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Relationship of CD4 Cell Counts and History of Diarrhea with *Cryptosporidium sp.* on HIV/AIDS Patients in West Sumatra Indonesia

Goldha Faroliu[1]; Nuzulia Irawati[2]; Netti Suharti[1]
[1]Faculty of Medicine, Andalas University, Padang 25138, Indonesia
E-mail address: goldhavaroliu@gmail.com
[2]Parasitology Laboratory, Faculty of Medicine, Andalas University, Padang 25138, Indonesia
E-mail address: nuzuliairawati03@gmail.com

Abstract
Opportunistic infections are infections that occur due to a decrease in the immune system. This infection can be fatal in people with weak immune systems, especially people with HIV/AIDS. *Cryptosporidium sp.* is one of the opportunistic intestinal protozoa that is generally asymptomatic and is self-limited in immunocompetent individuals, but in individuals with an immune deficiency often results in chronic diarrhea, causing dehydration and malnutrition which can increase mortality and morbidity in HIV patients. Observational study with cross-sectional design on 50 fecal samples of HIV/AIDS patients. *Cryptosporidium sp.* infections performed by staining Ziehl Neelsen, and looking at the relationship of a history of diarrhea with *Cryptosporidium sp.* The results obtained 2 respondents (4%) positive infected with *Cryptosporidium sp.*, There is a significant relationship between CD4 cell counts with *Cryptosporidium sp.* infection, p-value=0.001 ($p<0.05$) and there is a significant relationship between *Cryptosporidium sp.* infections with a history of diarrhea, p-value=0.045 ($p<0.05$).

***Keywords**: Cryptosporidium sp.*, history of diarrhea, HIV/AIDS, CD4 cells.

1. Introduction

Opportunistic infections are infections that occur due to a decrease in the immune system (Agarwal, *et al*., 2015). Such infections generally do not cause disease in people with a normal immune system but can be fatal in people with weak immune system such as in people with HIV/AIDS. HIV is a type of virus that attacks the human immune system. CD4 T lymphocyte cells are the target cells for HIV infection, a decrease in the number and function of CD4 T cells due to the cytopathic effect of HIV infection, with a decrease in immune status (CD4) so various microorganisms such as bacteria, viruses, and protozoa tend to grow and multiply causing secondary infections and even fungal infections (Mandal, 2008).

According to data from UNAIDS (United Nation Programme on HIV/AIDS) an increase in cases of HIV/AIDS in the world. In 2018 around 37.9 million HIV patients were discovered in the world, with 1.7 million new patients and 770,000 dying from AIDS. Based on their background, HIV/AIDS patients come from commercial sex workers (6%), injecting drug users (12%), homosexuals (17%), transgender peoples (1%), sex worker clients (18%), and prisoners (46%). Most HIV/AIDS patients are in the Eastern and Southern Africa Region with a number reaching 20.6 million patients. While Asia Pacific increased from the third position in 2017 to second place in 2018 with the number of patients reaching 5.9 million, with 310,000 new patients, and 200,000 suffering from AIDS. Backgrounds of HIV/AIDS patients in Asia Pacific include commercial sex workers (8%), injecting drug users (13%), homosexuals (30%), transgender peoples (2%), sex worker clients (25%), and prisoners (22%) (UNAIDS, 2019).

The cumulative number of HIV infections in Indonesia reported up to June 2018 is 640,443 people, 46,000 patients are new patients and 38,000 are reported to have died from AIDS, this number has increased compared to the last 10 years, while West Sumatra ranks eleventh. Reports of cases of HIV infection by the

West Sumatra Provincial Health Office up to 2018 was 1890 patients. Based on the report from the Padang City Health Office in 2018, in 2017 there were 370 HIV cases, the number of HIV/AIDS cases increased compared to 2016, which was 300 cases and 30% of these cases were caused by Male Sex Men (MSM) / gay. AIDS cases were 93 cases and also increased from the previous year by 56 cases (UNAIDS, 2018; Health Ministry of Indonesia, 2018)

HIV progressive infection will eventually cause a progressive decline in immunity due to the disruption of CD4 T lymphocytes. So that the marker of progression from HIV disease in addition to clinical symptoms, shown by the rapid decline in the number of CD4 T lymphocytes due to cell lysis by HIV virus. And the decrease in the number of CD4 T lymphocytes is also caused by the occurrence of CD4 T cell apoptosis, where CD4 T lymphocytes commit suicide when stimulated by an activating signal. CD4 T lymphocytes are no longer able to divide, resulting in a phenomenon called anergy and the formation of the syncytium, where uninfected CD4 T lymphocytes fuse with infected cells thereby eliminating many uninfected cells (Murtiastutik, 2008; Kumar, 2009).

With a decrease in immune status (CD4), various microorganisms such as bacteria, viruses, and protozoa tend to grow and multiply causing secondary infections and even fungal infections. Nevertheless, fungal infections can also occur together with infections due to bacteria, viruses, and protozoa (Mandal, 2008)

Cryptosporidiosis is a zoonotic disease caused by a parasite of the genus *Cryptosporidium* which is a pathogenic organism. *Cryptosporidium sp*. can cause acute diarrhea in humans or animals by infecting the small intestine. In individuals with immune deficiencies often result in mild to severe symptoms resulting in chronic diarrhea and can increase in the mortality rate of HIV patients because of their role in opportunistic infections in AIDS patients (Prasetyo, 2015).

