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Effect of ovarian dysfunction induced ovariectomy and Porphyromonas gingivalis induction to risk of metabolic syndrome: *in vivo* study

Efeito da disfunção ovariana por meio de ovariectomia e indução de Porphyromonas gingivalis no risco de desenvolvimento de síndrome metabólica: estudo *in vivo*

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ABSTRACT

Periodontal diseases and metabolic syndrome are related to complicated multifactorial conditions. However, the relationship is not yet evident. Estrogen insufficiency might correlate to this condition, possibly caused by ovarian removal and *Porphyromonas gingivalis* (*P. gingivalis*) infection. This study aimed to evaluate the effect of ovarian dysfunction caused by ovariectomy and *P. gingivalis* infection to metabolic syndrome development. This study was an experimental laboratory study using female rats Sprague Dawley Strain. Animal models were divided into four groups: control, ovariectomy (OVX), ovariectomy-periodontitis (OPG), and periodontitis (PG). The purpose of every treatment in each group was to induce ovarian dysfunction. The OVX group was undertaken ovaries removal surgery. PG was performed *P. gingivalis* induction. Therefore OPG was a combination of ovariectomy and *P. gingivalis* induction. Blood was drawn and observed on days 0, 3, 7, 14, 21, and 28. The blood sample was examined for uric acid, cholesterol, glucose and estrogen. The collected data were all statistically examined. All treatment groups presented body weight and blood biochemical observation significantly higher than the control group, except total cholesterol ($p<0.05$). Moreover, most variables presented a correlation between groups to body weight and biochemical blood indicators, except blood uric acid level ($R>0.5$). The metabolic syndrome was triggered by ovarian dysfunction brought on by *P. gingivalis* infection after ovariectomy. They both took the same risk. Even *P. gingivalis* induction made metabolic syndrome in the group of animal models which underwent ovariectomy worse.

KEYWORDS

Estrogen deficiency; Metabolic syndrome; Ovariectomy; Ovarian dysfunction; *P. gingivalis*.

RESUMO

Doenças periodontais e síndrome metabólica estão relacionadas a condições multifatoriais complicadas. No entanto, a relação ainda não é evidente. A insuficiência de estrogênio pode estar correlacionada a essa condição, possivelmente causada pela remoção dos ovários e infecção por *Porphyromonas gingivalis* (*P. gingivalis*). Este estudo teve como objetivo avaliar o efeito da disfunção ovariana causada pela ovariectomia e infecção por *P. gingivalis* no desenvolvimento da síndrome metabólica. Este foi um estudo experimental de laboratório utilizando ratos fêmeas da linhagem Sprague Dawley. Os modelos animais foram divididos em quatro grupos: controle, ovariectomia (OVX), ovariectomia-periodontite (OPG) e periodontite (PG). O objetivo de cada tratamento em cada grupo foi obter disfunção ovariana. O grupo OVX foi submetido à cirurgia de remoção dos ovários; no grupo PG foi realizada a indução de *P. gingivalis*; e no grupo OPG foi feita uma combinação de ovariectomia e

indução de *P. gingivalis*. O sangue foi coletado e observado nos dias 0, 3, 7, 14, 21 e 28. A amostra de sangue foi examinada para ácido úrico, colesterol, glicose e estrogênio. Os dados coletados foram todos examinados estatisticamente. Todos os grupos de tratamento apresentaram peso corporal e observações bioquímicas sanguíneas significativamente maiores do que o grupo controle, exceto o colesterol total ($p<0,05$). Além disso, a maioria das variáveis apresentou uma correlação entre os grupos com o peso corporal e indicadores bioquímicos sanguíneos, exceto o nível de ácido úrico no sangue ($R>0,5$). A síndrome metabólica foi desencadeada pela disfunção ovariana causada pela infecção por *P. gingivalis* após a ovariectomia. Ambos apresentaram o mesmo risco. Mesmo a indução por *P. gingivalis* piorou a síndrome metabólica no grupo de modelos animais que foram submetidos à ovariectomia.

PALAVRAS-CHAVE

Deficiência de estrogênio; Síndrome metabólica; Ovariectomia; Disfunção ovariana; *P. gingivalis*.

INTRODUCTION

A metabolic syndrome is a cluster group of metabolic interference, including dyslipidemia, visceral obesity, atherogenic, hyperglycemia, and hypertension, frequently associated with cardiovascular diseases and type 2 diabetes mellitus risk [1]. The prevalence of this syndrome is an individual who presents obesity, physical inactivity, aging, hormonal alteration, and genetics [2]. This syndrome correlates to inflammation, such as periodontal diseases [3]. Several studies reviewed metabolic syndrome and periodontal diseases were complex multifactor disorders and exhibited correlation [4–6]. However, the relationship between periodontal diseases and metabolic syndrome remains unclear and needs advanced investigation.

