RESEARCH ARTICLE

ANALYSIS OF STRESS LEVELS OF DENTAL STUDENTS USING ELISA BASED SALIVARY CORTISOL LEVELS AND COMPARING WITH SMART PHONE S-HEALTH SENSOR

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ABSTRACT

Background: Stress in students during examination is always associated with high levels of anxiety. Salivary cortisol is used as a biomarker to assess stress levels. The aim of the present study is to evaluate Salivary Cortisol levels and also recording and analyzing the heart rate, oxygen saturation and stress in dental students using the sensor and S-Health software provided in the smart phone.

Materials and Methods: The study is carried out on 40 BDS students, who are appearing for their university practical oral examination. Their average age ranged between 22-25 years. The concentration of cortisol in the saliva (µg/dl) is determined by using a salivary enzyme linked immunoassay (ELISA) kit. Subjects are evaluated for two times, one during relaxed state and another during stressed state. Heart rate, oxygen saturation and stress levels will be measured using Smart phone S-HEALTH sensor.

Results: Salivary Cortisol levels at relaxed state were 4.4950±2.22422 and at stressed state were 5.4025±2.51034. Heart rate at relaxed state was 100.42±15.20777 and at stressed state were 103.88±15.58465. Oxygen saturation was almost normal both at relaxed state stressed state. Maximum number of subjects in relaxed state showed low levels of stress and in stressed state showed high levels of stress. Comparison of Salivary Cortisol levels, Heart rate, oxygen saturation and stress levels between relaxed and stressed state showed no statistical significance.

Conclusion: Advantage from this study to the society is once the stress levels are recognized by self-assessment, skills for stress resistance should be implemented. An efficient relaxation program as well as counselling services with meditation programs, and regular yoga should be done to enhance their academic performance.

INTRODUCTION

Stress in students during examination is always associated with high levels of anxiety. Salivary Cortisol is used as biomarker to assess stress levels. The dental undergraduate course has often been described as one of the highest and most demanding of courses. It has been reported that dental students express significant stress symptoms during examination and are more anxious showing higher levels of depression.1

Previous studies have found that examinations and the pressure of clinical training are among the most important causes of stress in dental students. During university examination psychophysiological and autonomic reactions follow such as a freezing response, the fight or flight response or the alarm or stress response.2 Typical physiological changes occur in anxiety states such as increased heart rate, elevated pulse volume, enhanced tonic and phasic skin conductance, a galvanic skin response, or the stimulation of production of different hormones like catecholamine, adrenocorticotropic, cortisol, prolactin, and thyroxin. Hardly any studies have been done evaluating stress using S-health software present in smart phone which works on skin conductance.3, 4

Therefore this study aims to evaluate Salivary Cortisol levels and also use a technology for recording and analyzing the heart rate, oxygen saturation and stress in dental students using the sensor and S-Health software provided in the smart phone during their university practical oral exam and compare between both the methods.

MATERIALS AND METHODS

Study Design

Subjects will be assessed for stress parameters and Salivary Cortisol levels at two times. One in the relaxed state (with no examinations in preceding two weeks and coming two weeks) and another in the stressed state (on the day of University practical oral examination).
Subjects
Forty Dental students (Third and Final year BDS) who will be appearing in the final university practical oral exam are recruited for the study after written informed consent. Their average age ranged between 22-25 years. Students with the history of neurological or psychiatric disorders, taking medicines affecting emotional status and endocrinological profile, tendency of gingival bleeding or addicted to tobacco or alcohol will be excluded. The study protocol was approved by the institutional ethics committee.

Stress Parameters
Heart rate, oxygen saturation and stress levels will be measured using Smart phone S-HEALTH sensor.

METHOD
Index finger will be placed for 1 min on the sensor present behind the smart phone- Samsung S7 edge. Android S-health software has three parameters: Heart rate, oxygen saturation and stress level. Once the software measures, the values appear on the screen.

Heart rate is expressed in beats per minute, oxygen saturation in percentage and stress levels are expressed as mild, moderate, neutral, severe, and very severe based on the amount of stress.

Saliva Sample Collection
Subjects will be instructed in advance not to eat or drink anything except water one hour before saliva collection to minimize possible food debris and stimulation of salivation. To obtain samples of stimulated saliva, students will be asked to chew sugarless chewing gum for five minutes and then saliva samples will be collected at the same time of the day (between 10:00 am to 11:00 am) both during relaxed state and stressed state. Samples will be stored in the deep freezer at –80°C and transported to the lab for analysis within forty-five minutes of the collection of the sample.

Salivary Cortisol Assay
Salivary cortisol will be measured by ELISA method (manufactured by DRG, Germany). ELISA of all the samples was done by the same person with technical expertise who will be blinded for the results of scoring and performance.

Statistical Analysis
Data analysis was done using SPSS version 16.0, software. All the data will be expressed as mean±SD. Comparison of stress levels at relaxed and stressed state using Paired’t’ test and group comparison will be done using One Way analysis of variance (ANOVA). A probability value less than 0.05 (P<0.05) will be considered as significant for all statistical tests applied.

RESULTS
Salivary Cortisol levels at relaxed state were 4.4950±2.22422 and at stressed state were 5.4025±2.51034. Comparison of salivary Cortisol levels between relaxed and stressed state showed no statistical significance(Table 1).

