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Chymase level in dengue virus infection with or without positive Non-Structural 1 (NS1)

Menik Kasiyati[1a*], Jusak Nugraha[2b], Hartono Kahar[2c]

[1] Postgraduates School of Immunology Airlangga University Surabaya, Indonesia
[2] Department of *Clinical Pathology* Faculty of Medicine Airlangga University/Dr. Sutomo General Academic Hospital, Surabaya, Indonesia

[a] Email address: rifani.2010@gmail.com
[b] Email address: jusak.nugraha@yahoo.com
[c] Email address: hartono1@gmail.com

HIGHLIGHTS

There were no significant differences in chymase levels between groups NS1 (+) and NS1 (-)

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A B S T R A C T

Thrombocytopenia, leukopenia, and monocytosis are laboratory parameters in the diagnosis of dengue virus infection. In addition to monocyte cells, mast cells also play a role in the innate immune response, where degranulation of mast cells will occur, which will secretion the active vaso mediator, Chymase. Chymase has a role in increasing vascular permeability resulting in plasma leakage in patients with dengue virus infection to determine the number of platelets, leukocytes, monocytes and chymase levels in patients with dengue infection in the acute phase. The platelet count mean in NS1 (+) was 132,140 cells / mm3 and the platelet count in the NS1 group was (-) 176,000 cells / mm^3. The mean leukocytes NS1 (+) showed results of 4,350 cells / mm^3 and NS1 (-) 5,250 cells / mm^3. The mean monocyte NS1 (+) monocyte count was 8.26%, and NS1 (-) group was 8.76%. There were no significant differences in platelet counts, leukocytes and monocytes between NS1 (+) and NS1 (-) (P value> 0.05). The mean Chymase NS1 (+) 23.48, NS1 (-) 23.05 ng / mL and the control group 1.47ng / mL.

Corresponding Author:
Menik Kasiyati
Jurusan Analis Kesehatan, Poltekkes Kemenkes Yogyakarta
Jl. Ngadinegaran MJ III/62, Yogyakarta, Indonesia

1. INTRODUCTION

World Health Organization (WHO) estimates that 40% of the world's population of around 2.5 billion people living in tropical and sub-tropical regions are at risk of contracting dengue virus infection. In Indonesia, this disease has been occurring every year for 45 years.[1] This virus consists of four types of serotypes (DENV1, DENV2, DENV3 and DENV4).[2] Dengue virus infection in humans results in a varied spectrum of clinical manifestations. Most DENV infections are asymptomatic or have mild symptoms, with a small proportion experiencing Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). Clinical manifestations for DHF and DSS include dengue fever, thrombocytopenia, vascular permeability and plasma leakage.[3]

The innate immune response awakens immediately after the infected Aedes mosquito inoculates DENV into the skin. Immune cells that are targeted by DENV are myeloid groups, namely phagocytic cells, including dendritic cells and monocyte cells. Monocytes and DC cells will be recruited significantly to the skin that has DENV infection. Monocytes and DCs can detect the presence of DENV using pathogen recognition receptors (PRRs), including TLRs, RLRs and CLRs. Activation of PRRs by DENV will activate intracellular pathway signaling which will induce inflammatory cytokines and anti-viral immunity through the production of type I Interferon (IFN) namely IFN α and IFN β.[4]

Like Langerhans dendritic cells, mast cells also interact with DENV in the early stages of infection. However, cell mast interactions with DENV differ from the type of Antigen-Presenting Cell (APC), which is the target of infection. Mast cells will experience degranulation within a few minutes after being exposed to DENV and will produce cytokines in the next few hours. Degranulation of mast cells will produce inflammatory mediators.[5] Chymase from mast cells will support increased vascular permeability both indirectly with the production of bradykinin, as well as the possibility of direct mechanism.[6,7] Chymase can cause degradation of junction cells, which can cause increased permeability.[8] The mechanism of action of the chymase is evident that tight junction protein (for example, Claudin 4, Claudin 5 and Occludin) are closely related to chymase both directly and indirectly. It is clear that the degradation of several proteins supports inflammation by migrating leukocytes through the endothelium.[9,10]

2. MATERIALS AND METHOD

The sample was collected from patients with dengue virus infection in Dr. Darsono Hospital, Pacitan. Inclusion criteria included patients aged 17-60 years, having fever for 2-7 days, NS1 examination results showing positive (+) and NS1 (-) diagnosing dengue virus infection in medical records and willing to sign informed consent. While the exclusion criteria are patients with dengue fever infection who meet the inclusion criteria with asthma, urticaria and diarrhea. The sample was 59 patients during January - March 2019. Patients were taken for blood on the first day of hospital admission for NS1 examination and complete blood examination in the laboratory. The serum is collected from patients who have diagnosed DF / DHF based on the rules of the World Health Organization (WHO) and examined Chymase levels at the Pathology Laboratory of the Gadjah Mada University in Yogyakarta. Approval of ethical clearance was obtained from the Faculty of Dentistry of Airlangga University Surabaya with Number 004 / HRECC.FODM / I / 2019. NS1 examination was carried out with the Panbio early detection brand rapid test. Chymase examination is done using Human Chymase 1, Mast Cell ELISA KIT from Bioassay Technology Laboratory.

3. RESULTS AND DISCUSSION

The number of samples obtained was 59 samples consisting of 49 samples with positive NS1 results (+) and ten samples with negative NS1 (-). The results showed that the NS1 (+) group was dominated by a male as much as 67.3% (33 people), while female 32.7%. There was a significant relationship between sex and dengue fever, where the results showed that men had a risk of 4.99 times higher than a woman.[11] Moreover, men have relatively higher work activities and mobilization than women.[12]

Table 1. Characteristics of DHF patients

Characteristics (n = 59)	Non-Structural (NS) 1		P value
	Positive (+)	Negative (-)	
NS1 Test	49	10	-
Gender			0,301**
Male	33 (67,3%)	5 (50%)	
Female	16 (32,7%)	5 (50%)	
Age Group			0,178*
Late adolescent (17-25)	19 (38,8%)	1 (10%)	
Young adult (26-35)	10 (20,4%)	6 (60%)	
Late adult (36-45)	10 (20,4%)	0	
Early elderly (46-55)	10 (20,4%)	3 (30%)	

Grouping characteristics by age, in the NS1 (+) group, was dominated by late adolescents (17-25 years), as many as 38.8% (19 people). This is in line with the results of the 2017 study by Deborah and Joshi in 2018, showing that there was an increase in the distribution of dengue fever in adolescents and adults. Age is one of the internal factors related to a person's behaviour or community. Age is related to daily activities inside and outside because *Aedes Sp* has a biting habit in the morning and evening.[11] The results of different tests for the sex group and age between NS1 (+) and NS1 (-) showed that there were no significant differences. This happens because NS1 affects the severity of dengue disease but does not depend on the sex and age of the host.

Table 2. Blood Test Result of DHF Patients

Characteristics (n = 59)	Non-Structural (NS) 1		P value	Normal Value
	Positive (+)	Negative (-)		
Platelet Count (cell/mm^3)				150.000 -
Mean ± SD	132.140±69.285	176.000±53.337	0,064*	450.000
Modus	156.000 12.000 s/d	74.000 74.000 s/d		
Max-Min	378.000	187.000		
Leucocytes Count (cell/mm^3)				3.800 - 11.000
Mean ± SD	4.350 ± 1,95	5.250 ± 1,28	0,172*	
Modus	2	2.8		
Max-Min	1.500 s/d 9.500	2.800 s/d 7.870		
% Basophil				0-1
Mean ± SD	0,15 ± 0,17	0,40 ±0,00	0,000**	
Modus	0,1	0,4		
Max-Min	0,0 s/d 0,9	0,4		
% Monocyte				2-8
Mean ± SD	8,26 ± 3,19	8,76 ±3,28	0,592**	
Modus	5,10	8,70		
Max-Min	4,10 s/d 18,30	5,50 s/d 16,90		
% Lymphocyte				25-40
Mean ± SD	22,3 ± 11,96	27,7 ± 9,78	0,084**	
Modus	10,3	14,7		
Max-Min	7,3 s/d 52,9	14,7 s/d 44,2		

The results of peripheral blood tests will change during dengue infection. However, the results showed that there was no significant increase in the leukocyte cell counts in the NS1 (+) group showing the results of 4,350 cells/mm3 and NS1 (-) 5,250 cells/mm3. The results of leukocyte count

still in normal value, which is consistent with the Jatmiko study, 2018 where this was due to the recent course of the disease (the fever of the research subjects averaged four days) and the degree of disease that was not severe. Leukopenia in dengue virus infection occurs due to severe bone marrow suppression and occurs in infections that last longer and close to the healing phase.[13]

The results of platelet count in the NS1 (+) group experienced a slight decrease of 132,140 cells/mm3, while in the NS1 (-) group it was still within the normal range of 176,000 cells/mm3. The different platelet test between NS1 (+) and NS1 (-) groups shows the results of the value of P> 0.05, which means there is no significant difference. This can occur because many things affect the occurrence of thrombocytopenia in patients with dengue virus infection, including bone marrow suppression, platelet sequestration, megakaryocyte infection, destruction of platelets due to the presence of antiplatelet autoantibodies, and platelet apoptosis. The interaction between these factors and the use of K3EDTA anticoagulants could be the cause of no significant differences between NS1 (+) and NS1 (-).[11,14]

The results of a differential count examination, especially the number of lymphocytes and basophils did not increase. Monocyte cell counts had a slight increase where on the NS1 (+) group monocyte count was 8.26% and NS1 (-) group was 8.76%. This is consistent with the study of Tsai et al., 2017, which states that monocytosis (a significant increase in the number of monocytes) occurs starting on the fifth day. The number of monocytes will continue to increase on the sixth and seventh days after a fever. Monocytes are the main target of the dengue virus, the dengue virus that enters the monocytes will increase cross-reactive antibodies by antibodies which increases in number. Monocytosis, along with thrombocytopenia, is the initial guide and is consistent in the diagnosis of dengue and severe dengue markers.[12] Activated platelets are containing the dengue virus form aggregations with monocytes during the critical stages of the disease. This shows the possible role of cleansing the virus and the causes of thrombocytopenia implemented by innate phagocytic immune cells. Sequential sequences of events including low platelet counts and transient monocytosis, increasing phagocyte activity during dengue virus infection, can cause the release of large numbers of dissolved factors to induce DHF.[15]

The results of different tests on complete blood test parameters did not show a significant difference. This happens because the infection of the dengue virus is still in the initial phase, so excessive destruction has not occurred.[11]

