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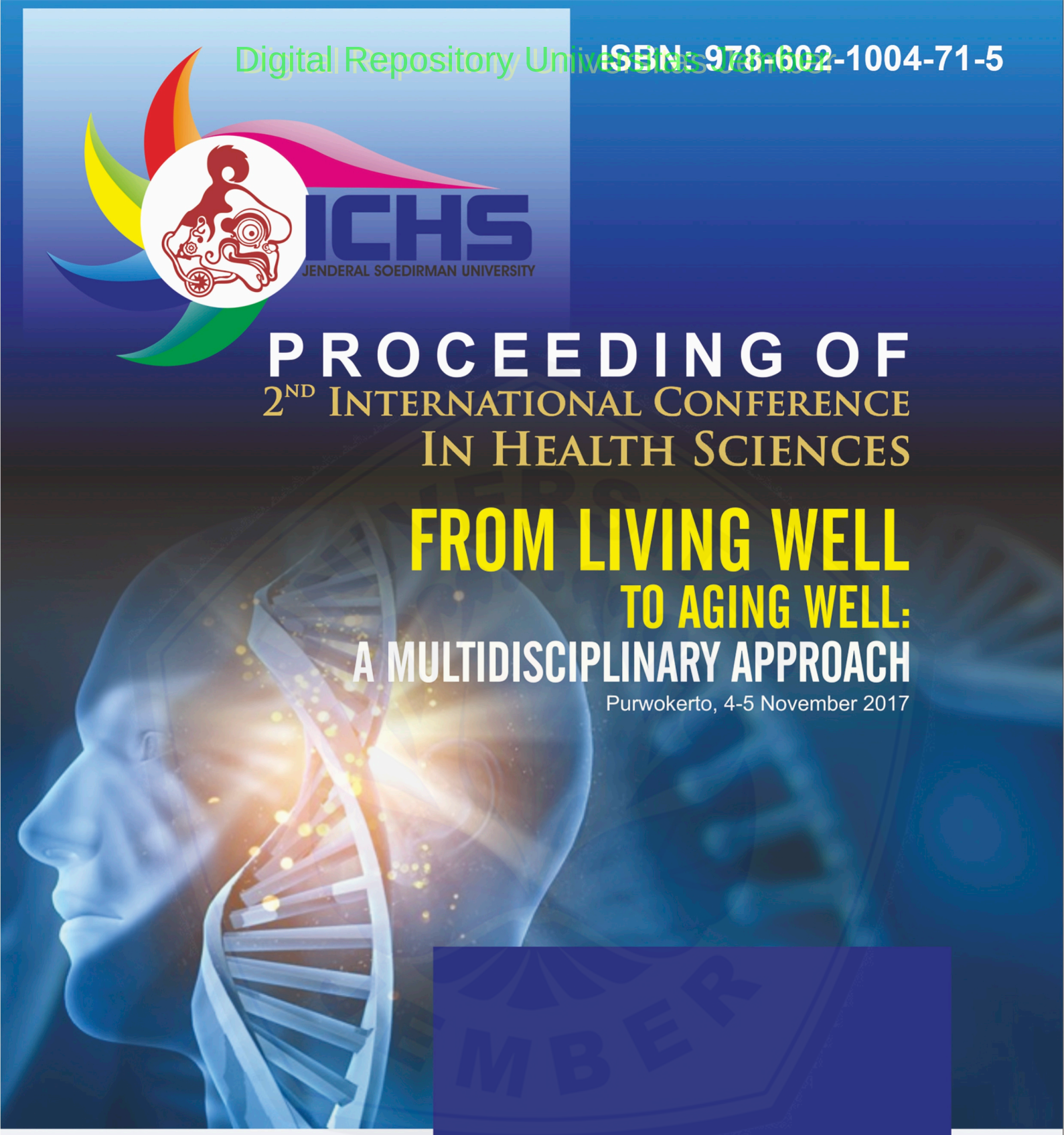
JENDERAL SOEDIRMAN UNIVERSITY

PROCEEDING OF
2ND INTERNATIONAL CONFERENCE IN HEALTH SCIENCES

FROM LIVING WELL TO AGING WELL: A MULTIDISCIPLINARY APPROACH

Purwokerto, 4-5 November 2017



UNIVERSITY OF JENDERAL SOEDIRMAN
INDONESIA
2018

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International Conference in Health Sciences (ICHS)