Most women over forty-five years old risk getting metabolic syndrome and periodontal diseases. Several studies correlated those disorders with aging and hormonal changes related highly susceptibility to inflammation, such as gingival inflammation and periodontitis, [7-9]. This population is entering the menopause phase, which decreases ovarian, hypothalamus-pituitary-gonadal axis function, and sex steroid hormone, particularly estrogen [10].

Ovaries are the main organs producing estrogen. This hormone affects both reproductive and other organs, such as the liver, adipose tissues, and periodontal tissue [11]. Moreover, estrogen also involves in several metabolisms, such as insulin regulation [12], immune cell activation and regulation [13], and anti-inflammation agents [14]. Estrogen level alteration stimulates several disorders and inflammation severities [15]. However, this statement has been controversial until now. Several previous studies exhibited opposite results each other [16-19].

Besides the aging process affecting estrogen levels in circulation and the activity, bacterial infection stimulates estrogen production disturbance, leading to ovarian dysfunction [20]. *Porphyromonas gingivalis* (*P. gingivalis*), as the major periodontal pathogen, presents several virulence factors [21]. The virulence factors, especially lipopolysaccharide (LPS) might down-regulates estrogen, thereby interrupting ovary steroidogenesis [20]. *P. gingivalis* was suspected of ovarian function through systemic inflammation and oxidative stress, which impacted on metabolism process, especially glucose, lipid, and uric acid regulation. However, an explanation about it required further investigation. This study aimed to evaluate effect of ovarian dysfunction caused by ovariectomy and *P. gingivalis* infection to metabolic syndrome development. This study used ovariectomy and *P. gingivalis* induction to get ovarian dysfunction or premature menopause. Apart from that, there are many methods for modeling periodontitis. However, this study used a model of periodontitis induced by *P. gingivalis* because this method better describes the condition of chronic periodontitis caused by periodontal pathogens, especially *P. gingivalis* [22]. Ovariectomy mimics oophorectomy or ovaries removed surgically, while *P. gingivalis* induction induced systemic inflammation and possibly triggered premature menopause. In this study, ovariectomy was used as a comparison to determine whether induction of *P. gingivalis* also produced the same effect as menopause models.

MATERIAL AND METHODS

Animals

This study was approved and conducted by the Health and Research Ethics Committee of the Dental Faculty, Gadjah Mada University. This study followed national and international guidelines for the care and welfare of laboratory animals.

This experimental laboratory study used rats (Rattus norvegicus) Sprague Dawley Strain, aged 6 to 8 weeks, female, and 150-200 grams. The rats adapted to constant room temperature and relative humidity on a 12-h day and night cycle, with direct access to food and water (diet and water ad libitum). Animal models were divided into four groups: control (without any treatments), ovariectomy group (OVX), periodontitis group (PG), and ovariectomy-periodontitis group (OPG). All of the treatment groups were performed to get ovarian dysfunction. The control group in this study was used to determine the baseline value (standard value) of the animal models. This OVX group manipulated animal models to mimic menopause or ovarian dysfunction. Animal models of OVX groups were given 3 days before blood sampling was carried out for the recovery period after ovariectomy surgery and to avoid the impact of surgery on blood test results. The PG group showed mimicking periodontitis due to the induction of major periodontal pathogens. Meanwhile, the OPG group is mimicking menopause accompanied by periodontitis due to the induction of major periodontal pathogens. In the OPG group, induction of p. gingivalis is given after 3 days post-surgery for the recovery period.

The grouping of animal models was carried out randomly. However, in this study, there were difficulties due to the non-uniformity of the body weight of the animal models, so one or two animal models that had body weights outside the average affected the results of the body weight of the model animals.

Preparation of *P. gingivalis* Suspension

P. gingivalis was obtained from *P. gingivalis* stock (Porphyromonas gingivalis ATCC 33277, Thermo Fisher Scientific, USA). *P. gingivalis* stock was inoculated on solid Brain Heart Infusion Agar (BHI-A) (Oxoid, Thermo Fisher Scientific, USA). After that, it was put in a desiccator for 2x24 hours with CO_2 gas pack (Oxoid, Thermo Fisher Scientific, USA) to make anaerobic condition. Subsequently, one ose of *P. gingivalis* on BHI was taken and put in 2 mL of Brain Heart Infusion Broth (BHI-B) (Oxoid, Thermo Fisher Scientific, USA). Afterwards, the suspension was homogenized using vortex (Thermo Fisher Scientific, USA) for 30 seconds and then incubated in a desiccator with CO_2 gas pack at 37° C for 24 hours. The growth marked the turbidity of BHI media, then it was diluted with sterile aquadest, shaken till homogenous and measured the concentration manually with 1.5 of Mc. Farland standard 2.10^9 cells/ ml of concentration [23].

