Stress Level Caused by Orthodontic Forcein Wistar Rats

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Abstract:
Background: Inflammation process initiates tooth movement in orthodontic treatment and causes pain in some cases. Some patients experience stress and anxiety during orthodontic treatment. This condition leads body to secrete several hormones. One of them is cortisol. The aim of this study is to observe the effect of stress caused by orthodontic force based on the level of cortisol hormone in Wistar rats within several days.

Materials and Methods: A total of 28 male Wistar rats were divided into 4 groups. Group 1 had no orthodontic intervention (n=7), group 2 to group 4 were given orthodontic force by fixing separator in maxilla incisor, corresponding to the period of the experiment, i.e. 1, 3 and 7 days. At the end of each experimental period, Wistar rats were euthanized and blood plasma was collected.

Results: Group 2 (day 1) demonstrated higher cortisol level than control group with different mean value of 21.2 (p=0.003). Both group 3 and 4 showed decreasing cortisol levels (p=0.310 and p=0.009). Overall, experimental showed significant mean differences of cortisol level between groups (p<0.05).

Conclusion: Orthodontic force causes pain leading to cortisol hormone secretion. Decreasing cortisol levels were observed in groups with longer period of orthodontic force intervention due to decreasing pain during lag phase.

Key Words: Stress; Orthodontic force; Cortisol.

Date of Submission: 17-06-2020
Date of Acceptance: 03-07-2020

I. Introduction
Orthodontic treatment is a field of dentistry that deals with facial aesthetic, function, and oral health. Orthodontic treatment has 2 types, removable orthodontic and fixed orthodontic. Fixed orthodontic produces more complex tooth movements compared to removable orthodontic that they speed up the process of tooth movement.

There are several types of active components in fixed orthodontic, namely separator, archwire, elastic, and spring. Separators are used to make space between two adjacent teeth. Tooth movement in orthodontic treatment is achieved through alveolar bone remodeling and periodontal tissue in response to mechanical forces. Tooth movement is initiated by inflammation process.

Inflammation is characterized by several clinical signs. One of them is pain. Pain is a common complication during orthodontic treatment. Stress triggered by orthodontic pain will increase cortisol hormone production.

Vandeska et al. conducted a study in Wistar rats which were divided into 3 groups and observed for 21 days. Samples were given interventions with stress induced by orthodontic force and foot shock. They concluded that the highest cortisol level was seen the first day after application of orthodontic force.

Destruction occurs continuously in the periodontal tissue as cortisol loses the ability to inhibit the inflammatory response, resulting in bone resorption, tissue damage, loss of attachment, and delayed wound healing. Dewi et al. in their study about phenomenon of orthodontic tooth movement concluded that cortisol hormone increased osteoclast activity, which resulting in excessive resorption that will inhibit tooth movement. The aim of this study is to observe the effect of stress caused by orthodontic force based on the level of cortisol hormone in Wistar rats within several days.

II. Materials And Methods
This research is a quasi-experimental study using a comparison group or control time series design.

Study Design: Quasi-experimental study.
Study Location: Department of Biology-Faculty of Mathematics and Science Universitas Sumatera Utara.

DOI: 10.9790/0853-1907010811
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Sumatera Utara, Integrated Laboratory-Faculty of Medicine Universitas Sumatera Utara.

Study Duration: April to May 2019.
Sample size: 28 male Wistar rats.
Sample size calculation: Sample size was estimated by formulation of Sastroasmoro & Ismael. The sample size obtained was 7 male Wistar rats for each group.

Subjects and selection method: Male Wistar rats weighing between 150-250g were allowed to acclimatize for 7 days before the study.

Group 1 (n=7) – no orthodontic intervention.
Group 2 (n=7) – fixing separator in maxilla incisor for 1 day.
Group 3 (n=7) – fixing separator in maxilla incisor for 3 days.
Group 4 (n=7) – fixing separator in maxilla incisor for 7 days.

Inclusion criteria:
1. Intact maxilla incisor.

Exclusion criteria:
1. Female Wistar rats.
2. Broken incisor.
3. Noticeable physical deformity.
4. Die before the end of experimental period.
5. Already had intervention in another study.

Procedural methodology
Blood samples were collected by cardiac puncture under ketamine anesthesia at the end of experimental period for each group. EDTA-Na2 is used as an anticoagulant. The samples were centrifuged with 1000rcf at 2-8°C for 15 minutes. Afterwards, cortisol level was calculated by using Mouse COR (Cortisol) ELISA Kit (Fine test, China).

Statistical analysis
The data was analyzed using Anova Repeated Measurement.

III. Result

Table 1 shows the mean value of cortisol level for each group. Increasing cortisol level after placement of separator can be seen in group 2. The highest mean value of cortisol level in Wistar rats was found on group 2 (51.4 ± 35.2), while the lowest was found on group 4 (20.4 ± 9.5).

