



ORIGINAL ARTICLE

Vitamin E supplementation reduces stress levels from orthodontic force in Wistar rats (*Rattus norvegicus*)

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KEYWORD

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Abstract *Background:* Orthodontic tooth movement is mediated by the inflammation process. Inflammation induces pain and increases the level of cortisol hormone as it triggers stress. The aim of this research was to observe the effects of vitamin E (VE) supplementation in reducing stress levels from orthodontic force in Wistar rats (*Rattus norvegicus*).

Methods: Wistar rats ($n = 56$) were divided into two groups: group 1 as the control group, and group 2 as the experimental group (VE group). VE supplemented for 14 days prior application of the separator as an orthodontic force. Each group was divided into four subgroups ($n = 7$), corresponding to the duration in days that force was applied, i.e., 0, 1, 3, and 7 days. Stress were measured by cortisol levels, and inflammation were measured by interleukin-1 beta (IL-1 β) levels in blood plasma.

Results: The VE group had lower cortisol levels than the control group, and significant found on days 3 and 7 ($p = 0.026$ and $p = 0.037$). The cortisol level in the VE group decreased faster, begin-

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ning on day 1, whilst the control group occurred on day 3. Statistical analysis of IL-1 β levels found insignificant differences between the two groups.

Conclusion: Vitamin E helps reduce stress caused by orthodontic force due to tooth movement.

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1. Introduction

Orthodontic tooth movement is based on a process called inflammation which releases chemical mediators such as prostaglandins and proinflammatory cytokines, i.e. interleukin-1 beta (IL-1 β), nitric oxide (NO) and tumor necrosis factor (TNF) (Kobayashi and Horinuki, 2017; Krishnan and Davidovitch, 2006). IL-1 β is one of the main cytokines secreted from the microvesicles of plasma membranes and commonly contributes to the 'host defense' against infections and injuries (Lee et al., 2004; Vandevska-Radunovic and Murison, 2010).

Orthodontic inflammation has clinical signs of discomfort can be observed before pain is perceived by the patient (Gibbins, 2018; Shroff, 2016). Discomfort and pain starts four hours after placement of a separator, and increases within 24 h and decreases approximately seven days later (Giannopoulou et al., 2006; Long et al., 2016). Pain can trigger stress and stimulate cortisol hormone released (Vandevska-Radunovic and Murison, 2010).

Studies have shown that several types of VE have anti-inflammatory properties by altering cytokine production (Esenlik et al., 2011; Jiang, 2014). Tocotrienol and α -tocopherol (VE) supplementation were able to maintain the corticosterone level at the nonstressed value under "restrained" conditions in rats (Nur Azlina and Nafeeza, 2008). Based on this finding, the authors hypothesized that VE might also reduce the stress condition and the inflammation caused by orthodontic force.

The current study is a continuation of a previous study which observed the orthodontic tooth movement by distance measurements and cellular numbers (Sufarnap et al., 2020). The purpose of this study was to evaluate the effect of VE supplementation on reducing stress induced by orthodontic force in Wistar rats. This study is the first to our knowledge to investigate this hypothesis.

2. Materials and methods

2.1. Animals and groups

The Ethics of the animal research were approved by The Animal Research Ethics Committees with the approval number of 0128/KEPH-FMIPA/2019. The research was an experimental study with a time series control design and generally enforced with ARRIVE guideline.

Fifty-six healthy male Wistar rats; 150–250gr were selected, housed and husbandry at the Biology Department - Faculty of Mathematics and Science (FMIPA), Universitas Sumatera Utara (USU); and followed the ARRIVE guidelines (Fawcett, 2012). Each polycarbonate cage contained 3 or 4 animals.

They were fed a standard pellet diet and tap water. A dark cycle with a temperature of 25–30 °C was maintained for a minimum of 12 h.

The experimental rats (n = 56) were divided into two groups and chosen by simple random sampling based on the rat's social behaviors. Group 1 served as a control group (tails marked black), and group 2 served as the vitamin E (VE) group (tails marked red). Each of the groups was further divided into four subgroups (n = 7), corresponding to the number of days orthodontic force lasted, i.e., 0, 1, 3 and 7 days.

2.2. Experiments

The VE group was given dl- α -tocopherol acetate supplement (Santa E-Sanbe®) at a dose of 60 mg/kg orally. The control group was given water orally as a placebo. Both interventions were administered with a gavage needle each morning for 14 days before a separator was applied; the interventions continued for the duration of the experimental periods.

Stress was induced with orthodontic force by placing a rubber separator between rat's upper incisor teeth; this moment was counted as the baseline (day 0). Measurements and samples were collected at days 0, 1, 3 and 7. The rats were euthanized with ketamine (Hameln Pharma Plus GmbH, Germany) at a dosage of 80 mg/kg and xyla® (Interchemie, Holland) at 10 mg/kg.

