Differences in Caspase-3 Expressions of Liver Organs and Spleen in Animals Model Rattus Norvegicus Infected with Candida Albicans and Candida Non Albican Fungus

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Keywords: Apoptosis, Caspase-3, *Candida Albicans*, *Candida Non Albicans*.

Abstract: Invasive candidiasis is an opportunistic infection as the leading cause of morbidity and mortality in immunocompromised patients. Candida albicans is the dominant species causing all cases of invasive candidiasis. However, other species namely Candida Non Albicans also play a role. High-grade Caspase signifies the hemeostasis system can not maintain the organ system causing multiple organ dysfunction syndrome (MODS). Caspase-3 plays a role in the process of apoptosis in extrinsic processes. The aim of this study was to look at caspase-3 expression in the liver and lien of experimental animals of Rattus norvegicus indicating the severity of Candida albicans infection and Candida non albicans. The healthy rats were randomly selected and injected the peritoneum in quadrant 3 to 1 ml (PZ, Suspension C. Albicans, C. Non Albicans) in each group of experimental animals. Mice were observed for 24 hours. After 24 hours of surgery for the removal of liver and liver organ. The organ tissue of the mouse was fixed into the formalin buffer and tissue preparation was performed for Caspase-3 Immunohistochemistry. The median values of the Caspase-3 liver index of C. albicans leprosy group (12.5), C. Non Albicans (10), and the control group (9.5) median value of caspase-3 organ index of C. albicans (31), C. Non Albicans group (28.5), and control group (4).

1 INTRODUCTION

Invasive candidiasis, including candidemia, and hepatosplenic candidiasis in these three decades has been a life-threatening opportunistic infection and a major cause of morbidity and mortality in critically ill patients. Crude mortality rate of patients with invasive candidiasis between 35% and 60%, ICU patients with candidemia have a higher mortality rate than non-ICU patients (Calderone, 2012).

Candidemia is the most common clinical manifestation of candida species. The development of new medical therapies, chemotherapy, increased invasive medical procedures, the emergence of human immunodeficiency virus (HIV) and AIDS, and the use of broad-spectrum antibiotics, leads to increased immunity of many species of candida albicans or candida non albicans, especially in patients with prosthetics, children or post-operative critical patients, and patients with various types of solid organ transplants. Despite major advances in anti-fungal therapy, invasive candidiasis remains a sustainable public health problem (Calderone, 2012).

Candida infections of the liver and spleen are referred to as hepatosplenic syndrome candidiasis (chronic disseminated candidiasis). The liver becomes the target of infection in systemic fungal infections through several mechanisms. First, a fungus of an appropriate size, such as a portion of the histoplasm capsulatum, is taken up by reticuloendothelial cells. Second, because of the volume of blood flow through the liver, the fungus that is spread through the bloodstream (blood stream infection) will infect the liver. Third, certain fungi, especially candida albicans and candida non albicans, allegedly penetrate the gastrointestinal mucosa of a severely ill patient and spread directly to the liver through the portal vein before attacking other organs. Another mechanism in which the fungus can cause damage to hepatocyte cells is

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through mycotoxicosis resulting from the toxicity of the fungus. Aflatoxin, patulin, and ochratoxin A enzymes produced by fungi can cause liver necrosis or cirrhosis (Juan, 2008).

This condition can trigger apoptosis characterized by increased caspase 3 in various innate and adaptive immune cells. The occurrence of apoptosis in various vital organs (spleen, lung, kidney and liver) by candida albicans and non-albicans candida can not be explained. While the incidence of candidemia continues to increase where nearly 15% of patients are treated in intensive care with severe sepsis candidemia (Yapar, 2014).

Increased apoptosis was associated with increased candida attachment to neutrophil plasma membrane, indicating that the increase in apoptosis caused by candida was associated with increased apoptotic enzyme activity interacting with cell membranes, with increased caspase activity 3. Membrane receptors from the Fas/CD95 family transmitted apoptotic signals in response to attachment to their physiological ligand, including Fas ligand and tumor necrosis factor (Rotstein, 2000).

We will report differences in caspase-3 expression in liver and spleen organs of Rattus norvegicus animals infected with C. albicans and C. Non Albicans fungus.

2 MATERIALS AND METHOD

Animals Model

Mouse Rattus norvegicus with criteria of normal/healthy male rats not affected by infection is characterized by agile movement, 3-month-old rat, 200-250 gram weight.