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Dr.sc hum Budi Aji, SKM., MSc
Dr. Saryono, S.Kp., M.Kes
Mekar Dwi A, S.Kep, Ners., MKep., PhD
Dr. Muhamad Salman Fareza, M.Si
Hendry Wasito, Sfarm., MSc., Apt
Yunita Sari, S.Kep., M.Kes., PhD
Dr. Ngadiman, M.Kes

Perancang Sampul:
Panitia

Penata Letak:
Panitia

Diterbitkan oleh:
UNIVERSITAS JENDERAL SOEDIRMAN
Jalan Prof. Dr. H.R. Boenyamin 708 Purwokerto
Kode Pos 53122 Kotak Pos 115
Telepon 635292 (Hunting) 638337, 638795
Faksimile 631802
www.unsoed.ac.id

Dicetak oleh:
BPU Percetakan dan Penerbitan
Universitas Jenderal Soedirman
Telepon: (0281) 626070
Email: unsoedpress@yahoo.com

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Dean's Welcome Note

Dear all the conference participants,

Welcome to 2nd International Conference in Health Sciences 2017. In this very good occasion I would like to extend my warm greeting to you all, the speakers as well participants. It is a great honor for Faculty of Health Sciences to host this international conference. This becomes our positive contribution in facilitating the spread of research work from scientist and practitioner in health sciences. It is also our way to provide scientists and practitioners with an understanding of key issues in health and nursing, medical treatment and health technology, health policy and health services, health promotion as well as economic, social cultural and ethical aspect of health.

Knowledge and research will always walk side by side that we cannot gain new knowledge without conducting research. All those attempts act as a respond to the increase of health demand in our community. I hope in this conference which entitled *" From Living well to Aging Well : a multidisciplinary approach"*, we will obtain new knowledge about health sciences from many perspectives.

I do hope you enjoy your stay in Purwokerto and be able to see the beauty of this city. And most important, you can build a good networking with other participants which will be benefit for your field and research area.

Best Wishes,
Dr. Warsinah, M.Si., Apt

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Conference Chair's Welcome Note

Dear Delegates,

On behalf of the organizing committee, we are pleased to welcome you to Purwokerto, Indonesia for the 2nd International Conference in Health Sciences (ICHS) 2017, held this year by Faculty of Health Sciences, University of Jenderal Soedirman. Theme of this event is *"From Living Well to Aging Well: a Multidisciplinary approach"*. We raise this special theme since the population ageing is a global issues which interesting for researchers, academic, policy makers, practitioners and governments. We want to have more people around the world are living longer healthier lives than our previous generations.

In this event, we are delighted to welcome this year's Plenary speakers : Representative of Ministry of Health Republic Indonesia, Prof.Dr. Gert Storm (Department of Pharmaceutical Sciences, Faculty of Science, Utrecht University), Prof. Jing-Jy Wang (Professor & Chair Department of Nursing, College of Medicine, National Cheng Kung University, Taiwan) and Chalermpol Chamchan, Ph.D. Assist. Prof. (IPRS Mahidol University, Thailand) and also Indonesian speakers.

We would also like to take this opportunity to thank the organising team for all their hard work to make this event run smoothly.
I know just how much time and effort goes into making such an event happen.

I hope all of us are going to enjoy this conference. Thank you for attending the meeting!

Kind regards,
Siwi Pramatama Mars Wijayanti, Ph.D

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The 2nd International Conference in Health Sciences (ICHS)

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School of Nursing, Faculty of Health Sciences, UNSOED

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School of Pharmacy, Faculty of Health Sciences, UNSOED

Hendri Wasito, S. Farm., MSc., Apt
School of Pharmacy, Faculty of Health Sciences, UNSOED

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Ovarian Failure Affected Leukocytes Profile in Peripheral Blood and Gingival Fluid (In vivo Study)

Agustin Wulan Suci D[1], Tantin Ermawati[2], Bangun Febrianto[3]
[1,2,3] Biomedical Department, Dental Faculty, University of Jember
Jl. Kalimantan 37, Jember, Indonesia
agustinwulan.fkg@unej.ac.id; tantin.ermawati@gmail.com

Abstract

Ovarian failure can be caused by ovariectomy which induces estrogen deficiency. Estrogen influenced leukocytes as immune and inflammation cells systemically and locally, and leukocytes also contribute to ovarian function in order to be located in ovaries tissue. The aim of the study was to investigate the impact of ovarian failure to leukocytes profile in peripheral blood circulation and gingival fluid. This study was experimental laboratory study approved by the Health and Research Ethics Committee of Dental Faculty, Gadjah Mada University. The animal models have undergone ovariectomy which removed their ovarian bilaterally. Blood sampling from retro orbital plexus and gingival fluid were taken before and 3, 7, 14, 21 and 28th days after ovariectomy procedures. Total leukocytes counted manually. The result showed that total leukocytes in peripheral blood were higher than in gingival fluid. Moreover, there were significant differences between leukocytes profile in peripheral circulation and gingival fluid based on the periods, except in basophils cells counting. The conclusion was ovarian failure affected leukocytes profile in peripheral blood circulation and gingival fluid.

