no significant associations were found between stress levels and salivary flow rate, calcium, phosphate, or magnesium concentrations ( $P=0.473$ ,  $0.527$ ,  $0.411$ , and  $0.073$ , respectively). The relationship between stress levels and dental caries severity was also non-significant ( $P > 0.05$ ). Despite this, certain correlations between salivary parameters and caries indices were noted within different stress level groups. **Conclusion:** The study highlights a significant inverse relationship between stress levels and salivary pH, suggesting that stress may contribute to adverse oral health outcomes. However, other salivary parameters and dental caries severity showed no direct association with stress levels. These findings emphasize the importance of stress management in promoting better oral health among dental students.

**Keywords:** Caries, Dental Students, Saliva Ph, Saliva Minerals, Stress.

## INTRODUCTION

It is very clear Students in universities are at high risk of exposure to mental health issues and distress [1]. According to studies, colleges are incredibly rigorous and stressful learning environments [2]. Although work stress can lead to a beneficial effect by encouraging motivation, when it exceeds a person's ability to manage, it can have an adverse effect on well-being. It may lead to burnout [3]. Five categories of dental stressors have been identified: the living environment, individual characteristics, academic environment, academic aspects, and clinical variables [4]. Dental caries is one of the disorders that affects people the most often, it is described as "a biofilm-mediated, diet-modulated, multivariate, non-communicable, dynamical disease leading in net demineralization of dental hard tissues"[5]. Whenever a person is exposed to a stressful time, the psychological response to this circumstance activates the sympathetic nervous system and the hypothalamic-pituitary-adrenocortical (HPA) axis, resulting in the secretion of a significant amount of stress hormones, primarily catecholamine and cortisol, which reduce salivary flow and consequently alter the saliva's oxidation-reduction capacity. Reduced salivary flow reduces the preventive properties of saliva, increasing the incidence of dental caries [6, 7]. The study of saliva has generally been critical since saliva contains all of the ions typically seen in human fluids. Saliva's capacity to buffer acids is vital for regulating the oral environment's pH levels. Bicarbonate ions play a significant role in establishing saliva's pH and buffering capacity, which helps protect teeth from acid attacks caused by bacteria. Due to the presence of calcium, phosphorus, and other inorganic ions, saliva also plays a crucial role in preserving the integrity of dental tissues

since this environment is known to promote the remineralization of incipient lesions or demineralized enamel zones. Thus, calcium and phosphorus in saliva constitute a protective barrier against tooth dissolution [8]. The current study aimed to determine the effect of levels of stress in dentistry students on the dental caries index and the saliva's biochemistry.

## MATERIALS AND METHODS

**Participants:** The study included 208 dental students currently enrolled in the dental department at Al-Zahrawi colleges. The sample size was determined according to Nunally and Bernstein [9].

**Data Collection:** The participants completed a standardized survey to assess their stress levels according to the self-answer Dental Environment Stress (DES) questionnaire [10, 11], and caries experience was determined according to the WHO standards [12]. Saliva samples were collected from each participant using standard collection techniques and expressed as milliliters per minute (mL/min). The samples were analyzed for pH (using a digital pH meter) and mineral content, including calcium, phosphate, and magnesium, using Mindray BS-120 automated biochemistry analyzer (China).

**Data Analysis:** The mineral content data and survey results were analyzed using SPSS 22 (IBM, USA) to determine the relationship between salivary mineral properties, stress levels, and dental caries severity.

Ethical Approval: The study has been granted ethical approval by the Al-Zahravi college committee, ensuring that it meets the necessary standards of research ethics involving human subjects. A consent form was acquired from each of the participant before their involvement in the study.

## Results

The distribution of subjects participating in the study is described in table 1. The participants included in the study were 48 (23.76%) 4<sup>th</sup>-grade and 154 (76.24%) 5<sup>th</sup>-grade dentistry students and did not show a significant difference when subdivided according to stress level into mild, moderate, and severe ( $\chi^2=3.201$ ,  $p=0.202$ ), they were also statistically non-significant when distributed according to gender ( $\chi^2=6.500$ ,  $p=0.051$ ).

