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Coffee Consumption Modulated The Level Of Adrenalin Blood Serum In Periodontitis Rat Model

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Abstract:
Background*. Adrenalin was supposed to have an anti-inflammatory effect; hence adrenalin concentration in blood serum might be lower in inflammatory diseases. Coffee contains caffeine that is thought to have the potency to increase adrenalin secretion in inflammatory diseases. This study aimed to analyze the effect of coffee consumption on adrenalin blood levels in periodontitis rat model.*
Materials and Methods. *This in vivo experimental study used the post-test-only control group design. Wistar male rats (Rattus novergicus) were divided into four groups (four rats/group) i.e. 1) Periodontitis (no coffee), 2) periodontitis + coffee, 3) Coffee (no periodontitis), 4) Control (no coffee, no periodontitis). The Periodontitis rat model was made by injecting periodontitis bacteria Phorpyromonas gingivalis in the buccal mucosa of the lower molar tooth three times a week for 4 wk. A single dose (0.6 mL d^{-1}) of robusta roasted coffee ground infusion (equal to one cup per day for humans) was given to each rat in the coffee group, for 28 d. At the end of the study, all of the rats were sacrificed and blood was drawn from intracardial. Blood serum adrenalin concentration was analyzed using Enzyme-linked immunosorbent assay (Elisa).*
Results*. The Periodontitis group showed significantly lower serum adrenalin ($p<0.05$) than others. Coffee consumption in periodontitis rats affected a significantly higher adrenalin blood serum level ($p <0.05$).*
Conclusion. *Coffee consumption could replenish the level of blood serum adrenalin which decreases in periodontitis. Further studies are needed to elucidate the modulation mechanism of coffee-adrenalin-inflammation.*
Key Words: *Caffeine; Elisa; Inflammatory disease; Phorpyromonas gingivalis; Robusta coffee*

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I. Introduction

Adrenalin or epinephrine (a catecholamine) was supposed to have an anti-inflammatory effect. Adrenalin down-regulates the pro-inflammatory activities of neutrophils[1]. Another study reported epinephrine regulation on anti-inflammatory and pro-inflammatory cytokines[2]. Catecholamine synthesis exhibits anti-inflammatory effects in human rheumatoid arthritis synovial cells in vitro[3]. Epinephrine administration caused a significant change in inflammation-related gene expressions[4]. Those studies raised the suspicion, that there might be low levels of adrenalin in inflammatory diseases, including periodontitis (a chronic bacterial inflammatory disease that affects tooth-supporting tissue).

The association of adrenalin and periodontitis was not fully understood. Periodontitis was reported to metastasize into the blood systemic circulation and was associated with inflammatory diseases in other organs apart from periodontal tissue and systemic diseases such as cardiovascular diseases, rheumatoid arthritis, diabetes mellitus, etc.[5,6,7]. There was a report about the adrenal crisis provoked by dental infection[8]. Another study showed that periodontitis patients presented modifications for serum or urinary markers of renal dysfunctions, suggesting that periodontitis might influence renal function[9]. Considering the potency of periodontitis metastasis, it was suspected that periodontitis invasion probably could affect the adrenal glands and influence the synthesis of adrenalin.

Coffee contains caffeine that was thought to increase adrenalin secretion, and therefore it was emerging a question, whether coffee can modulate adrenalin, in conditions of chronic inflammatory disease (such as periodontitis), since it might cause adrenalin reduction. The caffeine content of coffee is thought to be related to blood pressure through increased adrenalin[10]. Although many studies have investigated experimentally coffee's effect on blood pressure, however, the effect of coffee on adrenalin was rarely discussed. In addition, the role of adrenalin in inflammatory diseases and the modulation mechanism of coffee-adrenalin-periodontitis are still not clear. To contribute to the explanation of this phenomenon, the present research aimed to study the effect of coffee consumption on blood serum adrenalin in inflammatory disease, i.e. periodontitis.

II. Material And Methods

Materials. Pure Robusta ground coffee was purchased from the factory of State Coffee Plantation *PTPN XII*, East Java, Indonesia. Periodontitis bacteria was *Porphyromonas gingivalis* (ATCC 33277). A kit of Enzyme-linked immunosorbent assay (Elisa), namely EPI (Epinephrine/Adrenalin) was purchased from Elabscience, and Wistar rats from The Centre of Veterinary Research (*Pusvetma*) Surabaya, Indonesia.