Keywords: ovarian failure, leukocytes, peripheral blood, gingival fluid

Introduction

Ovarian failure is a pathological condition impacting ovarian and signed by dysfunction of ovaries, particular disturbance of sex steroid hormone production. The ovarian failure occurred before 45 years old is termed primary ovarian failure or premature ovarian dysfunction that induces early menopause. Ovarian failure can be caused and induced by bilateral oophorectomy or ovaries removal bilaterally. Ovariectomy is well-established technique in animal model to mimicking early menopause in human which removes ovaries unilaterally or bilaterally.[4, 6, 21, 25]

Ovarian failure induced ovariectomy can also cause sex hormone deficiency, especially estrogen. Estrogen is established sex hormone which not only in reproductive tissue, but it also influences to nonreproductive tissues, such as immune system, skeletal, vascular, and periodontal tissue. Therefore estrogen has receptors in many tissues. In immune system, estrogen regulates both innate and adaptive immune system. In innate immune system, estrogen regulates production number, chemotaxis, and infiltration neutrophil and macrophage which both cell type leukocytes are also played role in inflammation process. In adaptive immune, estrogen down regulates lymphocytes response which lymphocytes are chronic inflammatory cells. Those showed that estrogen also played as anti-inflammatory agent.[14, 24, 25]

Moreover, leukocytes as immune and inflammatory cell contribute in ovarian function. Leukocytes are located and contain in each ovaries tissue. Although they have difference function, the regulation is similar which regulated by estrogen. Furthermore, the leukocytes also influence estrogen excretion.[5] This may be caused presence of estrogen receptors on leukocytes and tissue. However, it is still unclear.

This became basic of recent study to investigate effect ovarian failure induced ovariectomy to leukocytes profile which not only in systemic circulation (peripheral blood),

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but also in local circulation which represented by gingival fluid. Although gingival fluid is transudate or exudates fluid originated periodontal tissue, the production and composition are also affected systemically. The objective of study was to investigate impact of ovarian failure to leukocytes profile in peripheral blood circulation and gingival fluid. It was not only to know effect estrogen deficiency-induced ovarian failure to leukocyte profile, but it is also utilized as biological marker and screening marker of morbidity and mortality of ovarian failure, so women can improve their life expectancy.

Materials and Methods

Animals and Adaptation

This study was experimental laboratory study using white rats (*Rattus norvegicus*) Sprague Dawley Strain. This study was approved by the Health and Research Ethics Committee of Dental Faculty, Gadjah Mada University. The criteria of rats were 11 to 12 weeks old, female, 200-250 g body weight. Before the treatment, all of the animal models were adapted to laboratory environment for 7 days. The rats were kept at constant room temperature and relative humidity under a 12-h day and night cycle, with free access to food and water (diet and water ad libitum).

Surgical Procedure (Ovariectomy)

This procedure was aimed to get ovarian failure and early menopause model. We used dorsal ovariectomy bilaterally. The rats were anesthetized with ketamine/ xylazine (80/10 mg/kgBW) intramuscular (Sigma Aldrich, Singapore). The area of surgery was with disinfectant. A small transverse dorsal incision of 0.5 – 1.0 cm was made with surgical scalpel blade no. 11 on the left and right incision. The ovary and associated fat were easily located and exteriorized by gentle retraction, bound with silk ligature, and then ovaries were removed. The wound was closed in two layers (muscle and skin) using sterile sutures. Finally, antibiotic powder was put on surgical wound.[8]

Peripheral Blood and Gingival Fluid Sampling

Peripheral blood and gingival fluid samples were taken before ovariectomy and on 3rd, 7th, 14th, 21st, and 28th days post ovariectomy. Blood sampling was from retro orbital plexus 1.5-2.0 cc. The gingival fluid was taken from buccal area of maxillary molar of rats. In gingival fluid sampling, the fluid was obtained by collecting gingival fluid using paper point #20 and 20 mm length that inserted in buccal area of maxillary molar for 30 seconds. Total leukocytes from peripheral blood were undergone manually using *improved Neubauer* chamber. Therefore differential counting also used manual method with Giemsa staining.[23]