Table 2 describes the mean levels of salivary analysis parameters distributed according to the stress level. It showed that pH levels were significantly higher ( $p=0.032$ ) in students with mild stress levels ( $6.288\pm0.097$ ) compared to moderate ( $5.978\pm0.100$ ) and severe ( $5.883\pm0.103$ ) stress levels, while other parameters (Flow rate, Ca,  $PO_4$ , and Mg) did not show a significant difference. Caries severity is described in table 3.

The results did not show a significant difference in caries severity when distributed according to stress level. The classification of the caries experience index (DMFT, DMFS, and its components DS, MS, and FS) is described in table 4. The results did not show a significant difference in DMFT and DMFS ( $p=0.777$  and  $0.930$  respectively), and also in DS, MS, and FS ( $p=0.781$ ,  $0.074$ , and  $0.830$  respectively).

**Table 1:** Distribution of Subjects by Study Grade, Gender, and Stress Level

| Variables   | Category        |          | N   | %     | Chi-Square | P Value     |
|-------------|-----------------|----------|-----|-------|------------|-------------|
| Study Grade | 4 <sup>th</sup> | Mild     | 14  | 29.17 | 3.201      | 0.202<br>NS |
|             |                 | Moderate | 22  | 45.83 |            |             |
|             |                 | Severe   | 12  | 25.00 |            |             |
|             |                 | Total    | 48  | 23.76 |            |             |
|             | 5 <sup>th</sup> | Mild     | 34  | 22.08 | 6.500      | 0.051<br>NS |
|             |                 | Moderate | 60  | 38.96 |            |             |
|             |                 | Severe   | 60  | 38.96 |            |             |
|             |                 | Total    | 154 | 76.24 |            |             |
| Gender      | Males           | Mild     | 30  | 25.86 |            |             |
|             |                 | Moderate | 56  | 48.28 |            |             |
|             |                 | Severe   | 30  | 25.86 |            |             |
|             |                 | Total    | 116 | 57.43 |            |             |
|             | Females         | Mild     | 18  | 20.93 |            |             |
|             |                 | Moderate | 26  | 30.23 |            |             |
|             |                 | Severe   | 42  | 48.84 |            |             |
|             |                 | Total    | 86  | 42.57 |            |             |
| Total       |                 | Mild     | 48  | 23.76 |            |             |
|             |                 | Moderate | 82  | 40.59 |            |             |
|             |                 | Severe   | 72  | 35.65 |            |             |

**Table 2:** Descriptive and Statistical Test of Salivary Analysis among Stress Levels.

| Variables | Mild |       |                | Mod. |       |                | Severe |       |                | F     | P     |
|-----------|------|-------|----------------|------|-------|----------------|--------|-------|----------------|-------|-------|
|           | N    | Mean  | Standard Error | N    | Mean  | Standard Error | N      | Mean  | Standard Error |       |       |
| PH        | 24   | 6.288 | 0.097          | 41   | 5.978 | 0.100          | 36     | 5.883 | 0.103          | 3.575 | 0.032 |
| Flow rate | 24   | 1.467 | 0.159          | 41   | 1.698 | 0.125          | 36     | 1.717 | 0.152          | .753  | 0.473 |
| Ca        | 17   | 3.862 | 0.246          | 33   | 4.003 | 0.165          | 25     | 4.195 | 0.186          | .647  | 0.527 |
| $PO_4$    | 17   | 6.955 | 0.651          | 33   | 6.487 | 0.407          | 25     | 7.358 | 0.493          | .900  | 0.411 |
| Mg        | 17   | 1.689 | 0.022          | 33   | 1.743 | 0.010          | 25     | 1.744 | 0.022          | 2.714 | 0.073 |

