Stressful Life Events In Relation to Dental Caries and Selected Salivary Constituents Among Secondary School Students in Baghdad City

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ABSTRACT

Background: Stress is the reactivity of the body to forces of a deleterious nature, infections and various abnormal states that tend to disturb its normal physiological equilibrium; it is described as adverse emotions or reactions to unpleasant experiences; thus, any real or perceived physical, social, or psychological event or stimulus that causes bodies to react or respond have deleterious effects on the general and oral health. The aims of this study were to assess the severity of dental caries among the students with different categories of stressful life events in relation to physiochemical characteristics of whole stimulated saliva.

Materials and Methods: The total sample is composed of 300 students (males only) aged 17-18 years old, who are randomly selected from 10 school in the First Al-Karkh/Baghdad. The total sample classified into three categories (less stress, more stress, accumulative stress) according to stressful life events scale (SLE); the sub sample consist of 60 students who are randomly selected from the total sample each category composed of 20 students. Diagnosis and recording of dental caries was assessed according to Decay, Missed, Filled surface index (DMFS) of Muhlemann (1976). Stimulated salivary samples were collected from the 60 students then measuring salivary flow rate; and chemically analyzed to determine salivary interlukin-6 (IL-6), cortisol, and total protein. All data were analyzed using SPSS version 21.

Results: Results recorded the highest mean rank value of [DMFS] was among the accumulative stress category of stressful life events scale followed by more stress category, the lowest mean rank value was among less stress category with statistically high significant difference (P<0.001). For the severity of dental caries (DS) Fraction was higher among the accumulative stress category than more stress and less stress categories respectively (P<0.001). According to the grades of lesion severity, for all fractions the higher mean rank values was among accumulative stress category with no statically differences except for (DS) was significant (P<0.05). The data from salivary analysis showed that, the highest values of salivary total protein and (IL-6) were among the accumulative stress category, followed by more stress then the lowest value among less stress category, while the cortisol showed the opposite picture, however all these differences were not significant. DMFS correlated positively with flow rate among less stress and accumulative stress categories and negatively with more stress category, while for (DS) correlated negatively with flow rate with highly significant for more stress category and accumulative category and positively related with significant for low stress category. The salivary constituents showed negative correlation with (DMFS) for all categories of stressful life events scale except for IL-6 and cortisol were positively correlated for accumulative category with non-significant difference.

Conclusion: The study revealed that, stressful life events have a significant deleterious impact on the oral and dental health including caries experience as well as the effect on the normal levels of salivary constituents.

Keywords: Stressful life events, Stress, Dental caries, Cortisol, Total Protein, IL-6.

