LPS HELICOBACTER PYLORI CAUSATIVE AGENT OF THE INCREASE OF TNF-α, VCAM, E-SELECTIN AND MMP-8 AS INDICATOR OF ENDOTHELIAL DYSFUNCTION

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ABSTRACT

Objective of the study: Analyzing the transformation that occurs in the experimental animal’s endothelium infected by Helicobacter pylori. Cytokine which has a role in endothelial dysfunction will be analyzed and comparison between the experimental animal that has been infected by Helicobacter pylori and the experimental animal that is not infected by Helicobacter pylori was pursued. Method of the study: The research is categorized as Experimental research using Rattus norvegicus as an object of research. It consists of two group. First group is a control group and the second one is intramuscularly induced by LPS Helicobacter pylori three times with 3-day interval. Afterward, the indicator of endothelial dysfunction, TNF-α, e-selectin, MMP-8 and VCAM will be analyzed by using method of immunohistochemistry. The result of research: After conducting the Mann-Whitney T-Test and Spearman’s Rho Correlation Test, it is concluded that there is significant difference between the control group and the group with treatment (TNF-α, p=0.001, VCAM, p=0.008, MMP-8, p=0.00, e-selectin, p=0.008). It can be seen that there is a good correlation between TNF-α and e-selectin. Conclusion: LPS Helicobacter pylori encouraged lesion in arterial endothelium and finally causes atherosclerosis.

Keywords: LPS Helicobacter pylori, Endothelial dysfunction, Inflammation, Infection.

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INTRODUCTION

In the world, cardiovascular disease is the second leading cause of death. In the United States reported that the atherosclerosis occurs first among the four residents, and was the cause of death by 42% (S Connor, 2001). While in Indonesia, deaths from coronary heart disease ranks first, about 42.9% (N Zubir, 1993). Factor of atherosclerosis has been widely known triggers such as: hypertension, smoking, diabetes mellitus, hyperlipidemia, hypercholester-emia, and genetic factors. Despite all efforts to prevent the triggering factors has been conducted, but the incidence of cardiovascular disease has not decreased. Some researchers reported a link between infection, inflammation with atherosclerosis (Benitez RM, 1999; Bertrand ME, 1999; Ameriso SF, 2001; Connor S, 2001; Mach F, 2002; Mayr M, 2003; Chmiela M, 2003; Sargowo, 2004). The study states that chronic inflammation and infections play a role in the initiation and progression of atherosclerosis. Chronic infection of some viruses and bacteria like Cyto Megalo Virus and Chlamydia pneumoniae can cause atherosclerosis turns out (S Connor, 2001; NeureiterD, 2003). It also reported that Helicobacter pylori can activate both leukocyte and endothelial cell transforming and smooth muscle cells, which in turn will stimulate atherosclerosis. Osawa H in 2001, conducts research serologically reported that there is a relationship between Helicobacter pylori infected patients with coronary heart disease based on endothelial dysfunction. But until now the mechanism of endothelial dysfunction that occurs in Helicobacter pylori infection is unclear.

In developing countries, the incidence of chronic infection in the stomach caused by Helicobacter pylori is big enough (Daniel, 2003). In the United States estimated that 10% of Helicobacter pylori infection occurs at age 20-29 years, while in Africa almost 90% of children infected with Helicobacter pylori bacteria. (Frenck, 2003) Although no data are valid, but is expected in Indonesia, the frequency of chronic gastritis due to Helicobacter pylori infection is high. In Padang, West Sumatra reported 73.4% of anti-Helicobacter pylori (+) were found in patients with chronic gastritis (Zubir N, 1993). Given the frequency of patients with Helicobacter pylori is high, then the mechanism of atherosclerosis in patients infected with Helicobacter pylori is necessary to explain, since the mechanism is very important as a basis for prevention of atherosclerosis in patients
infected by _Helicobacter pylori_. If it does not get serious attention, the incidence of atherosclerotic disease are feared to have increased sharply. In Indonesia on gastric _Helicobacter pylori_ infection is high enough, there should be a study linking between _Helicobacter pylori_ infection with the process of atherosclerosis, especially endothelial dysfunction.