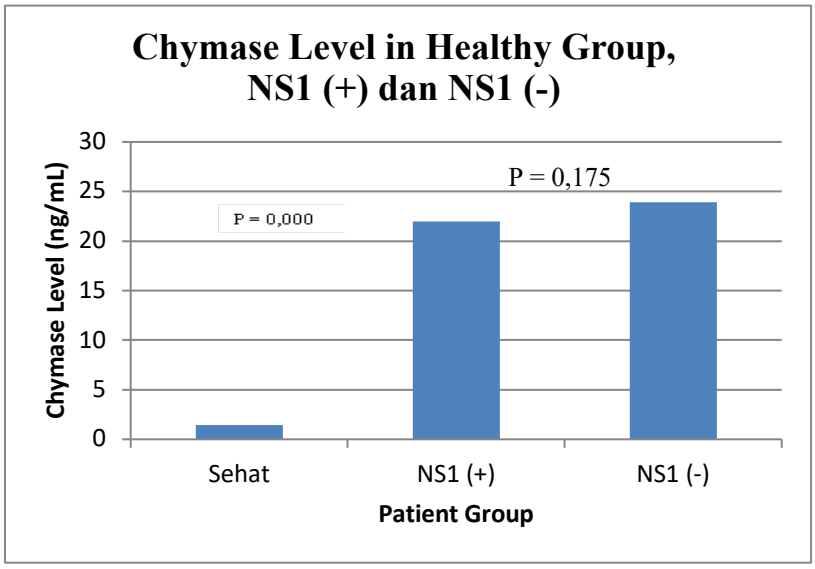

Figure 1. Chymase Levels in the Control Group, DHF Patients with NS1 (+) and NS1 (-)

Chymase levels in patients in the NS1 positive (+) group (n = 49) showed no significant difference in the NS1 negative group (n = 10). From the figure 1, it can be explained that the chymase level in the NS1 (+) group has an average that is almost the same as the NS1 (-) group. Based on statistical tests with the one-way ANOVA test obtained a value of P value> 0.05 which can be concluded that there is no significant difference between the chymase levels in the NS1 (+) and NS1

(-) groups. The results showed a significant difference between chymase levels in the control group with NS1 (+) and between the control group and NS1 (-) group.

Mast cells can produce many vasoactive mediators that have a function in inducing vascular permeability. Some of them are stored and can act almost instantly on the vascular endothelium, TNF, protease (tryptase and chymase) and heparin. Other vasoactive factors include leukotriene, prostaglandin, VEGF. With their activation, the factors released by mast cells act together to promote the degradation of junction cells, plasma leakage and oedema in tissues. The results of the study are not following the research reported by previous researchers that chymase levels will increase very significant in DF (tenfold) and DHF (thirty-fold) in the acute phase. This happens because other vasoactive factors besides chymase can degrade junction cells and cause plasma leakage in DHF patients.

4. CONCLUSION

There were no significant differences in chymase levels between groups NS1 (+) and NS1 (-). Significant differences in chymase levels were found between the control group with NS1 (+) and the control group with NS1 (-) in dengue virus infection. The decrease in platelets occurs very slightly below normal, with a slight increase in the number of monocyte cells.

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CONFLICT OF INTEREST

We declared in this work not any conflict of interest.

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The potency of bay leaves extract (Syzygiumpolyanthum) as anti-inflammation in Rattus norvegicus induced with Complete Freund's Adjuvant

Dwi Candra Buana[1a*], I Ketut Sudiana[2b], Jusak Nugraha[3c]

[1]Post Graduates School of Immunology, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Clinical Anatomy, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[3]Department of Clinical Pathology, Dr. Sutomo General Academic Hospital, Surabaya, Indonesia

[a] Email address: an_dra2112@yahoo.com
[b] Email address:ik.sudiana@yahoo.com
[c] Email address: jusak.nugraha@yahoo.com

HIGHLIGHTS

Bay leaves extract (Syzygiumpolyanthum) can prevent inflammation in rat (Rattus norvegicus) induced with Complete Freund's Adjuvant (CFA)

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A B S T R A C T

The using of herbal plants in Indonesia as medicine based on traditional knowledge. One of the herbal plants that believed has medication effect is bay leaves *(Syzygiumpolyanthum)*. Bay leaves contain a flavonoid that can prevent inflammation. The study aims to prove the potency of bay leaves extract to prevent the inflammation, which is indicated by decreasing the paw edema thickness in rat induced with Complete Freund's Adjuvant (CFA). This study used 25 animal models of male rat *(*Rattus norvegicus*)* strain Wistar that divided into five groups, one group for a normal group (no treatment), one group for positive control which was injected with CFA sub plantar and given treatment with Na CMC and three test groups which is got three different doses of bay leaves extract peroral and the doses were 100 mg/kg BW, 300 mg/kg BW, 400 mg/kg BW, and injected with CFA into the sub plantar. Bay leaves extract was given one hour per oral before injected with CFA. The measurement of paw edema was done at 24 hours after CFA injection. The result of this study showed that bay leaves extract gave the anti-inflammation effect to the rats which were injected with CFA. It is concluded that bay leaves extract containing flavonoid plays a role in inhibit cytokines proinflammatory synthesis.

Corresponding Author:

I Ketut Sudiana
Department of Clinical Anatomy, Faculty of Medicine, Universitas Airlangga,
Jl. Mayjend Prof.Dr.Moestopo No.47,Kec.Tambaksari,Surabaya 60132
Email: ik.sudiana@yahoo.com

1. INTRODUCTION

Inflammation is a response of the host to protect itself from infection and tissue damage which involve of immune cells and gathering of plasma protein to the area of infection and tissue damages by removing the injurious stimuli as well as initiate the healing process for the tissue and also vasodilatation of blood vessels.[1,2] Inflammatory process involves a series of phenomena that can be triggered by various stimuli such as infectious agents, ischemia, antigen-antibody interactions and thermal or injury caused by other physical agents.[3] This series of events leads to the release of damage-associated molecular patterns (DAMPs) then stimulate TLRs on macrophage inducing upregulation of cytokines TNF-α and IL-1β by way of NFκB activation.[4] The inflammation is characterized with five cardinal signs namely redness, swelling, heat, pain, and loss of function.[5]

Bay leaves is mostly distributed in the tropic and subtropic area especially in Southeast Asia including Indonesia and Malaysia.[6] Bay leaves usually use as a traditional medication for treating diabetes mellitus, hyperuricemia, hyper cholesterol, gastritis, hypertension, diarrhea and rheumatism.[7] Bay leaves contain of tannin, flavonoid, alkaloid and essential oil whereas flavonoid has an active role as anti-inflammation by inhibiting of transcription factors such as NF-κB.[8,9] Previous study showed that bay leaves extract with concentration 200% could reduce the inflammation to the rats that were dipped with phenol 50%.[10] Flavonoid based the chemical features are divided into subclasses: flavonols, flavones, flavanones, flavanols, anthocyanins, isoflavones, chalcones and dihydrochalcones.[11]

Complete Freund's Adjuvant (CFA) is a mixture of paraffin oil and surfactant with heat-killed *Mycobacterium tuberculosis* or *M. butryicum* in which aqueous antigen solutions are emulsified.[12] CFA has been used to induce inflammation and study inflammatory pain in several animal models, and it attempts to mimic human conditions of persistent or chronic inflammatory pain. Role of CFA is concluded that adjuvant activity from sustain release of antigens from the oily deposit and stimulation of local innate immune response causing delayed hypersensitivity reaction that can react with intense inflammatory reaction and hyperalgesia at the site of injection.[13] Therefore, this study aimed to prove the potency of bay leaves extract to prevent the inflammation, which is indicated by decreasing the paw edema thickness in rats induced with CFA.

2. MATERIALS AND METHOD

This study used adult male Wistar rats aged 12 weeks, weighing 200-250 gram, were acclimated one week before the experiment in a room with constant temperature (25-26 °C). Food and water were available *ad libithum*, and all the procedure were approved by Biochemical Laboratory of Medicine Faculty of Universitas Airlangga. This study was approved by the Ethical Committee of Dental Medicine Faculty, Universitas Airlangga 066/HRECC.FODM/III/2019. The data were analyzed by calculating mean and standard deviation.

Bay leaves derived and determined from Material Medika, Batu, Indonesia. Phytochemical screening test for bay leaves included was standardized of flavonoid. Stages to be performed were following: 500 g powder of bay leaves is macerated with 3L of ethanol 70% for 24hours, after maceration by mixing the processed bay leaves into rotary evaporator for 90 minutes (3 hours) then we get 370 mL of solution then added 8% of amylum (29.6 gram) then we get 50 grams dry extract of bay leaves. From the extract has done Phytochemical screening to know the presence of flavonoid in this extract given.

The Phytochemical screening was performed through Shinoda's test. The one mg bay leaf extract is added with 0.5 mL of hydrochloric acid and magnesium metal, and then reddish colouration appeared to confirm the presence of flavonoid. Inflammation model in this study used CFA (Invivogen) 0.1 mL mixed with phosphate buffer saline (PBS) 0.1 mL. This solution was injected into right hind paw sub plantar. Animals were then divided into five groups (n = 5/group) named normal group/ no treatment (Kn), CFA-control group (Kk), CFA with peroral prevention of bay leaves extract group (Kp1, Kp2, Kp3) with dose 100 mg/kg BW, 300 mg/kg BW and 400 mg/kg BW. The bay leaves extract was given per oral one hour before CFA induction. The measurement of hind paw edema used digital calliper at 24 hours after CFA injection.

The mean of group kn recorded lowest amongst all because of no experimental method was applied. Group Kk with CFA injected and Kp1 with 100 mg/kg BW mean is higher as compared to others with CFA and bay leaf higher dose i.e. ≥ 100 mg/kg BW subsequently we have to increase to dose to achieve the results. Consequently, dose increased to 300 and 400 mg/kg BW hence the

mean of group kp2 and kp3 are decreased. We can still evaluate the higher dose, i.e. ≥ 400 mg/kg BW, to achieve the maximum results. The dose 400 mg/kg BW is effective to inhibit the inflammation as compare to other compered groups in the study hence a future study is recommended to prolong the treatment and duration to observe the effects. We also applied Levene's test for homogeneity, i.e. one-way ANOVA in table 2. There is a difference between the control group and treatment groups, because of non-homogeneity of data, we used Tamhane posthoc multiple comparisons. Tahmane test results show that CFA applied to group and treatment with dose 400 mg/kg BW has a significant effect determined by p-value, which is $p \leq 0.05$. Hence it can be concluded that there is a difference between the control group with treatment group having applied dose of 400 mg/kg BW.

3. RESULTS AND DISCUSSION

There are many studies discussing the inflammation and flavonoids[14] and bay leaf and flavonoids[15] and its role in diseases.[16] The role of flavonoids and how to decrease inflammation by using it is discussed in details by Levya-Lopez.[17] According to Levya-Lopez[17] flavonoid can function in propelling the activation of NFkB so the synthesis of pro-inflammation cytokine, i.e. TNF-α can be inhibited. The flavonoids are also correlated with the structure-activity and have the ability to inhibit neutrophil degranulation.[2] Hence, we can assume that through neutrophil degranulation, we can identify inflammation easily. In view of discussion and explanation by Rathee[2] we can thus, assume that the current study hypotheses the functions of bay leaves as the anti-inflammation agent.