Surgical procedure (Ovariectomy)

The OPG and PG groups were prepared for ovariectomy procedures. They were anaesthetized with ketamine/xylazine (80/10 mg/kgBW) intraperitoneally (Sigma Aldrich, Singapore). After anesthetization, the dorsal area of animal models was disinfected with povidone-iodine. On the right side of the dorsal, approximately 1-1.5 cm of the spine was performed with a small transverse incision (0.4–0.6 cm) using surgical scalpel blade no. 11 on the right side. After the peritoneal cavity was accessed, the adipose tissue was pulled away until the right uterine tube and the ovary, surrounded by a variable amount of fat, were identified. The ovary and associated fat were located and exteriorized by gentle retraction. The uterine horn was bound, and then the ovaries were cut. After that uterine horn was reimplanted in the peritoneal cavity. The wound was closed by sterile sutures. The procedure is repeated for the left ovary through the same incision. Povidone iodine was applied to the area to disinfect the skin after suturing. A significant degree of aseptic procedure was maintained throughout the operation. After surgery, the rats were housed individually in cages for a week (7 days) to allow recovery and then re-grouped in their home cages [24].

P. gingivalis induction (Periodontitis Model)

P. gingivalis induction was undergone under light UV protection to prevent bacteria from being transmitted to a human. *P. gingivalis* induction was performed in OPG and PG groups. Each animal model was injected with 0.05 ml *P. gingivalis* suspension, suspected of having

a concentration of 2.10^9 cells/ ml dissolved in saline. The bacteria were injected into the distolingual and distobuccal gingival sulcus area of the mandible first molar. The induction was repeated every three days for 19 days. The animal model was x-rayed to find out that the mouse model had experienced periodontitis, and X-ray results showed alveolar bone resorption (Figure 1). In the OPG group, the *P. gingivalis* induction was taken seven days after the surgical procedure of ovariectomy [24].

Body weight measurement

Body weight measurements in this study were only to evaluate the health and safety level of animal models after receiving treatment. Apart from that, body weight is also associated with systemic changes due to metabolic syndrome.

Blood sampling

The blood sampling for each group was various because the end of the treatment for each group was different. However, the collection period remained the same for each group. The blood collection was carried out a day before and on the 3rd, 7th, 14th, 21st, and 28th day after the treatment. So, each animal model in each group was taken blood collection six times, except the control group, which was taken once (Figure 2). Before blood sampling, the animal models fasted for 8 hours. Blood sampling was from the infraorbital plexus, about 5-7.5% of body weight or about 0.8-1.0 cc. Blood collection with this volume was highly considered because it was the safest and did not cause stress and weakness in animal models. So that after taking blood, the animal stayed alive, and the recovery process was fast.

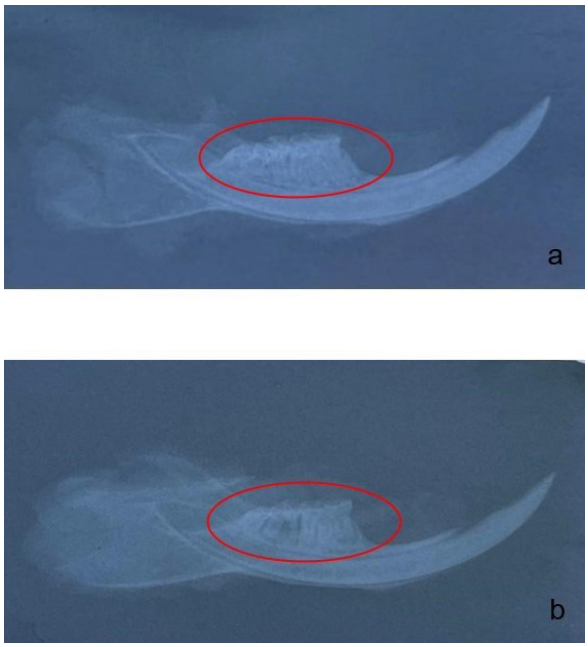

Figure 1 - Radiographic of the mandible. a) did not indicate periodontitis; b) periodontitis. Periodontitis was characterized by bone resorption between the first, second and third molars.

Blood was directly measured glucose, cholesterol, and uric acid and stored in tubes without anticoagulants aimed to get the serum. Measurement of glucose, cholesterol, and uric acid utilized GCUmeter (easy touch, Taiwan). Therefore, the serum for estrogen level analysis. The estrogen level used estradiol ELISA kit, which all of the procedures followed the manual of ELISA kit (BT-Lab, USA). All obtained data were analyzed with one-way analysis variance ($p<0.05$), multiple comparisons ($p<0.05$), and Pearson's correlation ($r=1$). However, before analysis, all data was tested for normality using Saphiro-Wilk analysis ($p>0.05$) and homogeneity using Levene test ($p>0.05$).

Blinding (masking)

Five researchers conducted this study, and all researchers were involved during the research process. The blinding process was carried out from the randomization of the grouping of animal models. Then, the researcher applied the experimental animals according to the treatment given to the experimental animals. To avoid blinding, when collecting blood and measuring serum biochemical and estrogen levels, only researchers in the treatment section knew the coding on blood tubes. In contrast, researchers in the data analysis section did not know the code. This step was done to avoid manipulation of research results.