Based on reports from the period 1999 to 2004 (Benitez RM, 1999; Ameriso SF, 2001; Mach F, 2002; Cmiela M, 2003 and Sargowo, 2004) which states that there is linkage between infection, inflammation with atherosclerosis. Other researchers (Osawa H, 2001) suggests that _Helicobacter pylori_ infection can trigger atherosclerosis, it is necessary to study the link between infection with the incidence of atherosclerosis. In this research, the object should be observed is the change in blood vessels in humans, but due to technical reasons, to get a sample of blood vessels of patients who experience chronic gastritis caused by _Helicobacter pylori_ is not easy, so in this research using experimental animals as a model. Experimental animals used were white rats of the genus _Rattus norvegicus_. While the lesion is given to trigger endothelial dysfunction is lipopolysacharida (LPS) from _Helicobacter pylori_ bacteria.

In this experiment, to make of _Rattus norvegicus_ in the infection, conducted by LPS _Helicobacter pylori_ enter multiple times by intramuscular injection. LPS injections several times intramuscularly into the animal's body will theoretically captured by macrophages. When this is done repeatedly, the macrophages will become active. Active macrophages would release several pro-inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α. These mediators then that induces increased vascular permeability. TNF-α and IL-1 plays a role in inducing increased expression of adhesion molecules on vascular endothelium, TNF-α induces endothelial E-selectin expressing the essential role of neutrophil adhesion, whereas IL-1 induces increased expression of VCAM, where VCAM essential role on monocyte and lymphocyte adhesion.

In addition IL-6 and TNF-α also has a role in inducing bone marrow to produce leukocyte, so that at the apparent increase in inflammatory leukocyte (leukocytosis) (Kuby, 2000). IL-8, are neutrophil chemotakтик factor (NCF), will lead to neutrophil migration into the peripheral (Holston, 1997), with the E-selectin, neutrophil will stick to the surface of endothelial and neutrophil to secrete an enzyme that is the Matrix Metallo Proteinases colagenase or MMP-8. MMP-8 will lead to lesions in blood vessels, and endothelial dysfunction will occur. Endothelial dysfunction disorder resulting formation of NO (nitric oxide), which decreased NO production would lead to increased oxidation of LDL. (NO role is to protect LDL from oxidation reactions). This process will further develop into atherosclerosis.

Inflammation caused by LPS into the body and the production of pro-inflammatory cytokines such as TNF-α and IL-1 produced by activated macrophages/distress causes of stress for endothelial cells, and will produce a protein in order to protect from threats known as stressors Heat Shock Proteins (HSP). (Ranford JC, 2000), however due to the stress exposure continuously, the presence of HSP will trigger endothelial cells to produce adhesion molecules, such as VCAM, and E-selectin. (Galdiero M, 1997) Heat Shock Proteins control the IL-1 and TNF-α in the process of gene transcription into a protein, namely E-selectin and VCAM. (Verdegaal ME, 1996). _Helicobacter pylori_ bacteria have be able to express HSP 60 and HSP 60 are on the cell surface, (Cao P, 1998) and because LPS is part of the germ _Helicobacter pylori_, the LPS as endotoxin, can also directly induce endothelial cells such as inflammatory disorders that occur above, which endothelial cells will experience distress and will trigger further production of HSP which will produce adhesives molecules as well as control of TNF-α and IL-1 gene transcription in the process to become adhesive molecules.

So, _Helicobacter pylori_ LPS was injected at the guinea pig will pass through two lines, the first past the macrophages, which were given repeated LPS will be caught by macrophages and macrophages will be activated and will then secrete IL-1, TNF-α, IL-8 and will trigger endothelial cells to express adhesives molecules which ended with the MMP-8 is produced by monocyte and neutrophil. The second path through Heat Shock Proteins. Inflammation caused by pro-inflammatory cytokines will cause stress for endothelial cells and endothelial cells that distress will produce Heat Shock Proteins (HSP). In addition, the bacteria _Helicobacter pylori_ LPS for 60 and has in addition to HSP can be excreted into the cell surface, LPS _Helicobacter pylori_ as endotoxin can directly trigger endothelial cells to remove the HSP. _Helicobacter pylori_ LPS directly induces endothelial cells can cause distress, because the bacteria _Helicobacter pylori_ stressors are repeated, endothelial cells are experiencing distress will produce HSP. Role of heat shock proteins produced are: (1) triggers endothelial cells produce adhesion molecules, and (2) control of TNF-α and IL-1 gene transcription in the process become adhesive molecules.