INTRODUCTION

Stress is the most important concept that underlines all the attempts to understand the influences of social experience, events and physiological factors on the general health and oral health (1). Stress was originally derived from the Latin word “stringi” which means, “to be drawn tight”. It is “an inharmonious fit between the person and the environment, one in which the person’s resources are taxed or exceeded, forcing the person to struggle, usually in complex ways, to cope” (2). One of the theories that try to explain influence of stress on health revealed that, if people perceive an event as more than they could deal with and exceed the resources available to them, then only at this point do they perceive stress that could lead to disease (3). A subject exhibits stress response or not depends upon the factors, including coping behaviors, genetic predisposition, concomitant stressors, level of social support and their lifestyle factors (4). There are two kinds of stress: acute and chronic: Acute stress (Short-term stress) is typically intense, flares quickly, and disappears quickly such us stress response that prepare the immune system for challenges like infection imposed by stressor. Although chronic stress may not feel as intense, it can linger indefinitely and wreak silent havoc on body’s systems (5). In fact, stressful life events across multiple domains have been shown to increase over the course of adolescence (6). This has been associated with increases in depression, anxiety, low self-esteem, and behavioral
problems (7). Stressful life events detract from self-care behavior, consequently, the existence leads to the neglect of the duties of the medical care, including oral health (8). Some health psychologists have studied the effects of significant life events, people who have had major life changes (loss of spouse or other close relative, loss of job) have a higher incidence of cardiovascular diseases and early death than those who do not (9). Other researcher had evaluated the effects of clusters of life events and their possible influence on mental and physical health (10). Because of the circumstances experienced by the people in the Iraqi environment, particularly adolescents it was necessary to adopt an accurate scale to measure the relationship of these non-natural events with oral health; so the researcher adopted stressful life events scale (SLE), which is specific scale designed to measure the stress of life events by using questioners which consisted of (30) items distributed to four categories (Loss, Social rejection, Poverty, Loss of safety), for each event two alternatives (passed, not passed), so the students have to answer for each event pass or not (11). Adolescents face various transitions and challenges, including puberty, poverty, schools changes, investment in peer, and changes in relationships with family (12). However, each of these events has the potential to become sources of stress; in general, adolescence is considered important for oral health, because majority of individuals during this period have tendency to gain independence in making personal and diet related choices. Moreover, Iraqi Children had been exposed to fighting in the streets, passing dead bodies on the way to school, seeing relatives and friends killed or severely injured, and other actions of war and occupation (13). Psychological health is recognized as a key public health issue for conflict- affected population (14). Dental caries is one of the most prevalent oral diseases of public health concern affecting all ages including adolescents (15). Oral health behaviors are established and habits are formed during adolescence, heavily influenced by the social environment related to peers (16). The relation between stress and increased susceptibility of dental caries was demonstrated in a several studies (1,3,17,18). The protective functions of saliva and the association between oral disease and psychological factors is considered. It is surprising how psychological stress can alter the defense systems in saliva. Psychological stress produce change in salivary flow and components (19). The relation between stress and salivary flow rate was controversy, some studies showed that the salivary flow rate does not change during stress responses (20), while other found that reduce salivary flow rate in relation to psychological conditions (21). On the other hand many studies suggested that salivary total protein level increase in response to psychological stress by number of mechanisms (22,23,24). Saliva is a mirror to the general health condition that reflects various systemic changes in the body (25).Where the composition of saliva immediately reflects the sympathetic and parasympathetic nervous systems, hypothalamic-pituitary-adrenal (HPA) axis and immune system response to stress (26); levels of salivary IL-6 and other proinflammatory cytokines can be directly stimulated by stressful experiences and depression other negative emotions. Indeed, both physical such as exercises and psychological stressors can provoke increases in proinflammatory cytokines (27). Cytokines are closely associated with the pathogenesis of inflammation in oral soft tissues (28,29), and evidence indicates that they contribute to the initiation and progression of dental caries (30). Saliva contains free, biologically active cortisol as opposed to total cortisol present in serum or plasma and the concentration of cortisol in saliva is independent of the salivary flow rate (31,32). Salivary cortisol is capable of affecting local, mucosal immunity and oral microbial flora, and that mucosal immune competence affects bacterial colonization and growth (33). It affects the performance of some immune system cells (34). Basal salivary cortisol and cariogenic bacteria were the strongest predictors of dental caries, and from a theoretical perspective, salivary cortisol could plausibly suppress mucosal immunity against cariogenic bacteria (35,36). In Iraq, several studies were conducted regarding the relation between dental disease with salivary total protein, cortisol and interleukine-6. Yet, no previous Iraqi study conducted to search stressful life events in relation to dental caries, salivary total protein, cortisol and interleukin 6 among 17-18 years old secondary school students in Baghdad City/Iraq. For all these explanations, this study was designed.