The measurement of hind paw edema thickness used a digital calliper and was done at 24 hours after CFA injection because subcutaneous injection in right hind paw of CFA induces a more prolonged swelling that becomes maximal at 24 hours and persists for at least seven days.[18,19]

Table 1. Hind Paw Edema Thickness (in mm)

	Minimum	Maximum	Mean	
	Statistic	Statistic	Statistic	Std. Error
kn	2.25	2.28	2.2620	0.00583
kk	5.49	5.70	5.5880	0.03904
kp1	4.69	6.01	5.3680	0.20958
kp2	3.71	5.42	4.9300	0.31056
kp3	4.48	4.94	4.7460	0.10127

Noted: Kn = (Normal); kk = (CFA Injected); kp1= (treatment with 100 mg/kgBW); kp2 (treatment with 300 mg/kgBW); kp3 (treatment with 400 mg/kgBW)

From the Table 1 showed that the mean of each treatment group showed that all variances dose of bay leaves extract has the capability to inhibit the inflammation than a group of CFA with no treatment (Kk) and a group of kp3 showed the lowest mean than the other treatment groups.

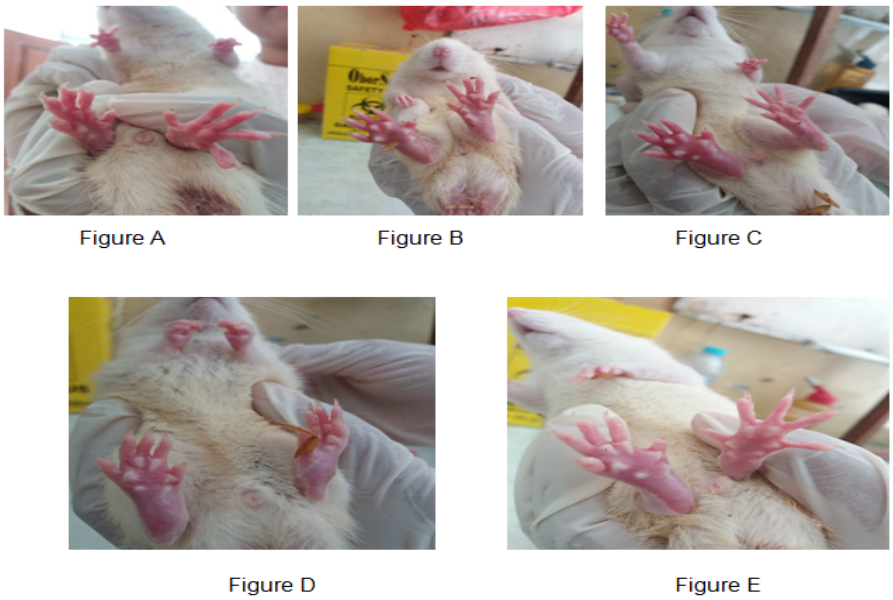

Figure A Figure B Figure C

Figure D Figure E

Figure 1. Hind paw edema of Wistar rats after 24 hours CFA induction

Noted:
Figure A: Hind paw of a normal rat with no treatment (Kn).
Figure B: Hind paw edema of rat with CMC Na and CFA induction (Kk).
Figure C: Hind paw edema of rat with bay leave extract with dose 100 mg/kg BW and CFA induction (Kp1).
Figure D: Hind paw edema of rat with bay leave extract with dose 300 mg/kg BW (Kp2).
Figure E: Hind paw edema of rat with bay leave extract with dose 400 mg/kg BW (Kp3).

From figure 1 showed the inflammation of right hind paw on each group, from figure E showed less inflammation after 24 hours of CFA induction, it means that dose 400 mg/kg BW has a possibility to inhibit the inflammation than no treatment group (kp3)

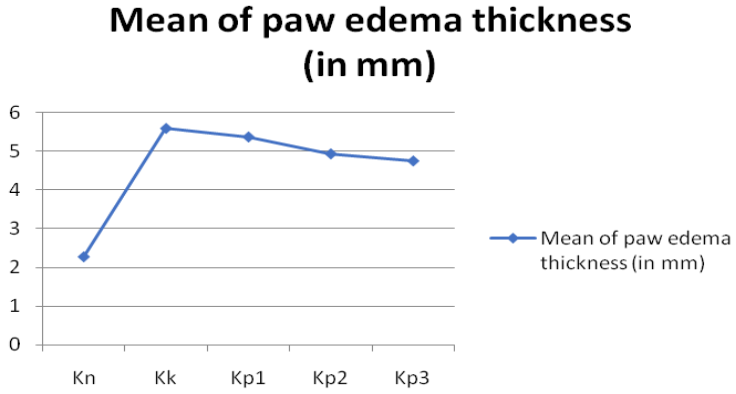

Figure 2. Graphic decreasing of paw edema thickness after 24 hours CFA injection

From figure 2 showed that there is increasing of mean value from Kn group to Kk group, but it also shows that there is decreasing of mean value from Kk group and all variances dose of treatments and Kp3 group shows the lowest mean value than the other treatment groups.

Table 2. Test of Homogeneity of Variances (for treatment)

Levene Statistic	df1	df2	Sig.
2.954	4	20	0.045

The table 2 is explaining homogeneity of variance for treatment through Levene's, and the result shows that there is no homogeneity noted in these groups. Thus, data was not homogeneous; we keep using posthoc multiple comparisons using Tamhane.

Table 3. ANOVA (Treatment)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.801	4	8.950	58,810	0.000
Within Groups	3.044	20	0.152		
Total	38.845	24			

To see the difference between the group, we applied the One-Way ANOVA test, it shows the value of $p<0.05$ indicates the significance of the current study, from table 3 shows that between the CFA applied to group and treatment 400 mg /kg BW is significant with a value of p = 0.005 (p <0.05), so that means it has a difference between the control group and the treatment group 400 mg /kg BW, from the result based on table 4 using Tamhane Post Hoc Multiple Comparison shows that there was difference result on each group of treatment with bay leaves extract (Kp1, Kp2, Kp3) compared with the CFA group (Kk) and the group of Kp3 showed the stronger inhibitory activity against inflammatory paw edema with value of $p=0{,}005$ ($p<0{,}05$). It means that dose 400 mg/kg BW is an effective dose to inhibit the inflammation as compare to other compered groups in the study.

Table 4. Post Hoc Multiple Comparison

Tamhane		Sig
Kk	Kn	0.000
	Kp1	0.988
	Kp2	0.656
	Kp3	0.005

CFA consist of mineral oil, containing a suspension of whole or pulverized heat-killed mycobacteria and its adjuvant activity results from the sustained release of antigens from the oily deposit and stimulation of a local innate immune response causing a delayed hyper-sensitivity reaction with an intense inflammatory reaction at the side of injection.[13] A previous study showed that sub-plantar injection of CFA resulted in a significant elevation in IL-1B as a proinflammatory cytokine.[20] The paw edema thickness of treatment group (Kp1, Kp2, Kp3) when compared to normal group (Kn) still have big difference in value (figure 1) because subcutaneous injection of CFA induced inflammation is still in the acute phase (0-10 days) is caused by various mediators such as histamine, serotonin, kinins and prostaglandins, released by leukocytes that migrate to the affected region and provoked a vasculo-exsudatif phenomena responsible edema.[21] When CFA was injected subplantar into paw, then it will make DAMPs forming to the protein of cell and cytoplasm. One of the receptor that binding to DAMPs is *a toll-like receptor* (TLR) then the inflammation cells which is inside the cytosol will activate MyD-88 then MyD-88 will activate TRAF-6 then TRAF-6 will activate IRAK-1 and IRAK-4, these 2 part will activate NFκB then NFκB will translocate to the cell nucleus to induce DNA to synthesize proinflammation cytokines like TNF-α and IL-1β.[22]

The anti-inflammation action of bay leaves extract might be related to flavonoid as antiinflammation. The activity of flavonoid in the inflammatory response include the inhibition of transcription factor-like NFkB and activating protein-1 (AP-1).[17] Flavonoid also inhibits of cyclooxygenase (COX) which has an important role as inflammatory mediator and is involved in the release of arachidonic acid that could be affected of starting point for general inflammatory response because it is a precursor for the biosynthesis of eicosanoids like prostaglandins and prostacyclin.[2] When the transcription factor is inhibited then it would be affected to the synthesis of pro-inflammatory cytokines like TNF-α, IL-1β and IL-6 and IL-8 and indicated with inhibiting leukocyte migration and edema formation.[3,9,23]

4. CONCLUSION

Bay leaves *(Syzygiumpolyanthum)* extract with dose 100mg/kg, 300 mg/kg BW and 400 mg/kg BW were applied and found 400 mg/kg BW effective in preventing inflammation in rats with induced CFA. Bay leaves extract contains flavonoid that may inhibit NFκB, which has important role in cytokines proinflammatory synthesis like TNF-α dan IL-1β.

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CONFLICT OF INTEREST

We declared in this work, not any conflict of interest.

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Total Lymfosit Count (TLC) with CD4 in HIV/AIDS Patients at Kupang

Adrianus Ola Wuan[1a*], Ayorince Herlinalt Gloria Banunu[2b], Norma Tiku Kambuno[1c]

[1]Department of Medical Laboratory Technology, Poltekkes Kemenkes Kupang, Indonesia
[2]Laboratory of Clinical Pathology, RSUD. W.Z. Johannes, Kupang, Indonesia

[a] Email address: lamabelawaa@ymail.com
[b] Email address: in05layauw@yahoo.com
[c] Email address: norma.kambuno@gmail.com

HIGHLIGHTS

Total lymphocyte count is in line with the increase in CD4 counts

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Human Immunodeficiency Virus (HIV) is a retrovirus originating from the retroviridae family of the genus lentivirus that infects and damages cells that have a molecule Cluster of Differentiation 4 (CD4), especially T lymphocytes that have receptors with high affinity for HIV. Total lymphocyte count / TLC has been proposed as an alternative guide to CD4 in limited health facilities. This study aims to determine the correlation between Total Lymphocyte Count (TLC) and CD4 in HIV/AIDS patients in the W.Z. Johannes Kupang hospital. The type of this research was observational analytic with a cross-sectional design. The study was conducted on 121 samples of patients who performed CD4 examination and Total Lymphocytic Count (TLC) in the laboratory of W.Z.Johannes Kupang Hospital. The Spearman correlation test shows a significance value of 0,000 with a Spearman correlation value of 0.799. Based on the results of this study it can be concluded that there is a significant correlation between Total Lymphocyte Count and CD4 and shows the direction of positive correlation with a very strong relationship, where the increase in the number of Total Lymphocyte Count is in line with the increase in CD4 counts.

