Effect of various inductions of sleep deprivation stress on proinflammatory cytokine levels in gingival crevicular fluids of white male Wistar strain rats (*Rattus novergicus*)

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ABSTRACT

**Background:** Stress that is induced by sleep deprivation can modulate the damage of periodontal tissue by elevating the levels of proinflammatory cytokines (i.e. IL-1β and TNF-α). The effects of sleep deprivation can be resolved with sleep recovery. Gingival crevicular fluid (GCF) is fluid in sulcular gingiva which acts as an oral biomarker for evaluating periodontal abnormalities. **Purpose:** The aim of this study was to determine the effect of various induction methods of sleep deprivation stress on cytokine levels in GCF of white male Wistar strain rats (*Rattus novergicus*). **Methods:** The study method was true experimental with a posttest-only control group design. Thirty male Wistar rats were randomly divided into five groups: paradoxical sleep deprivation (PSD), total sleep deprivation (TSD), partial sleep deprivation with sleep recovery for five days (PSD+SR), total sleep deprivation with sleep recovery for five days (TSD+SR) and a healthy control group. Data were analysed via one-way ANOVA to determine differences between groups. **Result:** The results showed the highest level of IL-1β and TNF-α was found in the PSD group. One-way ANOVA analysis showed significant differences (p<0,05) of IL-1β level between PSD and control groups, PSD and PSD+SR groups and PSD and TSD+SR groups; in contrast, the analysis of TNF-α levels showed significant differences (p<0,05) between PSD group to control group, PSD to PSD+SR group and TSD to TSD+SR group. **Conclusions:** There is an effect of various induction methods of sleep deprivation stress on proinflammatory cytokines (IL-1β and TNF-α).

Keywords: paradoxical sleep deprivation; proinflammatory cytokines; sleep deprivation; total sleep deprivation; sleep recovery

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INTRODUCTION

Stress is a common problem in modern life, causing a significant decrease in the number of sleep hours among adults and children. Epidemiological data show that sleep disorders and short duration of sleep have negative impacts on human physical health.1

Sleep deprivation causes an increase in lymphocyte activation, so that it produces more proinflammatory cytokines (IL-1, IL-6, IL-17 and TNF-α), thereby increasing the risk of inflammation or damage to tissue, including periodontal tissue. In addition, due to an imbalance between the formation and elimination of reactive oxygen species (ROS), sleep deprivation is also related to the mechanism of oxidative stress. However, the impact of sleep deprivation can be corrected by sleep recovery, which results in a decrease in lipid peroxidase and free radical productionm, thus restoring antioxidant activity in inhibiting oxidative stress.2–5

Proinflammatory cytokines play an important role in inflammation and bone resorption; therefore, they are important parameters in periodontal research. The presence of IL-1β in gingival crevicular fluid (GCF) can stimulate the occurrence of alveolar bone resorption. TNF-α produced by macrophages and lymphocytes has the same effect as IL-1β. Tumor necrosis factor-α (TNF-α) can stimulate cell proliferation and differentiation such as osteoblasts and osteoclasts. The ability of TNF-α to stimulate the production...
of enzymes that damage the matrix and bone recession activities plays an important role in damage caused by periodontal disease.6,7

Gingival crevicular fluid is a product of cells (transudates) found in the gingival sulcus originating from post-capillary venules from the gingival plexus, and it can be influenced by an inflammatory response in the area around the gingival sulcus. Cytokines detected in GCF can be used as oral biomarkers in evaluating periodontal disease conditions or in periodontal treatment results.7 Research on the effect of various induction methods of sleep deprivation stress on proinflammatory cytokine levels, IL-1β and TNF-α levels in GCF of white male Wistar strain rats (Rattus norvegicus) has never been done before; therefore, the authors are interested in exploring this topic.

