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Research Article

IDENTIFICATION OF CORONARY ATHEROSCLEROTIC LESIONS IN RAT INDUCED BY INTRAVENOUS CANDIDA ALBICANS

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ABSTRACT

Objective: This study aimed to identify the sign of coronary atherosclerosis lesion in rat induced by intravenous *Candida albicans*.

Methods: This research was an experimental laboratories (*in vivo*) that used 15 male Wistar rat, divided into three groups of the control, the first treatment group injected by *C. albicans* intravenously with concentration of the 10^{-10} cells/mL and the second treatment group injected by *C. albicans* intravenously with concentration of the 10^{-12} cells/mL. 0.2 mL of *C. albicans* were injected to the first and second treatment group on 1st, 4th, 9th, 16th, and 23rd day. On the 5th week, rats were sacrificed, taken its hearts that contained the coronary artery. Rat's heart had been fixed; histological preparations were made and painted with Picrosirius Red and Sudan IV, observed by a microscope.

Results: The artery walls of the first and second treatment group were thicker than the control group. Lesions and disposition lipids of the first and second treatment group were more frequent than the control group.

Conclusion: Intravenous *C. albicans* can increase the risk of coronary atherosclerosis.

Keywords: Atherosclerotic lesion, *Candida albicans*, Coronary artery, Systemic candidiasis.

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INTRODUCTION

Atherosclerosis is the largest health problem in the world [1]. Atherosclerosis occurs in arteries including the aorta and coronary arteries, femoral, iliac, carotid intera, and cerebral. Atherosclerosis in the coronary arteries is the main trigger of death because it can cause heart failure in pumping blood which can stop the blood supply throughout the body [2]. The pathogenesis of atherosclerosis is unidentified in certain yet. More than 20 epidemiological studies conducted in many countries to this day prove that there is a connection between the food and the lipid atherosclerosis [3,4].

Lipid peroxidation, due to free radical activity, plays an important role in the development of atherosclerosis. Free radicals in the presence of oxygen may cause degradation (peroxidation) of lipids within plasma and organellar membranes. Oxidative damage is initiated when the double bonds in unsaturated fatty acids of membrane lipids are attacked by oxygen-derived free radicals particularly by OH [5]. Oxidative damage of lipid, especially low-density lipoprotein (LDL) in the arterial wall, can lead proliferation and migration of smooth muscle cells [6].

In the last few years, it has been developed a paradigm that infection with bacteria, viruses, and other microorganisms contributes to the formation of plaque atherosclerosis [3]. Fungal infections *Candida albicans* are one of the suspected infections which contribute to the formation of plaque atherosclerosis [7,8]. Opportunistic pathogen *Candida* is a species that can cause infections in the superficial mucosa to spread to the blood vessels, causing injury to the endothelium and stimulates autoimmune [9].

This research shows that there is a connection between candida infections with lesions of atherosclerosis that is supported by the results of research which states that there is DNA of *C. albicans* in 58% of coronary heart disease patient samples identified by the method of polymerase chain reaction (PCR) [10]. 67.7% of patients with stenosis the coronary artery have a *Candida* colony in its oral cavity [11]. Although it is well known that *C. albicans* is associated with atherosclerosis, there are only few studies talking about the experimental causal effects of the role of *C. albicans* in atherosclerosis. This raises the writer's desire to conduct an experimental study to determine the effect of *C. albicans* on the formation of atherosclerotic lesions.

METHODS

This study has been approved by the Medical Research Ethics Committee of the Faculty of Medicine, University of Jember. The object of the study was 15 healthy male Wistar rats, with the age of 3–4 months. Rats were divided into three groups: A control group (no treatment), *Candida* 1 (injected with *C. albicans* in the concentration of 10^{-10} cells/mL), and *Candida* 2 (injected with *C. albicans* in the concentration of 10^{-12} cells/mL) with each group of five rats. Standard feed was given daily.

Induction of *C. albicans* intravenous

Rats had been adapted, treated according to the group. Injection of *C. albicans* (ATCC 10231) was performed on the vein of the tail (lateral vein) in about 0.2 mL at days - 1, 4, 9, 16, and 23.

Histological sample preparation

At week 5, the rats were sacrificed, performed thoracic surgery and the heart which contained coronary arteries were taken. After that, coronary arteries were fixed using frozen section cutting method which then was painted using Picrosirius Red and Sudan IV with counter Mayer's hematoxylin. Preparation of blood smearing with Giemsa was used to see leukocyte cells that are a sign of systemic candidiasis.

Atherosclerosis parameters

The observations of atherosclerosis included coronary artery wall thickness, presence of cap, and lipid deposition. Wall thickness and

atherosclerotic cap detection were observed in histological preparations with Picrosirius Red painting using a ×400 light magnification microscope. The thickness of the coronary artery wall (μm) was measured from the intima tunica to the media. The morphology of cap was illustrated by a bumpy surface on the inside (luminal) of the coronary arteries. Detection of lipid deposition was performed on histological preparations with Sudan IV painting and observed it using a light microscope at 1000x magnification. Lipid deposition was characterized by red color on coronary artery preparations.