In the research of Wahdini *et al* (2016), *Cryptosporidium sp*. is the most parasite found in the feces of HIV patients. In the research of Masarat (2012) found 80% of cases of cryptosporidiosis in HIV patients. In 2017 the examination of *Cryptosporidium sp*. in HIV/AIDS patients at Dr. M. Djamil Padang Hospital about the relationship between CD4 cell count and *Cryptosporidium sp.* through microscopic examination, 22 of 42 samples were detected by *Cryptosporidium sp.* oocysts. The study also found that the tendency for these infections is directly proportional to the lower CD4 cell count (Masarat, *et al*., 2012; Wahdini, *et al*., 2016; Handayani, 2017).

2. Materials and Methods

2.1 Instruments

Fecal samples of HIV/AIDS patients at Dr. M. Djamil Padang Hospital Indonesia, methanol, carbol fuchsin, alcoholic acid, and malachite.

2.2 Materials

Microscopic examination with Ziehl Neelsen's acid-resistant staining: fecal sample pots, cotton swabs, object glass, cover glass, spiritus, staining rack, emersion oils, and microscopes.

2.3 Methods

Each respondent signed the informed consent and each respondent's secondary data was collected from the medical record, namely CD4 cell count, take ARV therapy / no, and HIV risk factors (heterosexual, gay, sex workers). Each respondent was asked to collect a fecal sample using a ±5 grams sample pot provided. Furthermore, the samples were taken to the Parasitology Laboratory of the Faculty of Medicine, Andalas University for examination.

Ziehl Neelsen's examination was carried out using a microscope. Make preparations by taking the fecal with size of green beans with a cotton swab and blotted on glass objects, allow to dry at room temperature. Fixation above the flame of spiritus. Fixation with methanol for 5 minutes. Dry at room temperature. Place the preparations on the staining rack. Drop the carbol fuchsin until it floods the entire surface of the preparation for 15 minutes then washes with running water. Drop the alcoholic acid solution (HCl-methanol) for the decolorization process until there is no more carbol fuchsin coloring for 3 minutes, then rinse again using running water. Drop the malachite solution over the entire surface of the preparation for 30 seconds to 1 minute. Wash the preparation again with running water. Dry it on its side in the open air. Give one drop then cover with a glass cover, and observe the oocyst under a microscope at 1000x magnification. Oocysts will appear pink to red with a size of 4-6 µm (measurements are made with a microscope equipped with a micrometer lens).

2.4 Data Analysis

Bivariate analysis was performed to see the relationship between the respondent's CD4 cell count and *Cryptosporidium sp.* infections on Ziehl Neelsen staining. Variables with ratio and nominal data were tested with Independent Sample T-Test, while data with both nominal variables were tested with Chi-Square. Significant relationship if the p-value < 0.05.

3. Results and Discussion

In this study, 50 respondents with HIV/AIDS were obtained from the VCT Polyclinic Dr. M. Djamil Padang Hospital. Distribution of patients with a CD4 count of ≤200 cells/µL as many as 25 people (50%) and >200 cells/µL as many as 25 people (50%), with the lowest CD4 count of 5 cells/µL, respondents with symptoms of diarrhea were 4 people (8%), and with a history of diarrhea in the last 2 weeks of 11 people (22%). In this study, respondents who were positive infected with *Cryptosporidium sp.* had a history of diarrhea in the past 2 weeks.

Table 1. Relationship of CD4 Cell Counts with *Cryptosporidium sp.* infections

Result	Mean ± SD CD4 (cells/µL)	*p of Variances*	*p-value*	N
Positive	150 ± 0	0,027	0,001	2
Negative	206,79 ± 110,668			48

In this study the results of the examination of *Cryptosporidium sp.* using Ziehl-Neelsen, a positive total of 2 respondents (4%) was obtained with a CD4 cell count of ≤200 cells/µL. The average CD4 cell count in positive respondents infected with *Cryptosporidium sp.* was 150±0 cells/µL, while the average CD4 cell count in respondents who were negative for *Cryptosporidium sp*. infection was 206.79±110.668 cells/µL, from the results of statistical tests using the Independent Sample T-Test to determine the relationship between CD4 cell counts and *Cryptosporidium sp*. infection with p-value=0.001 (p<0.05) showed a relationship between CD4 cell count and *Cryptosporidium sp.* infection. With a significant difference between CD4 cell counts in respondents who were positive infected with *Cryptosporidium sp.* with respondents who were negative infected with *Cryptosporidium sp.*

In accordance with the research of Gupta *et al*., from 100 respondents of HIV patients found 4 respondents (4%) infected with *Cryptosporidium sp.* and 4 of these respondents had a CD4 cell count of ≤200 cells/µL, (Gupta, *et al*, 2013).