This study aimed to explore the mechanism of endothelial dysfunction in _Helicobacter pylori_ LPS.
Specifically this study aims to prove the increased expression of VCAM on blood vessel endothelial *Rattus norvegicus* after *Helicobacter pylori* induced by LPS in intra muscular, to prove the increased expression of TNF-α in fibroblasts surrounding blood vessels *Rattus norvegicus* after *Helicobacter pylori* induced by LPS in intra muscular, to prove the increased expression of E-selectin on vascular endothelial *Rattus norvegicus* after *Helicobacter pylori* induced by LPS in intra muscular, and to prove the increase in MMP-8 in vascular endothelial *Rattus norvegicus* after *Helicobacter pylori* induced by LPS in intra muscular.

**MATERIALS AND METHODS**

This research was conducted from July 2006 until July 2007, using rats *Rattus norvegicus*. This study is purely experimental because there is replication, randomization, and treatment and control groups, with the design of post test only control group design. (Campbell, 1966). As the population is white Wistar rats. The sample used in this study were male *Rattus norvegicus*, age 10-15 weeks, weight between 200-300 grams. From the calculation formula in 1985 Higgins & Klinboum obtained n = 5. Methods of measuring the variables used in this study were immunohistochemistry, because the observations made by the researchers was a local reaction. This research was conducted at several places such as FK GRAMIK Pathobiology Laboratory, Unair and Animal Biochemistry FK room, Airlangga University. This research was conducted September 2006 s/d September 2007. Giving *Helicobacter pylori* LPS intra muscular done every three days during the 3 -4 times. Tissue taken were fixed with formalin buffer. The tissue was processed and checked with the methods Immunohistochemistry. Doing preliminary research, that is one month prior to the research conducted. Number of guinea pig: 5 tails. The method is similar to the method of research. LPS dose given was 1 tg/kg BW, 2.5 tg/Kb BB, and 5 tg/Kb BB. New results differ in the provision of 5 tg/Kb BB. Also examined the distribution of TNF-α and VCAM.

**RESULTS**

![Figure 1. Cross section of heart veins *Rattus norvegicus*, magnification 400 X. DV: venous blood vessel walls are thin. Using immunohistochemical staining.](image)

![Figure 2. *Rattus norvegicus* heart slice preparations without treatment. Immunohistochemical staining, using monoclonal antibodies against TNF-α. Magnification 400 X. N: cells that showed a negative reaction](image)

![Figure 3. *Rattus norvegicus* heart slice preparation. With immunohistochemical staining, using monoclonal antibodies against TNF-α. 400x magnification. N: cells that showed a negative reaction. P: cell that showed a positive reaction](image)
Figure 4: Preparations *Rattus norvegicus* heart tissue incision. With immunohistochemical staining, using monoclonal antibodies against VCAM. Magnification 400x. N: cells that showed negative reaction. DA: the walls of blood vessels (arteries). E: erythrocytes.

Figure 5. *Rattus norvegicus* heart slice preparation. With immunohistochemical staining, using monoclonal antibodies against VCAM. 400x magnification. P: indicates a positive reaction. DA: the walls of blood vessels (arteries). E: erythrocytes.

Figure 6. *Rattus norvegicus* cardiac slice preparations. With immunohistochemical staining, using monoclonal antibodies against E-selectin. 400x magnification. N: negative reaction. DA: the walls of blood vessels (arteries). E: erythrocytes.

Figure 7. *Rattus norvegicus* heart slice preparation. With immunohistochemical staining, using monoclonal antibodies against E-selectin. 400x magnification. P: indicates a positive reaction. N: indicates negative reaction. DA: the arteries. E: erythrocytes.

Figure 8. *Rattus norvegicus* cardiac slice preparations. With immunohistochemical staining, using monoclonal antibodies against MMP-8. 400x magnification. DA: the walls of blood vessels (arteries). E: erythrocytes.

Figure 9. *Rattus norvegicus* cardiac slice preparations. Staining with immunohistochemistry, using monoclonal
antibodies against MMP-8. 400x magnification. P: cell that showed a positive reaction. N: cells that showed a negative reaction. DA: the walls of blood vessels (arteries). E = erythrocyte.

