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The Comparison Leukocytes Profile in Peripheral Blood and Gingival Tissue after Consume Anchovies (In Vivo Study)

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ABSTRACT

Background: Oral Allergy Syndrome (OAS) is used to ingestion food containing high protein.Anchovy represents high protein food, which is suspected it stimulates OAS. Leukocyte profile in peripheral blood and tissue can determine diagnostic of OAS. This study was aimed to compare leukocyte profile in gingival tissue and peripheral blood after consuming anchovy.

Methods: This study was an experimental laboratory with only a post-test control group design. This study used doses 0.5 to 4gram given every day and per two days. The blood sampling was taken from orbitalis sinus before treatment, on the third and seventh days after treatment. After that, the blood was made blood smear by Wright staining. Therefore gingival sampling was taken on the third and seventh day after treatment. Then, the gingival sample was prepared for histology and stained by hematoxylin-eosin. Both the preparation were observed under light microscope by 1000 magnification.

Results: Most of the leukocytes types significantly enhanced on the third day ($p \leq 0.05$), except neutrophils. The highest leukocytes alteration presented by animal model treated 2 gram/ 200gram bodyweight anchovies. The patterns of eosinophils, basophil,and monocytes altered significantly on the third and seventh day in each group ($p \leq 0.05$). Moreover, there presented significant differences between animal models which consumed anchovies per day and per two days, especially animal models which consumed 4gram/ 200gram body weight ($p \leq 0.001$). There were no significant correlations between the leukocyte profile in the gingival tissue and peripheral blood ($R<0.1$).

Conclussion: Anchovies consumption could induce an oral allergic reaction.

Keywords: allergy, anchovies, inflammation, leukocytes profile, oral allergy syndrome

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INTRODUCTION

Food allergy is used to describe an excessive immune response to food, especially protein. Protein is the most of food allergen. The IgE will bind the epitope protein of food, and then it activates eosinophile and basophile in peripheral blood.Excessive protein intake stimulates overreaction caused by food protein-IgE binding which the manifestations are called allergy.[1], [2] One of allergy clinical manifestation presentsin oral cavity, such as itching and swollen in lips, palate, and tongue; dry mouth; and halitosis. These manifestations are called Oral Allergy Syndrome (OAS).OAS represents one of a global problem that is generally caused by food allergy. The prevalence is about 10 to 30% of adults and 40 % of children.[3]

OAS is used to ingestion food containing high protein, such as nuts, fish, eggs, and cow milk.[4]Anchovy representsthe fish type, which is suspected it stimulates OAS. The previous study exhibited that Filipinos experienced food allergy that is majority caused by mackerel and anchovy.[5] Several studies related anchovies to parasite allergy.[6], [7] Therefore, studies about the relationship between anchovies consumption to oral allergy syndrome have been inadequately explored. Although many studies described that anchovies are high-quality food for bone and teeth due to calcium level contained in that fish,[8] the anchovies need further study to explore the drawback to human health.

Leukocyte profile is used to a screening tool to determine diagnostic a disease or disturbance. Leukocytes produce mediators and cytokines involved in inflammation and allergy. Leukocyte profile usually utilizes peripheral blood to detect the alteration. However, in some cases, such asallergy, the leukocytes profile is also determined from tissue. Peripheral blood and tissue present difference leukocyte profile, because the leukocytes have different behavior when they present in circulation and tissue. Aforementioned, this study was aimed to compare leukocyte profile in gingival tissue and peripheral blood after consuming anchovy. This study analogized that anchovy as food allergen stimulating OAS.

METHODS

This study was an experimental laboratory with only a post-test control group design. This study was approved by the Ethics Commission of Medical Faculty, Sultan Agung University, Semarang (No. 210/IX/2013/Komisi Bioetik, allowed on September 24, 2013). This study used white rats (Rattus norvegicus) Wistar Strain. Determination of anchovies based on conversion doses human to rat, which indicated that the minimum dose was 1,028 gram/ 200gram bodyweight. This study used doses 0.5 to 4gram given every day and per two days.

The blood sampling was taken from orbitalis sinus before treatment, the third and seventh days after treatment. After that, the blood was made blood smear by Wright staining. Therefore gingival sampling was taken on the third and seventh day after treatment. Then, the gingival sample was prepared for histology and stained by hematoxylin-eosin.

Both the preparation were observed under light microscope by 1000 magnification.