MATERIALS AND METHODS

This study used a true experimental approach with a post-test-only control group design for white male Wistar strain rats (Rattus norvegicus) aged 3–4 months with a weight of 200–300 grams. The rates were given various inductions of sleep deprivation stress with paradoxical model sleep deprivation (PSD) with 20 hours given stress and four hours rest for five days; total sleep deprivation (TSD) with 24 hours given stress and no rest for five days; paradoxical sleep deprivation was continued with sleep recovery for five days (PSD + SR); and total sleep deprivation was continued with sleep recovery for five days (TSD + SR). All stress models used a single platform method (SPM) tank measuring 23 x 23 x 35 cm where one rat in a flowerpot was equipped with muscle atonia which gave a shock effect automatically every 10 minutes in experimental animals.8

The subjects of this study were 30 white male Wistar strain rats (Rattus norvegicus) aged 3–4 months with a weight of 200–300 grams obtained from the Pharmacology and Therapy Department, Faculty of Medicine, Universitas Gadjah Mada. All experiments conducted in this study have been approved by the Ethics Commission of the Faculty of Medicine, Universitas Jenderal Soedirman No. Ref. 4704/KEPK/X/2018. The experimental animals were randomly divided into five groups: PSD (KI) group, TSD group (KII), PSD + SR group (KIII), TSD + SR group (KIV), and healthy control group (KV); each group consisted of six white rats.

All experimental animal samples were acclimatised for seven days before the induction of various sleep deprivation methods. After acclimatisation, groups I and III were given PSD stress treatment for 120 hours, and groups II and IV were given TSD stress treatment for 120 hours, while group V was not treated with stress. Groups I, II and III then returned to their original state while groups IV and V continued with sleep recovery for 120 hours and then returned to their original conditions.

After the induction of stress deprivation was completed, GCF was taken using filter paper with a size of 2.55 mm x 14.19 mm with a thickness of 0.16 mm with the intracrevicular absorption method. Previously, filtered paper was carried out by gas ethyleneoxide (EOG) sterilization to prevent bacterial or fungal contamination that could affect variables. The GCF retrieval method was carried out by controlling saliva with cotton in the area around the anterior gingival of the mandibula, inserting filter paper into the gingival sulcus at a depth of 1 mm in the anterior gingival of the mandibula of the white male Wistar strain rats for 30 seconds. GCF retrieval was carried out after the treatment was completed, and the extraction time was adjusted to the circadian rhythm of the rats at 07.00–10.00.

Filter paper containing GCF was immediately inserted into an eppendorf tube containing 200µl of Phosphate Buffered Saline (PBS) (Gibco®, USA) with a pH of 7.4; it was then homogenised using a vortex mixer. GCF was stored at -20°C or allowed to melt at 2–8°C, and then centrifuged from 2,000 to 3000 rpm for 20 seconds. Measurement of IL-1β and TNF-α levels was carried out using the enzyme-linked immunosorbent assay (ELISA) sandwich method which was read on an ELISA reader with an absorbance wave of 450 nm. Data were tabulated and tested for normality by the Saphiro-Wilk and Levene test for its homogeneous. Data were then analysed by one-way ANOVA to determine differences between groups.

RESULTS

The results of the study on the measurement of IL-1β levels revealed mean levels of IL-1β of experimental animals in each group described in Figure 1. Figure 1 shows that the highest mean of IL-1β level experimental animals is in the PSD group with a mean of 453.05 ± 48.81 pg/L. The lowest mean of IL-1β levels was found in the PSD + SR group: 380.40 ± 24.65 pg/L.

The results of the Shapiro-Wilk and Levene statistical analysis showed that the data of each group were normally distributed and homogeneous. The statistical test continued using the parametric one-way ANOVA test, and the result of the significance obtained is p<0.05. These results indicate the effect of sleep deprivation treatment on IL-1β levels. A post-hoc LSD test was carried out to determine if there are significant differences between each treatment in the sample groups.

Table 1 shows groups with significant results (p<0.05), a significant control group for the PSD group and vice versa. The PSD group was significant for the PSD + SR group and vice versa. The TSD + SR group is significant for the PSD group and vice versa. In contrast, the TSD group did not show significant results (p>0.05) for all groups.

The results of the study on the measurement of TNF-α levels obtained mean levels of TNF-α in each group as described in Figure 2. Figure 2 shows that the highest mean of TNF-α level in experimental animals was in the PSD group, with a mean of 377.89 ± 69.00 ng/ml. The lowest
Figure 1. Mean of IL-1β levels in experimental animals.

Figure 2. Mean bar chart of TNF-α levels in experimental animals.

Table 1. Post-Hoc LSD test, mean IL-1β levels in sleep deprivation stress treatment in each group

<table>
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<td>TSD</td>
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Notes: * p<0.05

Table 2. Post-Hoc LSD test, mean of TNF-α levels in sleep deprivation stress treatment in each group

<table>
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<tbody>
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<tr>
<td>TSD+SR</td>
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</table>

Notes: *p<0.05
mean of TNF-α levels was found in the PSD + SR group: 217.31 ± 26.40 ng/ml.