Corresponding Author:
Adrianus Ola Wuan
Department of Medical Laboratory Technology, Poltekkes Kemenkes Kupang
Jl. Farmasi Kelurahan Liliba Kec Oebobo, Kupang, Nusa Tenggara Timur, Indonesia
Email: lamabelawaa@ymail.com

1. INTRODUCTION

Based on Health ministry reported cases of Human Immunodeficiency Virus (HIV) from 2005 to December 2017 amounted to 280,623 people. The cumulative number of AIDS from 1987 to December 2017 was 102,667 people.[1,2] The highest number of cases of infection were DKI Jakarta with 51,981 people, followed by East Java with 39,633 people, Papua 29,083 people, West Java 28,964 people and Central Java with 22,292 people.[2,3]

Based on data from the Directorate General of Disease Prevention and Control, the Ministry of Health of the Republic of Indonesia in 2017 in East Nusa Tenggara Province the

number of HIV infections reported up to December 2017 was 3,464 people and AIDS cases were 1,965 people.[2,4]

HIV is a *retrovirus* originating from the *retroviridae* family of the *genus lentivirus* which infects and damages cells that have Cluster of Differentiation 4 (CD4) molecules, especially T lymphocytes that have receptors with high affinity for HIV.[5] This viral infection results in a continuous decline in the immune system.[6,7] The main target of HIV infection is through CD4 cells. Normal CD4 cell count ranges from 500 - 1600 cells / mm^3 of blood. CD4 cell count is a way to assess the immunity status of people with HIV-AIDS (ODHA). CD4 examination completes a clinical examination to determine patients who need *prophylactic IO* and antiretroviral therapy.[1]

Several studies have shown that to monitor the condition of HIV patients can use several parameters including delayed-type hypersensitivity responses, haemoglobin (Hb)[8], total lymphocyte count (TLC), serum albumin levels, and body mass index.[9,10,11] The gold standard for CD4 examination is to use a flow meter, but it requires expensive equipment, so it is difficult to obtain in areas with limited facilities.[10,12,13] WHO's anti-retroviral therapy guidelines state that if CD4 count cannot be obtained, the total lymphocyte count (TLC) will be used as a replacement.[14]

General Hospital Prof. DR. W. Z. Johannes Kupang has a special clinic for Counseling and Testing (Voluntary Counseling and Testing / VCT) as well as Care, Support and Treatment (Care, Support and Treatment / CST), namely the VCT CCT Sobat Clinic. A number of Patient visit at the VCT Sobat clinic at General Hospital W.Z.Johannes in 2018 were 9,244 people, with 229 HIV positive cases. The total cumulative ODHA who get ARV therapy in CST Sobat clinic until September 2018 was 627 people. Monitoring of CD4 count can be done at Laboratory of General Hospital Prof. DR. W. Z. Johannes Kupang, but the availability of reagents is very limited. Examination data shows that in 2017 Laboratory of General Hospital Prof. DR. W. Z. Johannes Kupang can only perform 70 CD4 examinations, whereas in 2018 only 50 reagents are received. This amount is very lacking, considering that the CD4 examination is not only used to determine the start of treatment in new patients but also is used to assess the success of ARV treatment. Decreased CD4 cell count results in reduced TLC as evidenced by Resindra et al., that TLC can be used as a replacement check for CD4 cell count in monitoring HIV infection.[15] The results of research conducted by Sanjaya's et al., also support the existence of a moderate correlation of TLC with CD4 cell count in all aspects of study.[16]

There are differences in the results of the research found both conducted abroad and in Indonesia as well as the limited research on the relationship between TLC and CD4 in Indonesia, especially in East Nusa Tenggara. Akinola et al., states that there is no strong correlation between TLC and CD4 (female: r = 0.473; male r = 0.384).[17] Irianto et al., also states There is a weak correlation between the number of lymphocytes with the number of CD4 cells, so the calculation of the number of lymphocytes cannot be used as an alternative in monitoring therapy.[18] The purpose of this study was to find the correlation between Total Lymphocyte Count (TLC) with CD4 in HIV/AIDS patients at General Hospital Prof. DR. W. Z. Johannes Kupang.

2. MATERIALS AND METHOD

This research was conducted at the Clinical Pathology Installation General Hospital W. Z. Johannes Kupang in April 2019. The sample study was data on HIV/AIDS patients who performed routine blood/total lymphocyte count and CD4 counts and obtained 121 patients. Data was collected and analyzed using SPSS for Windows software.

This study was an analytic observational study with a cross-sectional design. The sample of this study were patients diagnosed with HIV from the VCT / CST Clinic of General Hospital Prof. DR. W. Z. Johannes Kupang, who conducted routine CD4 and blood/lymphocyte examinations in the laboratory of the General Hospital Prof. DR. W. Z. Johannes Kupang. This research has received ethical approval through the ethics committee of the Faculty of Medicine, Gadjah Mada University with number: KE / 223 / EC / 2016.

The criteria of the sample were patients diagnosed with HIV from the VCT / CST Clinic of General hospital Prof. Dr. W.Z. Johannes Kupang, which conducts routine CD4 and blood/lymphocyte examinations in the laboratory. We used secondary data in the form of

patient medical records recorded General Hospital Prof. DR. W. Z. Johannes Kupang. The variables studied in this study were CD4 count that was calculated using the *PIMA Analyzer* with cell / µl unit and Total Lymphocyte Count (TLC) calculated by the *Automated Hematology Analyzer Sismex XN 550* with cell / µl unit. *Pearson* correlation test was used to find the correlation between *Total Lymphocyte Count* and CD4 count if the data were normally distributed. If the data are not normally distributed, then the *Spearman* correlation test would be used.

3. RESULTS AND DISCUSSION

This research was conducted at the Clinical Pathology Installation General hospital Prof. DR. W. Z. Johannes Kupang in April 2019. The sample study was data on HIV/AIDS patients who performed routine blood/total lymphocyte count and CD4 counts and obtained 121 patients. Data was collected and analyzed using statistical software.

Table 1. Distribution of Samples based on CD4 Count and *Total Lymphocyte Count*

Characteristics		Man		Female	
		Total	Percentage (%)	Total	Percentage (%)
CD4 Count					
	> 500	5	6.4	7	16.3
	350 – 499	5	6.4	6	13.9
	200 – 349	11	14.1	11	25.6
	< 200	57	73.1	19	44.2
	Total	78	100	43	100
Lymphocyte Count					
	< 1000	33	42.3	16	37.2
	> 1000	45	57.7	27	62.8
	Total	78	100	43	100

Based on table 1, the percentage of total lymphocyte count in this study is more than 1000 cells / µl, while the percentage of CD4 is less than 200 cells / µl. After grouping based on CD4 count, in the CD4 group that is more than 350 cells / µl, and the total lymphocyte count still exceeds 1000 cells / µl. Meanwhile, in the CD4 group that is less than 200 cells / µl, there is 95.9% of the study sample with a total lymphocyte count that is less than 1000 cells /µl. This is probably due to the fact that no definitive information was obtained from the study samples regarding the patient's infection phase, whether people with HIV were in the acute infection phase or were still in the latent infection phase where most lymphocyte counts were still within normal limits, but there had been a decline in CD4 counts.[12] These results also provide a general picture that the decrease in total lymphocyte count mostly occurs only in patients with CD4 count that is less than 200 cells / µl.

Table 2. *Spearman* Correlation Test Results

Variable	*N*	*Correlation Coefficient*	Sig.
Total Lymphocyte Count	*121*	0.799***	0.000
CD4	121		

The results of statistical analysis with the Spearman correlation test on the entire study samples showed that there was a significant correlation between Total Lymphocyte Count and CD4 ($p = 0.000$). They showed the direction of a positive correlation with a very strong relationship ($r = 0.799$).

The existence of a positive correlation between *Total Lymphocyte Count* (TLC) with CD4 in HIV/AIDS patients is based on the theory that a decrease in *Total Lymphocyte Count* (TLC) can cause a decrease in CD4 count. CD4 is a part of lymphocytes. Lymphocytes consist of two subtypes, namely T lymphocytes and B lymphocytes. B lymphocytes will be converted into plasmocytes to form antibodies in the humoral defence system. T lymphocytes which are primary cells of the cellular defence system that can be divided into helper T cells (CD4) and

suppressor T cells (CD8). CD4 cells are the largest part of T lymphocytes, which is 34-67%.[3,19,20]

In HIV infection, selective reduction in CD4 cells is found due to the direct effects of the virus or the body's immune response. This selective reduction is due to the tropism of the virus to infect CD4 cells. As part of lymphocytes, reduction of CD4 cells count will also decrease *Total Lymphocyte Count.*[19] Coinfection with other diseases such as Hepatitis B, Hepatitis C, and tuberculosis will further reduce the patient's immune system so that it will cause a CD4 cell count to decrease.[20,21] This situation is also called an opportunistic infection.[22,21]

The results of this study did not differ significantly from research conducted by Sanjaya, et al., on 350 subjects at Hospital of Dr. Hasan Sadikin Bandung which divided by WHO clinical-stage, and then the data were analyzed using Pearson correlation analysis and Fisher's r-to-z transformation. Results of the study obtained a good and statistically significant correlation between the number of CD4 lymphocytes and the number of lymphocytes at all stages of HIV infection.

The results of research conducted by the Sanjaya et al[17], have a slight difference which states that in every clinical-stage, they have moderate correlation, whereas in this study the statistical test results show a strong correlation. This is because, in this study, researchers only tested in general, and only divided the characteristics of the research based on sex.

A positive correlation between lymphocyte count and CD4 lymphocyte count is also shown in the study of Swati AF, et al.[2] The results of this study indicated a decrease in CD4 lymphocytes count followed by a decrease in the lymphocytes count. The study of Swati AF, et al. has a difference with this study in which retrospective data collection is performed on the medical records of children who have HIV who are hospitalized and subjects are controlled by several variables such as sex, age, (Body Mass Index) BMI, haemoglobin level, leukocytes count, and platelets.[2]

The results of this study differ from those of Akinola et al., In which the study states that there is no strong correlation between TLC and CD4 (women: r = 0.473; men: r = 0.384).[17] The difference from this study lies in the study sample, where Akinola et al. take study samples from patients with HIV-1 antibodies but have not yet started the ARV therapy. Others study states the same thing that there is a strong correlation between CD4 and TLC, but that correlation weakens when patients were divided into groups according to their CD4 count.[6,13]

The results of strong correlations in this study were also possible because this study only analyzed the correlation of total lymphocyte count and CD4 count in general, without dividing in detail from age group, clinical-stage, duration of ARV therapy or other factors affecting total lymphocyte count and CD4 count. The limitations of this study are expected to be a consideration for understanding the results of this study.

4. CONCLUSION

There was a significant correlation between *Total Lymphocyte Count* and CD4. The results of this study also showed the direction of positive correlation with a very strong relationship, where the increase in the *Total Lymphocyte Count* was in line with the increase in CD4 count.