RESULT

Table I existed to evaluate the characteristics of animal models before treatment. Before treatment, animal models enclosed varying body weights, estrogen levels and blood biochemistry. Even though the experimental animals were selected randomly, when measuring body weight, each group had the same normality and homogeneity values regarding body weight, serum levels of estrogen, glucose, cholesterol and uric acid ($p>0.05$). In measuring body weight, the OPG group (212.13 ± 14.44) had the heaviest body weight among the other treatment

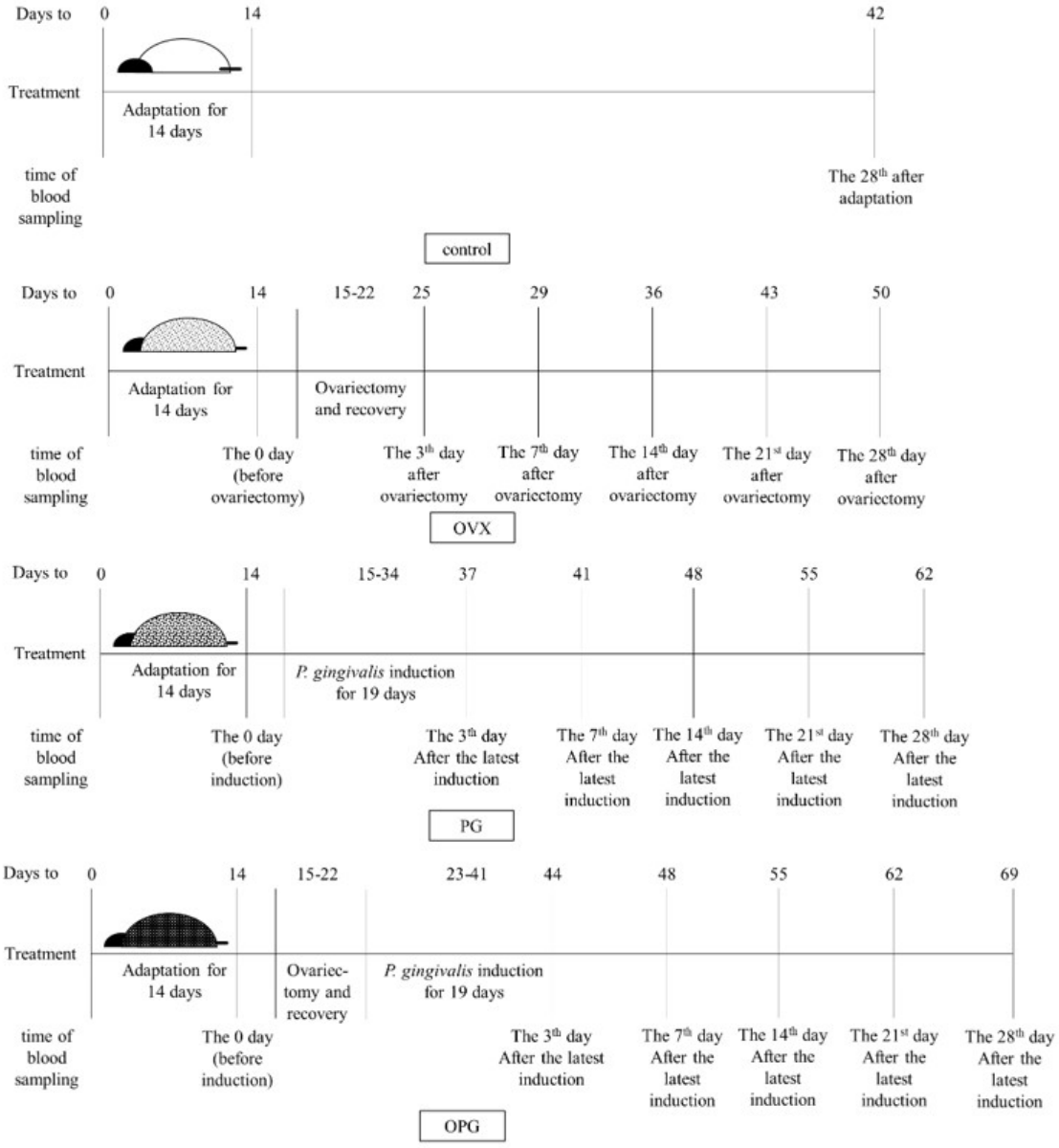

Figure 2 - Illustration of the treatment period on animal models for each group. OVX, ovariectomy group; PG, induction *P. gingivalis*; OPG, ovariectomy and induction *P. gingivalis*.