The results of the Shapiro-Wilk and Levene statistical analysis showed that the data were normally distributed and homogeneous. The statistical test continued using the parametric one-way ANOVA test, and the results of significance obtained was p<0.05. These results indicate that there is an effect of sleep deprivation treatment on TNF-α levels. Post-hoc LSD tests were carried out to determine if there are significant differences between each treatment in the sample group.

Table 2 shows groups with significant results (p<0.05), which is the significant control group for the PSD group and vice versa. The PSD group was significant for the PSD + SR group and vice versa. The TSD group was significant for the PSD + SR group and vice versa. In contrast, the TSD + SR group did not show significant results (p> 0.05) for all groups.

DISCUSSION

Based on the results of the study, the mean of IL-1β level was highest in the PSD group, while the lowest mean of IL-1β level was found in the PSD + SR group. This indicates that sleep deprivation stress has an impact on IL-1β levels, and the administration of sleep recovery after sleep deprivation treatment can reduce IL-1β levels. When sleep deprivation occurs, endogenous and endotoxin factors will induce the production of proinflammatory cytokines, including IL-1, TNF-α, and nuclear factor kappa B (NF-κB). Furthermore, proinflammatory cytokines along with neurochemicals play an important role in the regulation of sleep in the non-rapid eye movement sleep (NREM) phase, resulting in an increase in proinflammatory cytokines in the NREM phase and will decrease during the rapid eye movement sleep (REM) phase. This is in line with the results in this study, wherein the mean of IL-1β level of the PSD group was significant (p <0.05) for the control group who were not given stress exposure; hence, in the control group, there was no increase in IL-1β levels.9–11

Sleep deprivation can be divided into acute sleep deprivation and chronic sleep deprivation. Acute sleep deprivation is a long period of an awake condition, whereas chronic sleep deprivation is an accumulation of sleep deprivation that occurs over several days. According to Landolt et al. (2014),5 partial sleep deprivation (PSD) is included in acute deprivation (short-term), whereas total sleep deprivation (TSD) is included in chronic sleep deprivation (long-term). Stress exposure can have an effect on the immune response, where acute stress deprivation sleep can induce innate immunity, antigen presentation, antibodies and cytokine production.12,13

IL-1β levels in the PSD and TSD groups in this study did not show a significant difference (p> 0.05), although IL-1β levels in the PSD group were higher than the IL-1β levels in the TSD group. This shows that sleep deprivation stress in the PSD group can produce more IL-1β than the TSD group. This condition is caused by the immune response in the acute phase of sleep deprivation activating Toll-like receptors (TLR), which will trigger gene transcription in NF-κB and increase production of IL-1β and other proinflammatory cytokines, so that IL-1β levels in the PSD group are higher than in TSD groups. In addition, interleukin 1 (IL-1α and IL-1β) is a key mediator in the acute phase response to infected hosts.9,14

Some studies have suggested that sleep can play an anti-stress role through the mechanism of inhibiting the HPA axis. Sleep recovery can improve the impact of sleep deprivation by returning the HPA axis interaction to normal. Sleep deprivation conditions can activate the HPA axis, playing an important role in the occurrence of stress, inducing an increase in the production of IL-1β, and returning to normal after sleep recovery.15–17

IL-1β levels in this study had significant results (p<0.05) in the PSD group against the PSD + SR group. The IL-1β levels in the PSD + SR group decreased significantly when compared with the PSD group. This shows that the administration of sleep recovery in the PSD + SR group succeeded in reducing levels of IL-1β. Sleep recovery can improve the effects of sleep deprivation by returning the HPA axis interaction to normal, reducing the hormone cortisol so that it results in a decrease of CRH. Sleep deprivation will stimulate the activation of the HPA axis and induce the CRH hormone, which is the main regulator in the wake condition, inhibiting the non-REM (NREM) and REM sleep phase.14,18,19

This study also showed significant results (p<0.05) in TSD + SR groups for the PSD group, as the mean of IL-1β levels in TSD + SR group was lower than the PSD group. This suggests that the administration of sleep recovery exposure in the TSD + SR group also reduced IL-1β levels. The PSD group was included in the acute stress category so that levels of IL-1β produced were higher when compared with the TSD + SR group, which was included in the category of chronic stress to be then given additional treatment, namely sleep recovery.