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CONFLICT OF INTEREST

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Potential of Lactobacillus casei shirota's strain against the biofilm-forming of Salmonella Spp: an in vitro study

Annisa Rizka Fauziah[1a*], Meiskha Bahar[2b], Aprilla Ayu Wulandari[3c]

[1] Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta, Indonesia
[2] Departement of Microbiology, Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta, Indonesia
[3] Departement of Pharmacy, Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta, Indonesia

[a] Email address: annisarizka263@gmail.com
[b] Email address: meiskha27@gmail.com
[c] Email address: april.ayu83@gmail.com

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Biofilm of *Salmonella* spp. is formed through the expression of biofilm genes associated with proteins (bapA) regulated by curli synthesis genes (csg) which carry out adhesion, colonization, maturation, and dispersion on the surface of the intestinal epithelium. This study aimed to determine the antibiofilm activity of *Lactobacillus casei* Shirota'S strain (LcS) as an inhibitor of *Salmonella spp.* biofilm formation in vitro. This research was a true experimental study using Microtiter Plate 96 wells Biofilm Assay method. The sample used was the suspension of *Salmonella* spp. The treatment was in the form of adding a LcS suspension with a concentration series of 10^{-1};10^{-2}; 10^{-3};10^{-4}; and 10^{-5}. Biofilm measurements were carried out using a microplate reader and obtained quantitative data in the form of Optical Density at a wavelength of 595nm. The results of this study showed that LcS suspension has antibiofilm activity ranging from 10^{-5} concentrations with a percentage of 36.58% ($p<0.05$). The results of exometabolism LcS can reduce *Salmonella* growth. Exopolysaccharide (EPS) and sortase-dependent proteins (SrtA) of LcS form barriers as competitive adhesion in inhibiting pathogenic biofilm formation.

Corresponding Author:

Annisa Rizka Fauziah/Meiskha Bahar
Fakultas Kedokteran UPN "Veteran" Jakarta
Jl. RS Fatmawati, Pondok Labu, Jakarta Selatan, Indonesia, 12450
Email: annisarizka263@gmail.com/meiskha27@gmail.com

1. INTRODUCTION

Food contamination by pathogenic bacteria is an important public health problem that causes world mortality. Salmonella is transmitted through contaminated animals to humans. The animal is then processed into food (food-borne disease), causing Salmonellosis.[1] Salmonellosis is divided into two types namely typhoid which consists of typhoid fever and paratyphoid fever, the second notified is usually caused by Salmonella serovars that do not have specific hosts. The Salmonella enterica serotype group Enteritidis and Salmonella enterica serotype Typhimurium are the two Salmonella serotypes that are most commonly transmitted from animals to humans in most parts of the world.[1]

Every year one in 10 people get sick, and 33 million people lose their health in the world due to foodborne disease. Salmonella is one of the four main causes of global diarrhoea disease caused by contaminated food.[1] Centers for Disease Control and Prevention (2019) estimates Salmonella is a bacterium that causes about 1.2 million diseases, 23 thousand hospitalizations, and 450 deaths in the United States each year. Indonesia is one of the developing countries that has health problems such as typhoid fever.[2] Based on the results of "Riset Kesehatan Dasar" (Riskesdas) in 2007, the prevalence of typhoid fever reached 1.7% with typhoid morbidity reported at 81.7 per 100,000 population in Indonesia.[3]

Biofilm formation is a natural phenomenon for most bacteria through cell-to-cell communication called Quorum Sensing (QS). The process forms biofilms on the biotic surface, namely intestinal epithelium or abiotic, which are cutlery.[4] Salmonella biofilms that contaminate food can enter the body and can reach the intestinal epithelium if the amount exceeds the average infective dose can cause clinical or subclinical infections in humans is 10^5-10^8 Salmonella, but for Typhi, serotypes can reach 10^3. Biofilm Associated Proteins (Biofilm Associated Proteins (bapA) can increase the attachment of Salmonella to the intestinal epithelium and issue QS signals for colonization and form biofilms as a form of Salmonella's defence against adverse conditions such as pH, unstable temperatures and reduced nutrition.[4]

Biofilm control can be done in three ways, namely in physics, chemistry, and biology. Biofilm control in biology can use microbiological interactions, one of them by using bacteria that act as antibiofilm.[5] Biofilm control with Lactic Acid Bacteria (LAB) biosurfactants or probiotic bacteria can reduce the expression level of biofilm-related genes and block the release of signalling molecules in the QS system as a therapeutic agent to prevent infecting biofilms.[6]

The results of other studies previously stated that Conjugated Linoleic Acids (CLAs) results from critical metabolism as an anti-pathogenic substance from Lactobacillus secreted at 24, 48, and 72 hours of incubation significantly ($p < 0.05$) capable of suppressing the formation of Salmonella typhimurium biofilms by damaging cells membrane of pathogenic bacteria.[7] *Lactobacillus* is also able to form a biofilm protector as a barrier to prevent the attachment of pathogenic bacteria and as a competitor in the attachment of intestinal epithelium.[8]

Probiotic bacteria are bacteria that are beneficial to the body. Probiotics can produce antimicrobial substances that function as agents that can suppress the growth of enteric pathogenic bacteria. The genus Lactobacillus has the potential as a probiotic agent, which maintains the health of intestinal epithelium including being able to produce antimicrobial substances, antagonistic power against enteric pathogens that can survive at low pH, and resistant to bile salts.[9]

Lactobacillus casei Shirota strain (LcS) is a type of probiotic bacteria that have been scientifically proven to have health benefits. LcS can produce antimicrobial substances called bacteriocin consisting of acidoline, acidophylline, and lactosidin which have the ability as a broad spectrum against positive Gram and negative Gram bacteria.[10]

There are many mechanisms performed by LcS probiotics in the intestine, namely immune modulation, lactic acid production (resulting in a decrease in local pH) and competitive adhesion or transfer of pathogenic bacteria.[11] LcS is used as a probiotic bacterium in fermented milk that is circulating in the community and is widely consumed by various groups, and LcS in these research has not been tested biologically, so that researchers are interested in examining the potential of *Lactobacillus casei* Shirota strain as an inhibitor of biofilm formation *Salmonella* spp.

2. MATERIALS AND METHOD

This type of research is a pure experimental study (True Experimental) with posttest only control group design and uses a 96 wells microplate assay biofilm method to determine the activity of Lactobacillus casei strains of Shirota strain.

The tools used are Microplate reader, Incubator, Autoclave, anaerobic jar, glass object, Wells microplate 96 flat bottoms, Eppendorf micropipette and 200 µl tip size, Yellow Tip box. The materials used were isolates of Lactobacillus casei strain Shirota (Department of Microbiology FKUI), Salmonella spp. Isolate, Trypthon Soya Broth (TSB), Nutrient Agar (NA), Methanol 96%, Crystal violet 1%, Aquadest, NaCl 0.9% sterile, 0.5 McFarland, Acetic Acid 33%.

The sample of Lactobacillus casei Bacteria Shirota strain test was a bacterial suspension in fermented milk products in the Pondok Labu area, South Jakarta. Bacteria derived from bacterial suspensions were etched on NA media, then incubated for 24 hours at 37ºC in anaerobic atmosphere in anaerobic jar and stored in the refrigerator.

Salmonella bacterial test samples came from a pure culture of Salmonella Shigella Agar (SSA) from the microbiology laboratory, Faculty of Medicine, UPN "Veteran" Jakarta. Salmonella growth on SSA media is characterized by a black colony.

Lactobacillus suspension is taken 2-3 bacterial colonies from bacterial stock then input into the 40 mL TSB media, then incubated for 24 hours at 37°C, standardized turbidity using standardization <300x10^6 CFU / mL McFarland using the turbidimetry method, then take 1 mL, entered with standardization of <300x10^6 CFU / mL McFarland using the turbidimetry method into the tube and added 9 mL NaCl to the tube, then shake until homogeneous, the first tube diluting 10^{-1}. The 1 mL pipette from 10^{-1} is then put into a test tube containing 9 mL of diluent solution and is called the 10^{-2} second dilution. Do the same thing until the last dilution is 10^{-5}.

Salmonella suspension is taken 2-3 bacterial colonies from the bacterial stock and then input into a 40 mL TSB medium, then incubated for 24 hours at 37° C. Then take 100 µl, each inserted into a tube containing 10 mL TSB, then incubated for 3-5 hours at 37° C. Dilute with 0.9% NaCl until the bacterial density is equal to 0.5 McFarland standard (<300x10^6 CFU / mL).

Minimum Inhibitory Concentration (MIC) is done by giving 1 mL of Salmonella spp suspension into 9 mL of LcS suspension with each tube containing a different concentration of LcS. The concentration was carried out by the dilution method five times the dilution series, namely 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, then incubated for 24 hours at 37ºC, then the suspension mixture was planted and spread on selective media Salmonella Shigella Agar (SSA) and incubated 24 hours again, after incubation, the number of colonies was measured with a plate counter.

Evaluation of Salmonella biofilm formation. This evaluation is to see and prove the biofilm formation of Salmonella formed in microplates wells, put in 75 µL TSB and 25 µL bacterial suspension (<300x10^6 CFU / mL) and then incubated for 24 hours at 37° C. After incubation, the microplates were then rinsed using sterile water, and the wells on the microplate were stained with 1% violet crystal for 15 minutes, then rinse.[12]

Evaluation of Lactobacillus casei Biofilm Formation Shirota's Strain (LcS). Evaluation to see the formation of biofilms from LcS put 100 µL TSB and 100 µL bacterial suspension (<300x10^6 CFU / mL) into the microplate wells and then incubated for 24 hours at 37° C. After incubation, the microplate was then rinsed three times using NaCl and dried, gave 200 µL 96% methanol and incubated at room temperature for 10 minutes, then discarded the liquid methanol from the well and dried, and the smear on the microplate was stained with 0.1% crystal violet during 10 minutes, then rinsed three times with NaCl, dissolve biofilm with 33% acetic acid, then place it on the microplate reader to find out the optical density results at a wavelength of 595 nm.[12]

A total of 100 µL of liquid TSB media was added to the microplate wells, then 50 µL of Lactobacillus bacterial suspension was added first and continued with 50 µL of Salmonella, covered and incubated for 24 hours at 37° C. After incubation, the mixture is removed, then the microplate is washed with NaCl and dried. Microplate give 200 µL of 99% methanol and incubate for 10 minutes at room temperature, then remove the methanol liquid from the well and dry, and the microplate stained with 0.1% crystal violet for 10 minutes, then rinse three times with NaCl, dissolve it with NaCl, dissolve it biofilm with 33% acetic acid. The negative control was only given 100 µL TSB liquid media and 100 µL Salmonella bacterial suspensions in microplate wells. Positive controls were given 100 mL of Salmonella bacterial suspension and 100 mL of ceftriaxone. Solvent control was given 200 µL NaCl, and media control was given 200 µL TSB liquid media, then place it on the microplate reader to find out the optical density results at a wavelength of 595 nm.[12]

The test is carried out with three comparisons, and in the test group, the percentage of biofilm inhibition is calculated from the OD value which is processed using the following formula (*Clinical and Laboratory Standards Institute*, 2012):

$$\text{percentage of biofilm inhibition} = \frac{(C\text{-}B)\text{-}(T\text{-}B)}{(C\text{-}B)} X\ 100\% \quad (1)$$[13]

Note:
C = *Optical Density* (OD) 595 nm control media,
B = OD_{595} *negative controls*,
T = OD_{595} *treated wells*.