Fungus

Fungus C. albicans and C. Non albicans obtained from the Installation of Clinical Microbiology RSUP Dr. Sutomo Surabaya. Created a concentration of 105 CFU bacteria with Phosphate-buffer saline (PBS).

Sample

The healthy rats were randomly selected and labeled each group consisting of 4 mice. Inject the fungus on the peritoneum in quadrant 3 to 1 ml (PZ, Suspension C. albicans and C. Non Albicans) in each group of animals, try. Rats are observed for 24 hours. After 24 hours, surgery for the removal of liver and spleen. Rat organ tissues were fixed into the formalin buffer and tissue preparation (formalin fixed and paraffin embedded section) was performed.

Immunohistochemistry Caspase-3

Cut sections at 4um and place on pre-cleaned and positively charged microscope slides. Heat in a tissue-drying oven for 45 minutes at 60°C. Wash slides 2 times in Xylene for 3 minutes. Wash slides in Xylene 1:1 with 100% ethanol for 3 minutes. Wash slides 2 times in 100% ethanol for 3 minutes. Wash slides 2 times in 95% ethanol for 3 minutes. Wash slides in 70% ethanol for 3 minutes. Wash slides in 50% ethanol for 3 minutes. Rinse slides gently with running distilled water for 5 minutes. Boil slides in 0.01M sodium citrate buffer (pH6) at 100°C for 15-20 minutes. Remove the slides from heat and allow them to stand at RT in buffer for 20 minutes. Rinse twice with TBST for 5 minutes. Block with endogenous peroxidase with 3% hydrogen peroxide for 30 minutes. Block with 5% serum or BSA for 2 hours. Drain blocking buffer from slide. Incubate slides with the diluted primary antibody overnight at 4°C with gentle agitation. Wash slides 2 times with TBST for 5 minutes. Incubate slides with diluted conjugated secondary antibody for 2 hour at RT with gentle agitation. Wash slides 2 times with TBST for 5 minutes. Develop with chromogen for 10 minutes. Wash slides in distilled water for 1 minute. Counterstain (if required). Dehydrate when using a chromogen substrate that is alcohol insoluble by washing slides in 80%, 95%, 100% and Xylene each for 1 minute at RT. Mount coverslips.

3 RESULT

Spleen Organ

The result showed that the average number of caspase-3 cells in the lymph organ, treatment group in C. albicans infection was higher than treatment group in C. non albicans infection and higher than the control group (Figure 1).

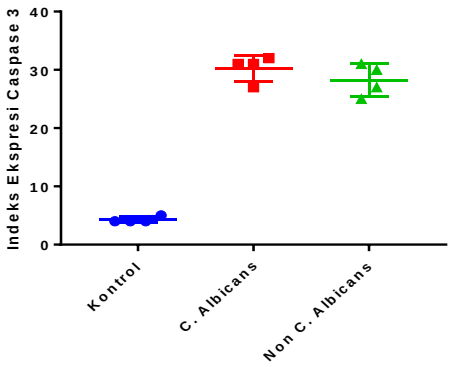

Figure 1 : Box-Plot Number of Cells Experiencing Caspase-3 in the Spleen.

The highest and lowest median values were C. albicans (31), C. non albicans (28.5), and control group (4), respectively. The description of terapoptotic cells observed under a light microscope with 100x objective lens enlargement in 10 field of view is as follows:

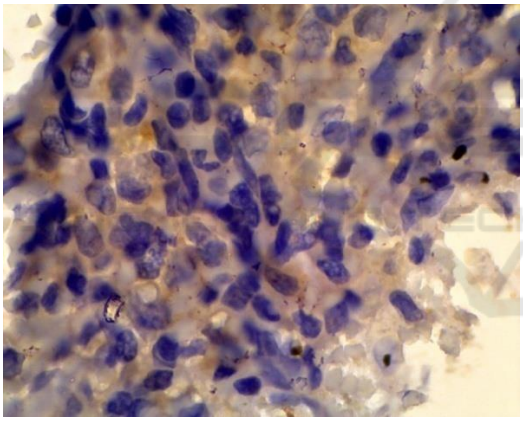

Figure 2 : Normal Cell Overview of the Spleen Tissue (Rattus norvegicus) Control Group.