Study Design and Experimental Animal. This in vivo experimental study used the post-test-only control group design. The experimental animal was a Wistar male rat *(Rattus novergicus*), with a body weight of around 200 g. Prior studied all of the rats were acclimated to ensure that they were healthy and well-adapted to the laboratory environment. The research protocol was approved by the Research Ethical Committee of the Faculty of Medicine Universitas Jember (No.1100. H.25.1.11/KE.2016). A total of 16 rats met the inclusion criteria and then were divided into four groups (four rats/group), i.e. i) Periodontitis (no coffee), ii) periodontitis + coffee, iii) Coffee (no periodontitis), iv) Control (no coffee, no periodontitis).

Periodontitis rat model. Periodontitis rat models were created according to the method described by Susilawati et.al. with some modifications[11]. In doing so, rats in the periodontitis model were injected using periodontitis bacteria 0.05 mL *Phorpyromonas gingivalis* (2×10^9 CFU mL^{-1}) in the buccal mucosa of the lower molar tooth three times per week for 4 wk. The occurrence of periodontitis was confirmed by the sign of gingival swelling and the existence of alveolar bone resorption that was indicated radiographically (at the end of the study).

Coffee preparation and treatment. Regular Robusta ground coffee was used in this study for practical reasons because Robusta coffee was more commonly found in communities. A single dose of ground coffee infusion (equal to one cup per day for humans) was given to each rat in the coffee group, for 28 d. The coffee infusion was made according to the receipt of the coffee factory in the brochure. Briefly, to make a coffee infusion, a teaspoon (10 g) of ground coffee was poured into 200 mL of boiled water, steered for ten seconds, and then let at room temperature to allow the separation between sediment and supernatant. The supernatant was then collected, and saved in the refrigerator until before being analyzed. The dose for rats was determined based on the body weight ratio between adult human (70 kg) and rat (200 g). This calculation yields a dose for each rat namely 0.6 mL/rat/day. The coffee infusion was given to rats using stomach sondage to make sure a proper dose was given for each rat.

Blood serum preparation. At the end of the study, rats were fasted for 10 h and were sacrificed using intraperitoneal injecting of ketamine 100[11]. After that, the animals were fixed in such a manner and performed thorax surgery to visualize the cardiac organ. Then, whole blood was drawn as much as ± 6 mL from intra-cardiac per rat. The whole blood tubes were placed in the ice box container before being centrifuged. Furthermore, whole blood was immediately centrifuged at 300 rpm for 10 min. The supernatant (blood serum) was separated and kept in refrigeration (-20°C) until before being analyzed.

Adrenalin measurement. Adrenalin levels in blood serum were analyzed according to the instructions in the brochure of the ELISA kit. In brief, a total of 50 µL standards or samples was added into the micro-ELISA plate that has been pre-coated with EPI (primary antibody), and incubated for 1 h at 37°C. After washing the unbound sample or standard from the plate, 50 µL biotinylated detected antibody (seconder antibody) was added to each microplate well and incubated for 45 min at 37 °C. After washing (three times), each well was added with 100 µL conjugate SA-HRP (Streptavidin-Avidin-Horseredish Peroxidase) and was incubated for 30 min at 37°C. Furthermore, after washing the excess conjugate, each well was added with 90 µL substrate reagent Tetramethyl benzidine (TMB) and incubated for 15 min at 37 °C. The enzyme-substrate reaction is terminated by the addition of 50 µL stop solution and the color change is measured using a spectrophotometer at a wavelength of 450 nm ± 2 nm. The concentration of adrenalin in the samples was then determined by comparing the optical density (OD) of the samples to the standard curve.

Data analysis. The data (serum adrenalin concentration) were expressed as a mean ± standard deviation (X ± SD) and analyzed by One Way ANOVA followed by Least Significant Difference (LSD).

III. Result

This study confirmed the occurrence of periodontitis (in rats in periodontitis group) that was characterized by alveolar bone resorption. Figure 1. shows the rat's jaws, in which the gingival tissues had been peeled to visualize the alveolar bone. The alveolar bone resorption was confirmed by the apical migration of the alveolar bone crest, clinically and radiographically.

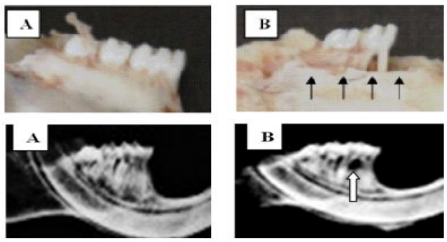

Figure 1. The Periodontitis rat model was characterized by alveolar bone resorption, clinical examination (above), and radiographic examination (below). A. The control group did not exhibit periodontitis (no alveolar bone resorption) B. Periodontitis group demonstrated alveolar bone resorption indicated by apical migration of alveolar crest (arrow).