Gingival fluid needed special treatment before it was observed. The paper point was taken in 0.5 ml Eppendorf tube and diluted with 200 µL PBS 2.0 M. The supernatant was put in other Eppendorf. For total leukocytes, the fluid taken was same with peripheral blood manually using *improved Neubauer* chamber. For differential counting leukocytes, 20µL the supernatant was put on slide and then it was dried. After that, it was stained with Giemsa.[12, 23]

All of the data leukocyte profile was analyzed by analysis variants, multiple comparisons and correlation with 95 % significance ($p<0.05$).

Result

Table 1 illustrated trending pattern of leukocytes profile in peripheral blood and gingival fluid of ovarian failure rats induced ovariectomy during 28th days. Leukocytes profile in peripheral blood was significantly higher than in gingival fluid. Furthermore, all of the leukocyte types were detected in peripheral blood. While in gingival fluid was only

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monocytes not detected. Eosinophil and basophil in peripheral blood had similar percentage in gingival fluid. However, lymphocytes in peripheral blood had higher percentage than neutrophil in peripheral blood, while lymphocytes in gingival fluid had lower percentage than neutrophil in gingival fluid.

Based the counter time of observation, there were significant differences leukocytes profile ovarian failure rats induced ovariectomy ($p<0.05$), except basophil, eosinophil, and monocytes in gingival fluid ($p>0.05$). Almost on 28th-day, the leukocyte profiles in peripheral blood and gingival fluid had the highest value, especially in gingival fluid the value of total leukocytes was higher followed with percentages of neutrophil and lymphocytes. Although on 28th days basophil and eosinophil were the highest percentage in peripheral blood, the total leukocyte on 28th days was the lowest during observation, because basophil and eosinophil had minor percentage in leukocyte total percentage peripheral blood. The highest total leukocyte in peripheral blood was on 7th days and followed with monocytes. Neutrophil and lymphocytes in peripheral blood had different pattern which neutrophil had the highest percentage on 3rd days and lymphocytes was on 14th days (table 1).

Table 1. Leukocytes Profile in Peripheral Blood Circulation and Gingival Fluid of Ovarian Failure Rats Induced Ovariectomy (n=5)

	Counter-Time						P
	0 days	3rd days	7th days	14th days	21st days	28th days	value
				Peripheral blood			
WBC	7420±139.64	9380±1055.11	9410±1944.67	8050±916.52	7120±1771.16	6430±1260.26	0.006[a*]
Ba	0.20±0.45	0.80±0.84	1.00±1.23	0.00±0.00	2.20±1.64	2.60±1.82	0.000[b*]
Eo	1.40±0.55	2.00±1.00	1.20±1.10	0.80±1.10	2.60±1.52	3.00±0.71	0.019[a*]
Seg	24.40±0.55	29.00±1.58	27.20±0.84	20.40±1.52	20.20±1.79	15.60±1.14	0.000[a*]
Mo	21.40±2.88	19.60±3.36	28.40±4.16	24.20±2.49	22.80±5.26	25.76±4.69	0.024[a*]
Lym	44.20±2.59	49.40±1.67	52.60±1.67	57.00±1.87	53.60±1.52	54.40±2.07	0.000[a*]
				Gingival Fluid			
WBC	120±57.01	220±67.08	490±74.16	290±263.15	520±210.95	630±405.59	0.009[a*]
Ba	0.00±0.00	0.40±0.55	0.00±0.00	0.20±0.45	0.20±0.45	0.00±0.00	0.350[b]
Eo	0.00±0.00	0.40±0.55	0.40±0.55	0.00±0.00	0.40±0.55	0.20±0.45	0.389[b]
Seg	1.80±0.84	4.00±1.23	6.40±1.14	2.20±0.84	5.80±1.92	7.40±1.14	0.000[a*]
Mo	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.467[b]
Lym	0.40±0.55	1.00±1.00	1.60±0.89	1.00±1.00	2.00±0.71	2.40±0.55	0.004[a*]

Data were expressed as mean (SD, standard deviation) for all variables.
P value was significant value of mean difference within groups based on the counter time
[a] was derived from one-way analysis of variance ($p<0.05$); [b] was derived from Kruskal Wallis analysis ($p<0.05$)
* significantly different between the groups ($p<0.05$)
n, number of study subjects in each group; WBC, total leukocytes number (cell/mm3); Ba, basophile (%); Eo, eosinophil (%); Seg, neutrophil (%); Mo, monocytes (%); Lym, lymphocytes (%).