The administration of sleep recovery to PSD and TSD treatment in the PSD + SR and TSD + SR groups in this study had almost the same results, which showed a non-significant difference (p>0.05), although the IL-1β levels in the PSD + group SR show lower results than the TSD + SR group. It demonstrated that administering sleep recovery may successfully reduce levels of IL-1β after treatment of PSD and TSD. Sleep recovery can restore body performance, especially in the REM cycle recovery, where REM sleep deprivation for more than 72 hours can cause increased levels of IL-1β, IL-6, IL-17A, and TNF-α in rats.16,17

Changes in TNF-α levels due to stress deprivation sleep have been widely demonstrated in previous studies. Based on the results of this study, sleep deprivation stress has an impact on the levels of TNF-α, where the highest levels of TNF-α were highest in the PSD group, and the...
lowest levels of TNF-α were found in the PSD + SR group. Sleep deprivation stress plays a role in modulating cytokine production, one of which is TNF-α. The body’s response to stress can change the homeostasis system because stress will induce activation of the HPA axis and sympathetic nervous system. The activation of the HPA axis will cause glucocorticoid production in the adrenal cortex. Stress exposure that occurs continuously has an effect on adrenaline response quickly, so glucocorticoids experience a rapid increase in exposure to acute stress, whereas, in chronic stress, the body can control these conditions so that glucocorticoid production decreases and can be lost. In chronic sleep deprivation, there is an increase in cortisol levels which causes desensitisation of glucocorticoid receptors, resulting in a decrease in glucocorticoid levels.\textsuperscript{5,14,20}

This study showed a higher increase in TNF-α levels in the PSD group than in the TSD group. However, the results of the post-hoc LSD analysis did not show a significant difference (p>0.05) between the two groups. This shows an increase in TNF-α levels in the PSD group which can be seen when compared with the TSD group. As in the IL-\textsuperscript{1β} pattern, stress exposure PSD treatment is included in the category of acute stress induction, where the immune response in the acute phase of sleep deprivation will activate receptors such as toll-like receptors (TLR) that will transcript genes at NF-\textalpha and increase production of TNF-α and other proinflammatory cytokines.\textsuperscript{9,21}

Similar to IL-\textsuperscript{1β}, TNF-α plays a role in the sleep regulatory mechanism in the hypothalamus and locus coeruleus (LC) in the NREM phase. The administration of sleep recovery treatment in experimental animals can reduce the activity of the HPA axis so that it can reduce TNF-α levels. This study had significant results (p <0.05), namely in the PSD group for the PSD + SR group, where TNF-α levels in the PSD + SR group decreased after being given a sleep recovery treatment. This shows that exposure to sleep deprivation stress with the treatment of PSD + SR has been successful in reducing TNF-α levels. Sleep recovery can improve the effects of sleep deprivation by returning the interaction from the HPA axis to normal. A decrease in activation of the HPA axis can reduce the production of TNF-α.\textsuperscript{16,22,23}

The administration of sleep recovery in the PSD + SR group in this study was also significant (p<0.05) for the TSD group. The PSD + SR group had lower TNF-α results when compared with the TSD group. This happens because the administration of sleep recovery in the SR PSD + group can reduce levels of TNF-α. Sleep can reduce substances that can stimulate TNF-α production and can inhibit the corticotropin-releasing hormone (CRH). Reduction in CRH secretion will reduce activation of the HPA axis so that TNF-α levels decrease.\textsuperscript{15}

The PSD + SR and TSD + SR groups showed almost the same results so that there was no significant difference (p>0.05). TNF-α levels in the TSD + SR group were higher when compared to the PSD + SR group. This happens because the PSD + SR group still has time to sleep every day and also gets additional time for sleep recovery so that the levels of TNF-α in the PSD + SR group are lower than the TSD + SR group. Subjects who were given TSD for 24 hours and 48 hours full, and then given a sleep recovery treatment for 24 hours, will recover from the sleep deprivation by 72% for TSD for 24 hours and have 42% for TSD for 48 hours.\textsuperscript{24}

In conclusion, the induction of various stress deprivation stress methods has an impact on the levels of proinflammatory cytokines namely IL-\textsuperscript{1β} and TNF-α in GCF white male Wistar strain rats (Rattus norvegicus). The administration of sleep recovery after the induction of sleep deprivation stress has an effect on levels of IL-\textsuperscript{1β} and TNF-α in GCF white male Wistar strain rats (Rattus norvegicus).

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