**Table 3:** Descriptive and Statistical Test of Caries Severity among Stress Levels.

| Vars. | Mild |                | Moderate |                | Severe |                | F     | P     |
|-------|------|----------------|----------|----------------|--------|----------------|-------|-------|
|       | Mean | Standard Error | Mean     | Standard Error | Mean   | Standard Error |       |       |
| D1    | 3.33 | 0.378          | 3.73     | 0.335          | 3.25   | 0.322          | 0.623 | 0.537 |
| D2    | 1.88 | 0.361          | 2.07     | 0.275          | 2.11   | 0.314          | 0.135 | 0.874 |
| D3    | 0.37 | 0.192          | 0.63     | 0.073          | 0.53   | 0.103          | 1.292 | 0.277 |
| D4    | 1.00 | 0.462          | 1.13     | 0.229          | 0.81   | 0.324          | 0.230 | 0.795 |

**Table 4:** Mean Levels of caries Experience Index According to Stress Levels.

| Vars. | Mild  |       | Moderate |       | Severe |       | F     | P Value |
|-------|-------|-------|----------|-------|--------|-------|-------|---------|
|       | Mean  | SE    | Mean     | SE    | Mean   | SE    |       |         |
| DS    | 6.96  | 0.607 | 7.29     | 0.563 | 6.75   | 0.577 | 0.247 | 0.781   |
| MS    | 0.51  | 0.445 | 0.97     | 0.234 | 1.58   | 0.307 | 2.911 | 0.074   |
| FS    | 3.46  | 0.619 | 3.68     | 0.647 | 4.03   | 0.574 | 0.187 | 0.830   |
| DMFS  | 10.93 | 0.912 | 11.94    | 0.763 | 12.36  | 0.870 | 0.072 | 0.930   |
| DMFT  | 10.99 | 0.896 | 11.88    | 0.725 | 11.54  | 0.877 | 0.253 | 0.777   |

Table 5 illustrates the correlation between salivary parameters and caries severity. The results of flow rate showed a significantly correlated with caries severity (D4) positively ( $r=0.509$ ,  $p=0.001$ ) in moderate

stress level and a positive correlation with D1 ( $r=0.442$ ,  $p=0.007$ ) in severe stress level. Levels of  $PO_4$  were correlated with caries severity negatively (D2) ( $r=-0.451$ ,  $p=0.024$ ).

**Table 5:** Correlation between Salivary Analysis and Caries Severity by Stress Levels.

| Stress level |           | D1     |       | D2     |       | D3     |       | D4     |       |
|--------------|-----------|--------|-------|--------|-------|--------|-------|--------|-------|
|              |           | R      | P     | R      | P     | R      | P     | R      | P     |
| Mild         | Flow Rate | 0.111  | 0.606 | -0.049 | 0.822 | -0.058 | 0.788 | -0.003 | 0.987 |
|              | PH        | -0.255 | 0.229 | 0.353  | 0.091 | 0.013  | 0.953 | -0.162 | 0.448 |
|              | Ca        | 0.249  | 0.335 | 0.136  | 0.604 | -0.132 | 0.612 | 0.206  | 0.427 |
|              | $PO_4$    | 0.019  | 0.944 | 0.269  | 0.296 | 0.172  | 0.508 | 0.489  | 0.047 |
|              | Mg        | -0.088 | 0.737 | -0.289 | 0.261 | 0.191  | 0.463 | 0.245  | 0.344 |
| Mod.         | Flow Rate | -0.003 | 0.983 | 0.253  | 0.110 | 0.280  | 0.076 | 0.509  | 0.001 |
|              | PH        | -0.047 | 0.770 | 0.054  | 0.739 | -0.103 | 0.523 | 0.043  | 0.789 |
|              | Ca        | -0.055 | 0.761 | -0.209 | 0.242 | -0.027 | 0.883 | 0.224  | 0.211 |
|              | $PO_4$    | 0.222  | 0.214 | 0.041  | 0.821 | -0.012 | 0.949 | 0.066  | 0.714 |
|              | Mg        | -0.083 | 0.648 | -0.019 | 0.915 | 0.144  | 0.424 | 0.188  | 0.295 |
| Severe       | Flow Rate | 0.440  | 0.007 | -0.003 | 0.986 | 0.028  | 0.872 | 0.002  | 0.989 |
|              | PH        | -0.128 | 0.456 | 0.002  | 0.993 | 0.192  | 0.263 | 0.070  | 0.687 |
|              | Ca        | 0.120  | 0.567 | -0.161 | 0.441 | 0.098  | 0.642 | -0.016 | 0.940 |
|              | $PO_4$    | -0.261 | 0.207 | -0.451 | 0.024 | 0.215  | 0.302 | 0.362  | 0.075 |
|              | Mg        | 0.098  | 0.643 | -0.133 | 0.528 | -0.025 | 0.906 | 0.033  | 0.874 |