RESULT

Table 1 revealed that the general characteristic of animal models before they consumed anchovies. Each group exhibited similar characteristics($p \geq 0.05$). The biological parameters indicated that the levels presented in the normal range. Thus, this study could utilize animal models as the object of study.

Table 1. Characteristic of Animal model (n=20)

Variable	A	B	C	D	P-value
Body weight (gram)	267±14.49	281.6±23.16	284.8±14.27	275.8±5.97	0.284
Systole (mmHg)	123.33±2.89	123.67±1.53	122.33±2.52	122±2	0.139
Diastole (mmHg)	89.67±0.58	88±3.00	87.67±2.52	88.1±2.78	0.432
Total Leukocytes (/cmm)	7620±149.3	7120±1040.8	7440±562.8	8090±1194.4	0.059

Data represented mean and standard deviation; P-value, the significant value within the group which is analyzed the analysis of variance test; A, 0.5 gr/200gr body weight; B, 1gr/200gr body weight; C, 2gr/200gr body weight; D, 4gr/200 gr body weight

Leukocytes profile represented one parameter of blood complete count. There are two kinds of examination, the total number, and differential counting. The screening diagnostic of diseases usually utilizes the leukocyte profile examination. This study observed leukocytes profile in peripheral blood and gingival tissue. Based on the period of observation, this study revealed that differential counting examination in peripheral blood and gingival tissue presented similar patterns in all of the group, exception neutrophil count. However, this study only investigated the leukocytes profile in gingival tissues were the third and seventh days. Most of the leukocytes types significantly enhanced on the third day ($p \leq 0.05$), except neutrophils. Beside it, the highest leukocytes alteration presented by animal model treated 2 gram/ 200gram bodyweight anchovies, as can see in figure 1.