The results are then analyzed with the Anova oneway hypothesis test.

3. RESULTS AND DISCUSSION

Identification of *Lactobacillus casei* Shirota strain bacteria by the Gram staining method was seen in a microscope with the results of identification of the form of bacilli, single arrangement, purple colour, and Gram-positive properties. Identification of Salmonella bacteria was seen in a microscope with the results of the identification of the form of bacilli, single arrangement, red colour, and negative Gram.

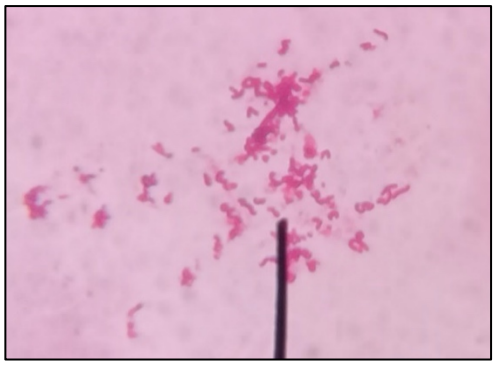

Figure 1. Lactobacillus Microscopic Identification Results (Gram Staining)

Table 1 shows that group 10^{-1} had the most significant growth inhibition than the other groups. In contrast, group 10^{-5} showed the lowest number in inhibiting the growth of Salmonella spp. in each group with different concentrations. The higher the concentration of LcS suspension, the less the amount of Salmonella colonies that grow in SSA.

Table 1. Results of Measurement of Total Growth of Salmonella Spp. on the MIC

Group	Number of Salmonella sp. colonies Within 0.1 millilitres of bacterial suspension
10^{-1}	43.6
10^{-2}	46
10^{-3}	80.4
10^{-4}	125
10^{-5}	161

Salmonella spp is a bacterium that has a flagella that functions to move and attach to the surface of an object, when the process of biofilm formation flagella will tend to stick to the surface, thus forming a biofilm ring that will be seen on the surface of the object.[4] Figure 1 shows a purplish-blue biofilm ring on the surface of the microplate wells. The picture shows the top view seen throughout the microplate wells are blue, to see the shape of the ring can only be seen in the side view picture. Evaluation to see the formation of biofilms from Salmonella in each microplate wells, if the microplate remains purplish-blue, then a biofilm has been formed in the microplate wells.[12]

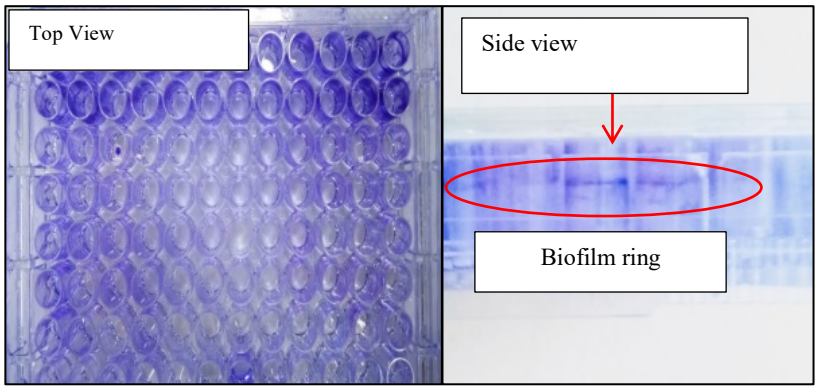

Figure 2. Evaluation of biofilm formation

Evaluation of the formation of LcS biofilms was carried out to monitor the formation of biofilms and LcS growth without the suspension of Salmonella spp. through optical density values. The results obtained were OD values in the form of planktonic and biofilm in the dilution method.

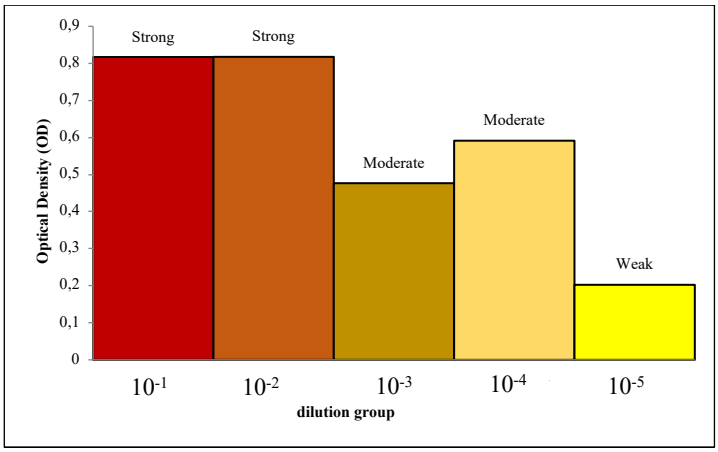

Figure 3. Results of Optical Density for LcS Biofilm Formation

The results of the formation of LcS biofilms can be classified according to the magnitude of OD values compared to OD values of media control (ODC), i.e. wells that only contain liquid media. According to Borges et al. (2012), the classification of biofilms according to OD values is as follows, if (OD≤ODC) then categorized as non-biofilm producers, if (ODC <OD≤2 × ODC) then classified as weak biofilm producer, if (2 × ODC <OD ×4 × ODC) then categorized as moderate biofilm producer, and if (4 × ODC <OD) then classified as keen biofilm producers.[14] Figure 2 shows the LcS OD values at 10-1 and 10-2 dilutions forming a strong biofilm, the OD LcS values at 10-3 and 10-4 dilutions form a moderate biofilm, and the LcS OD values at 10-5 dilutions form a weak biofilm. This shows that the higher the concentration of LcS bacterial suspension, the greater the OD value produced.

Lactobacillus antibiofilm activity can be seen from the value of optical density with different bacterial conditions namely planktonic and biofilm contained in the microplate wells of each test group and control group.

Table 2. Results of Optical Density (OD) of All Groups on Microplate 96 Wells with Two Repetitions

Group	Optical Density Results for All Groups			
	Planktonic		Biofilm	
	1	2	1	2
Positive Control	0.19433	0.18544	0.20809	0.24558
Negative Control	1.47607	1.47002	0.50370	0.51246
Test group				
10-1	1.22957	1.25635	0.48704	0.46604
10-2	1.26903	1.30692	0.44372	0.43266
10-3	1.29641	1.31218	0.38462	0.39403
10-4	1.31553	1.34341	0.33899	0.33365
10-5	1.33861	1.35872	0.28161	0.29158
Media Group	0.07075	0.07078	0.15655	0.16113
Solvent Group	0.04016	0.04012	0.10534	0.16334

Table 2 shows that positive control containing Salmonella bacterial suspension with Ceftriaxone antibiotics showed lower planktonic and biofilm OD values compared to the test group, indicating that Salmonella was still sensitive to the antibacterial effect of Ceftriaxone. The negative group only contained Salmonella bacteria with the media showing the highest planktonic and biofilm OD values of the other groups, and this indicates that Salmonella can form biofilms totally without the intervention of the antibacterial effects of ceftriaxone or LcS suspension. Media groups and solvent groups showed the lowest OD values of all groups because they did not contain bacteria in the wells and showed no biofilm formation.

The planktonic test group at the highest concentration ie group 10^{-1} showed the lowest OD value compared to other planktonic test groups. The lowest concentration in group 10^{-5} shows the highest OD value compared to other planktonic test groups, and the conclusion is that the growth of Salmonella in planktonic form decreases with increasing concentration of LcS suspension illustrated as shown in Figure 4.

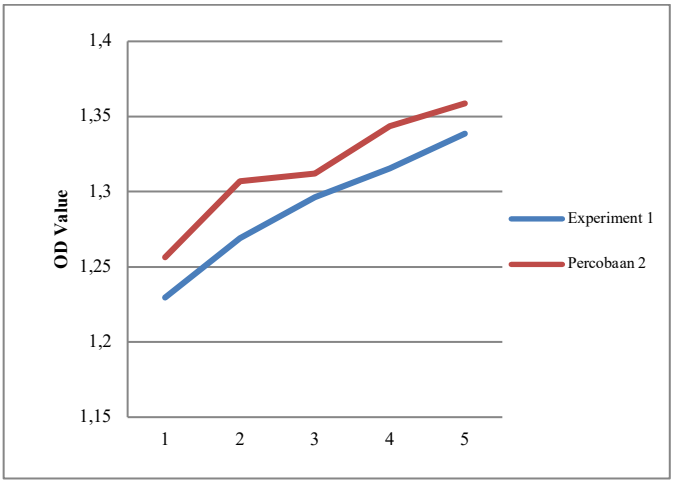

Figure 4. Results of Optical Density Inhibition of Formation of Salmonella Biofilm spp.

The results of OD values for Salmonella biofilm inhibition are expressed as percentages, to find out how much the percentage of LcS inhibition. The percentage of LcS inhibition against Salmonella was obtained based on the calculation of the formula between two bacterial suspensions with different concentrations because this research method used two concentrations with two types of bacteria that have different characteristics and characteristics.

Table 3. Percentage Results of Lactobacillus casei Inhibition of Shirota Strain Against Formation of Salmonella spp.

Group	Percentage of LcS Inhibition Against Salmonella Biofilm	
	Test 1	Test 2
10^{-1}	95.20%	86.79%
10^{-2}	82.72%	77.29%
10^{-3}	65.70%	66.29%
10^{-4}	52.55%	49.10%
10^{-5}	36.02%	37.13%

Table 3 shows the group with 10^{-1} concentration had the highest percentage of inhibition compared to other groups. The group with a concentration of 10^{-5} had the lowest percentage of inhibition compared to other groups. These results can be concluded that the higher the concentration, the higher the percentage of inhibition of LcS bacteria against Salmonella.

The value of optical density biofilm has a variety of results at each concentration. Concentration 10^{-1} produces an average value of optical density 0.47654 with an average value of the percentage of inhibition of 91.00%, at a concentration of 10^{-2} produces an average value of optical density of 0.43819 with an average value of the percentage of inhibition of 80.01%, at concentration of 10^{-3} produces an average value of optical density 0.389325 with an average value of the percentage of inhibition of 66.00%, at a concentration of 10^{-4} produces an average value of optical density of 0.33632 with an average value of the percentage of inhibition of 50.83%, at concentration of 10^{-5} produces an average value of optical density 0.286595 with an average value of the percentage of inhibition of 36.58%.

Lactobacillus suspension in concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} can inhibit the formation of Salmonella spp biofilms. With the average percentage of the minimum inhibitory power found at a concentration of 10^{-5} that is equal to 36.58% and the average percentage of the maximum inhibitory power is found at a concentration of 10^{-1} that is equal to 91.00%. Increasing the concentration of Lactobacillus suspension can increase the activity of spending antibiofilm such as biosurfactants and exopolysaccharides so that the percentage of inhibition against pathogenic bacteria increases. The results of the study were also supported by the results of the Minimum Concentration Concentration (MIC) test. The results of this test indicate that there is a decrease in the number of colonies in the SSA agar media with higher LcS concentrations.