Data analysis

This study produces qualitative data (the presence of lipids and lipid deposition) and quantitative data in the form of measurements of coronary artery wall thickness. Qualitative data were analyzed by non-parametric difference Kruskal–Wallis test, followed by Mann–Whitney test data ($p<0.05$). The quantitative data were analyzed by parametric test of independent one-way ANOVA, then continued with *post hoc* test to know the group of samples that gave significant value ($p<0.05$).

RESULTS

Systemic candidiasis in experimental animals

C. albicans induction caused systemic infection that was proved by examination of leukocyte cells in blood smear with Giemsa staining, and it was observed with a ×400 magnification light microscope (Fig. 1). The number of leukocytes in the *Candida* 1 group was higher than in *Candida* 2 and control group.

Measurement of the coronary arterial thickness wall

The result showed that *Candida* 1 had the thicker average than the other groups. Data were analyzed with one-way ANOVA. It was found that coronary artery wall between groups was significantly different ($p<0.05$) (Table 1). *Post hoc test* showed that group *Candida* 1 was significantly different ($p<0.05$) compared to control group, but there was no significant difference between *Candida* 2 group with control and *Candida* 1 group (Table 2).

Observation of atherosclerotic cap and lipid deposition

Histological features show that groups of *Candida* 1 and 2 were found in morphology of lipid. All groups have a lipid deposition profile. The presence of cap is characterized by a bumpy formation of bulge on the inner side (luminal) of the coronary arteries (Fig. 2). *Candida* 1 group has the highest percentage of atherosclerotic cap and lipid deposition. Kruskal–Wallis test showed that there were significant differences between the groups ($p<0.05$) (Table 3), followed by Mann–Whitney test showed that *Candida* 1 group was significantly different to control ($p<0.05$), whereas *Candida* 2 group with control and *Candida* 1 did not differ significantly ($p>0.05$) (Table 2).

DISCUSSION

This study showed that the effect of *C. albicans* infection induced the formation of coronary atherosclerosis lesions. The degree of systemic candidacies infection is evidenced by the examination of leukocyte cells in the blood smear. Leukocyte cell identification results showed differences in the number of leukocyte cells between groups. The group *Candida* 1 had more leukocytes counts than the other groups; this was because the group of *Candida* 1 was injected by *C. albicans* with a higher concentration than the *Candida* 2 and control group. The increase in leukocytes occurred due to systemic infection of *C. albicans* relating to its role in the cellular and humored defenses of organisms against foreign substances [12]. Shi and Tokunaga report that *C. albicans* may result in endothelial injury and stimulate an autoimmune reaction [9].

Factors which are affecting the infection process of *C. albicans* are adhesion, spore changes to hyphae, and extracellular enzyme production. The adhesion of *C. albicans* in spore form involves the interaction between ligand and receptor in endothelial cells. The attachment and physical contact between *C. albicans* and endothelial cells will activate the MAP-kinase necessary for invasive hyphae development and biofilm development. The hyphae form and extracellular enzyme aspartyl proteinase will facilitate *C. albicans* to invade endothelial cells to form holes in endothelial cells. The invasion

Table 1: The average of coronary artery wall thickness (intima media)

Groups	N	Wall thickness average X±SD (μm)
Control	5	6.2±1.4
Candida 1	5	10.1±1.8
Candida 2	5	8.2±2.4
One-way ANOVA test Sig.(p)		0.002*

*Significant difference ($p<0.05$). Control: Not induced *C. albicans*, Candida 1: Induced *C. albicans* intravenous 10^{-10}cells/mL. *Candida* 2: Induced *C. albicans* intravenous 10^{-12}cells/mL

Table 2: *Post hoc* test for coronary artery wall thickness and Mann–Whitney test for atherosclerotic cap

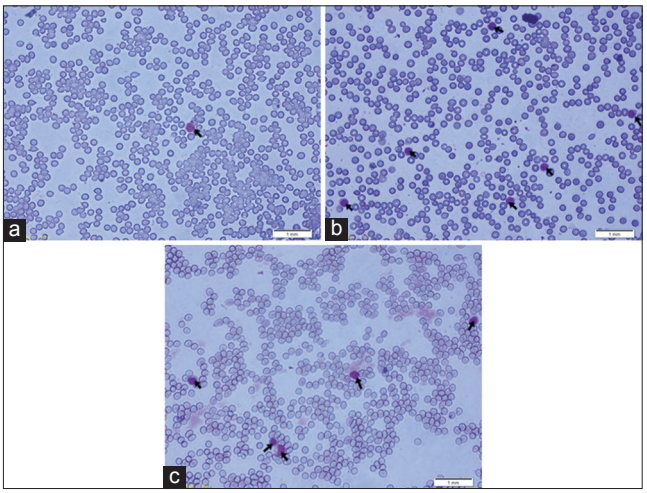
Groups	*Post hoc* test Sig. (p)	Mann–Whitney test Sig. (p)
Control-*Candida* 1	0.002*	0.002*
Control-*Candida* 2	0.157	0.234
Candida 1-*Candida* 2	0.187	0.105