When comparing salivary parameters to caries experience (Table 6), the Flow rate is shown to correlate negatively with the DS index ( $r=-0.398$ ,  $p=0.010$ ) at moderate stress level. pH levels are negatively

correlated with the MS index ( $r=-0.420$ ,  $p=0.006$ ) at the moderate stress level. In severe stress levels, calcium is shown to be positively correlated with the DS index ( $r=0.483$ ,  $p=0.004$ ).

**Table 6:** Correlation between Salivary Parameters and Caries Experience by Stress Levels.

| Stress level |           | DS     |       | MS     |       | FS     |       | DMFS   |       | DMFT   |       |
|--------------|-----------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|
|              |           | R      | P     | R      | P     | R      | P     | R      | P     | R      | P     |
| Mild         | Flow Rate | 0.019  | 0.929 | -0.067 | 0.757 | 0.070  | 0.744 | 0.028  | 0.897 | 0.018  | 0.933 |
|              | PH        | -0.069 | 0.749 | -0.012 | 0.954 | 0.125  | 0.561 | 0.033  | 0.879 | 0.006  | 0.979 |
|              | Ca        | 0.303  | 0.237 | 0.188  | 0.469 | 0.134  | 0.609 | 0.374  | 0.139 | 0.378  | 0.135 |
|              | $PO_4$    | 0.453  | 0.068 | 0.185  | 0.478 | -0.202 | 0.436 | 0.193  | 0.458 | 0.223  | 0.390 |
|              | Mg        | -0.060 | 0.820 | 0.158  | 0.544 | -0.156 | 0.551 | -0.085 | 0.746 | -0.175 | 0.501 |
| Mod.         | Flow Rate | -0.398 | 0.010 | 0.062  | 0.700 | -0.073 | 0.651 | 0.245  | 0.122 | 0.230  | 0.148 |
|              | PH        | -0.012 | 0.940 | -0.420 | 0.006 | -0.033 | 0.837 | 0.062  | 0.698 | 0.093  | 0.563 |
|              | Ca        | -0.053 | 0.770 | -0.150 | 0.406 | 0.120  | 0.245 | 0.203  | 0.257 | 0.124  | 0.492 |
|              | $PO_4$    | 0.152  | 0.398 | -0.104 | 0.566 | -0.005 | 0.979 | 0.092  | 0.609 | 0.002  | 0.990 |
|              | Mg        | 0.095  | 0.601 | -0.210 | 0.241 | -0.286 | 0.107 | -0.144 | 0.426 | -0.074 | 0.681 |
| Severe       | Flow Rate | 0.258  | 0.129 | -0.037 | 0.832 | -0.318 | 0.058 | -0.052 | 0.764 | -0.040 | 0.818 |
|              | PH        | 0.020  | 0.909 | 0.031  | 0.855 | -0.033 | 0.849 | 0.002  | 0.988 | 0.031  | 0.857 |
|              | Ca        | 0.483  | 0.004 | -0.038 | 0.856 | 0.229  | 0.270 | 0.095  | 0.650 | 0.138  | 0.510 |
|              | $PO_4$    | -0.048 | 0.900 | 0.245  | 0.238 | -0.094 | 0.657 | -0.010 | 0.962 | 0.001  | 0.997 |
|              | Mg        | 0.001  | 0.997 | -0.002 | 0.993 | 0.485  | 0.014 | 0.248  | 0.232 | 0.264  | 0.202 |