Table I - Characteristic of Animal Models Before Treatment

Variables	Control (n=7)	OVX (n=7)	PG (n=7)	OPG (n=7)	P value
Body Weight (gram)	170.21±4.36	179.67±20.4	201.66±13.27	212.13±14.44	0.179*[b]
P value[a]	0.199*	0.251*	0.085*	0.088*	
Serum Estrogen (pg/mL)	460.00±58.31	450.28±86.49	462.64±70.64	452.86±50.57	0.195*[b]
P value[a]	0.231*	0.157*	0.273*	0.116*	
Blood Glucose (mg/dL)	52.15±17.24	62.68±9.94	60.71±9.32	62.14±8.09	0.137*[b]
P value[a]	0.292*	0.876*	0.028	0.233*	
Total Cholesterol (mg/dL)	108.67±20.56	121.2±17.02	118.57±240.16	119.29±10.5	0.545*[b]
P value[a]	0.534*	0.278*	0.718*	0.079*	
Blood Uric Acid (mg/dL)	3.1±1.63	3.71±0.76	1.5±1.18	1.98±2.35	0.543*[b]
P value[a]	0.573*	0.086*	0.163*	0.018	

Data was expressed as a mean (SD, standard deviation) for all variables. P value described statistic analysis using a) Shapiro-Wilk test (p>0.05); b) Levene analysis (p>0.05). n, number of study subjects; OVX, ovariectomy group; OPG, ovariectomy and induced Pg group; PG, induced *P. gingivalis* group; *significant difference for normality and homogeneity analysis (p>0.05).

groups and controls, but it was not significantly different (p> 0.05). In measuring estrogen levels, which is one of the steroid sex hormones that control ovarian function, the results of the study showed that there was no significant difference in estrogen levels in the control and treatment groups (p>0.05). However, the estrogen levels in the PG group were the highest among the other groups (p>0.05). 462.64±70.64). Likewise, for glucose measurements, even though the control group had the lowest glucose levels (52.15 ± 17.24), the glucose levels in the groups were considered the same because there were no significant differences (p> 0.05). Even though the OVX had the highest cholesterol (121.2 ±17.02) and uric acid level (3.71±0.76), the results showed that there were no significant differences in total cholesterol and uric acid between any of the groups (p>0.05).

Figure 3 illustrated multiple comparisons regarding mean differences in body weight and blood biochemical parameters between the control, the treatment groups and the observation periods. The lined chart related to body weight revealed similar patterns among the treatment groups following the counter-time. The body weight of animal models inclined following the observation