MATERIALS AND METHODS

The sample consist of 300 students (males only) aged 17-18 years old, who are randomly selected from 10 secondary school in the First Al-Karkh/Baghdad. Oral examination and stressful life events were recorded for all students by mean of self recorded questionnaires
using stressful life events scale (SLE) which designed for this purpose, this scale characterized by validity and be appropriate for the Iraqi environment and able to measure different and various types of stress \(^{(1)}\). For salivary analysis, sub sample of 60 students (20 students selected randomly from each category). Caries severity was diagnosed by decay, missing, filling surface index (D\(_{1,2,3}\)MFS) according to criteria suggested by Muhlemann \(^{(37)}\). Plane mouth mirror and dental explorer were used. The collection of stimulated saliva from the total sample (300 students) were performed before the students answering questionnaire of (SLE) scale and under standard condition following instruction cited by Tenovuo and Lagerl \(^{(38)}\). Each participant was asked to sit in comfortable position and chew a piece of Arabic gum (0.5-0.7 gm) for one minute then all saliva was removed from mouth by expectoration, after that chewing was continued for 10 minute with same piece of gum and the salivary samples was collected in sterile screw capped tubes. Salivary flow rate was determined before centrifuged the samples after the foam was faded by dividing the total volume of collected saliva (stimulated) in milliliter on the total time of saliva collection in minute (ml/min) \(^{(39)}\). The samples of saliva were taken to the laboratory for centrifuge at 3000 rpm for 15 min; then the clear supernatant was separated by micropipette and was divided to 3 portions, stored at (-25 C) in a deep freeze until the time of biochemical analysis in the Teaching Laboratories in the Medical City Hospital and Poisoning Center. Total protein (mg/dl) determined by colorimetric method, Syrbio kit was used according to the manufactured instructions \(^{(40)}\). For determination of IL-6 in saliva, commercially available ELISA was used and performed as recommended in leaflet with kit (Salimetrics Company, USA) \(^{(41)}\). Measurement of salivary cortisol was done by means of High sensitivity, salivary cortisol enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding (Demeditec Company, Germany) \(^{(42)}\). Non-normally distributed variables were conveniently presented by median and mean rank. The Kruskal-wallis test was used to assess the statistical significance of difference between more than 2 groups. The Spearman linear correlation was used to assess the direction, strength and statistical significance of linear correlation between 2 quantitative variables, one of which being non-normally distributed. Data analysis was conducted by application of SPSS program version 21.

RESULTS

Table (1) represents caries experience (D\(_{1,2,3}\)MFS) according to categories of stressful life events. The highest mean rank value was among accumulative stress category followed by more stress category and less mean rank value was among less stress category with statistically highly significant difference (P< 0.001); the same picture was obtained for (DS) fraction. Mean rank values of MS component not significantly differs among the three groups of scale with lower mean rank among less stress group of students and higher for more stress group. For FS component, the accumulative stress group has mean rank value higher than other two groups followed by less stress group then the more stress group value. However, all these differences were not significant (P> 0.05). Table (2) illustrates the grades of caries severity (D\(_{1,2,3}\)) among study groups. The mean rank of all grades of lesion severity were higher among students with accumulative stress category of scale with no significant difference (P> 0.05) except the result of D\(_2\) since it shows higher mean rank among the students with more stress with statistically significant difference. Table (3) demonstrated mean rank values of salivary constituents among students according to stressful life events scale (SLE). Mean values of total protein were more among the students in accumulative stress category, followed by more stress category and least values for less category. Moreover, the same result was found for IL-6, while for salivary cortisol the opposite result was noticed; regarding salivary flow rate, mean rank values differ slightly among the students in all categories of (SLE) scale, with higher mean rank values among the students with accumulative stress category, followed by more stress and less stress respectively, however, all these differences were statistically not significant (P> 0.05). Table (4) demonstrated the correlation coefficient between salivary flow rate and caries experience according to the categories of (SLE) scale. DMFS in addition to caries severity (DS) represented by grades of lesion severity (D\(_1,2,3,d_4\)) were weak and positive correlated with flow rate among the students of less stress category; however all these correlations were not significant (P> 0.05) except for (DMFS, DS), it was significant; while among the more stress category negative correlations were noticed between flow rate and D\(_1, d_2,\) DS, and DMFS with statistically significant. The opposite result was noticed concerning D\(_1\) and D\(_4\) as a weak