Table 3 shows the group with 10-1 concentration had the highest percentage of inhibition compared to other groups. The group with a concentration of 10-5 had the lowest percentage of inhibition compared to other groups. These results can be concluded that the higher the concentration, the higher the percentage of inhibition of LcS bacteria against Salmonella.

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Salmonella biofilm spp. able to form with antigen virulence activity Biofilm associated proteins (bapA) are large cell-surface proteins needed for biofilm formation. Biofilms are encoded by the bapA gene and secreted through the type-I protein secretion system (bapBCD operon) which is located at

the end of the bapA gene. The expression of BAPA is coordinated with the genes encoding curli fimbriae and cellulose, through the action of curli synthesis genes (csgD). Both Long polar fimbriae (Lpf) and plasmid-encoded fimbriae (Pef) contribute to the initial steps of biofilm formation. The bapA gene is also a gene that lives in Salmonella. Salmonella produces O-antigen capsules that are regulated with fimbria and extracellular matrix cellulose.[4]

Biofilm biological control with these bacteria is different from other biofilm control. In this study, the value of optical density is influenced by two types of bacteria with different characters. The mechanism of LcS in inhibiting Salmonella as explained in the previous literature review, namely the exopolysaccharide and various other proteins released by LcS, so that it adheres to the surface of the wall as a protective barrier the same as when it is in the intestinal epithelium. This can affect the value of optical density biofilm in each well, and the optical density value is greater when LcS concentration is greater, inversely proportional to the shape of bacteria in a planktonic state that the optical planktonic value is getting smaller when LcS concentration is greater, this is because LcS produces exometabolite which acts as an antibacterial so that the growth of pathogenic bacteria is inhibited.[15]

Previous research stated that biofilms from *E. coli* O157: H7, *Listeria monocytogenes*, and *S. typhimurium* in Lactic Acid Bacteria (LAB) suspensions for 24, 48, and 72 hours were significantly reduced ($p < 0.05$). The use of probiotic biofilms can be an alternative approach to reduce the formation of pathogenic biofilms in the food industry, without giving risks to consumers. The application of competitive biofilms formed by LAB bacteria can produce natural antimicrobial substances and biosurfactants can provide new opportunities for controlling pathogenic bacteria and avoiding infectious diseases from food contamination. Development of protective biofilms with probiotic LAB present in food can help avoid the problem of contamination into the food chain.[8]

Lactobacllus casei Shirota's strain in fermented milk has two unique abilities in its role as inhibitors of the formation of pathogenic bacterial biofilms that infect humans. First, probiotic bacteria can adhere firmly to the surface of the intestinal epithelium and survive in the extracellular polymer matrix with exopolysaccharide (EPS) and sortase-dependent proteins (SrtA) components maintaining the barrier function as a competitive adhesion in inhibiting the formation of pathogenic biofilms. Secondly, bacteriocin is the result of Lactobacillus metabolism which can act as an antibacterial, besides bacteriocin, there are other Lomet's exometabolime results such as lactic acid, hydrogen peroxide can suppress the growth of Salmonella.[16] Bacterial biofilms can form on biotic or abiotic surfaces. *Lactobacillus casei* Shirota's strain (LcS) can attach to the surface of objects or human intestine in response to the formation of protective barriers against pathogenic bacteria. There are several genetic and environmental factors that influence the formation of these microbial structures in the digestive tract. Lucks gene and pheromone peptide plantaricin A (Plna) owned by Lactobacillus, have an important role in regulating interactions between microbes in the human intestinal system. The role of other Lactobacillus on the intestinal surface is to increase the expenditure of several proteins (Dnak, GroEL, ClpP, GroES and catalase) to stimulate immunomodulation.[17]

This study uses a microplate surface as a biofilm coating site which describes the attachment of biofilms on the surface of the intestinal epithelium. The difference is the inhibitory response of the formation of pathogen biofilms by Lactobacillus with favourable environmental conditions for the stimulation of additional inhibitory agents such as various proteins in the body and immunomodulation which can only occur in the digestive tract and can be done in vivo, whereas microplate provides an environment with laboratory standards by in vitro. The response is only obtained from the ability of Lactobacillus such as the removal of probiotic bacterial surface adhesion proteins and forming EPS as an attachment response, as well as the release of exometabolism as an antibacterial agent. This ability is carried out by Lactobacillus itself without the aid of immune system stimulation and protein from the body.[18]

4. CONCLUSION

Based on the results of the analysis and discussion of the research that has been done, it can be concluded Lactobacillus casei Shirota's strain has the potential to inhibit the formation of Salmonella spp biofilms. In vitro at the suspension concentration of Lactobacillus bacteria 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. The quantitative biofilm detection method using violet crystal staining on

microtiter plates has the result that the higher the concentration of Lactobacillus bacterial suspension, the higher the inhibitory power for the formation of Salmonella spp biofilms.

For the next research can be done with the field emission scanning electron microscopy method to see the structural differences produced by Lactobacillus and Salmonella in biofilms attached to the microplate wells. It measured the growth curve of Lactobacillus casei Shirota strain as an inhibitor of the formation of Salmonella spp. Biofilms, to determine the number of bacteria that can inhabit. Test other types of pathogenic bacteria that produce biofilms or can also test probiotic bacteria or bacteria that have other antibacterial properties. Test the potential of Lactobacillus casei Shirota strain as an inhibitor of the formation of Salmonella spp biofilms. In vivo and carry out other biofilm control tests, such as physics and chemistry.

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CONFLICT OF INTEREST

We declared in this work, not any conflict of interest.

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Effect of electromagnetic field on whole blood, biochemical and hormone level in human

Mehmet Cihan YAVAŞ[1a*], Veysi AKPOLAT[2], Özkan GÖRGÜLÜ[3], İbrahim KAPLAN[4]

[1] Department of Biophysics, Faculty of Medicine, Kırşehir Ahi Evran University, Kırşehir, Turkey
[2] Department of Biophysics, Faculty of Medicine, Dicle University, Diyarbakır, Turkey
[3] Department of Biostatistics and Medical Informatics, Faculty of Medicine, Ahi Evran University, Kırşehir, Turkey
[4] Department of Biochemistry, Faculty of Medicine, Dicle University, Diyarbakır, Turkey

[a] Email address: mcihanyavas@ahievran.edu.tr

HIGHLIGHTS

Magnetic fields constantly lead to changes in the biochemistry, hormone and whole blood parameters

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Hormone

A B S T R A C T

The electromagnetic field is increasing in our environment and these exposures cause concern**.** The aim of our study is to investigate the effects of magnetic fields generated by the hair dryers' devices used by women working in the same job on their serum biochemistry, whole blood and hormone values. The sixteen women working continuously in hairdressing salons were included in the study. Two groups of studies were designed: control (n:8, mean age: 22.25±6.04) and experimental group (n:8, mean age:23,62±6.67). The biochemical (median values of alanine amino transferase, aspartate aminotransferase, triglycerides and very low density lipoprotein were found high) and hormonal results of the experimental group were compared with the biochemical (cholesterol, low-density lipoprotein and very high-density lipoprotein had high median values) and hormonal results of the control group and no significant difference was found ($p>0.05$). When the whole blood parameters were examined, the white blood cells and mean platelet volume results of the experimental group were significant ($p<0.05$), while there was a meaningless difference between red blood cell, hemoglobin, hematocrit, mean corpuscular volume, red cell distribution width and platelet values ($p>0.05$). It is evident from the results that occupational exposure to magnetic fields constantly leads to changes in the biochemistry, hormone and whole blood parameters of the female.

Corresponding Author:
Kırşehir Ahi Evran
Faculty of Medicine, Department of Biophysics
Bağbaşı, 40100, Kırşehir, Turkey
E-mail: mcihanyavas@gmail.com
Phone: +90 386 280 2504

1. INTRODUCTION

In modern life it is now possible to see devices that produce man-made electromagnetic fields everywhere. With the development of the industry, the rapid increase of these tools and equipment has increased the anxiety of the public, and the first scientific studies on this field started in the 1970's.[1] The electric field, the magnetic field and the electromagnetic field generated by the electric home appliances and the devices used in the work places have become widespread with the increase in need.[2] Discussions continue in the scientific circles related to the biological effects of very low frequency electromagnetic fields on humans. The use of electric power in conjunction with urbanization and industrialization suggests recent study reports that are undesirable harmful effects on humans.[3] Studies on very low frequency magnetic fields are continuing. Epidemiological studies related to childhood leukemia of low-exposed magnetic fields (<1 microTesla) continue. More than 20 biophysical mechanisms related to these epidemiological studies have been investigated and still no causal link has been established.[4,5] It reports recent epidemiological studies. They investigated the relationship between the risk of leukemia in children, the use of pre-natal mothers' sewing machines, the use of stereo worship, the use of television sets and hair dryers. It has been emphasized that the sustained work and measurements made by the mouthpieces of the hair dryers may increase depending on the distance.[6]

2. MATERIALS AND METHOD

This work was carried out in approximately 100 hairdresser workplaces in Diyarbakir Office district. All participant surveys were conducted before blood samples were taken. Exclusion factors have been applied to female employees involved in the study (e.g., non-smoking, no metabolic, systemic and other illnesses). As a result of exclusion, the sixteen female employees randomly (control group: age; 23.62 ± 6.67; age: 22.25 ± 6.04) were grouped into 2 groups (n: 8). Women were allowed to drink no antioxidants, tea, juice until about 3 hours before blood was taken from the employees. Blood was collected and maintained at -20 ° C until analyzed by centrifugation at 300 rpm for 10 min. Serums were analyzed with fully automated analyzers. Biochemical serum results were analyzed using the photometric method with the Abbott Architect C16000 (Illinois, United States) instrument. Hormone results were analyzed using the electrochemiluminescence method with a Roche Cobas 601 instrument. The whole blood results were analyzed using the Abbott CELL-DYN Ruby instrument with multi-angle polarized light scatter separation and laser measurement technology. All participants were informed about the study and received their consent for the study. The study continued after the approval of the Ethics Committee of the Dicle University.

Specific features of hair dryers used in the study are; AC, 200-240 Volt, 50-60 Hz, 1800-2200 Watts. Reference measurement methods are taken into account and the arithmetic mean of the measurements is calculated. Our measurements were taken at a distance of 10 cm and found an electric field of 73.5 V / m and a magnetic field of 4.61 μT, respectively. The electromagnetic field was measured by aid of a Spectran device NF5035 (AARONIA AG, Strickscheid, Germany), using the method of six-minute measurement proposed by the International Commission on Non-Ionizing Radiation Protection (ICNIRP).

The Kolmogorov-Smirnov test was used to analyze whether the data obtained in the study were normal distributions. As a result of the analysis, biochemical values were not normally distributed ($p<0.05$). Complete blood and hormone values provide normality assumption ($p>0.05$). Mann Whitney U test, a nonparametric test, was used to compare biochemical values that did not imply normality hypothesis. Independent t test was used to compare whole blood and hormone values, which provided normality hypothesis. Participants were asked whether they had any complaints, and the distribution of the data according to the groups was converted into a quotient table. The values in the contingency table were analyzed by the Fisher Exact Chi square test. Statistical analysis of the study was conducted with SPSS 21. 0 (IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp., USA).