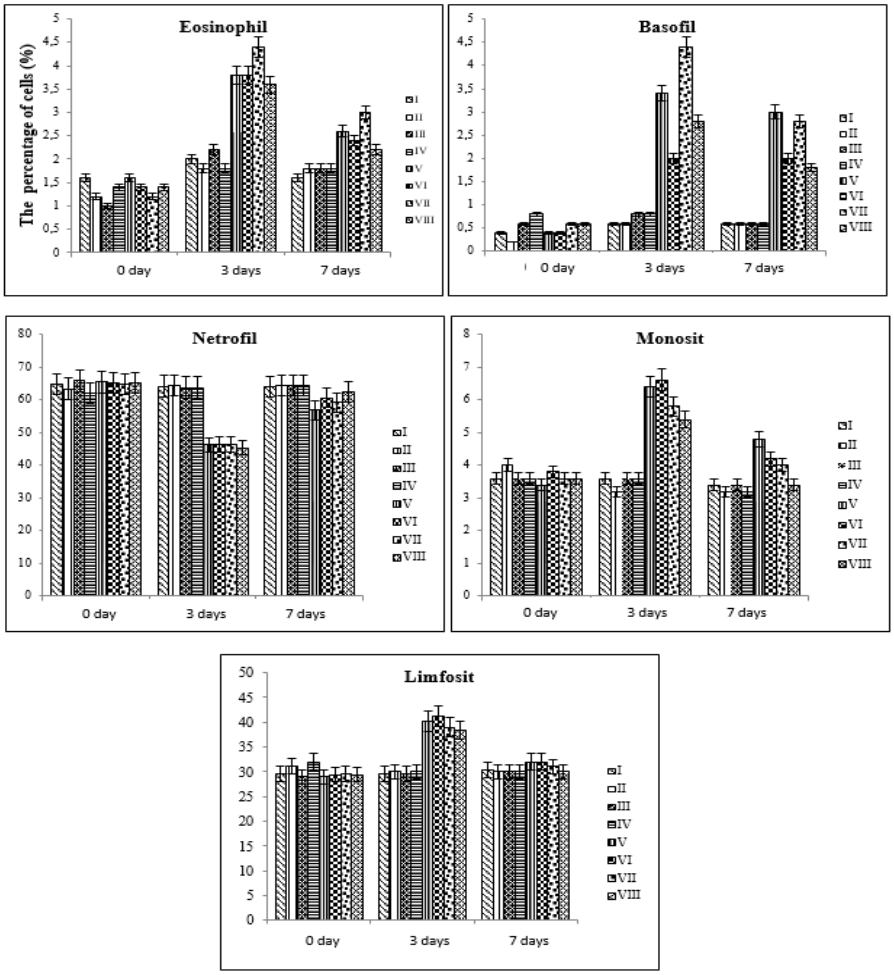

Figure 1. Leukocytes profile in peripheral blood of animal model

Data represented mean and standard error; Data were analyzed by two way ANOVA and multiple comparisons ($p \leq 0.05$); I, 0.5gr/200 gr body weight given per day; II, 0.5gr/200 gr body weight given per two days; III, 1gr/200 gr body weight given per day; IV, 1gr/200 gr body weight given per two days; V, 2gr/200 gr body weight given per day; VI, 2gr/200 gr body weight given per two days; VII, 4gr/200 gr body weight given per day; VIII, 4 gr/200 gr BB body weight given per two days.

However, the recent study presented insignificant alteration in animal models consumed per day and per three days in each dose ($p \geq 0.05$). Figure 1 show that animal models consumed 0.5 gram and 1 gram/ 200 gram body weight per days were as similar than animal model consumed 1 gram and 1.5 gram/ 200 gram body weight per days ($p \geq 0.05$). In the recent study, leukocytes profile were not only investigated in peripheral blood, but it was also investigated in tissue, included gingival tissue. Observation in tissue determines allergy respond and allergy type. Figure 2 indicated leukocytes profile in the oral mucosa (gingival and vestibule mucosa) presented similar patterns, except macrophage and lymphocytes.