2.3. Blood sampling, cortisol and IL-1 β analysis

Blood samples were collected through cardiac puncture with anticoagulant (EDTA) and transferred to the Terpadu Laboratory (Medical Faculty-USU) within 30 min at 4 °C. The procedures continued with centrifugation (Eppendorf) for 15 min/1000 gr of force. The plasma was then stored at –80 °C until all samples were collected.

Enzyme-linked immunosorbent assay (ELISA) analysis of cortisol levels was performed using a Mouse COR (Cortisol) Kit (Fine Test, China), and IL-1 β levels were determined by using a Rat IL-1 β ELISA Kit (Elabscience, USA).

2.4. Statistical analysis

IBM SPSS Statistic version 26 was used for statistical analysis. The distribution of data based on the Shapiro-Wilk test; normally distributed for cortisol data ($p > 0.05$); were not normally distributed for IL-1 β data ($p < 0.05$).

Independent t-tests were used to carry out comparisons between groups at different times for cortisol levels. The analysis also compared the cortisol levels between time intervals by

using ANOVA General Linear Model-Repeated Measures (ANOVA GLM-RM) for each group. Levene's test was measured prior to the ANOVA statistic, and the homogeneity variances for both groups were equal at each time measurement with $p > 0.05$, except on day 3 ($p = 0.017$). The IL-1 β level data were analyzed with the Kruskal-Wallis test to compare the groups at each measurement time point.

3. Results

3.1. Cortisol levels

The average cortisol levels in both groups after orthodontic force can be seen in Table 1 and Fig. 1, the values showed that all subgroups in the VE group had lower cortisol levels than the control group, but the difference was significant only at day 3 and day 7 ($p < 0.05$).

Data analyzed for the time interval (Table 2) from day 0 to day 1 revealed significantly increased of cortisol levels. On day 1 to day 3, cortisol levels significantly decreased only in the VE group, and the cortisol level continue decreased significantly below the baseline level at day 7.

3.2. Interleukin-1 beta (IL-1 β) levels

The overall average level of IL-1 β showed that the VE group had lower levels than the control group, but there were no significant differences between the two groups at all time points which were analyzed with Kruskal-Wallis test (Table 3 and Fig. 2). Due to the insignificant IL-1 β level results, analysis would be meaningless if continued with the post hoc test.

4. Discussion

Daily physiological stress occurs during all social life activities (Almeida et al., 2009). For this reason, the VE supplemented time of this experiment started before the orthodontic force was applied and was the reason why the rats were separated based on their social behavior.

The stress induced in this research was created by application of orthodontic force, which stimulated secretion of proinflammatory cytokines. Proinflammatory cytokines are represented by the level of IL-1 β in Wistar rat blood plasma, which was expected to indicate whether local inflammatory conditions would affect the whole-body system due to psycho-

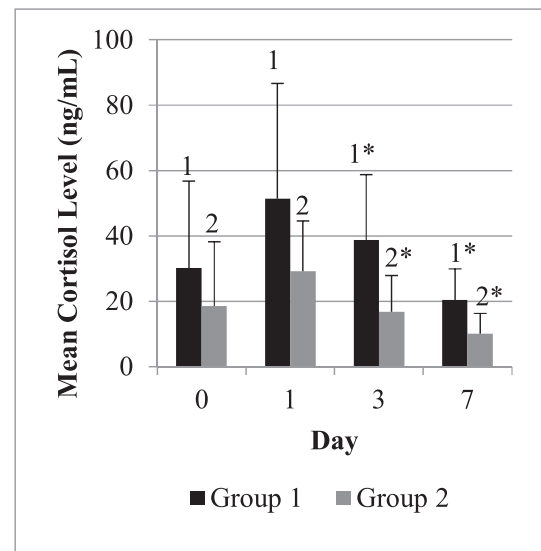


Fig. 1 Mean value of cortisol for group 1 versus group 2. An asterisk denotes significant differences found between groups ($p < 0.05$).

logical stress (Almeida et al., 2009; Ibrahim et al., 2012; Lee et al., 2015).

The highest cortisol levels in both groups occurred at day 1 (24 h) after orthodontic force was applied. This period was a peak time of orthodontic pain due to the proinflammatory cytokines released to the alveolar bone and the periodontal ligament (Ertan Erdiñç and Dinçer, 2004). This was also a peak time of cortisol release; after stress is stimulated by any trigger (Andrade et al., 2018).

From day 3 to day 7, the IL-1 β level increased, while the cortisol level decreased. Cortisol hormones have another function as an anti-inflammatory agent. This process occurs as homeostasis from the hypothalamic-pituitary-adrenocortical (HPA) axis to balance and terminate the inflammatory-stress condition and it also has a function for the animals to be able to adapt and cope with longer stress conditions (Davidson et al., 2006; Hannibal and Bishop, 2014).