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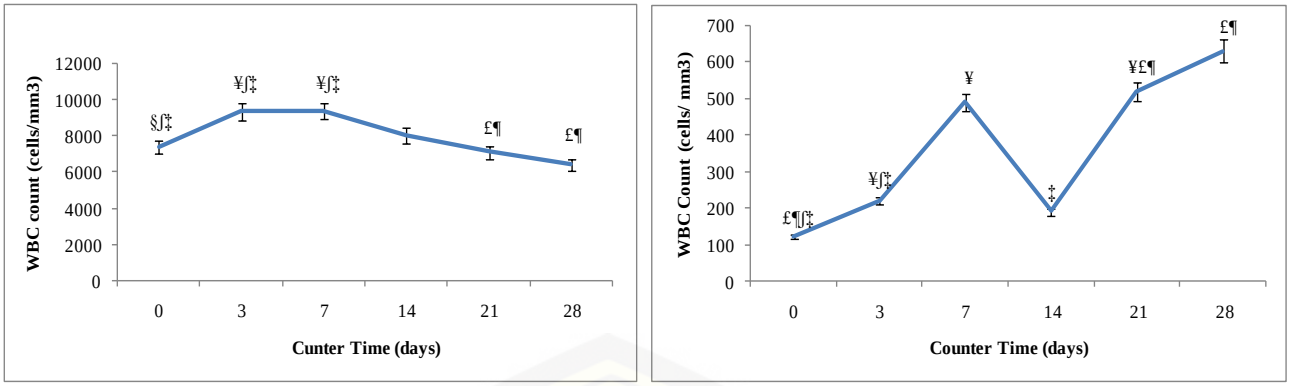

Figure 1. Total leukocyte based on counter time of ovarian failure rats induced ovariectomy, a. in peripheral blood, b. in gingival fluid

Data were presented mean and standard errors and significant difference of multiple comparison tests. *Significant difference inter-group on all counter time ($p<0.05$); ¶ significant difference inter-group on 0 days ($p<0.05$); £ significant difference inter-group on 3rd days ($p<0.05$); ¥ significant difference inter-group on 7th days ($p<0.05$); § significant difference inter-group on 14th days ($p<0.05$); ∫ significant difference inter-group on 21st days ($p<0.05$); ‡ significant difference inter-group on 28th days ($p<0.05$).

Figure 1 illustrated line graph about total leukocytes trending in peripheral blood and gingival fluid during 28 days of ovarian failure rats induced ovariectomy. Total leukocytes in peripheral blood had different pattern of gingival fluid. Total leukocytes in peripheral increased on 3rd and 7th days, after that it gradually declined till to 28th days. Almost total leukocyte in peripheral blood had significant difference inter-group ($p<0.05$), except on 14th days ($p>0.05$). Although total leukocytes on 14th, 21st, and 28th gradually declined, there was no significant difference between the groups ($p>0.05$) (Figure 1a).

Total leukocyte in gingival fluid was more fluctuant than in peripheral blood and it significantly increased on 28th days which is the peak of total leukocyte in gingival fluid. The total leukocyte was remarkably increased till 7th days then decline, after that, it significantly increased to 28th days ($p<0.05$). Almost the total leukocytes had no remarkable mean difference inter-groups to 7th and 14 days ($p>0.05$).

Figure 2 described differential counting of ovarian failure rats induced ovariectomy in peripheral blood and gingival fluid following 28th days. There are difference pattern leukocytes in peripheral blood and gingival fluid. Almost leukocyte types had similar patterns which they increased to 28th days, except neutrophil. While neutrophil and lymphocytes in gingival had similar patterns which they gradually increased to 7th days, and then they decreased on 14th days. After that, they went up on 28th days at the peak of their percentage. Lymphocytes in peripheral blood were the highest percentage leukocytes in peripheral blood followed neutrophil and monocytes, while basophil and eosinophil was the lowest percentage, less than 5%. Whereas basophil and eosinophil in gingival fluid was the lowest percentage, less than 1%, and their percentage were more stable.