**RESULTS**

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positive not significant correlation with flow rate (P> 0.05). Concerning the accumulative stress category, weak negative and not significant correlations for D2, D3 and DS with flow rate except it was significant for D1, while for D1 and DMFS, positive weak correlations were found. However all these correlations were found be not significant (P > 0.05). Table (5) illustrates the correlation coefficient between caries experience (DS, DMFS) and salivary constituents according to categories of (SLE) scale. Results revealed weak negative not significant correlation between salivary total protein and (DMFS) for all categories, while for (DS), the opposite results was found, all these correlations were not significant (P > 0.05). Concerning salivary IL-6, the correlations were weak and not significant except for accumulative stress category it was weak positive, for (DS), the correlations were weak positive not significant except for less stress it was negatively correlated. Regarding salivary cortisol, for all the correlations were in negative direction and significant only for (DMFS) among less stress, while for accumulative stress category it was weak positive. Table (6) demonstrates the correlation coefficient between total protein and salivary constituents according to categories of (SLE) scale. For less stress, correlations were weak and in positive direction except a negative correlations with IL-6 was noticed with statistically not significant (P > 0.05). Concerning more stress category, the direction was positive except for flow rate was in negative direction, with only highly significant correlation with IL-6. For accumulative stress category, the direction of correlations were positive except for cortisol it was negative, with statistically not significant (P > 0.05).

**DISCUSSION**

Stress may tend to have a negative effect on immunological response of body, which represents an important example of mind body interaction that leading to an imbalance between host and microorganism which influence oral environments and subsequent development oral diseases (8,43). In this study, the experience of dental caries among accumulative stress category was significantly higher than other categories of (SLE) and the increased dental caries among stressful individuals agrees with other study (3,18). This could be attributed to different bacterial levels were found under stress and relaxation conditions, which supports the concept that stress may contribute to dental caries and relaxation may have an anticariogenic effect by affecting the immune system and compromising host resistance to cariogenic bacteria by increasing salivary catecholamines and corticosteroids (23); also unhealthy emotional eating habits usually associated with stressful individual leading to frequent snacking and more intake of sugar containing diet (17,44). By impaired performance of self-care habits and neglect oral hygiene measure leading to poor oral hygiene creating unfavorable environment mainly for cariogenic bacteria (45). Also reducing salivary secretion may greatly influence the ability of saliva to flush microorganisms (cariogenic bacteria), substrates and maintain oral cleanliness which leads to subjective oral dryness (46), even if the results of cortisol and salivary flow rate were almost equal between three categories, the decrease in salivary flow rate and increase in the cortisol level among the students of accumulative category have been occurred and return to normal level, because of this study measured chronic effects of stress events and situations before six months ago, also the effect of psychological stress on salivary cortisol concentrations was transient and gradually decreases after stressor, same results found by (47); since dental caries is a chronic disease, it is likely that there was a role for salivary constituents change in progression of disease. Although the mean rank of salivary flow were found in the present study to be not significantly differ among different categories but still salivary flow rate was in significant negative correlation with (D1 and D2) and highly significant with (DS) among students with more stress, this results in line with other studies (48,49). This could be attributed to that higher the flow rate, faster the clearance, higher the buffer capacity and prevention of oral infection and dental caries (50). While among less stress category, flow rate was significant positive correlation with (DS and DMFS), this could be attributing to normal caries experience among students with less stress and adolescents in general with relatively unchanged flow rate , in addition, flow rate may have little bearing on caries activity at a specific point in time and one-time determination of salivary flow rate may not be a comprehensive evaluation of salivary flow functions (51). Salivary total protein was found positively related to caries severity (DS), the same results were found in other studies (52); while other study found that total protein related negatively with dental caries experience (DMFS) (48). However, Total salivary proteins may have both protective and detrimental properties. Thus, salivary proteins...
can be known as “double edged” swords, adhesins and agglutinins play a detrimental role by increasing the colonization of microorganisms (55). Moreover, when total protein level of saliva elevated, which leads to elevated saliva viscosity and reduced saliva quantities which in turn lead to decrease salivary cleansing action of saliva (54). Other explanation for the increase in caries experience with increase SLE could be due to increase in salivary interleukin-6 (IL-6) among students with accumulative stress category, this result line with other study (30). Cytokines are important in immunity and inflammation (55), however, it is known that proinflammatory cytokines play role in molecular mechanism connected to dental caries, but the details remain unexplained. However, IL-6 correlated positively with dental caries experience (DMFS) and caries severity (DS) in accumulative and more stress category, this result was parallel with other studies (30,56). On the other hand, chronic stressful life events are risk factors for contracting depression, the pathophysiology of which is strongly associated with impairments in serotonergic (5-HT) neurotransmission (57), which is later linked to the high carbohydrate intake. Salivary cortisol reported to be negatively correlated with (DS and DMFS), this result agree with other studies (59,60), while among accumulative stress (DMFS) was positive correlation, which agree with other studies (36,60); however, all these differences were not significant. The current study revealed a highly significant positive correlation between salivary total protein and IL-6 among more stress and accumulative stress categories this result in line with other study (61). IL-6 initiates and up-regulates inflammation, triggers the release of acute phase proteins (62), however, the exact mechanism by which total salivary protein level increase in relation to IL-6 in saliva was not clear, but this relation may be attributed firstly, to that salivary total protein level increase in response to psychological stress by numbers of mechanism (22,23,24). However, concerning the relations between salivary constituents, IL-6 was reported to be negatively correlated with cortisol, this results in line with other study (63), which may be attributed to repeated episodes of acute or chronic stress can cause a state of Allostatic Load (64). While the result of present study showed that a weak negative relation between salivary cortisol and total protein this in line with other studies (23,58), this could be attributed to high catecholamine levels are associated with stress, which can be induced from psychological reactions or environmental stressors, which is in turn effects on saliva secretion by significantly reduces the total protein and α-amylase concentration (65). Moreover, salivary cortisol and total protein α-amylase changes are independent to each other.