Table 1. The observation on the examination of variables

<table>
<thead>
<tr>
<th>Var</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
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<td>1.4800</td>
<td>1.17913</td>
<td>10.7840</td>
<td>3.30598</td>
</tr>
<tr>
<td>VCAM</td>
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<td>0.83666</td>
<td>6.400</td>
<td>2.70185</td>
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<tr>
<td>e-Selektin</td>
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<td>0.65192</td>
<td>16.0780</td>
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<td>MMP-8</td>
<td>1.4900</td>
<td>1.5001</td>
<td>14.090</td>
<td>2.580</td>
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</tbody>
</table>

From the data analysis, data distribution from the results of this study is normally distributed (p> 0.05), is a TNF-α., VCAM and MMP-8, while E-selectin is not normally distributed (p <0.05), so to test different E-selectin non-parametric test of differences. From the different test results (t-test) obtained, TNF-α., VCAM., MMP-8 between the control group with treatment groups showed highly significance (p <0.05). From the results of Mann-Whitney difference test result, E-selectin between the control group with treatment groups showed highly significance (p <0.05). From the test results obtained by the correlation between TNF-α with E-selectin, correlated very strongly/positive (**)

DISCUSSION

As many as 50% patients found no risk factors for atherosclerosis are unclear, various studies and observations made by experts suspect there are factors that can not be detected. (Vita JA, 2002) Many factors that trigger cardiovascular disease, among other characteristics of the atherosclerotic lesion containing cholesterol from arteries, fibrosis and infiltration of inflammation. Formerly known atherosclerotic disease due to accumulation of fat in the blood vessel walls, and is currently known that the inflammatory response in blood vessel formation is involved in the process of atherosclerosis. (Ross R, 1999, Libby P, 2001).

The process of atherosclerosis begins early endothelial dysfunction, followed by the deployment of lymphocytes, the formation of macrophages, lipid deposition, proliferation of smooth muscle cells and extracellular matrix synthesis. The interaction of all the characteristics of the plaques that formed atherosclerotic (Ross R, 1999). Chronic inflammatory process in atherosclerosis evidenced by the discovery of marker of inflammation, such as CRP (C-reactive protein), cytokines (IL-6, IL-8 and TNF α), adhesive molecules (ICAM), E-selectin are increased in blood plasma. (Altman R, 2003).

Atherosclerosis initially regarded as a natural disorder, caused by the degeneration process, which arises due to the additional time and age. Today the study of atherosclerosis states that atherosclerosis is a pathological condition of the walls of arteries that is initiated by the emergence of a chronic lesions in the walls of blood vessels or endothelial. Body's response to endothelial lesions in the form of inflammatory reaction. So the actual process of inflammation is a mechanism of homeostasis, adaptive, protective response to danger from cell damage. The primary mechanism of leukocyte inflammatory process starts, including monocytes, polymorphonuclear neutrophils (PMN) and lymphocytes B and T. In this process, the cells interact with endothelial cells, especially at the existing receptors on the surface of endothelial and will then trigger the inflammatory cytokines. The main purpose of the process to eliminate and dispose of foreign substances as well as restore the original network. In this study begins the process of atherosclerotic lesions in endothelial events. So this study focused events that endothelial dysfunction occurs in bacteria Helicobacter pylori infection.

LPS or lipopolysaccharide is a layer that surrounds the outside of the membrane of gram-negative bacteria. LPS is composed of a lipid part, called lipid-A and binds to part of the polysaccharide. Part of the polysaccharide consists of three components, the inner core, outer core and the O-specific chain, every chain has the structure and function of their own. For example, the O-specific chain has a role in the activation of complement by bacterial receptor, whereas the lipid A, in each gram-negative bacteria have varying composition. Differences in the composition of the chain are in clusters hexosamine, chain length and degree of acetylation and phosphorylation. Lipid A is responsible for the pathophysiological changes on gram-negative bacterial infection. Lipid A is known to Endotoxin. Helicobacter pylori has a lipid-A is not phosphorylated and has a long chain fatty acids. The structure will be able to suppress the immune response of the host, so that Helicobacter pylori infection will continue. (Corcoran A, 2005).