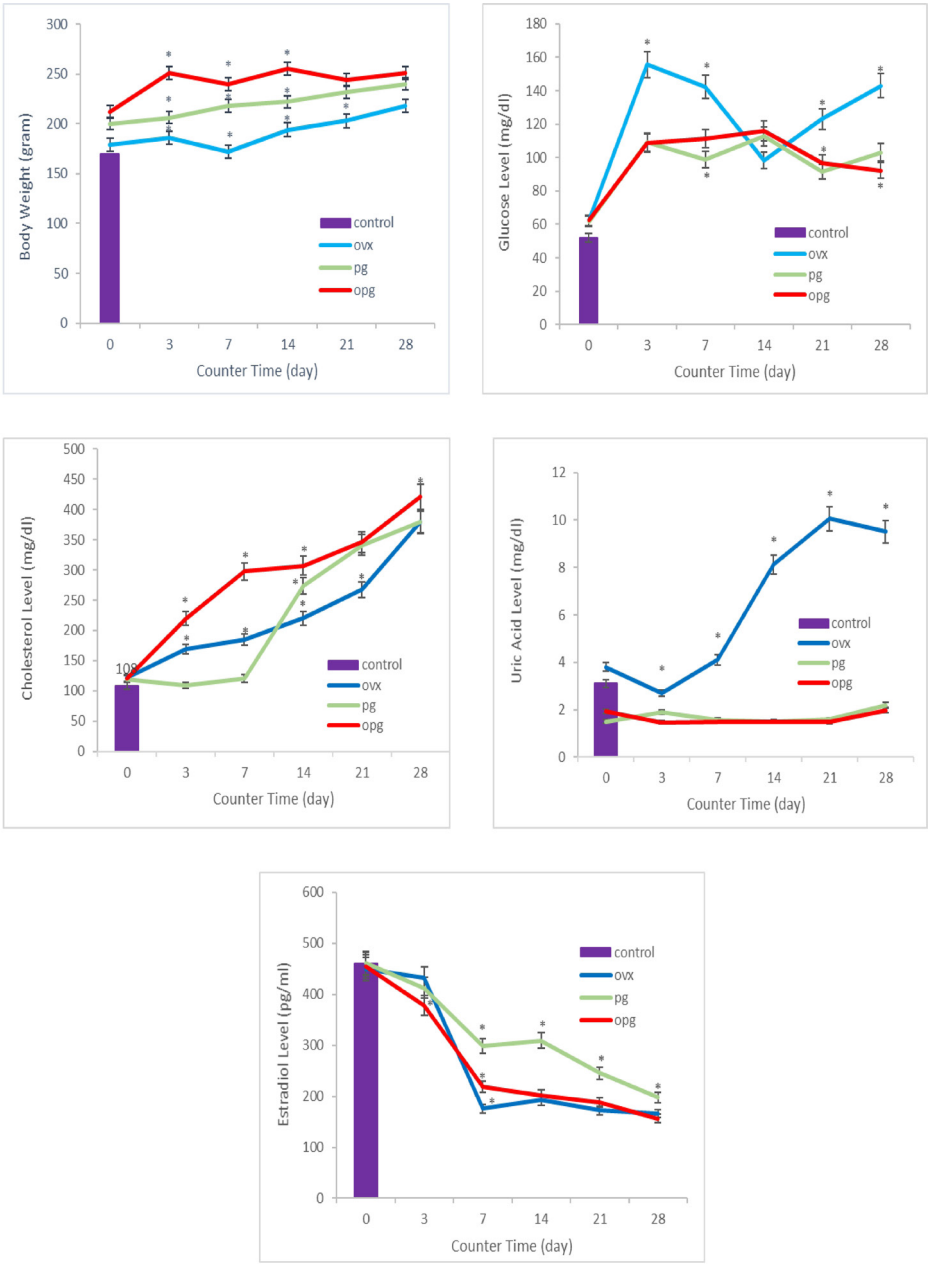

Figure 3 - Body Weight, Blood Biochemical Parameters, and Estradiol Level Based on Counter-time of Ovarian Dysfunction. a) Body Weight, b) Blood Glucose Level, c) Total cholesterol, d) Blood Uric Acid Level, and e) Estradiol Level. Data presented were included mean, standard errors. Data were analyzed with multiple comparison test. *, significant different between the groups and counter time (p<0.05).

time, except for the OPG group, which presented more fluctuation than the other treatment groups. In treatment groups, there were significant differences between the control group, several observertion periods and several treatment groups ($p<0.05$). In blood glucose levels, the alteration of blood glucose levels among the treatment group fluctuated more. However, the enhancement peak was presented on the third day, except the peak of the OPG group was on the seventh day. The blood glucose in the OVX and PG groups inclined on the 28th day, while the OPG group declined on the 21st and continued on the 28th.

Based on multiple comparison analyses, treatment groups' mean blood glucose levels comprised significant differences following the observation periods ($p<0.05$). However, there were no significant differences between the control and treatment groups at the beginning of observation ($p>0.05$). On the concomitant of observation time, the treatment groups significantly presented differences with control groups several times ($p<0.05$), but there were no significant distinguishes between treatment groups ($p>0.05$). The mean of Total cholesterol enhanced following the time of observation. The pattern of Total cholesterol alteration in the OVX group exhibited the same as the OPG group; the more extensive observation, the higher the blood glucose level. However, the peaks of treatment groups were on the 28th day. Therefore the PG group altered not significantly on the 3rd and the seventh days, following the time the level in this group increased. Concerning the blood uric acid level, most of the statistical analysis demonstrated there were no significant differences between the control and treatment groups following counter-time ($p>0.05$), except with OVX ($p<0.05$). Nevertheless, the pattern of the lined chart of OPG was likewise the PG group.

Ovarian dysfunction is associated with hormonal changes, including estrogen, in the form of decreased estrogen levels in the blood. Estradiol is the primary circulating estrogen form. The results showed that estradiol levels tended to decline over time. The highest serum estradiol levels occurred before treatment (0 days) in all groups (OVX=466.2±17.9; OPG=469.1±38.8; PG=468.4±39.4). After day 3, estradiol levels decreased gradually until day 28 ($p<0.05$).

Regarding correlation analysis in Table II revealed most of the variables presented correlation between groups, inflammation and estrogen level to body weight and blood biochemical indicators, excepting association between inflammation condition and estrogen level to blood uric acid level ($R<0.1$, $p>0.05$). The groups and inflammation variables demonstrated significantly positive correlation, which blood glucose level presented significantly positive moderate correlation to groups variable likewise inflammation condition to Total cholesterol. Interestingly, estrogen level obtained significantly negative strong correlation with body weight and blood biochemical parameters ($R>0.5$, $p<0.05$), except correlation with uric acid level. Moreover, the groups also revealed significantly negative strong correlation with blood uric acid level ($R>0.5$, $p<0.05$).