Table 1: Mean value of cortisol level in Wistar rats for each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (day)</th>
<th>Mean (X±SD) (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>Day-0</td>
<td>30.2 ± 26.6</td>
</tr>
<tr>
<td>2</td>
<td>Day-1</td>
<td>51.4 ± 35.2</td>
</tr>
<tr>
<td>3</td>
<td>Day-3</td>
<td>38.7 ± 20.0</td>
</tr>
<tr>
<td>4</td>
<td>Day-7</td>
<td>20.4 ± 9.5</td>
</tr>
</tbody>
</table>

Table 2 shows the mean differences of cortisol level in Wistar rats. Samples in day 1 experienced an increased cortisol level compared with the control group. The different mean value was statistically significant (p = 0.003). Decreased cortisol level was seen on day 3 compared to day 1. This decreased value was not significant (p = 0.310). Decreased cortisol level was also found on day 7 compared to day 3 and the different mean value was statistically significant (p= 0.009).

Table 2: Mean differences of cortisol level in Wistar rats

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Mean Difference (ng/mL)</th>
<th>Significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>21.2</td>
<td>0.003</td>
<td>0.045</td>
</tr>
<tr>
<td>1-3</td>
<td>12.7</td>
<td>0.310</td>
<td></td>
</tr>
<tr>
<td>3-7</td>
<td>18.3</td>
<td>0.009</td>
<td></td>
</tr>
</tbody>
</table>

*General Linear Model-Repeated Measure, p<0.05 – statistically significant
IV. Discussion

Table 1 shows that mean value of cortisol level is highest on day 1 with a value of 51.4 ± 35.2 and the lowest was found on the day 7 with a value of 20.4 ± 9.5. Increased cortisol level was statistically significant (p<0.05) on day 1. This proves that orthodontic force influences cortisol hormone secretion. Increased cortisol level during tooth movement is caused by pressure on the periodontal ligament of the tooth which produces an inflammatory response mediated by cytokines and prostaglandins[10]. This is accordant to previous study by Aksoy et al. concluded that cortisol levels increase after the installation of molar bands[11].

Table 2 shows significant mean differences of cortisol level between groups (p<0.05). The mean value of cortisol level decreased on the third day, then decreased significantly on the seventh day (p<0.05). This is consistent with the research of Vandevska et al. who observed cortisol levels in Wistar rats. Their samples were divided into 3 groups which given foot shock and coil spring installation. Intervention was observed on day 3, 7, 13, and 21. Their study showed significant difference of cortisol level in the group received intervention of coil spring seen on the third day post-installation compared with the control group[8].

Movement of the teeth involves tension and pressure side, where inflammatory process takes place. Bone deposition (remodeling) takes place on the tension side. Inflammation is characterized by periodontal vasodilation and migration of leukocytes from blood vessels, where proinflammatory mediators including prostaglandins, growth factors, and cytokines are released[12]. This is supported by the work of Chami et al., who conducted research on cytokines in gingival crevicular fluid during tooth movement with aligner. The result of their study showed that proinflammatory cytokines, including IL-1, IL-6, and TNF-α increased on the first day after aligner installation[3].

Study of Chami et al. is accordant to our study where cortisol level increased significantly one day post-installation. Cortisol level increased due to inflammatory process that caused pain after device installation, namely the initial phase. Pain caused by the use of orthodontic devices is a stressor that influences cortisol hormone secretion[6].

Jameel et al. conducted a study on the effect of various stress models seen in cortisol levels of Wistar rats. Intervention was given by force to do swimming. Result of their study shows that cortisol level immediately increased significantly after 3 hours of stress application[14].

In the case with installation of orthodontic devices, cortisol level increased significantly in 24 hours after intervention. This is due to the movement of tooth in the initial phase. Tooth movement occurs quickly in this phase and it usually lasts for 24 hours to 2 days after installation. Then it decreases on day 3 to day 7 as it enters the lag phase. Lag phase is characterized by the formation of hyaline tissue where there is no tooth movement or slow tooth movement[16].

Bohl et al. also conducted a study of dental displacement in dogs by moving the left mandibular premolar with a close coil spring. Decreased tooth movement was observed on the fourth day. It was caused by the formation of hyaline tissue in the pressure region[16]. Their study is accordant with our research where cortisol levels decreased on the third day and decreased significantly on the seventh day as it entered lag phase. Pain decreases when there is only little tooth movement during lag phase, this is why cortisol levels decrease as well.

V. Conclusion

Orthodontic force influences cortisol hormone secretion. Orthodontic force causes pain leading to cortisol hormone secretion. Decreasing cortisol levels were observed in groups with longer period of orthodontic force intervention due to decreasing pain during lag phase.

Acknowledgement

This research was addressed to our Research Institution, Universitas Sumatera Utara who assisted and funded the completion of this study by TALENTA’s program funding in 2019, batched No.263/UN5.2.3.1/PPM/KP-TALENTA-USU/2019. We also send our gratitude to NurismaInarah, NurAfifah, and UswatulHusnaini for their assistance in this research.

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