REFERENCES

Table (1): Caries experience (DMFS) and its components (DS, MS, FS) (Median, Mean Rank) according to categories of Stressful Life Events scale.

** Highly significant P ≤ 0.01

<table>
<thead>
<tr>
<th>Stressful Life Events scale categories</th>
<th>Low level stress</th>
<th>More stress</th>
<th>Accumulative stress</th>
<th>Statistical differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Median</td>
<td>Mean Rank</td>
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<td>Median</td>
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<tr>
<td>---</td>
<td>---</td>
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</tr>
<tr>
<td>DS</td>
<td>8</td>
<td>118.9</td>
<td>10</td>
<td>158.3</td>
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<tr>
<td>MS</td>
<td>0</td>
<td>146.0</td>
<td>0</td>
<td>153.0</td>
</tr>
<tr>
<td>FS</td>
<td>0</td>
<td>155.6</td>
<td>0</td>
<td>144.4</td>
</tr>
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</table>

Table (2): Caries experience represented by grades of Lesion severity (D1-D4) (Median, Mean Rank) according to categories of Stressful Life Events scale.

*Significant P ≤ 0.05

<table>
<thead>
<tr>
<th>Stressful Life Events scale categories</th>
<th>Low level stress</th>
<th>More stress</th>
<th>Accumulative stress</th>
<th>Statistical differences</th>
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<td>Mean Rank</td>
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<td>Median</td>
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<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>D1</td>
<td>3</td>
<td>144.8</td>
<td>3</td>
<td>152.8</td>
</tr>
<tr>
<td>D2</td>
<td>3</td>
<td>132.4</td>
<td>4</td>
<td>160.5</td>
</tr>
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<td>D3</td>
<td>1</td>
<td>140.4</td>
<td>1</td>
<td>152.9</td>
</tr>
<tr>
<td>D4</td>
<td>0</td>
<td>141.0</td>
<td>0</td>
<td>150.7</td>
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Table (3): Salivary constituents and flow rate (Median, Mean Rank) according to categories of Stressful Life Events scale.