DISCUSSION

Metabolic syndromes are multiple and complex symptoms or disorders affecting metabolism alteration, and they magnify cardiovascular diseases and type 2 diabetes mellitus risks. They are frequently related to

Table II - Association between groups, inflammation condition and estrogen level to body weight and blood biochemical parameters

	Groups		Estrogen Level	
	R value	P value*	R value	P value*
Body Weight (gram)	0.724‡	0.001*	-0.637‡	0.026*
Blood Glucose level (mg/dL)	0.469†	0.054*	-0.728‡	0.007*
Total cholesterol (mg/dL)	0.517‡	0.028*	-0.716‡	0.009*
Blood Uric Acid level (mg/dL)	-0.763‡	0.000*	0.000#	1.000

Data was expressed as association of variables (R square value) and significant difference (P value) for all variables All of variables were analyzed with Pearson's correlation. *significant correlation between variables ($p<0.05$). #, no significantly correlation; †significant and moderate correlation. ‡significant and strong correlation; (-), negative correlation. Groups were treatment groups, including OVX (ovariectomy group), OPG (ovariectomy and induced Pg group), and PG (induced P. gingivalis group).

dysglycemia, dyslipidemia, visceral obesity, atherogenic and hypertension. These syndromes are mainly caused by obesity, physical inactivity, aging, hormonal alteration and genetics [1]. However, these are currently associated with inflammation, like chronic periodontitis primarily caused by *P. gingivalis* [21,25].

In this recent study, animal models were manipulated to mimic ovarian dysfunction or menopause, thereby ovariectomy and infectious agent induction utilizing *P. gingivalis*. This study demonstrated that there was metabolic syndrome risk in animal models. The syndrome was characterized by alteration of body weight, blood glucose level, Total cholesterol and uric acid level. Body weight observation was related to investigating the obesity status of animal models. Regarding the body weight, this study exhibited the body weight of animal models magnified following the observation time. Although there were body weight alterations, the body weight of animal models typically tended to be underweight regarding the age increase. Rats strain Sprague Dawley aged eight weeks present 200-220 grams [26]. However, based on the control group, the body weight of animal models of treatment groups was higher than the control group, and the OPG group was the heaviest. The body weight peak was on the 28th day of observation.

Also, this study revealed that significant changes in body weight were negatively correlated with estrogen levels. Compared to the larger animal models, these exhibited significant oestrogen levels. Possible causes of the estrogen deficiency that led to the stimulation of fat accumulation and the down-regulation of metabolism include ovariectomy and *P. gingivalis* infection. Moreover, estrogen insufficiency and bacterial infection-induced inflammation were identified as contributing factors to the severity of fat accumulation [27,28]. Ovariectomy causes estrogen shortage by imitating the onset of menopause by affecting reducing estrogen production [15,29]. This disorder affected visceral fat deposition and interfered with the metabolism of the liver and fat tissues [28]. Moreover, a lack of estrogen promotes hyperphagia and systemic inflammation. This ultimately resulted in obesity. As a sex steroid hormone, estrogen plays a crucial part in various homeostatic processes, metabolism operations, and the release of cytokines in fat tissues. One of the rules is to control fat deposition and reduce the danger of obesity [30,31].

Blood glucose level observation was aimed at investigating glucose dysregulation. This recent study exhibited that the level fluctuated following the time and tended to increase on the 28th day, except for the OPG group. Notwithstanding, the blood glucose level of the treatment group presented higher than the control group; blood glucose level enhancement could not be indicated as a diabetic condition. The blood glucose level in treatment groups was slightly higher than the physiologic value in rats. The standard value is 93.2±10.3 mg/dL [32,33]. It might be caused by the treatments (ovariectomy, *P. gingivalis* induction and combination) only induced hyperglycemia and influenced the glucose intolerance and regulation in the bloodstream. Nevertheless, it might not affect glucose metabolism and insulin regulation permanently. The mechanism might be related to the type of treatments, inflammation severity and estrogen deficiency. This suggestion was substantiated by correlation analysis. The analysis revealed a significantly positive correlation between blood glucose alteration and inflammation and a significantly negative correlation with serum estrogen level. *P. gingivalis* induction related to bacterial endotoxemia stimulated early hyperglycemia, representing a general sign of metabolic syndromes [34,35]. Moreover, *P. gingivalis* induction affected inflammation, in which the host released proinflammatory cytokine in response to *P. gingivalis* [21]. This response caused insulin resistance. The effect of insulin resistance on blood glucose level among treatment groups were suspected to be the same and depended on the sensitivity of the host response [36].

Total cholesterol level indicates lipid profile as a screening parameter for the risk of atherosclerosis and cardiovascular diseases. This study demonstrated that total cholesterol among treatment groups significantly enhanced following the period of observation, presenting a negatively significant correlation with serum estrogen level. The treatment might stimulate inflammation and sex steroid hormone alteration. Inflammation and hormone alteration possibly generated lipid metabolism disturbance. *P. gingivalis* stimulates the released proinflammatory cytokines as a host response, which the cytokines stimulate lipid discharge and hyperlipidemia [37].

In comparison, ovariectomy might influence estrogen production, and ovariectomy impaired lipid metabolism. Estrogen regulates cholesterol metabolism, accelerates cholesterol catabolism, enhances fatty acids oxidation and plays anti-inflammation [15,38,39]. Furthermore, estrogen deficiency stimulated ovariectomy generated triglyceride accumulation liver and impacted the enhancement of total cholesterol [40].