## Discussion

Stress, an intricate and multifaceted phenomenon, has been metaphorically characterized as a double-edged sword, possessing the capability to either invigorate and propel students toward optimal performance or diminish their effectiveness. Many prior research endeavors have revealed that dental education imposes substantial stress upon students [13-15]. These findings manifest as near-universal occurrences in various nations, irrespective of the distinct educational systems and curricula employed. The complete elimination of stress-inducing factors within dental education programs remains a formidable challenge. In order to develop into conscientious dental practitioners, students are required to attain elevated levels of knowledge and expertise, in addition to nurturing

benevolent dispositions towards the care of patient, all within a restricted time frame [16]. The current study aimed to investigate the correlation between stress levels in dentistry students and salivary parameters, including pH, flow rate, calcium (Ca), phosphate ( $PO_4$ ), and magnesium (Mg). Results of the current study indicated a significant inverse relationship ( $p=0.032$ ) between stress levels and salivary pH, which is in agreement with previous studies [17]. However, no significant correlation was found between stress levels and salivary flow rate, calcium, phosphate, or magnesium levels. The relationship between dental caries severity and stress levels was also non-significant. Our findings are consistent with those of Abdul-Ameer et al. [18] and Kadhum and Qasim [7], who reported that dental environment stress does not have a significant impact on caries experience or salivary flow rate [19]. Similarly, Al-Moosawi and Qasim

demonstrated that salivary flow rate does not correlate with stress levels [20]. The results of our study are also in line with these findings, as there was no significant difference in flow rate between the groups with mild, moderate, or severe stress. The negative association between stress levels and salivary pH found in the current study supports the notion that increased stress might negatively affect oral health. A decreased salivary pH can result in a more acidic oral environment, which could contribute to dental caries formation [20]. However, the current study did not find a statistically significant association between stress levels and dental caries severity. This suggests that other factors may play a more significant role in caries formation or that the relationship between stress and dental caries is more complex than previously thought. There are several proposed mechanisms that could explain the observed inverse relationship between stress levels and salivary pH.

One possible explanation is that stress may influence the autonomic nervous system, resulting in changes to the salivary gland function and secretion, ultimately leading to a decrease in pH [21]. Another potential mechanism is that stress could lead to increasing the cortisol levels, that which might alter the composition of saliva and contribute to the decrease in pH [22]. Furthermore, stress could impact oral health through indirect pathways. For example, stress has been shown to influence oral hygiene practices, dietary habits, and general health, all of which can indirectly affect oral health and dental caries risk. The complex interplay between stress and these factors could partly explain the lack of significant correlation between stress levels

and dental caries severity observed in our study. Interestingly, our study found some specific correlations between salivary parameters and dental caries indices under different stress levels. For example, the flow rate was observed to positively correlate with D4 at moderate stress levels ( $r = 0.509$ ,  $p = 0.001$ ) and with D1 at severe stress levels ( $r = 0.442$ ,  $p = 0.007$ ), while it negatively correlated with the DS caries experience index at moderate stress levels ( $r = -0.398$ ,  $p = 0.010$ ). Furthermore, calcium levels were observed to correlate positively with the DS index ( $r = 0.483$ ,  $p = 0.004$ ), and  $PO_4$  levels were negatively correlated with D2 ( $r = -0.451$ ,  $p = 0.024$ ) at severe stress levels.

These findings suggest that there might be some specific relationships between salivary parameters and dental caries indices under certain stress conditions, which warrants further investigation. It is important to note that although the current study did not find a significant association between stress levels and dental caries severity, it does not rule out the possibility that stress might still have an impact on oral health through other mechanisms. Instance, stress-induced changes in immune function could potentially contribute to an increased susceptibility to oral infections and inflammation, which in turn could impact dental caries risk [23, 24]. In conclusion, our study identified a notable negative connection between stress levels and salivary pH, which supports the idea that stress can adversely affect oral health. However, we did not find any significant relationships between stress levels and other salivary factors or the severity of dental caries. This implies that the link between stress and dental caries could be more complex than previously assumed.

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