<table>
<thead>
<tr>
<th>Stressful Life Events scale categories</th>
<th>Low level stress</th>
<th>More stress</th>
<th>Accumulative stress</th>
<th>Statistical differences</th>
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</thead>
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<td>Mean Rank</td>
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<td>Median</td>
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<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Total protein (mg/dl)</td>
<td>52.84</td>
<td>27.6</td>
<td>55.29</td>
<td>29.8</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.635</td>
<td>29.3</td>
<td>4.45</td>
<td>29.6</td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>2.880</td>
<td>31.6</td>
<td>2.74</td>
<td>30.5</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td>0.84</td>
<td>30.2</td>
<td>0.9</td>
<td>30.5</td>
</tr>
</tbody>
</table>
**Highly significant P ≤ 0.01**

**Significant P ≤ 0.05**

**Highly significant P ≤ 0.01**

**Significant P ≤ 0.05**

**Highly significant P ≤ 0.01**

**Significant P ≤ 0.05**

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**Significant P ≤ 0.05**

**Highly significant P ≤ 0.01**

**Significant P ≤ 0.05**

**Highly significant P ≤ 0.01**

**Significant P ≤ 0.05**

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**Table 4:** Correlation coefficient of salivary flow rate with caries experience (DMFS and DS) and grades of lesion severity according to categories of Stressful Life Events scale.

<table>
<thead>
<tr>
<th>Stressful Life Events scale categories</th>
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<th>Accumulative stress</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Total protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ds</td>
<td>0.30</td>
<td>0.170</td>
<td>-0.43</td>
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<tr>
<td>Ds</td>
<td>0.08</td>
<td>0.70</td>
<td>-0.54</td>
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<tr>
<td>Ds</td>
<td>0.26</td>
<td>0.210</td>
<td>0.005</td>
</tr>
<tr>
<td>DS</td>
<td>0.51</td>
<td>0.015*</td>
<td>-0.67</td>
</tr>
<tr>
<td>DMFS</td>
<td>0.49</td>
<td>0.019*</td>
<td>-0.19</td>
</tr>
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</table>

**Table 5:** Correlation coefficient between caries experience (DMFS, DS) and salivary constituents according to categories of Stressful Life Events scale.

<table>
<thead>
<tr>
<th>Stressful Life Events scale categories</th>
<th>Low level stress</th>
<th>More stress</th>
<th>Accumulative stress</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
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<tr>
<td>Total protein</td>
<td></td>
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</tr>
<tr>
<td>Ds</td>
<td>0.02</td>
<td>0.92</td>
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<td>Ds</td>
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<td>Ds</td>
<td>-0.05</td>
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<tr>
<td>Ds</td>
<td>-0.09</td>
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<tr>
<td>Cortisol</td>
<td>-0.21</td>
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<td>-0.06</td>
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<tr>
<td>DMFS</td>
<td>-0.40</td>
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**Table 6:** Correlation coefficient between salivary Total protein and salivary constituents according to categories of Stressful Life Events scale.

<table>
<thead>
<tr>
<th>Stressful Life Events scale categories</th>
<th>Less stress</th>
<th>More stress</th>
<th>Accumulative stress</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>IL-6(ng/ml)</td>
<td>-0.13</td>
<td>0.55</td>
<td>0.64</td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>0.08</td>
<td>0.71</td>
<td>0.31</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td>0.15</td>
<td>0.49</td>
<td>-0.24</td>
</tr>
</tbody>
</table>

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