*: Significant difference ($p<0.05$). Control: Not induced *C. albicans. Candida* 1: Induced *C. albicans* intravenous 10^{-10}cells/mL. *Candida* 2: Induced *C. albicans* intravenous 10^{-12}cells/mL

Table 3: Observations of cap atherosclerotic and lipid deposition on coronary artery

Groups	Atherosclerotic cap percentage (%)	Lipid deposition percentage (%)
Control	0	50
Candida 1	87.5	100
Candida 2	37.5	62.5
Kruskal–Wallis test Sig.(p)	0.002*	0.081

*: Significant difference ($p<0.05$). Control: Not induced *C. albicans. Candida* 1: Induced *C. albicans* intravenous 10^{-10}cells/mL. *Candida* 2: Induced *C. albicans* intravenous 10^{-12}cells/mL



(Fig. 1: Leukocyte cells, with Giemsa stain (amplification ×400) in rats blood smear. Control group (a), the number of leukocyte is normal. The increase in the number of leukocyte cells higher in *Candida* 1 group (b) than *Candida* 2 (c) (black arrow)

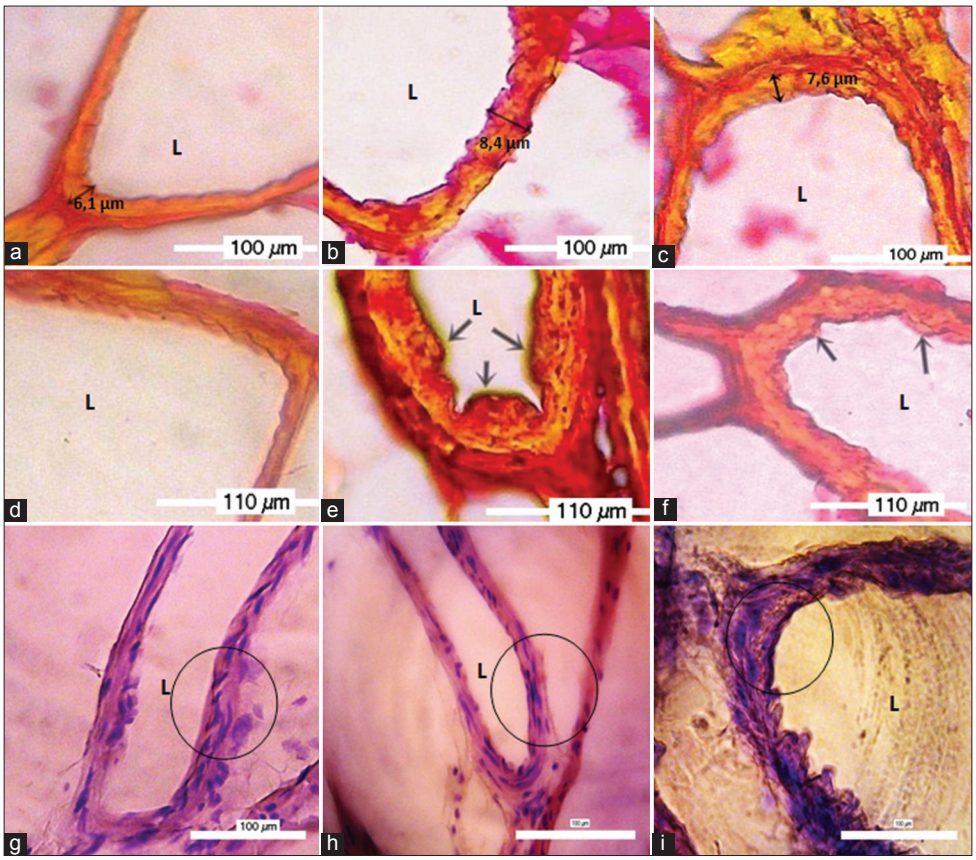


Fig. 2: Histological coronary artery using Picrosirius Red staining (×1000). Control group wall thickness of 6.1 µm (a). *Candida* 1 group wall thickness of 8.4 µm (b). *Candida* 2 group wall thickness of 7.6 µm (c). Atherosclerotic cap, in the control group (d) was not found. *Candida* 1 (e) and *Candida* 2 (f) were found atherosclerotic cap (black arrow). In the control group (g), *Candida* 1 (h) group and *Candida* 2 (i) group were found morphology of lipid deposition (dark circle) (Sudan IV staining; ×1000). L = Lumen

of hyphae leads to vascular inflammation that induces an increase in the number of leukocytes [13-15].