With the endotoxin LPS is a powerful trigger of the immune system of the host body. (MG Rittig, 2004) Endotoxin triggers the cell to synthesize a protein, endotoxin, in this case are as stressors on endothelial cells and will synthesize heat shock proteins (HSP) as a response to the stressor toxin. In addition to the toxin as a stressor that can trigger the cell to synthesize HSP, other conditions such as infection and inflammation can also trigger the cell to synthesize HSP.
Inflammatory reaction caused by LPS, is a defense mechanism against microorganisms. Of cells responsible for defense in the body is Macrophages that have a specific receptor recognition, in this case the introduction of LPS in gram-negative bacteria. Bond between the LPS with macrophages will result in macrophage release some chemicals that regulate the body's defenses, called cytokines. Cytokines released by macrophages include IL-1, IL-6, IL-8 and TNF-α. (Kuby, 2000). IL-1 produced by macrophages or monocytes in the body causing a host inflammatory manifestations include fever, increased leukocytes and leukocyte infiltration in areas of inflammation by enhancing lymphocyte and neutrophil migration into areas of inflammation. Also IL-1 can increase the settings on the endothelial expression of adhesive molecules, which will trigger endothelial VCAM produce. (Van der Poll T, 1997) VCAM or vascular cell adhesion molecule is a molecule that can cause adhesive lymphocytes, monocytes, eosinophils and basophil stuck/attached to the endothelium of blood vessels and can also cause monocytes attached to endothelial having atherosclerosis. (Wu TC, 2007). IL-6 is also produced by macrophages, formerly known as IFN-ß-2 which functions similar to IL-1, whereas the cytokine IL-8 was included in the group with low molecular weight peptides which have chemotactic properties and can improve the adhesion of PMN on vascular endothelium, also known as Neutrophil chemotactic factor (NCF). These molecules are produced by macrophages and vascular endothelial cells. (Ulgaard JO, 1998). TNF-α is one of the cytokines that play a role in systemic inflammation, are produced mainly by macrophages in response to stimulation of LPS, TNF-α would then trigger the endothelium to produce E-selectin. E-selectin, also known as CD62E, is the only adhesive molecules expressed by endothelial cells, plays an active role in the inflammatory process. In the process of inflammation, the role of E-selectin is a leukocyte kearea attract inflammatory cells. (Ryan U.S., 1992)

In this study, LPS given Helicobacter pylori to Rattus norvegicus repeatedly will cause inflammatory reaction, LPS will phagocitized by macrophages and the macrophages with repeated administration will become active. Macrophages are active pro-inflammatory cytokines will issue, among others, IL-1, IL-6, IL-8 and of TNF-α. IL-1 and IL-6 stimulates bone marrow to produce leukocyte and will place a condition known as leukocytosis. In addition to inducing bone marrow, IL-1 also induces endothelial VCAM to produce. While IL-8, which is a neutrophil chemotactic factor which attracts neutrophil exist in peripheral blood flow leading to and because there is an e-selectin pada endothelial, neutrophil will stick to the endothelium. Attached to the endothelial neutrophil will issue a proteinase enzyme known as MMP-8 (Matrix Metallo proteinases). MMP-8 is among protease, has the ability to damage the extracellular matrix proteins, resulting in lesions on the cell surface receptors. (Eisen A, 1968)

MMP-8 that occurs will cause lesions on arterial endothelium of blood vessels. This event is the beginning of atherosclerosis. The following series of events has been studied by many scholars, (Mach F, 2002; Cmiela M, 2003; Sargowo, 2004) that these lesions will decrease the production of nitric oxide or NO by the endothelium, so that the LDL is not protected from oxidation reactions. LDL would be formed through oxidation and oxidized LDL or ox-LDL. Ox-LDL to trigger endothelium to produce cytokines, such as monocyte chemotactic factor (MCF), monocyte colony stimulating factor (MCSF) and VCAM. MCF will result in peripheral monocytes attracted to and by the presence of IL generated by VCAM-1, monocyte will stick to the endothelial lesion in the region and into the intima. Monocytes in the intima/sub intima will turn into macrophages as a result of MCSF and will phagocytosize ox-LDL, will become foam cells (foam cells). Foam cells will produce IL-1 that was instrumental in triggering smooth muscle cells in the arterial vascular wall proliferate. Continuous proliferation will lead to thickening and rigidity of arterial smooth muscle, and eventually formed a condition called atherosclerosis. So in this study, once again, a series of events that scrutiny is the distribution of the cytokines TNF-α, MMP-8, E-selectin and VCAM are increased in the arterial wall Rattus norvegicus given LPS Helicobacter pylori compared with those not given LPS of Helicobacter pylori. All four of these factors determine the occurrence of lesions in the arterial endothelium, which will cause the sequence of events with the final result is the occurrence of atherosclerosis.