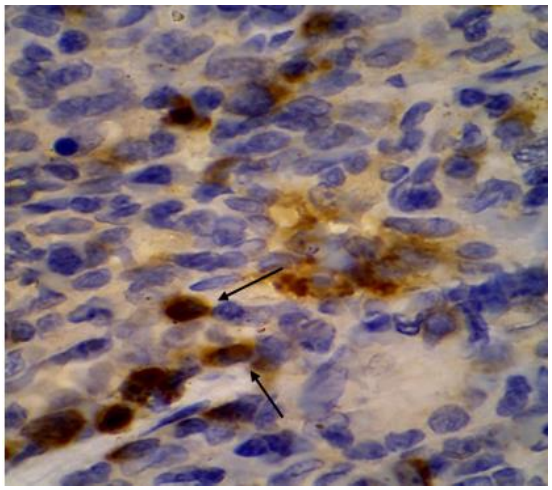

Figure 3 : Expression of Caspase 3 Lymphocyte Cells in the Spleen (Rattus norvegicus) tissue of C. albicans group at 1000x magnification. Brown cells are undergoing apoptosis.

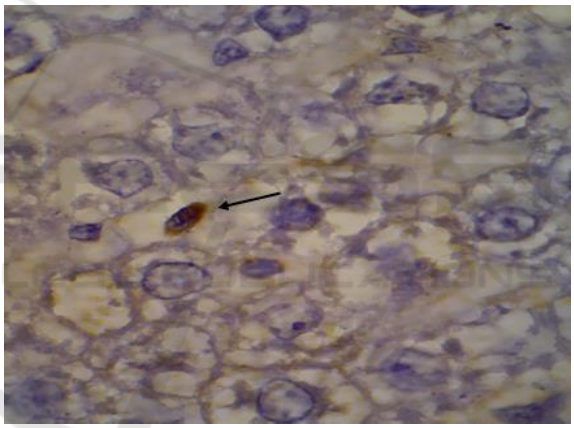

Figure 4 : Expression of Caspase 3 Lymphocyte Cells in Spleen Network (Rattus norvegicus) group C. non albicans at 1000x magnification. Brown cells are undergoing apoptosis.

Liver organs

From the research, it was found that the average number of caspase-3 cells in Liver organ, the treatment group in C. albicans infection was higher than the treatment group in C. non albicans infection and higher than the control group.

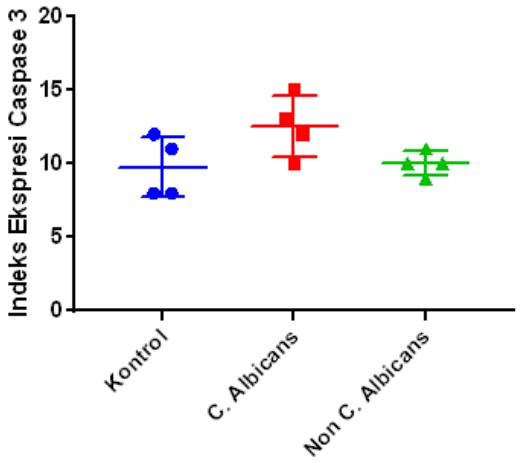

Figure 5 : Box-Plot Number of Cells Experiencing Caspase-3 on the Liver.

The highest and lowest median values were C. albicans (12.5), C. non albicans (10), and control group (9.5), respectively. The description of terapoptotic cells observed under a light microscope with 100x objective lens enlargement in 10 field of view is as follows:

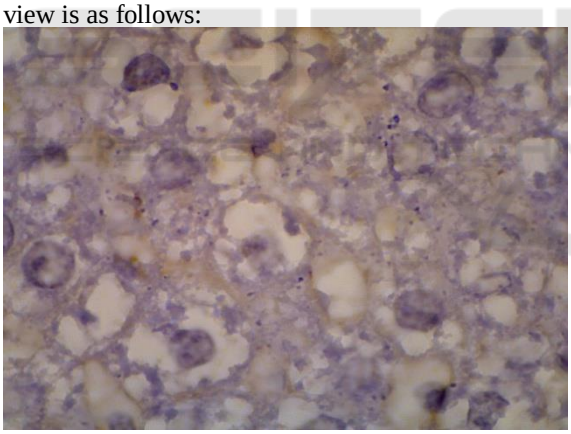

Figure 6 : Normal Cell Overview of the Liver Tissue (Rattus norvegicus) Control Group.