Uric acid acts as a potent natural antioxidant and free radical scavenger. It serves cellular defence against oxidative stress [41]. However, excessive uric acid leads to ischemia and perfusion of tissues [42]. A recent study showed that only the OVX group presented elevated uric acid levels. There was no correlation between uric acid alteration to serum estrogen level. Estrogen alteration might not affect uric acid fraction alteration [43]. The previous study associated significant uric acid alteration with obesity [44], while in this study, the animal model's body weight normally increased. Furthermore, the advanced age of animal models in this study did not influence uric acid levels. Although previous studies exhibited middle age, estrogen deficiency and periodontal disease increased uric acid levels [45].

This study revealed changes in estrogen levels in the treatment group. *P. gingivalis* infection might affect hormone production in the ovaries, such as during an ovariectomy. *P. gingivalis* encloses potent virulence factors that may influence the activity of estrogen synthesis and production in the ovaries, such as LPS. Several studies described that LPS reduced estradiol synthesis in the ovaries by decreasing the activity of the aromatase cytochrome P450 enzyme (CYP19A1). This enzyme is needed to convert androsterone into estradiol. LPS will modulate inflammation in the ovaries and increase the activity of CCAAT enhancer binding protein beta (CEBPβ), which plays a role in regulating cytokine expression and possibly the expression of aromatase, the pro-inflammatory cytokine IL-6 or TNFα which increases after LPS exposure can also regulate estradiol synthesis [46-48].

This study proposed the mechanism by which ovariectomy and *P. gingivalis* contribute to the development of metabolic syndrome. The first, metabolic syndrome, was related to the treatments' estrogen-altering effects. The treatments had a significant effect on serum estrogen levels, causing them to be substantially lower than in the control group. Reductions in estrogen levels induced ovarian dysfunction and metabolic disturbances in the treatment groups. In adipose, muscle, and liver tissues, estrogen controls lipid and glucose metabolism. When estrogen levels are insufficient, calorie intake and energy expenditure are impaired. It generates inefficient glucose utilisation, resulting in hyperglycemia and glucose dysregulation [12,30]. In addition, estrogen deficiency causes insulin sensitivity and resistance, which worsens hyperglycemia and disrupts immune cell activity. This condition stimulates macrophages and adipose tissue to produce pro-inflammatory cytokines. This condition performed oral microenvironment and periodontal disease severity tests conclusively [49-51].

Subsequently, disruption of glucose homeostasis also influences lipid metabolism in adipose and liver tissue. Both estrogen deficiency and disturbance of glucose metabolism will stimulate lipid deposition and accumulation of vascular and tissue. This accumulation will increase triglyceride, total cholesterol and low-density lipoprotein (LDL), enhancing cardiovascular disease risk. This hyperlipidemia will trigger leukocytes to be hyperactive to produce ROS. Imbalance ROS and endogen antioxidant lead to oxidative stress, insulin resistance and inflammation. Briefly, glucose and lipid metabolism inferences influence bidirectional. Moreover, these interferences stimulate systemic inflammation [52,53].

The second proposed mechanism is via the inflammation process. *P. gingivalis*, an infectious agent, caused systemic inflammation. This local induction spreads via the bloodstream and causes endotoxemia. The endotoxemia of the virulence factor of *P. gingivalis*, LPS, involves ovarian inflammation and dysfunction. LPS disturbs steroidogenesis and estrogen production. This interruption will lead to immune cell impairment to produce cytokines and ROS. Excessive cytokine and ROS also influence steroidogenesis and estrogen excretion. Finally, it causes estrogen deficiency and leads to the serial of metabolic interruptions [24,54,55].

The limitation of our study was using periapical x-ray to detect alveolar bone resorption, as the animal models got periodontitis, so it did not detect detailed bone destruction. Although, the radiographic showed that the induction was performed in distobuccal and distolingual of first

molar mandible caused expansion of damage to the mesial and distal second molar.

CONCLUSION

Briefly, ovarian dysfunction caused by ovariectomy and *P. gingivalis* infection might increase the metabolic syndrome development, especially cardiovascular disease and glucose intolerance, in which signed by increase glucose and cholesterol blood level. *P. gingivalis* induction not only leaded metabolic syndrome risk but also worsens metabolic syndrome in ovarian dysfunction-induced ovariectomy due to estrogen deficiency. However, this study required further studies to investigate the molecular and cellular mechanism of periodontal pathogen involvement in ovarian dysfunction-induced metabolic syndrome.

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Author's Contributions

AWSD: Conceptualization, Methodology, Resources, Investigation, Writing – Original Draft Preparation, Writing – Review & Editing. ZM: Data Curation, Formal Analysis, Software, Validation, Visualization. TE: Methodology, Validation, Visualization, Data Curation. AMP: Investigation, Project administration. ZH: Funding Acquisition, Supervision.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article

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Regulatory Statement

This study was conducted in accordance with all the provisions of the medical research oversight committee guidelines and policies of: Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia

The approval code for this study is: 0029/KKEP/FKG-UGM/EC/2022

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