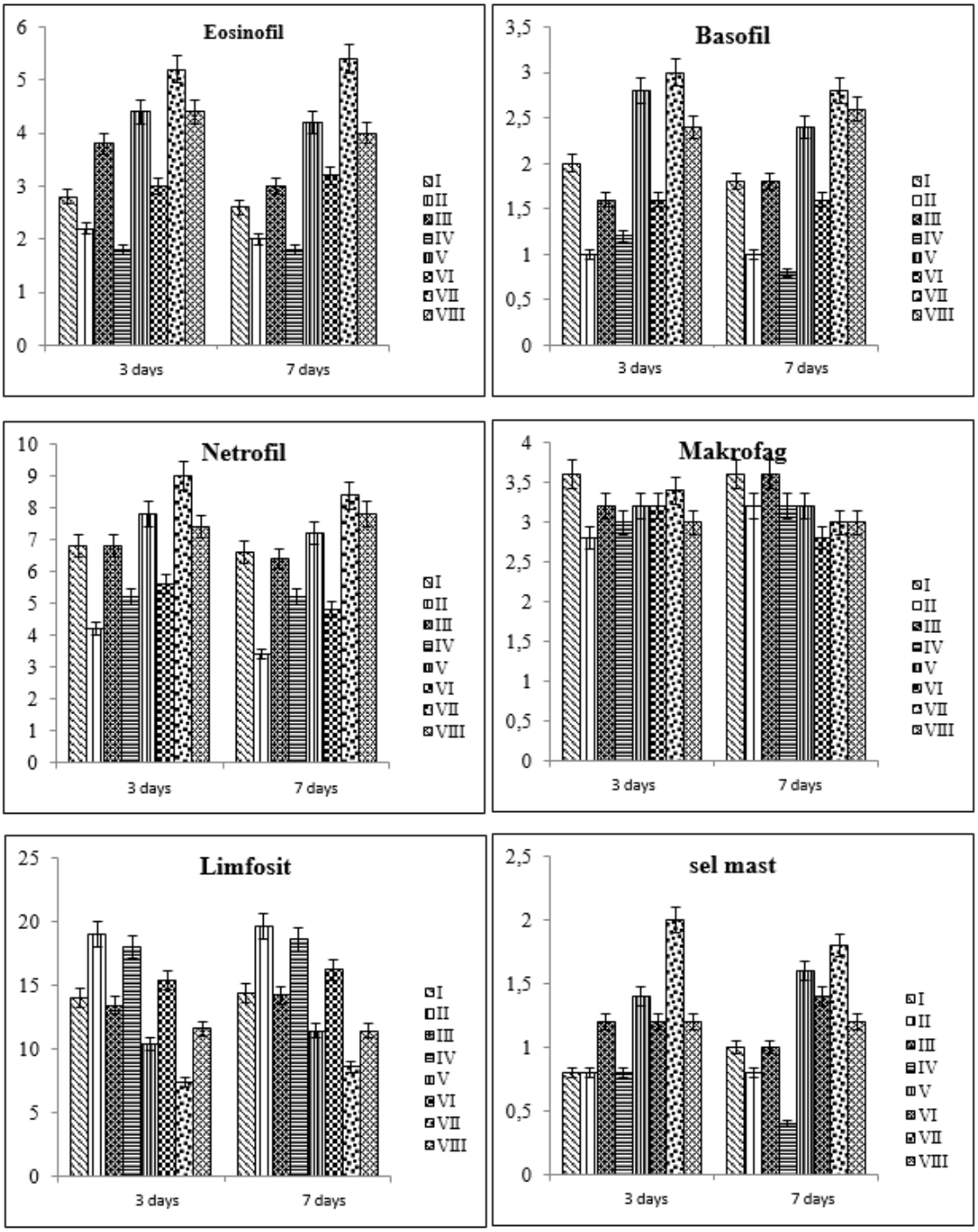

Figure 2. Leukocytes Profile in Gingiva Tissue

Data represented mean and standard error; Data were analyzed by two way ANOVA and multiple comparisons ($p \leq 0.05$); I, 0.5gr/200 gr body weight given per day; II, 0.5gr/200 gr body weight given per two days; III, 1gr/200 gr body weight given per day; IV, 1gr/200 gr body weight given per two days; V, 2gr/200 gr body weight given per day; VI, 2gr/200 gr body weight given per two days; VII, 4gr/200 gr

body weight given per day; VIII, 4 gr/200 gr BB body weight given per two days.

Table 2. Association Leukocytes Profile in Peripheral Blood, Dosis of Anchovies and Frequency of Anchovies Consumption

Variable	Dose [a]			Frequency [a]		
	B coeficient	R square	P value	B coefficient	R square	P value
Eosinophil	1.360	0.351†	0.000***	1.342	0.288‡	0.001**
Basophil	0.076	0.479†	0.000***	0.308	0.312†	0.001**
Netrophils	66.557	0.379†	0.000***	63.125	0.130‡	0.158
Monosit	3.302	0.244‡	0.007**	3.842	0.066#	0.473
Lymphocytes	29.071	0.274‡	0.007**	31.200	0.036#	0.695

[a]Linear regression test; ***, significantly correlation between variables (p<0.001); **, significantly correlation between variables (p<0.05); #, no correlation; ‡, weak correlation; †, significant and moderate correlation

Table 3. Association Leukocytes Profile in Peripheral Blood, Dosis of Anchovies and Frequency of Anchovies Consumption

Variable	Dose [a]			Frequency [a]		
	B coeficient	R square	P value	B coefficient	R square	P value
Eosinophil	1.857	0.541§	0.000***	3.652	0.062#	0.586
Basophil	1.096	0.434†	0.000***	2.050	0.053#	0.641
Netrophils	4.864	0.410†	0.000***	6.975	0.098#	0.389
Monosit	3.914	0.114‡	0.313	3.150	0.012#	0.915
Lymphocytes	18.089	0.508§	0.000***	13.000	0.079#	0.487
Mast	0.675	0.324†	0.003**	1.200	0.016#	0.886

[a] Linear regression test; ***, significantly correlation between variables (p<0.001); **, significantly correlation between variables (p<0.05); #, no correlation; ‡ moderate correlation; †, significant and moderate correlation;§, strong correlation

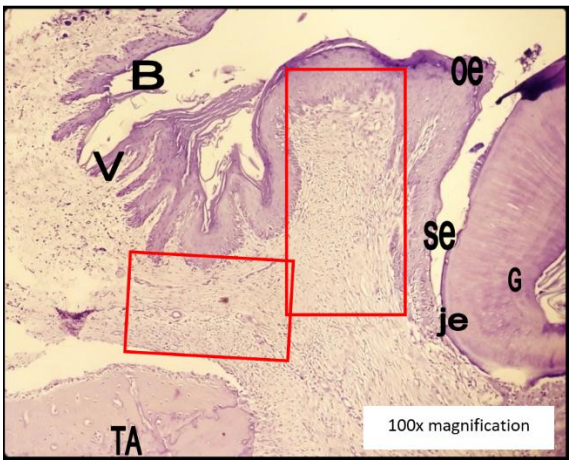

Figure 3. Observation Area of Leukocytes Counting in Gingival Tissue (red box)

B, buccal mucose; V, vestibulum; oe, oral epithelium; se, sulcular epithelium; je, junctional epithelium; G, gingival; TA, alveolar bone

Table 4 and figure 4 revealed the correlation between leukocytes in peripheral blood and gingival tissue. There were no significant correlations between the leukocyte profile in the gingival tissue and peripheral blood (R<0.1).