3. RESULTS AND DISCUSSION

It has been determined that gender has no significant effect on the averages of age. For this reason, the analysis did not need to be used as a covariate. The mean age of the women in the experiment group was 23.62 ± 6.67. The average daily usage time of women in the experimental group of the hair dryer is 361.25 ± 188.63 minutes.

The women in the experimental group worked on average 3.6 years in the hairdressing salon. When the distance to the body during the use of the hair dryers' machine was examined, it was determined that the women were trapped at an average distance of 15.00 ± 3.77 cm. The complaint of the women who participated in the survey are given in Table 1 according to the groups. According to the Fisher exact test statistics, the weakness complaints of the ladies were higher than the ladies in the control group ($p<0.01$). According to the Fisher exact test statistics, the fatigue complaints of the ladies were higher than the ladies in the control group ($p<0.05$).

Table 1. Fisher exact test results

Complaints	Headache		Irritability		Earache		Weakness		Fatigue		Forgetfulness		Eye Pain	
Yes / No	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Control group	4	4	6	2	8	0	6	2	6	2	6	2	6	2
Experiment group	1	7	4	4	5	3	0	8	1	7	4	4	1	7
Fisher exact chi square test	p=0.282		p=0.608		p=0.200		p=0.007*		p=0.041*		p=0.608		p=0.041*	

Note:
1. when compared with the control group of the experimental group (Fisher exact chi square test)
2. Symbol + for answer "yes"; symbol – for answer "no"

The explanatory statistics (mean ± standard deviation, median) and Mann Whitney U test values for female biochemistry values are summarized in Table 2. When the women's biochemical values were examined, the median values of the alanine amino transferase (ALT), aspartate aminotransferase (AST), triglycerides (TRIG), very low-density lipoprotein (VLDL) parameters of the experimental group were found higher in the control group. The median values of the cholesterol (CHOL), low density lipoprotein (LDL), ultra-low-density lipoprotein (UHDL) parameters of the control group were higher than the experimental group. According to Mann Whitney U test results, the differences between control and experiment groups in terms of all biochemical parameters are statistically insignificant ($p>0.05$).

The explanatory statistics (mean ∓ standard deviation) and independent t test results for the female hormone values are given in Table 3. According to these results, there was no statistically significant difference between experimental and control groups in terms of cortisol, estrogen and testosterone values in females ($p>0.05$).

The statistical analysis results of the whole blood values of the female are given in Table 4. According to these results, the RBC value of the experimental group is higher than the control group. The difference between the white blood cells (WBC) values of both groups is statistically significant ($p<0.05$). Mean platelet volume (MPV) value was higher in the experimental group than in the control group. The difference between the control group and the experimental group is statistically significant ($p<0.05$). Differences between red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), red cell distribution width (RDW) and platelet (PLT) parameters were statistically insignificant ($p>0.05$).

Table 2. Female's biochemical results

Parameters	References Ranges	Control Group		Experimental Group		Mann Whitney U Test	p Value
		Mean ± SD	Median	Mean ± SD	Median		
ALT (U/L)	10 – 35	12.25 ± 5.97	15.00	20.50 ± 16.91	12.50	21.50	0.268
AST (U/L)	10 – 40	15.25 ± 4.80	15.50	20.12 ± 16.64	14.50	27.50	0.632
CHOL (mg/dL)	112 – 200	169.12 ± 30.59	143.50	162.25 ± 22.48	153.00	26.00	0.528
LDL (mg/dL)	60 – 160	85.62 ± 28.00	82.50	80.00 ± 20.98	75.00	29.50	0.792
TRIG (mg/dL)	50 – 180	101.37 ± 92.42	93.50	126.37± 70.98	124.50	23.50	0.372
UHDL (mg/dL)	37 – 79	63.25 ± 7.47	41.50	57.00 ± 8.78	58.50	18.50	0.154
VLDL (mg/dL)	10 – 32	20.25 ± 18.38	19.00	25.25 ± 14.05	24.00	22.50	0.317

Note: Data are presented as mean ± SD and median. p>0.05 compared to control (Mann Whitney U test). ALT: alanine aminotransferase, AST: aspartate aminotransferase, CHOL: cholesterol, LDL: low-density lipoprotein, TRIG: triglyceride, UHDL: high-density lipoprotein, VLDL: very low-density lipoprotein.

Table 3. Analysis results of female hormone values

Parameters	References ranges	Control group (Mean ± SD)	Experimental group (Mean ± SD)	t-test	p value
Cortisol (µg/dl)	6.2 – 19.4	8.95 ± 4.07	6.64 ± 2.59	1.354	0.197
Estrogen (pg/ml)	7.6 – 43	105.29 ± 101.48	179.28 ± 133.07	- 1.250	0.232
Testosterone (ng/dl)	2.8 – 8	0.23 ± 0.06	0.32 ± 0.13	- 1.565	0.140

Note: Data are presented as mean ± SD. p>0.05 compared to control (Independent t test)

Table 4. Female's whole blood results

Parameters	References ranges	Control group (Mean ± SD)	Experimental group (Mean ± SD)	t-test	p value
WBC (K/µL)	3.2 – 12	6.83 ± 1.60	8.56 ± 1.61	-2.164	0.048
RBC (M/uL)	3.2 – 6	4.37 ± 0.17	4.29 ± 0.41	0.550	0.591
HGB (g/dL)	10 – 18	13.35 ± 0.81	13.30 ± 1.47	0.084	0.934
HCT (%)	30 – 55	37.52 ± 2.03	37.28 ± 4.15	0.145	0.887
MCV (fL)	98 – 120	85.71 ± 1.81	86.85 ± 3.91	-0.746	0.468
RDW (%)	9 – 18	15.32 ± 0.52	14.77 ± 0.50	2.137	0.051
PLT (K/uL)	150 – 500	318.50 ± 41.38	280.73 ± 96.44	1.018	0.326
MPV (fL)	0 – 15	7.72 ± 0.63	8.67 ± 0.87	-2.495	0.026

Note:
Data are presented as mean ± SD. p>0.05 compared to control (Independent t test). *p<0.05 compared to control group. **p<0.01 compared to control group. WBC: white blood cells, RBC: red blood cell, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, RDW: red cell distribution width, PLT: platelet, MPV: mean platelet volume.

Commercial salon-type hair dryers create a magnetic field after long-term operation. With this modeling studies, it has been emphasized that these hair dryers cause thermal effects and noises in the heads of people.[7] In his analysis of measurements made by Johansen; ELF-electromagnetic fields are becoming increasingly widespread and the magnetic field created by the hair dryer forms a magnetic field of 17.44 micro tesla at 5 cm, 0.12 micro tesla at 50 cm and 0.02 micro tesla at 100 cm.[2] The results of analyzes made by the World Health Organization show that hair dryer machines generate 80 V/m electric field at 30 cm distance, generate magnetic field 6-2000 microTesla at 3 cm distance, 0.01-7 microTesla at 30 cm and 0.01-0.03 microTesla 1 m distance.[8]

In a sustained study by Stronati et al. a short exposure to ELF magnetic fields of 1 mT (2 h) did not show any genotoxic effect in human blood cells. A statistically significant effect has been observed although there is an effect of exposure on cell proliferation, but at this point it is difficult to interpret this effect as biological effect.[9] Biophysical and biological mechanisms have not yet been fully elucidated as low-frequency magnetic fields can affect reproduction and development.[10]

Güler and his colleagues investigated the effects of vertical and horizontal electric field on Guinea pigs. It has been reported that there is no statistical difference in some serum biochemical parameters of the electric field applied in the study.[11] Daşdağ and his colleagues have shown that very low frequency electromagnetic fields do not change the hematological and immunological parameters in the work carried out on workers working in welding jobs.[12] In vivo studies of Heredia-Rojas et al. indicate that they may produce a clastogenic effect in mice after a magnetic field exposure at 60-Hz, 1.5 and 2.0 mT.[1] Alghamdi and colleagues investigated the short and long term effects of the electromagnetic fields of 0.49 W/kg and 0.72 W/kg SAR on the hematological parameters of mice at 900-1800 MHz frequency. Decrease in PLT value and increase in WBC value in the study were found to be consistent with our study.[13] Dogan and colleagues emphasized that electrical and magnetic fields generated by high voltage in the laboratory environment cause changes in trace element concentrations of rats.[14] Chater and colleagues investigated the effect of static magnetic field on pregnant rats. Chater and colleagues investigated the effect of static magnetic field on pregnant rats. While the findings of WBC, RBC and HGB in our study showed similar changes with our study, PLT changes were different. While AST and PLT were significant in Chater's study, AST and ALT parameters in our study were not statistically significant compared to the control group.[15]

In a study conducted by Çakir and colleagues on rats, it appears that very low frequency magnetic fields (3 hours a day, 0.97 mT, 50 and 100 days) reduce some blood count values.[16] In our study, similar changes were observed, and blood count values increased and decreased. WBC and MPV show significant change P <0.05).

Investigators have reported that exposure to electromagnetic fields on sex hormones of wistar female rats (4 h/day and 3 mT /day for six weeks) has an adverse effect.[17] Literature on the effects of highly low-frequency electromagnetic fields on cortisol is very limited. These few studies are short-term data.[3] Al-Akhras and colleagues reported that the 50 Hz 25 micro-tesla magnetic field had an adverse effect on the fertility of both male and female rats.[18]

In our study, we found that complaints of fatigue, fatigue and eye pain were found to be meaningful in employees who used a continuous hairdryer. When the biochemical findings of our study were analyzed, no significant change in all parameters was observed. When compared to the control group, the mean values of ALT, AST, TRIG and ULDL in the experimental group increased and CHOL and UHDL values decreased. Statistically no difference was observed when the hormone values were examined.

However, it was noticed that the estrogen level increased in the experimental group. A significant change in the WBC and MPV values was observed in the whole blood values of our study. Our findings suggest that some of the parameters in the sera of women may have been altered by the influence of the electrical and magnetic fields generated by the hair dryer. Most of the literature information that has been made suggests that very low frequency electromagnetic fields have a negative effect on biochemical, hematologic and other parameters.

4. CONCLUSION

Our work has drawn attention to the effects of electrical and magnetic fields on the organism, which are made by man-made electrical devices that are constantly used by professional workers. With this work, we may be able to protect ourselves from areas we are exposed to constantly without knowing it. The fact that the literature data on this area is too small that our study may be a reference. In our work, the effects of the people who spend a lot of time in hairdressing salons and the effects of the women using these devices on the biochemistry, hormone and whole blood parameters have been examined. We believe that our work will be a useful source of concern for community health and we emphasize the need for more work.

CONFLICT OF INTEREST

All authors state that there is no conflict of interest.

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