

Systemic Effects of Occlusal Disharmony on Corticosterone Serum Levels in *Rattus Norvegicus*

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Abstract

Background: Mastication process occurs over time and it can lead to occlusal reduction of the teeth. This causes reducing occlusal contact of teeth and it will lead to disharmony occlusion disorders. The occlusion disharmony is recognized by the body as a stressor that systemically affects the homeostasis in the body, especially the production of hormones.

Objective: The aim of this study was to determine the systemic effect of occlusal disharmony on corticosterone serum levels in *Rattus norvegicus*.

Method: The research method of this study used experimental design and conducted among 7 male *Rattus Norvegicus* in each groups. In intervention group, an occlusal reduction was ± 1 mm in all molars and for control group did not given any intervention. Moreover, at 1st day, 7th day, 14th day and 21st day was taken 2 cc of blood via infraorbital vein. The measurement of Corticosterone levels used Enzyme Linked Immunosorbent Assay (ELISA).

Result: Anova test results showed that corticosterone levels was significant difference between each groups ($p < 0.05$). Tukey HSD test showed there was a significant difference between intervention group and control group on 1st day, in intervention group on 7th day ($p < 0.05$).

Conclusion. The results of this study showed that there were differences of corticosterone serum levels among mice who experienced occlusion disharmony so it concluded that the dental occlusion disharmony had a systemic impact to corticosterone serum levels in *Rattus norvegicus*.

Keywords: Occlusal disharmony, Corticosterone, Stress, Trauma occlusion, Temporomandibular disorders

Introduction

The stomatognathic system is a unitary organ that has related functions each other. The organs are include mandible, maxilla, temporo mandibular joint (TMJ),

tooth structures and other supporting structures such as masticatory muscles, facial muscles also head and neck. The stomatognathic system plays an active role in mastication or chewing process. In normal conditions, there is relationship and integrity of masticatory components system such as the teeth, muscles, TMJ, lips, cheeks, palate, tongue and salivary secretions⁽¹⁾.

If there is a problem in one of component system, it will impacts on occlusion⁽¹⁾. Occlusion is a connection in jaw, where teeth of each jaw will connect on occlusion line curve. This occlusion is important for mastication process, speaking and swallowing processes. Occlusion is

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influenced by physiological and pathological conditions. The pathological condition of occlusion known as occlusal disharmony and it can be caused by several factors such as tooth loss, caries, attrition, periodontal tissue disorders, bruxism, and one-sided chewing habit (2). Moreover, occlusal disharmony problems also can be caused by systemic disorders such as cardiovascular disease, respiratory disorders, nutritional problems, diabetes, posture abnormalities and osteoporosis (3). However, that mechanism of conditions are need further research.

Previous studies showed that occlusal disharmony affected a series of homeostatic processes in body, especially the regulation of Corticosterone hormone and bone metabolism (4). This condition guests that occlusal disharmony as a stressor and affects physiopsychology of human, and stimulate the activity of hypopituitary system axis (5). This situation is possible to increasing adrenocorticotropic hormone (ACTH) secretion and it influencing corticosterone secretion, so it will suppressing the immune system (6). The immunology system can increase the inflammatory cytokines, such as interleukin (IL) -1, IL-6, tumor necrosis factor-alpha (TNF-α), and it activates the osteoclasts role. Osteoclasts are cells who scrapes the bone, dissolve bone and inhibit bone remodeling (7). This condition causes reduction of mineral levels in jaw. Therefore, if the occlusion disharmony problems are not immediately prevented or treated, within a certain time period it will possible cause systemic problems and reduce quality life of the patient.

The aim of this study was to determine the systemic effect of occlusal disharmony on corticosterone serum levels in *Rattus norvegicus*. The measurement of

corticosterone levels conducted at different time frames, it was to determining the trend if there was an increase in each variables. It is important to early detect and identify the abnormalities of bone and teeth caused by occlusal disharmony.

Materials and Method

This study used 7 male white rats of *Rattus norvegicus* in control group and intervention group, 8 weeks of old and 200-250 grams of weigh, and in good health status. Before conducted this research, all procedures obtained ethical research from ethics commission of Dental Faculty, Gajah Mada University, Yogyakarta. In intervention group, the researcher reduced occusal ± 1mm on molar teeth and prevented pulp perforation. The reduction procedure performed under anesthesia. While, the researcher didn't given any intervention in control group.