Table 4. Association Leukocytes Profile in Peripheral Blood and Leukocytes Profile in Gingival Tissue

Variable	Leukocyte Profile in Peripheral Blood		
	B coefficient	R square	P-value
Eosinophil	1.236	0.273‡	0.014**
Basophil	0.281	0.298‡	0.007**
Netrophils	65.850	0.150‡	0.183
Monosit	3.748	0.029#	0.800
Lymphocytes	32.822	0.134‡	0.235
Mast	0.669	0.147‡	0.193

[a] Linear regression test; ***, significantly correlation between variables (p<0.001); **, significantly correlation between variables (p<0.05); #, no correlation; ‡, weak correlation

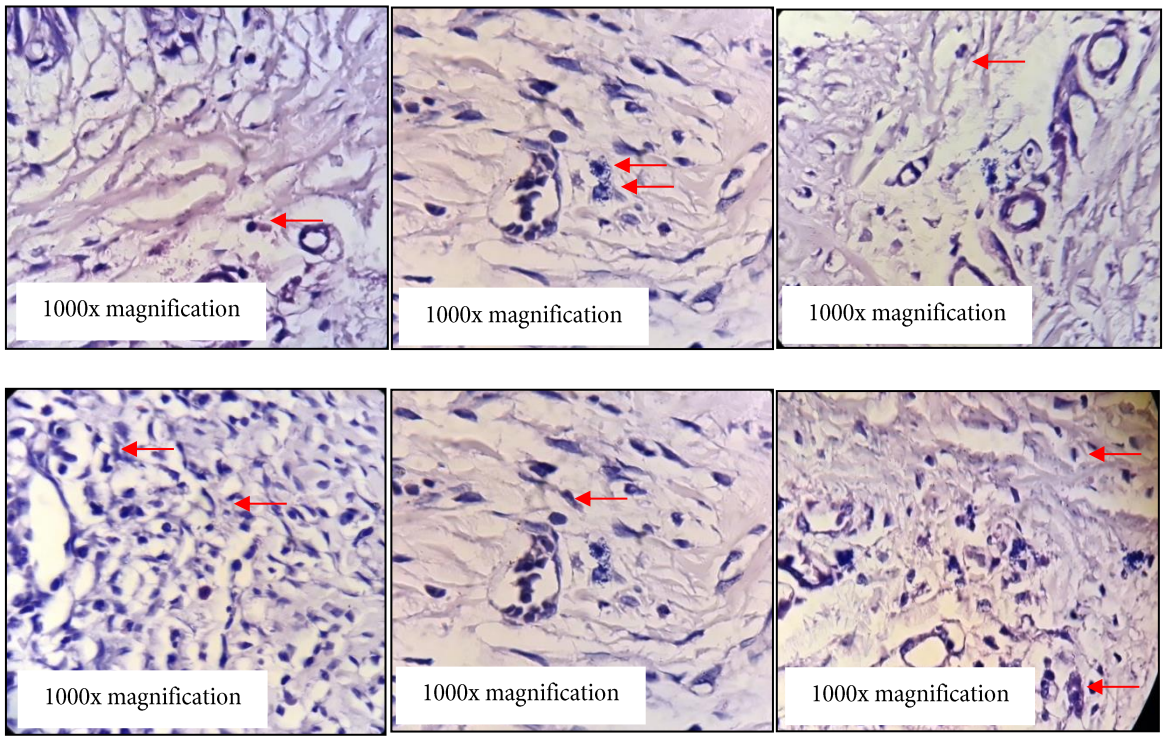

Figure 4. Leukocytes Profile in Gingival Tissue (HE staining, 1000x magnification)

A, eosinophil; B, basophil; C, segment neutrophil; D, macrophage; E, lymphocyte; F, degranulation mast cell

DISCUSSION

The digestive process determines allergy in the oral cavity. Intolerance in oral mucosa depends on oral mucosa integrity and gastrointestinal barrier activity. The gastrointestinal tract is the biggest immune organ which continuously experiences exogen antigen exposure, such as bacteria and chemical compound contained in food. This organ has epithelial barriers characterized by brush border, bile salt, and high acid. Moreover, it contains antibodies and immune cells. If there is disintegrate, antigens stimulate inflammation and allergy.[9], [10]