Supplementation with multivitamins and minerals as micronutrients has beneficial effects in boosting mood in daily life, especially vitamin B (Long and Benton, 2013). VE supplementation was given to rats 14 days before application of orthodontic force. Lower levels of cortisol were found at each time measurement in the VE group than in the control group, the time interval analysis (Table 2) showed that the VE group's levels decreased significantly faster, starting at day 1, while the control group decreased after day 3. Based on the research outcomes, VE could accelerate stress reduction induced by orthodontic force. Some studies have concluded that VE, with antioxidant and anti-inflammatory functions, could hypothetically reduce inflammation; unfortunately it might inhibited orthodontic tooth movement (Esenlik et al., 2011; Ibrahim et al., 2012; Jiang, 2014).

NSAID drugs mostly used to reduce the orthodontic pain and also inhibited the tooth movement (Diravidamani et al., 2012). In this research found that there were insignificant differences in IL-1 β levels between the two groups. Previous research coincidentally found that the osteoclast amounts in

Table 1 Means and differences of cortisol levels in group 1 versus group 2.

Day	Group 1 (X ± SD) ng/mL	Group 2 (X ± SD) ng/mL	P Value*
0	30.2 ± 26.6	18.57 ± 19.65	0.371
1	51.4 ± 35.2	29.25 ± 15.38	0.164
3	38.7 ± 20.0	16.85 ± 10.98	0.026*
7	20.4 ± 9.5	10.13 ± 6.22	0.037*

* Significant with p -value < 0.05 (Independent t -test).

Table 2 Observation of cortisol levels in group 1 versus group 2 based on time intervals.

Cortisol Level	Day 0 to 1		Day 1 to 3		Day 3 to 7	
	Group 1 (ng/mL)	30.2 ± 26.6	51.4 ± 35.2	51.4 ± 35.2	38.7 ± 20.0	38.7 ± 20.0
(X ± SD)						
p Value	0.003*		0.310		0.009*	
Group 2 (ng/mL)	18.5 ± 19.6	29.2 ± 15.3	29.2 ± 15.3	16.8 ± 10.9	16.8 ± 10.9	10.1 ± 6.2
(X ± SD)						
p Value	0.024*		0.003*		0.012*	

*Significant; *p*-value < 0.05 (ANOVA GLM-RM).

Table 3 Means and differences in IL-1β levels in group 1 versus group 2.

Day	Group 1 (X ± SD) ng/mL	Group 2 (X ± SD) ng/mL	P Value*
0	636.29 ± 390.38	584.71 ± 118.50	0.338
1	938.86 ± 275.87	1060.43 ± 431.49	0.565
3	767 ± 206.54	683.57 ± 167.53	0.338
7	902.71 ± 153.28	856.43 ± 423.61	0.338

* Significant; *p*-value < 0.05 (Kruskal-Wallis test).

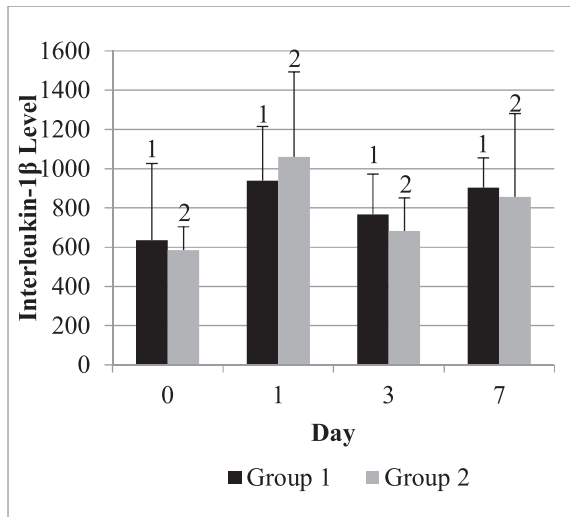


Fig. 2 Mean value of IL-1β group 1 versus group 2. No asterisk denotes insignificant differences found between the two groups according to the Kruskal-Wallis test.

the VE group showed insignificantly difference between groups, but for the distance measurement, a significant difference found that VE group had better tooth movement than the control group (Sufarnap et al., 2020).

The answer to the study hypothesis was that VE supplementation succeeded in reducing stress conditions without

inhibit inflammatory activities due to orthodontic force. The limitation of the research was that these results might not be definitive because the sample used the blood plasma to analyze the IL-1β level, which had been chosen due to insufficient amount of rat saliva or gingival crevicular fluid (GCF) in the preliminary study. Blood plasma used to analyze the cortisol level had higher amounts of cortisol than saliva and GCF (Dhama et al., 2019; Nayak et al., 2013). Further research should analyze the correlation of tooth movement to inflammation from GCF or by immunohistochemistry analysis.

5. Conclusions

The study concluded that VE supplementation during orthodontic treatment helped to reduce the stress effect without significantly inhibiting proinflammatory activities in the tooth movement process.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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