2 cc of blood serum was taken via infraorbital vein on 1st days, 7th day, 14th day and 21st day. Then the blood was put into a centrifuge tube and centrifuged at 1500 rpm for 20 minutes to get serum. The serum stored in deep freezer at -70 °C until all serum samples have been collected and are ready for corticosterone levels to be checked. The identification of corticosterone levels in serum used sandwich enzyme linked immunosorbent assay (ELISA) technique. The data analyzed by used One Way Anova and continued with Tukey HSD test with a significance p value <0.05.

Results

The results of this study showed that Corticosterone levels in serum become increased in intervention group on 7th day and become decreasing in intervention groups in 14th and 21st day (Table 1).

Table 1. Results of Corticosterone levels in serum (nmol/l)

| Group | Mean ± Standard Deviation | | | |
|--------------|---------------------------|-------------|------------|------------|
| | Day-1 | Day-7 | Day-14 | Day-21 |
| Intervention | 71.50±11,76 | 91.60±4.95 | 87.46±7.44 | 85.95±8.37 |
| Control | 70.38±12.65 | 71.56±16.52 | 74.01±8.17 | 74.64±3.84 |

The results of this study showed that the Corticosterone levels serum increased in treatment group on 7th day and it decreased in treatment groups on 14th and 21st day.

The Anova statistical test and continued with the Tukey HSD test to determine the average difference in each intervention groups. Anova test results showed that there was a significant difference in mean corticosterone levels of serum in experimental animals with p value = 0.003 ($p < 0.05$). The Tukey HSD test used to determine the differences of corticosterone levels between groups. The Tukey HSD test results showed that there was a significant difference of corticosterone levels between intervention group on 1st day and 7th day. The significant differences also showed between control group on 1st day and 7th day, and showed in intervention group on 7th day and control group on 7th day ($p < 0.005$).

The occlusal disharmony is a malocclusion which will reduce mastication function and lead to psychological stress⁽⁸⁾. Stress is psychophysiological response of an organism toward a disturbance or challenges⁽⁹⁾. Occlusal disharmony is recognized by a stressor body that affects a person's psychophysiology and stimulates the activities of axis hypopituitary system⁽⁶⁾. This condition can increasing the secretion of adrenocorticotrophic hormone (ACTH) which affects the corticosterone secretion⁽⁶⁾.

The results of this study showed that there was differences of corticosterone levels between control group and intervention group who experienced occlusal reduction as a form of occlusal disharmony. The increasing of corticosterone levels occurred on 7th day and decreasing on 14th and 21st day. In occlusal disharmony process, systemically the part of body (central nervous of brain system) received stressor. In the brain the stressor translated into a physiological process as a defense response from host. The part of brain stimulated hypothalamus, and play an important role to responding of stress. The hormonal system in hypothalamus start activate in HPA (hypothalamic-pituitary-adrenal) axis. The activated HPA axis way can stimulate the hypothalamus to secreting corticotrophic releasing hormone (CRH) into the hypothalamic-pituitary portal bloodstream⁽¹⁰⁾. CRH stimulates the anterior pituitary to secreting ACTH. ACTH will circulate in blood and throughout to body until to adrenal glands. ACTH will influence the adrenal cortex to secreting glucocorticoid hormones. One of the secretion of glucocorticoid hormones during stress is corticosterone. Corticosterone secretion as a stress hormone and it will increase as a

cellular physiological response⁽¹¹⁾.

The occlusal disharmony among experimental animals caused an increasing of corticosterone levels as an indicator of psychological stress⁽⁸⁾. The body's responses stress in 3 stages, first stage is warning reaction, second stage is resistance stage and third stage fatigue stage⁽¹²⁾. The highest level of corticosterone occurred on 7th day after occurred disharmony disorder. This assume that the resistance phase in intervention mice, where the pituitary secretes ACTH at their peak so the corticosterone secretion also reaches in highest level. Furthermore, the body's response will decline as a form of resistance to stress. This indicated that the decrease of corticosterone levels start at 14th and 21st day.