The recent study indicated that animal models represented the normal range of biological parameters. This study used the reference standard to compare baseline range level before treatment because this study did not use the control group/ baseline. Bodyweight in this study was by the reference, which the animal models aged 9-12 weeks old (white rats strain Spraque Dawley) were 229-328 grams. Therefore, systolic and diastolic blood pressure was in the normal range, which the blood pressure based on reference was 121/89±5 mmHg.By the same token total number of leukocytes, the normal range of total number leukocytes presents 9.4 ± 3.2 10^3/ cmm.[11]

This study indicated eosinophil, basophil, lymphocyte, and monocyte in peripheral blood and gingival tissue altered after animal models consumed anchovies. The duration, doses, and frequency of anchovy's consumption influenced the leukocytes profile alteration. It might represent intolerance signs (hypersensitivity reactions) to anchovy's consumption. Alteration of eosinophile and basophil generally indicated intolerance reaction to food. These cells are granulated, which the granules contain chemical mediators, such as histamine, prostaglandin, cytokine, and leukotriene. The mediators will induce inflammation reaction and food allergy.[12], [13]

Besides it, this study revealed there were mast cells in gingival tissue, especially animal models given 2gram/ 200gram body weight per day for seven days. It might reveal hypersensitivity in the tissue which indicated oral allergic syndrome. Mast cells obtain resident cells in connective tissue and are distributed in the microvascular bed of oral mucosa. These cells functions as host defense and tissue repair. However, the granules of these cells contain histamine and heparin. If the number is excessive, it will bind Ig E and induce degranulation and allergy reaction.[13], [14]

Leukocytes change both in the gingival tissue and peripheral blood of animal models consumed anchovies correlated with oral allergy syndrome risk. Allergy is hypersensitivity due to over response of specific antigen in the immune system to an allergen. Allergy manifestations depend on site and chronicity. Oral allergy syndrome is an allergy reaction in oral triggered by food.Oral allergy syndrome usually obtains in oral mucose, such as buccal mucosa, gingiva, and tongue.[15]This study exhibited that anchovies consumption induced leukocytes alteration in gingival and buccal mucosa. It might relate to the frequency or chronically, which per two days consumption more affected the alteration than per day consumption.

Anchovies are high protein food; however, anchovies contain histamine. The level in oil is about 33 mg/kg, which the range is 0 to 518 mg/kg. Therefore the level in dried anchovies is about 35 mg/kg (the range, 0-483 mg/kg).[16]

The histamine might activate eosinophil, tryptase, specific IgE, IgA, IgG, IL-4, and IFN-γ in blood. This reaction also induces the proliferation of eosinophile, basophil, mast cell in peripheral blood and tissue. These cells present granules which the cells will attack antigen release the granules, called degranulation.The degranulation triggers inflammation and allergy reactions because the granules contain inflammation cytokine, histamine, and leukotriene. [17]

Anchovy is one of the protein sources and other essential nutritional values, such as fatty acid, minerals, and vitamins. However, anchovy can induce allergy due to high protein composition. The high protein will stimulate the excessive response of immune system, although protein forms most of the human cells,so it should not stimulate allergy reactions. Otherwise, the protein which should be broken to be small units in GI tract reacts to Ig A and Ig E in intestinal mucosa. Beside it, the protein structures present instability due to the food storage, processing, and digestive process.[18]These processes cause protein denaturation which stimulates antigen formation and induces cell immune activation. Moreover, the processes induce conformation histidine and histamine, that both compounds play role in allergy triggered by seafood.[19]

Moreover, this study exhibited that eosinophile increased significantly. The eosinophilia might relate to parasitic allergy. The anchovies might containparasites. Several studies described that anchovy is the main source of the human anisakiasis in Italy which was signed by eosinophilia.[6], [7] Eosinophils are the granulocyte lineage from the hematopoietic system. Eosinophils, as antigen presentating cells, produce inflammatory mediators both in acute and chronic phase. The inflammatory mediators represent lipid-derived molecules, peptides, cytokine. Eoshophils also plays role in elimination of helmint and parasite by through degranulation. Moreover, eosniphils involve in homeostatic of immune response. They are one of the immune cells that play as antigen presentation; producer of lipid-derived, peptide, and cytokine mediators in acute and chronic inflammation; and helminth and parasite clearance by degranulation; and homeostatic immune responses.[12], [20]

CONCLUSSION

Briefly, anchovies consumption in this study could induce an oral allergic reaction. The reaction depended on consumption frequency and doses. However, this study requires further studies to know the molecular alteration, so that know about the mechanism, prevention and curative treatment due to impact of anchovies intake.