There was significant mean difference of leukocytes in peripheral blood statistically between the groups based on counter time ($p<0.05$), especially neutrophil and lymphocyte. Almost basophil percentage in peripheral blood of ovarian failure rats induced ovariectomy had significant difference following a long time, with 21st and 28th days, except basophil percentage on 21st which was significantly different to 0 and 14th days ($p<0.05$). Eosinophil percentage also had significant difference between 21st and 28th days ($p<0.05$), except on 3rd days which there showed no significant difference in all of the counter time ($p>0.05$). For monocyte percentage, the difference was significantly on 3rd and 7th days ($p<0.05$) and there

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was no significant difference on 14th days (p>0.05). There was no significant mean difference in percentage of basophil and eosinophil of gingival fluid statistically between the groups (p>0.05). However, neutrophil percentage in gingival fluid of ovarian failure rats induced ovariectomy had significant mean difference inter-group, especially on 0, 3rd and 7th days with 21st and 28th days and except on 14th days. Whereas lymphocyte percentage in gingival fluid showed there was significant difference between 0, 3rd and 14th days with 21st and 28th days.

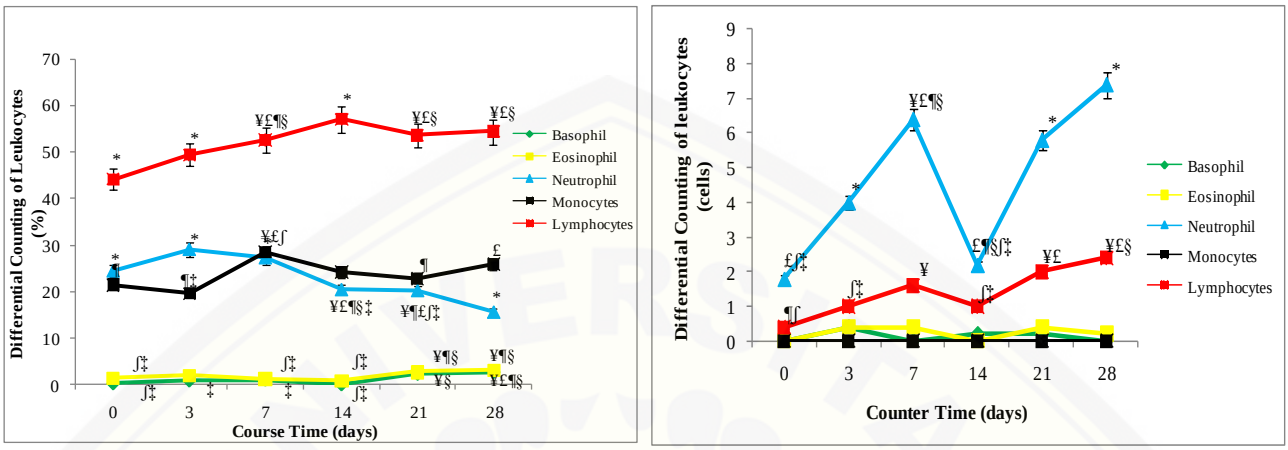

Figure 2. Differential counting in peripheral blood and gingival fluid based on counter time of ovarian failure rats induced ovariectomy

Data were presented mean and standard errors and significant difference of multiple comparison tests.

* Significant difference inter-group on all counter time (p<0.05); ¶ significant difference inter-group on 0 days (p<0.05); £ significant difference inter-group on 3rd days (p<0.05); ¥ significant difference inter-group on 7th days (p<0.05); § significant difference inter-group on 14th days (p<0.05); ∫ significant difference inter-group on 21st days (p<0.05); ‡ significant difference inter-group on 28th days (p<0.05).

Table 2. Association between Leukocytes Profile in Peripheral Blood Circulation and Gingival Fluid of Ovarian Failure Rats Induced Ovariectomy and Counter Time

Leukocytes profile	P value[a]	*r* value[b]	Leukocytes profile	P value[a]	*r* value[b]
Peripheral blood			**Gingival Fluid**		
WBC count	0.000[§]	0.779[‡]	WBC count	0.000[§]	0.599[†]
Basophil	0.002[§]	0.518[†]	Basophil	-0.382	0.057[#]
Eosinophil	0.020[*]	0.378[#]	Eosinophil	0.358	0.069[#]
Neutrophil	-0.000[§]	0.800[‡]	Neutrophil	0.000[§]	0.600[‡]
Monocytes	0.054[*]	0.292[#]	Monocytes	-	-
Lymphocytes	0.000[§]	0.745[‡]	Lymphocytes	0.000[§]	0.635[‡]