Role of heat shock protein big enough to endothelial dysfunction. Helicobacter pylori LPS given to Rattus norvegicus is a stressor for Macrophages, because LPS given repeatedly causes macrophages into distress. Macrophages that distress will issue a pro-inflammatory cytokines such as IL-1 and TNF-α as a form of stress response. Stress response of a cell can be eustress, the cell will respond to a stressor so as to achieve a new balance in the protective, or may be distress, which causes a balance disorder of cells, causing cell death. (Son, 2005)
Pro-inflammatory cytokines will cause a state of inflammation that can be a stressor for endothelial cells. Endothelial cells will respond by secreting heat shock protein. In endothelial cells, HSP triggers the formation of adhesive molecules, such as E-selectin and VCAM as well as control the pro-inflammatory cytokines, especially TNF-α and IL-1. (Ranford CJ, 2000) The presence of adhesive molecules produced by endothelial HSP induction and TNF-α and IL-1, endothelial dysfunction that occurs will be more real.

This study is purely experimental, because there are replication, randomization and control and treatment groups, with the design of post test only control group design, using statistical t-test and correlation test. As a guinea pig, white rat population is the type of the genus Rattus norvegicus male sex, adults aged between 10-15 weeks and weighing between 200-300 grams, Helicobacter pylori LPS administered intramuscularly three times at intervals of three days. Then Rattus norvegicus done deseksi, captured his heart and organs examined contained the arteries of the heart (coronary). Immunohistochemistry methods are observed and compared the distribution of TNF-α, E-selectin, VCAM and MMP-8 between Rattus norvegicus given LPS H. pylori and what does not. Results obtained in general it was found that TNF-α, E-selectin, VCAM and MMP-8 on LPS given Rattus norvegicus H. pylori obtained higher results than those not given LPS of Helicobacter pylori. In statistical calculations, generally obtained results are significant differences between the two. From the results obtained, showed that the bacteria Helicobacter pylori can cause increased TNF-α, MMP-8, E-selectin and VCAM on blood vessel walls of heart arteries. With the increase of the four factors, then can be expected to follow a lesion in the coronary artery endothelium, and will be followed by a series of subsequent processes that will lead towards atherosclerosis. All four of these factors, particularly, MMP-8 and E-selectin increased as a result of TNF-α. This means that if there are increase in TNF-α in peripheral blood flow, the concentration of MMP-8 and E-selectin on blood vessel walls of heart arteries will also be increased, and it can be assumed will happen atherosclerosis. Clinical applications, when TNF-α was found on examination of blood of patients with Helicobacter pylori infection, can be predicted will happen coronary artery endothelial lesions and atherosclerosis will occur.

Know the computation of TNF-α, in the control group obtained the results (X = 1.4800, SD = 1.79131) and in the treatment group obtained the results (X = 10.7840: SD = 3.30598) after the test was done using the statistical t-test, the results obtained between control group with treatment group had a very significant difference that is at (p <0.05). These results suggest that experimental animals induced by Helicobacter pylori-LPS intramuscularly, can trigger the activity of macrophages to secrete TNF-α. TNF-α then this will trigger the endothelium to secrete E-selectin, E-selectin is the adhesive molecules on neutrophils. Neutrophils that are trapped by molecular adhesives tried to escape by releasing an enzyme that is kolagenase/MMP-8 (Kuby, 2000). Collagenase can trigger damage to the vascular system. To protect against such damage to the macrophages to secrete TGF-SS1, and TGF-SS1 will trigger the formation of collagen to repair damaged collagen. If there is excess secretion of TGF-SS1, will trigger fibrosit forming collagen in blood vessel walls that ultimately excessive excess collagen will trigger abnormalities in the vascular system. (Nehra A, 1999).

It is known that the computation of TNF-α, in the control group obtained the results (x = 0.8000, SD = 0.83666) and in the treatment group obtained the results (x = 6.4000: SD = 2.70185) after the test was done using the statistical t-test, the results obtained between control group with treatment group had a very significant difference that is at (p <0.05). This shows that in experimental animals induced by Helicobacter pylori LPS-LPS intramuscularly, can trigger the activity of macrophages to secrete IL-1. IL-1 then this will trigger the endothelium to secrete VCAM, which is an adhesive molecule on monocytes (Kuby, 2000). Monocytes are trapped by adhesive molecules trying to escape by releasing an enzyme kolagenase/MMP-8 (Kuby, 2000). Collagenase will trigger damage to system degradation. To protect against such damage to the macrophages to secrete TGF-SS1 to trigger the formation of collagen in an effort homeostasis.