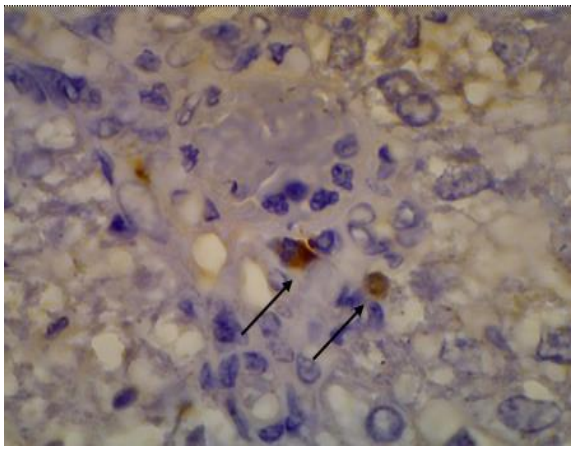

Figure 7 : Expression of Caspase 3 Lymphocyte Cells in Liver tissue (Rattus norvegicus) of C. albicans group at 1000x magnification. Brown cells are undergoing apoptosis.

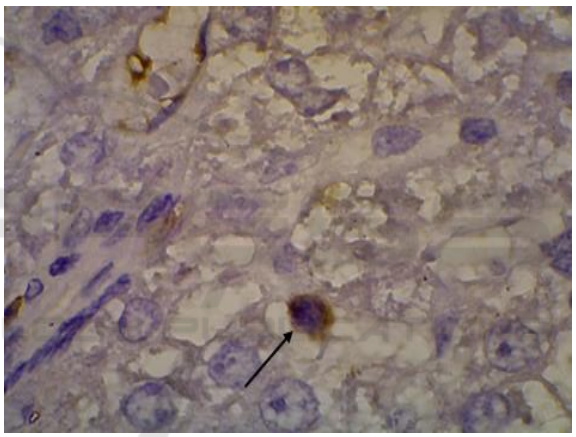

Figure 8. Expression of Caspase 3 Lymphocyte Cells in Liver tissue (Rattus norvegicus) group C. non albicans at 1000x magnification. Brown cells are undergoing apoptosis.

4 DISCUSSION

From the results of the research data in the controlled animals, C. Albicans and C. Non albicans all can survive within 24 hours. During infection, C. albicans forms colonies on the host with various environmental differences, e.g. availability of nutrients, pH, hypoxia and CO2 levels. One of the main abilities of C. albicans as a successful pathogen is the adaptability to successfully evolve under different host conditions. The microenvironment of hosts has a heterogeneous carbon source and, when migrating between different environments, C.

albicans can adapt to use alternative carbon sources simultaneously, for survival and virulence. The first step of the host defense system is to recognize pathogens that attack by the innate immune system. The introduction of pathogenic microorganisms occurs through the chemical characteristics possessed by these pathogens, these chemical characteristics are called pathogenic molecular patterns (PAMP). This PAMP will be recognized by specific immune receptor systems known as pathogen introduction receptors (PRRs). Therefore, the host is able to regulate immune defenses against pathogens-specific. The cell populations of the innate immune system involved in the introduction of C. albicans are monocytes, macrophages, dendritic cells and neutrophils. These cells express PRRs in different patterns, allowing hosts to make special defenses against C. albicans.

Candida cell wall consists of inner layers of polysaccharides (chitin, 1,3 β-glucan and 1,6-β-glucan) and an outer layer of glycosylated protein with mannan composing PAMP. The main PRRs involved in the introduction of C. albicans are Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). TLR2 recognizes phospholipomannans, TLR4 recognizes O-linked mannans, CLR dectin-1 recognizes β-glucans and macrofag manrose receptor CLR (MR) and dendritic (DC) cells Specific-ICAM3-grabbing non-integrin (DC Sign) recognizes N-linked mannans. Macrofage-inducible CLR (Mincle), expressed primarily in macrophages, is a receptor for C. albicans and plays a role in macrophage response to C. albicans. Recently, the activation of inflammation through the nucleotide-binding oligomerization (NOD) -like receptor (NLR) domain containing pyrin 3 (NLRP3) and protein-associated receptor 5 (MDA5) RigI-helicase (RLR) melanoma receptors has also been shown to be involved in host defense as anti-candida. MDA5 is known as a viral RNA identifier and plays a role in anti-viral immunity, but recently that MDA5 is also involved in anti-fungal defenses (Linda, 2018).