The results showed that there was an effect of *C. albicans* infection that was injected intravenously to the formation of coronary atherosclerosis lesion seen from three parameters, i.e., coronary artery wall thickness, the presence of cap coronary artery, and foam cell in coronary artery wall. The results of this study are supported by previous epidemiological studies that prove that fungal infection of *C. albicans* is associated with an increased risk of developing cardiovascular disease [10-11,16]. Jegier *et al.* explained that there is DNA *C. albicans* in 58% of samples of patients with coronary heart disease identified by the PCR method [10].

The measurement results showed coronary artery thickening in the *Candida* group compared to the control group. The thickening of the coronary arteries is due to endothelial dysfunction caused by endothelial injury. It happens because there is hyphae penetration of *C. albicans* into the blood vessels. Endothelial dysfunction will express certain adhesion molecules, which facilitate leukocytes, monocytes, and T-lymphocytes to attach to the arterial wall and penetrate the intima with the help of monocyte chemoattractant protein-I and T-cell chemoattractant. Monocytes will differentiate and become active macrophages, so they can secrete pro-inflammatory cytokines and growth factors that also play a role in the thickening of artery walls. Macrophages will perform phagocytosis in LDL-oxidized (ox-LDL) to form a foam cell. The presence of foam cells in the subintimal layer and the increasing number of monocytes or macrophages leads to adaptive thickening of artery walls [17,18]. The ratio of coronary artery wall thicknesses is not different significantly between the groups of *Candida* 2 and the *Candida* 1 and control groups; it is possibly due to the concentration of *C. albicans* in the group of *Candida* 2 is too low and close to the group of *Candida* 1 so that the systemic severity is less visible [19].

The results of the identification of coronary artery limbs in this study show that in the treatment group 1, it was found more cap than other groups. Cap in the arteries is formed due to the accumulation of inflammatory cells, foam cells, necrotic cells, calcium, and smooth muscle cells. Inflammatory cells, especially T-lymphocytes, are increased due to endothelial dysfunction caused by vascular inflammation. T-lymphocytes along with growth factor will induce the proliferation of smooth muscle cells which is the largest composition of the cap coronary artery (68%) [20,21]. At the time of atherosclerosis process progresses, endothelial cells, macrophages, and smooth muscle cells develop apoptosis or necrosis into necrotic cells that will make cap get worse. The process of calcification in the blood vessels also occurs and affects the formation of the cap, but this process is influenced by the age factor. Increasing age causes the calcium metabolism (calcium) disturbed. It makes much calcium circulates with blood (hypercalcidemia) and deposits in the blood vessel wall [22-24].

This study found morphology of lipid deposition in all groups, especially in the group Candida 1. Lipid deposition is an accumulation of *foam cells* formed due to the response of vascular inflammation caused by *C. albicans*. Vascular inflammation causes endothelial dysfunction which can cause the increase of endothelial permeability. This process makes the entry of LDL into the arterial wall, and it is oxidized by reactive oxygen species to be ox-LDL. Endothelial dysfunction increases pro-inflammatory cytokines IL-1 and TNF-α production. Those substances induce adhesion molecule interstitial cell adhesion molecule and vascular cell adhesion molecule-1 expression on the endothelial layer. This makes monocytes easier to attach to the

surface of endothelial. The attached monocytes migrate among cells on intercellular junction with the help of platelet endothelial cell adhesion molecule-1 to the subendothelial layer. Monocytes will differentiate into macrophage [25]. Ox-LDL will be phagocyted by macrophages through the scavenger receptor (predators) in the cell surface. That causes the formation of peroxide lipid facilitating the accumulation of cholesterol that forms the foam cell. The foam cells are the agents which form lipid deposition subendotel [26,27]. Lipid deposition is an early symptom of atherosclerosis and is triggered by several factors, one of the factors is age. This is the reason why the deposition of lipids was also found in the control group. Elderly age may increase the risk of oxidative stress that can decrease the bioavailability of nitric oxide (NO). The decreasing levels of NO cause vasoconstriction of blood vessels and endothelium to be more proaterogenic [28-30].

CONCLUSION

This research shows that an intravenous induction of *C. albicans* can increase of atherosclerotic lesions in the coronary arteries characterized by the thickening of the walls of the coronary arteries, cap morphology, and lipid deposition in the coronary arteries.

AUTHORS' CONTRIBUTION

Rendra Chriestedy Prasetya conducted this research, Nadie Fatimatuzzahro and I Dewa Ayu Susilawati planted and designed this study. The last, Ayu Prativia Yonenda supported the conduction of the study.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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