It is known that the computation of the E-selectin, on the control group obtained the results (X = 0.4000; SD = 0.65192) and in treatment group obtained the results (X = 16.0780: SD = 2.54193) Having performed a statistical test by using the Mann Whitney test, obtained results between the control group with treatment group had a very significant difference that is at (p <0.05). This shows that in experimental animals induced by Helicobacter pylori-LPS intramuscularly, can trigger the activity of macrophages to secrete TNF-α, TNF-α then this will trigger the endothelium to secrete E-selectin, as adhesive molecules on neutrophils (Kuby, 2000). Neutrophils attached to endothelial adhesion molecules will be released by enzyme kolagenase/MMP-8 (Kuby, 2000). Collagenase may damage the collagen in blood vessels that cause damage to the vascular system. To protect against such damage to the macrophages to secrete TGF-SS1, such as previous incidents above.
Note that the computation of MMP-8, the results obtained in the control group (X = 1.4900, SD = 1.50017) and the results obtained in the treatment group (X = 14.0900, SD = 2.58001) Having performed a statistical test by using t-test, the obtained result between the control group with treatment group had a very significant difference that is at (p <0.05). These results indicate that in experimental animals induced by Helicobacter pylori-LPS intramuscularly, can trigger the activity of macrophages to secrete TNF-α, TNF-α then this will trigger the endothelium to secrete e-selectin which is the adhesive molecules on neutrophils (Kuby, 2000). Neutrophils and Monocytes attached to the endothelial then be issued a MMP-8 (Kuby, 2000). Collagenase will damage the structure of the vascular endothelium, resulting in lesions or endothelial dysfunction. Based on these results, the neutrophil turns out to have very high activity to release the enzyme MMP-8, which would trigger damage to the vascular system. When the note, one of the causes of vascular lesions is the Matrix Metallo Proteinases (MMP). In blood vessels found in collagen type I, IV and VIII. Collagen type-1, a substrate of MMP-8, when an increase in MMP-8 would trigger vascular endothelial damage (Darnell, 1990). TNF-α have an important role to trigger the endothelium to secrete e-selectin, then test the correlation between the proficiency level in these variables. Controls have X = 0400, SD = 0.65192, and treatment X = 16.0780, SD = 2.54193. After using Spearman’s Rho correlation test, the results showed there was a strong positive correlation between TNF-α by e-selectin that is at (r = 0892). Given that e-selectin is a molecule found on the surface of endothelial adhesive, and to identify the expression of adhesive molecules in endothelial hard to do because they have to do a biopsy.

This action seems ethically difficult to be understood, for it was another breakthrough for researchers seeking to detect an increase in particular adhesive molecule E-selectin endothelial surface without making the network. According to Kuby, 2000, reported that the inflammatory reaction of TNF-α induces endothelial E-selectin expressing the essential role of neutrophil adhesion. The researchers sought to link between the increase in TNF-α by e-selectin, since TNF-α is a cytokine that can be released by the cells systemically. Therefore, if there was an increase of TNF-α, in the blood, the researchers will also assume an increase in endothelial E-selectin pada, which certainly increased E-selectin has a very large contribution to the vascular system damage.

**CONCLUSION**

This study describes the mechanism for endothelial dysfunction caused by infection from Helicobacter pylori bacteria, which can be seen from the results of the investigation as follows: VCAM expression on vascular endothelial Rattus norvegicus H. pylori induced by LPS intramuscularly larger than that are not induced in LPS. TNF-α expression in heart tissue macrophages Rattus norvegicus H. pylori induced by LPS intramuscularly bigger than that is not induced LPS. E-selectin expression of endothelial vascular in Rattus norvegicus H. pylori induced by LPS intramuscularly bigger than that is not induced LPS. Expression of MMP-8 in vascular endothelial Rattus norvegicus H. pylori LPS induced by intra muscular bigger than that is not induced LPS. H. pylori LPS given intramuscularly in Rattus norvegicus, can trigger a heart blood vessel endothelial dysfunction. Change E-selectin on endothelial surface positively correlated with TNF-α. Need to conduct further research on the role of TNF-α as one strong predictor of endothelial dysfunction. Development of therapy using anti-TNF-α to stop the process of endothelial dysfunction. Development of methods of prevention with immunization against Helicobacter pylori bacterial infection.

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