Analysis of blood serum showed that rats in the periodontitis group significantly ($p < 0.05$) demonstrated the lowest adrenalin level compared to other groups (Table 1). Coffee consumption in periodontitis rats affected a significantly higher adrenalin blood serum level ($p < 0.05$). Coffee consumption in normal rats (no periodontitis) and in periodontitis rats showed no significant difference ($p > 0.05$) in the serum level of adrenalin, and also when compared to the control group (no coffee, no periodontitis).

Table 1. The average adrenalin blood serum levels in rat groups

Groups	Adrenalin (pg mL^{-1})
	X ± SD
Periodontitis (no coffee)	227.88 ± 37.82
Periodontitis + coffee	316.31 ± 35.38[a]
Coffee (no periodontitis)	310.33 ± 32.36[a]
No coffee, no periodontitis	325.07 ± 14:52[a]

[a] Significant difference ($p < 0.05$) to the periodontitis group (ANOVA & LSD)

IV. Discussion

Adrenalin is an endocrine hormone, naturally released from the medulla of the adrenal glands. About 80% of the cells of the adrenal medulla secrete adrenalin[12]. In general, adrenalin is produced in response to stress that increases heart rate, strengthens the force of the heart's contraction and cardiac output, increases blood pressure, opens up the bronchioles in the lungs, and raises the blood levels of glucose and lipids among other effects.

In some studies, adrenalin was reported to have anti-inflammatory effects. Adrenalin down-regulates the pro-inflammatory activities of inflammatory cell neutrophils[1] by cAMP-mediated enhancement of the clearance of cytosolic Ca^{2+}. Other studies reported epinephrine regulation of anti-inflammatory and pro-inflammatory cytokines[12]. Catecholamine synthesis exhibits anti-inflammatory effects in human rheumatoid arthritis synovial cells in vitro and administration of EPI in a rat sepsis model inhibits the production of TNF-α possibly via the β2–adrenoceptor[3,13]. Epinephrine administration caused a significant change in inflammation-related gene expressions[14].

This study indicated that in conditions of inflammatory disease (periodontitis), serum adrenalin level was lower than normal. Some arguments could be stated: i) periodontitis might suppress the anti-inflammatory effect of adrenalin, ii) invasion of periodontitis might reach the medulla adrenal and affect the synthesis of adrenalin. Further studies were needed to confirm these notions since this is the first study to report the level of adrenalin in inflammatory diseases such as periodontitis.

Periodontitis is a chronic bacterial inflammatory disease characterized by the destruction of the tooth-supporting tissues, which is the leading cause of tooth loss[14]. Periodontitis is mainly caused by infection of Gram-negative bacteria *Porphyromonas gingivalis*[15]. Periodontitis was associated with systemic diseases such as cardiovascular disease, diabetes mellitus, preterm low birth weight, rheumatoid arthritis, and osteoporosis[5,6,7]. One of the possible mechanisms by which periodontitis might contribute to the pathogenesis of the systemic disorder is due to its potency of metastatic or spreading into the systemic circulation and reaching organs apart from the periodontal origin[15,16,17,18,19]. This bacterial metastasis might contribute to the destruction of body organs including the medulla adrenal glands. Further studies were needed to confirm this notion.

This study showed that coffee consumption modulated the level of serum adrenalin in periodontitis rat model. Coffee contains bioactive compounds that is rich of antioxidants that might counteract the oxidative destruction of cells or tissues[20,21,22,23]. And therefore, it might provide protection for the medulla adrenal gland

against periodontitis metastatic. In addition, some studies reported the anti-inflammatory effect of coffee[24,25]. This study proved that coffee might replenish the level of adrenalin, which decreased in periodontitis.

Coffee consumption equal to one cup per day did not affect the adrenalin serum level. This study proved there was no significant difference ($p > 0.05$) in adrenalin serum levels neither in the normal nor coffee group. Meanwhile, in the periodontitis group, coffee consumption provided a beneficial effect in improving serum adrenalin. Whether this phenomenon could have a similar effect on other inflammatory diseases, further studies were needed. In addition, the effect of coffee dose was needed to study as well, since a high dose of coffee might be detrimental to the adrenal gland and might affect adrenalin production.

V. Conclusion

Coffee consumption could replenish the level of blood serum adrenalin, which decreases in periodontitis. Further studies are needed to elucidate the modulation mechanism of coffee-adrenalin-inflammation.

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