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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 leadsbody to secrete several hormones. One of them is cortisol. The aim of this study is to observe the effect of stress caused by orthodontic forcebased 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 wereeuthanasized and blood plasma was collected.*
Results:*Group 2 (day 1) demonstrated higher cortisol level than control group with differentmean value of 21.2 (p=0.003). Both group 3 and 4 showed decreasing cortisol levels (p=0.310 and p=0.009).Overallexperimental showed significant mean differences of cortisol level between groups (p<0.05).*
Conclusion:*Orthodontic force causes pain leadingtocortisol 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; Orthodonticforce; Cortisol.*

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I. Introduction

Orthodontic treatment is a field of dentistry that deals with facial aesthetic, function, and oral health[1].Orthodontic treatment has 2 types, removable orthodontic and fixed orthodontic. Fixed orthodonticproduces more complex tooth movements compared to removable orthodontic that theyspeed up the process of tooth movement[2].

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[3].Tooth movement in orthodontic treatment is achieved through alveolar bone remodeling and periodontal tissue in response to mechanical forces[4].Tooth movement is initiated by inflammation process[5].

Inflammation is characterized by several clinical signs. One of them is pain[5].Pain is a common complication during orthodontic treatment. Stress triggered by orthodontic pain will increase cortisol hormone production[6,7].

Vendeska et al. conducted a study in Wistarrats 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 thatthe highest cortisol levelwas seenon the first day after application of orthodontic force[8].

Destructionoccurs 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[9].Dewi et al. in their study about phenomenon of orthodontic tooth movementconcluded that cortisol hormone increasesosteoclast activity which resulting in excessive resorption that will inhibit tooth movement[6].The aim of this study is to observe the effect of stress caused by orthodontic forcebased 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 ScienceUniversitas

Sumatera Utara,Integrated Laboratory-Faculty of MedicineUniversitas Sumatera Utara.
Study Duration :April to May 2019.
Sample size :28 male Wistar rats.
Sample size calculation :Sample size was estimated by formulation ofSastroasmoro& 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.
2. Normal interproximal gap.

Exclusion criteria:
1. Female Wistar rats.
2. Brokenincisor.
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 bycardiac puncture under ketamine anesthesiaat the end of experimental period for each group. EDTA-Na2is 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 levelfor each group. Increasing cortisol level after placement of separator can be seen in group 2. The highest mean value of cortisol level inWistar rats was found on group 2 (51.4 ± 35.2), while the lowest was found on group 4 (20.4 ± 9.5).

Table1 : Mean value of cortisol level in Wistar rats for each group

Group	Time (day)	Mean (X±SD) (ng/mL)
1 (control)	Day-0	30.2 ± 26.6
2	Day-1	51.4 ± 35.2
3	Day-3	38.7 ± 20.0
4	Day-7	20.4 ± 9.5

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). Decreasedcortisol level was seen on day 3compared to day 1. This decreased value was not significant(p = 0.310). Decreasedcortisol level was also found on day7 compared to day 3 and the different mean valuewas statistically significant (p= 0.009).

Table 2 : Mean differences of cortisol level in Wistar rats

Time (day)	MeanDifference (ng/mL)	Significance	p-value
0-1	21.2	0.003	0.045
1-3	12.7	0.310	
3-7	18.3	0.009	

*General Linear Model-Repeated Measure, p<0,05 – statistically significant

IV. Discussion

Table 1shows 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 levelwas statistically significant ($p<0.05$)on day 1. This proves that orthodontic forceinfluences cortisol hormone secretion.Increased cortisol levelduring 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 accordantto previous study by Aksoy et al. concluded thatcortisol levels increase after the installation of molar bands[11].

Table 2 shows significant mean differencesof cortisol level between groups ($p<0.05$).The mean value of cortisol leveldecreased on the third day, then decreased significantly on theseventh 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 3groups 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 receivedinterventionof 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]. Thisis 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 studyshowed thatproinflammatory cytokines, including IL-1, IL-6, and TNF-α increased on the first day after aligner installation[13].

Study of Chami et al. is accordant to our studywhere 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 ofWistar rats. Intervention was given byforce 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 itusually 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[15].

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 observedon 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 seventhday 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 leadingto cortisol hormone secretion. Decreasing cortisol levels were observed in groups with longer period of orthodontic force intervention due to decreasing pain during lag phase.

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