Heart rate at relaxed state was 100.42±15.20777 and at stressed state were 103.88±15.58465. Comparison of Heart rate between relaxed and stressed state showed no statistical significance (Table 2).

Salivary cortisol was almost normal both at relaxed state stressed state. Comparison of Oxygen saturation between relaxed and stressed state showed no statistical significance (Table 3).

Maximum number of subjects in relaxed state showed low levels of stress and in stressed state showed high levels of stress. Comparison of Stress Levels between relaxed and stressed state showed no statistical significance (Table 4).

### Table 1 Comparison of salivary cortisol levels between relaxed and stressed state.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>t</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary Cortisol Value at relaxed state</td>
<td>40</td>
<td>4.4950</td>
<td>2.22422</td>
<td>.35168</td>
<td>-1.711</td>
<td>.091</td>
</tr>
<tr>
<td>Salivary Cortisol Value at stressed state</td>
<td>40</td>
<td>5.4025</td>
<td>2.51034</td>
<td>.39692</td>
<td>11.711</td>
<td>.068</td>
</tr>
</tbody>
</table>

### Table 2 Comparison of Heart rate between relaxed and stressed state

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>t</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate</td>
<td>Relaxed state</td>
<td>40</td>
<td>100.42</td>
<td>15.20777</td>
<td>2.40456</td>
<td>-1.002</td>
<td>0.319</td>
</tr>
<tr>
<td></td>
<td>Stressed state</td>
<td>40</td>
<td>103.88</td>
<td>15.58465</td>
<td>2.46415</td>
<td>11.711</td>
<td>.001</td>
</tr>
</tbody>
</table>

### Table 3 Comparison of Oxygen saturation between relaxed and stressed state

<table>
<thead>
<tr>
<th>Oxygen saturation</th>
<th>At Stressed state</th>
<th>At relaxed state</th>
<th>Total</th>
<th>Chisquare</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Low</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.054</td>
<td>0.816</td>
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<tr>
<td>Total</td>
<td>2</td>
<td>38</td>
<td>40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4 Comparison of stress levels between relaxed and stressed state

<table>
<thead>
<tr>
<th>stress levels at stressed state</th>
<th>stress levels</th>
<th>low</th>
<th>medium</th>
<th>high</th>
<th>total</th>
<th>chi-square</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>4</td>
<td>9</td>
<td>9</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>medium</td>
<td>1</td>
<td>6</td>
<td>5</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td></td>
<td>2.513</td>
<td>0.642</td>
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<tr>
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<td>5</td>
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</tbody>
</table>

DISCUSSION
Many studies have been done to analyze the stress, anxiety or depression using salivary cortisol levels. However this study is a unique study which is conducted on Dental students and also stress is analyzed using skin conductance provided in a smart phone using software. In-addition stress levels were compared with salivary Cortisol levels which is also a non-invasive method.

The results of the present study showed that salivary cortisol levels were increased in stressed state when compared to relaxed state. However the difference was not statistically significant.

In-addition, heart rate and Oxygen saturation measured by smart phone Samsung S-HEALTH sensor did not show much difference between relaxed state and stressed state. Further, stress levels measured in relaxed state were maximum in low
levels, where as in stressed state, more number of subjects were in high levels of stress.

Vivian et al. 2003 has conducted a study to assess the difference in self-perceived stress before and after an academic assessment and its association with the marks scored and to study the association of salivary cortisol, Immunoglobulin A (IgA), and chromogranin A (CgA) with stress levels before and after the assessment.

Pani et al. in 2011 has conducted a study in Final-Year Saudi Dental Students to evaluate the stress using Salivary Cortisol as a Biomarker. Modified dental environmental stress (DES) scale was analyzed and correlated these findings, at various times in the semester, with the level of acute stress measured with salivary cortisol.

Singh et al. in 2012 has conducted a study on Effect of Examination Stress on Mood, Performance and Cortisol Levels in Medical Students. Mood parameters were assessed, using Depression Anxiety Stress Scale (DASS) scoring, and salivary cortisol levels using quantitative ELISA.

Sano et al. in 2013 conducted a study in which stress was recognised using Wearable Sensors and Mobile Phones. Stress was analysed using wrist sensor (skin conductance and accelerometer), mobile phone usage (short message service, call, screen on/off and location) and surveys (mood, stress, tiredness, sleep, general health, caffeinated beverage intake or alcohol and electronics usage).

Kumar et al. in 2016 has studied the effect of stress during university examination on the differential leucocyte count (DLC), Heart Rate (HR), and Blood Pressure (BP).

Numerous methods are brought forward to recognize stress levels. Smartphone sensor with S-health software which we used in this study has many advantages:

- Users always can carry anywhere,
- It is a non-invasive method, and self-assessment can be done at any point of time,
- It is easily assessable.

When stress is perceived negatively or becomes excessive, it leads to anxiety before and during examinations and ultimately affects their academic achievement. Also the students who are striving hard to perform well in the exam may be stressed. Thus students have to analyse their stress in order to prevent chronic diseases. Additionally, more emphasis should be placed on the importance of humanistic faculty-student relationships.

**CONCLUSION**

Stress is increasing day by day because of the competitive world. Examination stress has been reported to produce debilitating cognitive effects including difficulties with memory and recalling information. Advantage from this study to the society is once the stress levels are recognized by self-assessment, skills for stress resistance should be implemented. An efficient relaxation program as well as counselling services with meditation programs, and regular yoga should be done to enhance their academic performance.

**References**


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