[a] Pearson's correlation test; [b] linear regression test

[*] significantly correlation between variables (p<0.05); [§] significantly correlation between variables (p<0.001)

[#] significant but weak correlation; [†] significant and moderate correlation; [‡] significant and strong correlation

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According to correlation analysis (table 2), most of the variables had correlation between leukocytes profile and counter time (days). Although the correlation power was varied from each variable, total leukocytes, neutrophil and lymphocytes in peripheral blood and gingival fluid of ovarian failure rats induced ovariectomy had significant and strong correlation to counter time ($p<0.05$, $r>0.6$), except basophil in peripheral blood which had moderate correlation ($p<0.05$, $r\geq0.3$) and the other was weak correlation ($p<0.05$, $r\leq0.3$).

There was difference imaging between leukocytes in peripheral blood and gingival fluid. The recent study could not find monocyte in gingival fluid. Moreover, leukocytes in gingival fluid were not intact, the cell walls were lysis. So they were observed nucleus form and granules.

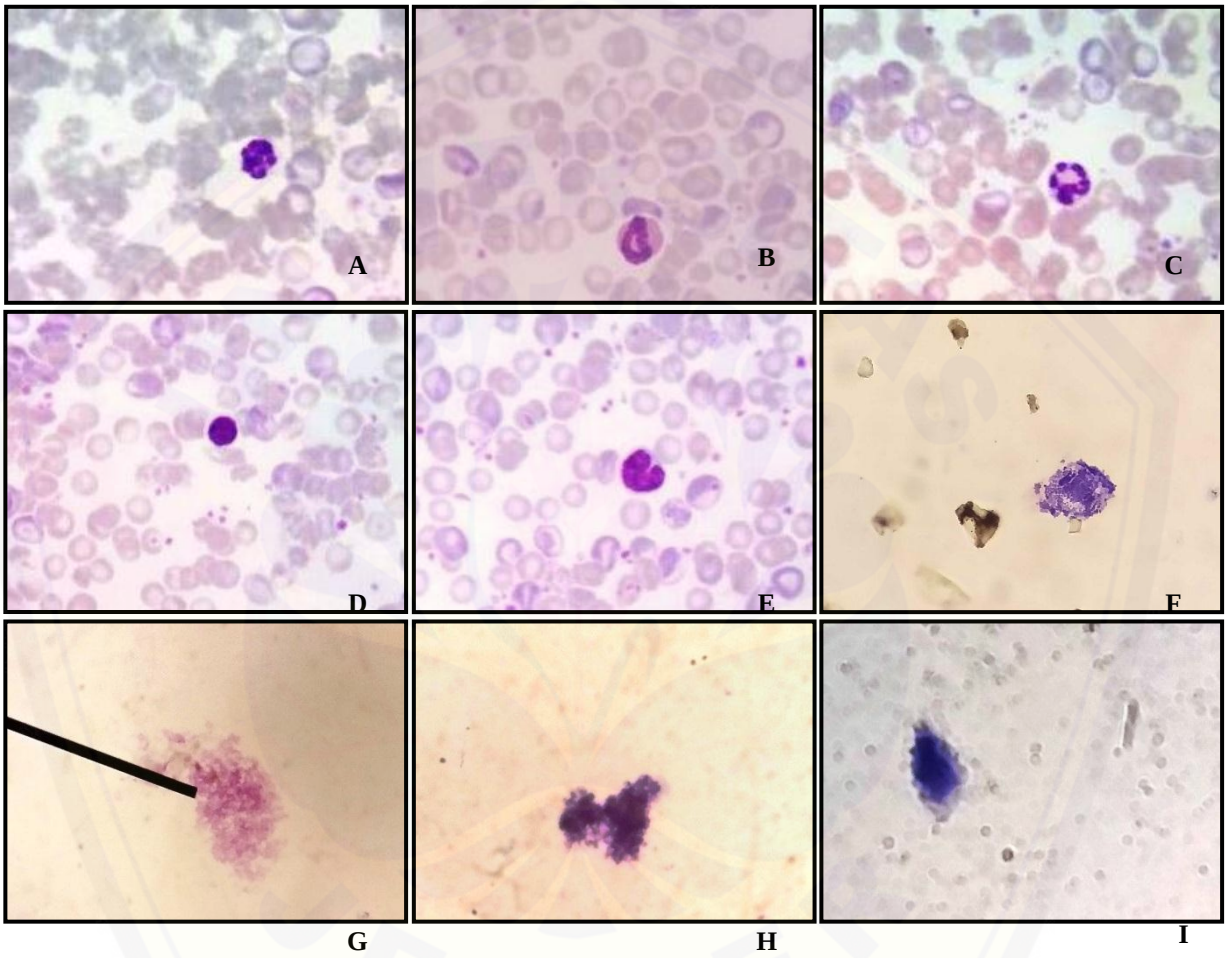

Figure 3. Morphology characteristic of leukocytes in peripheral blood and gingival fluid of ovarian failure rats induced ovariectomy