PAMP activation with specific PRR causes activation of the innate immune system, promoting the production of pro-inflammatory cytokines and chemokines, and activation of NLRP3 inflammation, which processes pro-interleukin (IL) -1β and pro-IL-18 into their active biologic form, inflammatory activation then the adaptive immune response will work. Furthermore, this pathway potentiates phagocytosis and killing of C. albicans, especially by neutrophils. Cell dendritic APC cells play an important role in the activation of response from T cells. The specific T-Helper cells for Candida (TH-cells) consist of TH1-cells and TH17-cells. TH1-cells are induced by IL-18 and produce interferon-gamma (IFN-γ). TH17-cells are induced by IL-1β and produce IL-17 and IL-22. IFN-γ is important for the activity of neutrophil fungicides and macrophages. IL-17 and IL-22 induce neutrophil recruitment and activate neutrophils and epithelial cells and induce the release of antifungal β-defensin. Activation through an intracellular MDA5 PRR induces a signaling pathway leading to the production of type I interferon. Recent studies have found that this interferon disrupts the cytokine response of adaptive defense cells, induced by C. albicans, from the TH17 response in response to TH1 response. The host microbiomal composition may have a significant impact on Candida colonization, invasion and host defense against Candida (Linda, 2018).

One of the markers of invasion of candida albicans is the change of yeasts into the hyphae (filaments). The change of yeast form to hyphae is strongly influenced by the host cell microenvironment detected by candida albicans during the invasion process. The ability to change morphology is an important factor in determining the infection and spread of candida albicans in host tissues. Mutant Saccharomyces cerevisiae and non-pathogenic candida albicans can not form hyphae and invade endothelial cells while pathogenic candida albicans can form germ tube and intracellular hyphae. The yeast form makes candida albicans easier to spread than the hyphae forms while the hyphae forms facilitate candida albicans to penetrate the host body (Gow, 2013).

Phagocyte cells, such as macrophages and neutrophils, are a major defense against fungal infections and are essential for preventing invasive candidiasis. Macrophages are phagocyte cells that play an important role in a major response to pathogens, maintaining tissue homeostasis, promoting and resolving inflammation and tissue repair processes. The relevance of phagocyte cells to C. albicans infection is widely studied for being able to swallow fungi and eliminate it, then if necessary, trigger recruitment and activation of other immune cells (Khan, 2010).

The phagocyte cells of C. albicans may experience apoptosis when subjected to various types of stress, such as antifungal drug treatment or exposure to antimicrobial peptides. Apoptosis in C. albicans phagocyte cells is characterized by chromatin condensation, DNA fragmentation and phosphatidylserine externalization, and Mca1's proteases also have been shown to play an important

role in apoptotic processes through farnesol and H2O2 in C. albicans phagocyte cells. The induction and execution of apoptosis involves a system that includes enzymes, regulatory genes and receptors. Among these systems there are caspases divided into 2 groups, namely caspase initiator (caspase-2, 8, 9, 10) and caspase executor (caspase-3, 6, 7). Among the 14 caspases present, caspase-3 is a key factor of apoptotic execution. Caspase-3 can enter the nucleus through the pores made by caspase-9, releasing substrates that cause DNA degradation. There are two pathways of apoptosis involving caspase, an extrinsic pathway that occurs when the apoptotic signal received by death receptor is strong enough. When the signal is not strong enough, it will be emitted to the mitochondria, the release of cythochrome-c, which will then activate caspase-3 to execute apoptosis. The second line is called the intrinsic path (Khan, 2010).

Positive caspase-3 cells in liver and spleen organ in experimental animals C. Albicans is higher than that of C. Non albicans infected animals caused by almost every component of the Candida cell wall having a role in the interaction between host and pathogen. The introduction of immune cells to Candida is initiated by PAMP involvement recognized by PRRs. This recognition event is dominated by the carbohydrate binding of the cell wall of the fungus. The secreted aspartyl proteinase (Saps) enzyme has been shown to have many important roles. Sap2 inactivates the H-Factor and CR3 and CR4 complement receptors in macrophages, thus mediating the release of C. albicans from recognition by innate hostate immune cells. Sap2 and Sap6 function to induce production of inflammatory cytokines by the host i.e. IFN type I, caspase-11 induction and activation of NLRP3 inflammasome. After Candida is absorbed by the host epithelial cells, Saps4-6 specific to hypha causes lysosomal permeabilization and triggers the dependent apoptosis of caspase-1. These results suggest that the ability of C. albicans to induce apoptosis early in macrophages can further induce or modulate the anti-inflammatory pattern of subsequent immune responses (Wu H, 2014).

5 CONCLUSION

Caspase-3 expression in fungal infections C. Albicans and fungal infections C. Non Albicans either in the liver or liver organ is higher than control. Caspase-3 expression in fungal infections C. albicans is higher than fungal infection C. Non Albicans is good in spleen or liver organ. These results suggest that the ability of C. albicans to induce apoptosis early in macrophages can further induce or modulate the anti-inflammatory pattern of immune responses thereafter.

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