The increasing of corticosterone levels in intervention mice due to stress and lead to occlusal disharmony than affected host immune system. The effect on immune system can increase inflammatory mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) which they activates the role of osteoclasts. The glucocorticoid stimulation secretes corticosterone, inhibits osteoblast activity and increases osteoclast activity⁽¹⁴⁾. Stimulated osteoclasts caused bone resorption, so it caused the extrication of calcium and phosphorus. The extrication of bone mineral will increases serum calcium and phosphorus levels⁽¹⁵⁾. In addition, glucocorticoids secretes corticosterone and inhibit calcium absorption in digestive tract⁽¹⁶⁾. This will reduce the bone remodeling process.

Based on the results of this study, it concluded that there were differences in corticosterone levels serum among mice who experienced occlusal disharmony. This suggests that the occlusal disharmony caused by occlusal reduction of teeth and it can causes stress and affects the corticosterone secretion.

Ethical Clearance: This study was obtained ethical clearance from the ethics commission of Science Faculty of Gajah Mada University, Yogyakarta.

Conflict of Interest Satatment: The authors declare that they have no conflict of interests regarding the publication of this paper.

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References

1. Suhartini. Fungsi Pengunyahan pada Sistem Pengunyahan (Masticatory Function in the Mastication System) Stomatognathic Jurnal Kedokteran Gigi (Stomatognathic Journal of Dentistry).2011;8 (3): 122-126
2. Sato, Slavicek. The Masticatory Organ and Stress Management. J Stomat Occ Med. 2008; 1: 51-57.
3. Teixeira F B, Luanna de Melo Pereira Fernandes, Patrycy Assis Tavarres Noronha, Marcio Antonio Raiol dos Santos, Wallace Goms-Leal, Cristiane do Socorro Ferraz Maia, et al, Masticatory Deficiency as a Risk Factor for Cognitive Dysfunction. Int. J. Md. Sci. 2014; 11 (2): 209-14.
4. Hwang YK, Chun JS, Yoo PD Ma JY, Hyun, BH, Kim SU, et al, Occlusal Reduction of Unilateral Molars Influences Change of Stress-Related hormones in Rats. Scand. J. Lab. Anim. Sci. 2014; 31 (2).
5. Taga H, Yukio Azuma, Kiyoshi Maehara, Shuichi Nomura. Effects of Changes in Vertical Occlusal Dimension on Heart Rate Fluctuations in Guinea Pigs. In Vivo. 2012; 26: 177-182
6. Guyton AC, Hall. Textbook of Medical Physiology. 10th Ed. New York: WB Saunders Company. 2007.
7. Corwin EJ. Patofisiologi. Jakarta: EGC. 2009.
8. Ekuni D, Yoneda T, Endo Y, Kasuyama K, Irie K, Mizutani S, et al, Occlusal Disharmony Accelerates The Initiation Of Atherosclerosis In Apoe Knockout Rats. Journal Lipids in Health and Disease. 2014; 13: 144
9. Breivik T, Thrane PS, Murison R, Gjerno P. Emotional Stress Effect on Immunity, Gingivitis and Periodontitis. 1996.
10. Yoshihara T, Taneichi R, Yawaka Y. Occlusal Disharmony Increases Stress Response in Rats. Neuroscience Letters. 2009; 452 (2): 181-184.
11. Liu W, Eunice Y. Yuen, Zhen Y. Regulation of the GDI-Rab4 Complex and Glucocorticoid-inducible Kinase (SGK) Pionic Acid (AMPA) Receptors via Serum-Amino-3-hydroxy-5-methyl-4-isoxazolepro Increases Synaptic. Biochemical Chemistry. 285:6101-6108.
12. Seyle, H. History and Present Status of The Stress Concept in Books of Stress Theoretical and Clinical aspect. Editor: Gold Belger and Broznitz S. 1982.
13. Walwadkar DS, Suryakar A.N., Katkam R.V., Kumbar K.M. and R.D. Ankush. Oxidative Stress and Calcium-Phosphorus Levels in Rheumatoid Arthritis. Indian Journal of Clinical Biochemistry. 2006; 21 (2) :134-137
14. De Nijs RNJTL. Glucocorticoid-Induced Osteoporosis in Rheumatic Disease. Thesis. University Medical Centre Utrecht. The Netherland
15. Saliba W, El Hadad B. Calcium and Phosphorus Homeostasis. Journal of the American Board of Family Medicine. 2009.
16. Canalis E, Mazziotti G, Giustina A, Bilezikian JP. Glucocorticoid-Induced Osteoporosis: Pathophysiology and Therapy. Journal of Osteoporosis. 2007;18(10):1319-28