The presented images were histology preparation with Giemsa staining under 1000x magnification. A. basophil, B. eosinophil, C. neutrophil, D. lymphocyte, E. monocyte in peripheral blood; and F. basophil, G. eosinophil, H. neutrophil, I. lymphocyte in gingival fluid

Discussion

In this study, leukocytes profile in peripheral blood was significantly higher than in gingival fluid. Furthermore, all of the leukocyte types were detected in peripheral blood. While in the gingival fluid was only monocytes not detected. It might be caused by leukocytes source in those sites were different which the peripheral blood was originated from the haemopoietic system of bone marrow and lymphoid and they were circulated in vascular, whereas leukocytes in gingival sulcus were derived from the systemic source that

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accumulated in periodontal tissue, especially in gingival sulcus. The leukocytes excreted to gingival pocket when there were permeability changes of gingival sulcus epithelium.[26, 29] In addition, leukocytes was only 2 % of gingival fluid component.[22]

Moreover, total leukocytes in peripheral blood were gradually declined to 28th days. It might premature ovarian failure induced ovariectomy suppressed leukocytes production and it might be impacted immune system suppression. On the contrary, the previous study showed that ovarian failure induced ovariectomy stimulated low-grade chronic inflammation that was manifest high inflammation response correlated increasing total leukocytes, however, the leukocytes functions decreased. Therefore premature ovarian failure induced ovariectomy would enhance morbidity and mortality due to immune system dysfunction.[2, 13] Hirokawa et al described that total leukocytes were affected by increasing of ages.[10] Furthermore, Wakf explained that alteration total leukocytes in menopause women were influenced by menopause phase. Total leukocyte in early menopause was higher than premenopause and lower than late postmenopause. In this study, we suggested the periods of counter time mimicking the menopause phase that 3rd and 7th day might be early menopause phase and after 14th days was late menopause.[1, 15, 19, 28]

Otherwise total leukocyte in gingival fluid was fluctuant and it tent to increase following the days of observation. The peak was on 28th day. It might be affected local and systemic factors. Ovarian failure rats induced ovariectomy was supposed modifying environment in periodontal tissue that influenced migration and excretion leukocyte from periodontal tissue to gingival sulcus area. Rahnama et al described that proportion leukocyte in peripheral blood and gingival fluid was difference which neutrophil was more accumulated in gingival fluid in order to protect periodontal tissue from injuries.[17, 22]

The percentage of leukocytes was different between peripheral blood and gingival fluid, which lymphocytes in peripheral blood had higher percentage than neutrophil in peripheral blood, while lymphocytes in gingival fluid had lower percentage than neutrophil in gingival fluid. It might be ovarian failure rats induced ovariectomy stimulated immune cell activity. Neutrophils constituted 91-97% of the immune system among all leukocytes in gingival fluid and the remaining were monocyte/ macrophage and lymphocytes.[22] The previous study showed that ovariectomy cause deficiency of sex steroid hormone, estrogen and progesterone. Those deficiencies altered accumulation leukocytes in gingival sulcus.[9, 16, 17]

This study also showed there were differences imaging of leukocytes in peripheral and gingival fluid which leukocytes cell wall were more intact than in gingival fluid. We suggested that leukocyte cell in gingival fluid had through gingival sulcus epithelial and the excretion process caused cells damage. Moreover, we supposed that leukocyte cells were lysed in order to phagocyte and chemotaxis function. Gingival crevicular fluid is an exudate secreted by gingival and it accumulates the crevices gingival. The concentrations of this fluid are influenced by inflammation.[22]

Conclusion

This recent study concluded that ovarian failure induced ovariectomy affected leukocytes profile in peripheral blood circulation and gingival fluid. Leukocytes profile might be effect in ovarian function, although it needed further study to know the effects. Moreover, leukocytes could be used as biological marker of ovarian function, and morbidity and mortality status in ovarian failure or menopause. However, this study needs further studies in order to know effect ovarian failure induced ovariectomy to leukocytes activities.

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