REFERENCES

1. S. Waserman, P. Bégin, and W. Watson, "IgE - mediated food allergy," *Allergy, Asthma Clin. Immunol.*, vol. 14, no. s2, pp. 1–11, 2018.
2. A. Barbaud, C. Poreaux, E. Penven, and J. Waton, "Occupational protein contact dermatitis," *Eur J Dermatol 2015*, vol. 25, no. 6, pp. 527–534, 2015.
3. C. M. Webber and R. W. England, "Oral allergy syndrome : a clinical , diagnostic , and therapeutic challenge," *Ann Allergy Asthma Immunol*, vol. 104, no. 2, pp. 101–108, 2010.
4. C. H. Katelaris, "Food allergy and oral allergy or pollen-food syndrome," *Curr. Opin. Allergy Clin. Immunol.*, vol. 10, no. 246–251, 2010.
5. M. F. Sharp and A. L. Lopata, "Fish allergy : In review," *Clin. Rev Allerg Immunol*, vol. 46, pp. 258–271, 2014.
6. L. Guardone, A. Armani, D. Nucera, F. Costanzo, and S. Mattiucci, "Human anisakiasis in Italy : a retrospective epidemiological study over two decades," *Parasite*, vol. 25, p. 41, 2018.
7. S. Mattiucci *et al.*, "Anisakiasis and reactions associated with anisakis pegreffii," *Emerg. Infect. Dis.*, vol. 19, no. 3, pp. 3–6, 2013.
8. E. A. Ekoningtyas and A. W. Suci-Dharmayanti, "Manfaat ikan teri segar (Stolephorus sp) terhadap pertumbuhan tulang dan gigi," *Odonto*, vol. 1, no. 2, pp. 52–56, 2014.
9. B. Chassaing, M. Kumar, M. T. Baker, V. Singh, and M. V.- Kumar, "Mammalian gut immunity," *Biomed J.*, vol. 37, no. 5, pp. 246–258, 2014.
10. G. Vighi, F. Marcucci, L. Sensi, G. Di Cara, and F. Frati, "Allergy and the gastrointestinal system," *Clin. Exp. Immunol.*, vol. 153, no. suppl 1, pp. 3–6, 2008.
11. Harlan, "Sprague Dawley Rat," *Taconic Biosci.*, pp. 1–11, 2015.
12. G. A. Ramirez *et al.*, "Eosinophils from physiology to disease : A comprehensive review," *BioMed Res. Int.*, vol. 2018, pp. 1–28, 2018.
13. D. Santoro and R. Marsella, "Animal models of allergic diseases," *Vet. Sci*, vol. 1, pp. 192–212, 2014.
14. A. Kabiraj *et al.*, "Mast cells in oral inflammatory lesions- a pilot," *World J. Pharmceutical Res.*, vol. 4, no. 4, pp. 787–797, 2015.
15. R. R. Kashyap and R. S. Kashyap, "Oral allergy syndrome : An update for stomatologists," *J. Allergy*, vol. 2015, pp. 1–6, 2015.
16. "Presence of histamine in anchovies." NSW Food Authority, Silverwater NSW, Australia, pp. 1–18, 2010.
17. K. D. Stone, C. Prussin, and D. D. Metcalfe, "IgE, Mast Cells, Basophils, and Eosinophils Kelly," *J Allergy Clin Immunol*, vol. 125, no. 2, pp. 1–16, 2010.
18. M. Masilamani, S. Commins, and W. Shreffler, "Determinants of Food Allergy," *Immunol. Allergy Clin. NA*, vol. 32, no. 1, pp. 11–33, 2012.
19. P. Visciano, M. Schirone, R. Tofalo, and G. Suzzi, "Biogenic amines in raw and processed seafood," *Front. Microbiol.*, vol. 3, no. 188, pp. 1–10, 2012.
20. A. Kovalszki, P. F. Weller, B. Israel, D. Medical, B. Israel, and D. Medical, "Eosinophilia," *Prim Care2016*, vol. 43, no. 4, pp. 607–617, 2017.