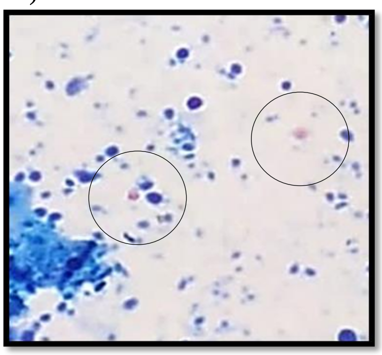

Figure 1. Microscopic Results No. 22 (1000x magnification)

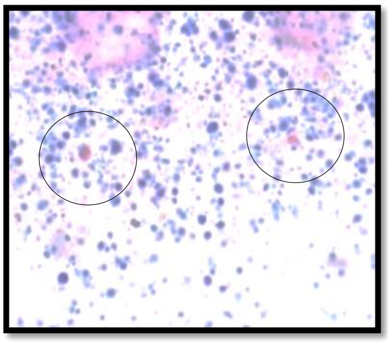

Figure 2. Microscopic Results No. 29 (1000x magnification)

Cryptosporidium sp. microscopically done with Ziehl Neelsen's acid-resistant staining. The Ziehl-Neelsen staining was chosen because it is a method choice that is quite simple, inexpensive, and provides quite high sensitivity. Ziehl-Neelsen's examination serves to determine the reaction of bacterial cell walls through a series of staining. Groups of acid-resistant bacteria and other microorganisms that have lipid content in cell wall are so thick that they cannot be colored with ordinary coloring reactions, but must be with acid-resistant staining and can maintain dyes when washed with a pale solution.

The sample preparation is fixed with methanol over a flame of spiritus, this heating fixation aims to widen the pores of the microorganism's lipid so that carbol fuchsin as a staining agent can enter. In giving the first color of carbol fuchsin, smear and thick cell wall microorganisms maintain it. Carbol fuchsin is a basic fuchsin dissolved in 5% phenol solution and gives a red color. Phenol is used as a solvent to help introduce dyes into microorganism cells. When the cells are able to absorb the color of carbol fuchsin, the cell wall will be closed again at its original temperature. So that before adding the alcoholic acid, wait up to 15 minutes. Next is the addition of alcoholic acid which functions to rinse or wear off dye (decolorization) on microorganism cells. When adding this alcoholic acid, the color of carbol fuchsin in thin cell wall microorganisms will fade because it is not able to bind strongly as acid-resistant bacteria and thick cell wall microorganisms. Then wash with running water to close the pores and stop blanching. Then the addition of a second dye is malachite which is a counter dye or secondary dye. This substance functions to dye back cells that have lost the main coloring after treatment with alcoholic acid. Malachite enters into thin cell wall microorganisms whose cell wall permeability is enlarged due to the lipid layer of thin cell wall microorganisms extracted by alcoholic acid, thus causing the cell wall of the thin cell to turn blue. In thick cell wall microorganisms, the cell walls are dehydrated by alcohol treatment, pores shrink, cell wall permeability and membranes decrease so that the methylene blue dye cannot enter so that microorganisms with thick cell wall will remain red in color.

Cryptosporidium sp. reported as a gastrointestinal opportunistic infection in people with HIV/AIDS and can increase mortality and morbidity especially in patients with low CD4 T-cell counts. With a decrease in immune status (CD4), various microorganisms such as bacteria, viruses, fungal, and protozoa tend to grow and multiply causing secondary infections, especially in individuals with HIV infection will be at risk of being infected with *Cryptosporidium sp.* if CD4 is ≤400 cells/µL. According to the study of Mitra *et al* (2016), respondents with CD4 counts ≤350 cells/µL were more infected with *Cryptosporidium sp.* compared to other parasites.

Table 2. Relationship of *Cryptosporidium sp.* with a history of diarrhea

Result	History of Diarrhea		Total (%)	*p*
	Present	No		
Positive (%)	2 (4%)	0 (0%)	2 (4%)	0,045
Negative (%)	9 (18%)	39 (78%)	48 (96%)	
Total	11 (22%)	39 (78%)	50 (100%)	

In this study, respondents who had a history of diarrhea in the last 2 weeks tested positive with *Cryptosporidium sp.* infections, 2 out of 50 respondents (4%) in patients with a history of diarrhea positively infected with *Cryptosporidium sp.* (Table 2), based on statistical tests showing a significant relationship between *Cryptosporidium sp.* infections with a history of diarrhea.

Cryptosporidium sp. in immunocompetent individuals, the duration of diarrhea ranges from 2-21 days. Oocyst removal can still take up to 8-50 days (average 12-14 days) after improvement in clinical symptoms. In immunocompromised diarrhea can occur more severe and longer, reported more than 4 months and years (3 years). According to the research of Wahdini *et al* (2016), *Cryptosporidium sp.* is the most parasite found in the feces of HIV patients.

4. Conclusion

There is a significant relationship between CD4 cell counts and *Cryptosporidium sp*. infections. There is a significant relationship between *Cryptosporidium sp.* infections with a history of diarrhea.

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