



## **Association between CD4 Level and Opportunistic Intestinal Protozoan Infections in Diarrhea Suffering HIV/AIDS Patients**

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**Abstract :** One of the major factors affecting the morbidity and mortality of HIV/AIDS patients is the immunity defect marked by decreased CD4 level. This immunocompromised condition causes the opportunistic intestinal protozoa which normally asymptomatic become symptomatic and lead to mild to severe and chronic diarrhea. The objective of this study was to find out the association between CD4 level and opportunistic intestinal protozoan infections in HIV/AIDS patients with diarrhea. A total of 39 HIV/AIDS patients suffering from diarrhea participated in this study. Each subject was examined for CD4 count in blood and collected their stool for microscopic examination to reveal opportunistic intestinal protozoa existence and species. Mean of CD4 count in HIV/AIDS patients with diarrhea was 44,49 cells/ $\mu$ l. The prevalence of opportunistic intestinal protozoan infection was 28,2%. The most common opportunistic intestinal protozoa were *Cryptosporidium parvum*, *Cystoisospora belli*, and *Cyclospora cayetanensis*, respectively. Bivariate analysis of this study shows no relation between variables. This study determine that there is no association between CD4 level and opportunistic intestinal protozoa infection in diarrhea suffering HIV/AIDS patients.

### **Introduction**

Around the end of twentieth century, the world of health was shocked by the emergence of a very dangerous and malignant disease, HIV/AIDS<sup>1</sup>. Acquired Immunodeficiency Syndrome (AIDS) is a retrovirus disease caused by HIV (Human Immunodeficiency Virus), characterized by severe immunosuppression which can cause opportunistic infections<sup>2,3</sup>.

The high severity and death rate of HIV/AIDS patients are caused by various factors, and one of them is that the management of patients is still inappropriate, including delay in diagnosing opportunistic infections, whilst opportunistic infections often deliver AIDS patients to death. Contrast to developed countries, HIV patients in developing countries tend to easily fall to AIDS stage and suffer from opportunistic infections, because the patients commonly live side by side with other infection patients of which incidence rate is still high<sup>4</sup>.

In Indonesia, the number of HIV infected patients and cases of AIDS continue to increase. The cumulative number of reported cases was 23,176 cases with a total of 8,553 death cases. Of these numbers, as many 8,103 cases were reported from North Sumatera province<sup>5</sup>.

Similar to other viruses, HIV can not survive and multiply by itself, thus must be living in host. Not all cells of the host can be infected by HIV, only those possessing CD4 receptors such as T-helper, monocyte, macrophage, and dendritic cells<sup>6</sup>. HIV infected patients will suffer immunity disorder signed with lower CD4 level in the blood, caused by TCD4 damage and disturbance of T-helper survival<sup>3</sup>.

Immunodeficiency causes patients more susceptible to diseases, leads to increased risk of opportunistic infections<sup>7</sup>. Gastrointestinal tract (GIT) infections were the most frequent infections in HIV/AIDS patients. Best estimation suggested that 50 to 93 percent of all patients would have GIT symptoms during the course of the disease<sup>8,9</sup>.

One that plays a role in causing digestive disorders in people with HIV/AIDS is opportunistic intestinal protozoans infection, which causes no complaint or symptom in normal patients, but can cause severe and chronic diarrhea in immunocompromised patients. Opportunistic protozoas that are often found in GIT are *Cryptosporidium parvum*, *Cystoisospora belli*, and *Cyclospora cayetanensis*, which are parasites of the coccidia class<sup>10</sup>. From the recent study, it has been known that the prevalence of opportunistic intestinal protozoa in North Sumatera was 10%<sup>11</sup>.

Based on the background mentioned above, the objective of this study is to find out the correlation between CD4 level and opportunistic intestinal protozoan infections in diarrhea suffering HIV/AIDS patients in North Sumatera Province.

## Experimental

This cross-sectional study was conducted at Haji Adam Malik (HAM) central hospital, a teaching and referral hospital located in Medan city, capital of North Sumatera province, from January to August 2017.

This study population consisted of HIV positive inpatients resided in integrated ward of HAM hospital who gave CD4 T-cell count at the first enrollment to monitor disease status. The inclusion criterias were suffering diarrhea and aged more than 18 years old and the exclusion criteria was diarrhea caused by anatomical abnormalities. A total of thirty nine consecutive subjects were included in the present study.

All diarrhea suffering HIV patients were visited in their ward. To each of the subjects, interview was held to explain about the study, and question about how long they had suffered HIV/AIDS, about the diarrhea symptom and history of taking antiretrovirus therapy (ART). After signing up the informed consent, each subject was provided with standard stool collecting container, labeled with the subject's name, expecting the subjects fill the container with about three grams or fifteen milliliter of solid or liquid stool respectively.

Short questionnaire was maintained which included; sociodemographic data (age,sex), and medical history (ART medication).

For CD4 count, three milliliter of blood was taken and stored in EDTA tube, then was analyzed with FACS count (Becto Dickinson).

For stool examination, after the subjects filled the containers with stool, the stool specimens were fixated with 10% formalin, and a lugol stained direct examination was carried out in the laboratory. Then the formol-ether concentration technique was carried out, and specimens were examined with the Kinyoun Gabbet modified acid faststaining technique.

The data of CD4 count and fecal protozoa examination were then statistically analyzed. Bivariate analysis was conducted to calculate the correlation between two variables, CD4 levels as an independent variable and opportunistic intestinal protozoan infections as a dependent variable.

Bivariate analysis with variables of categorical data was done by Chi Square test or Fisher's Exact alternative test. From bivariate analysis, p value, Odds Ratio and 95% Confidence Interval were calculated. To determine the significance of statistical calculations, the value of  $p < 0.05$  was used.

## Result

In this study, thirty nine samples were collected. Distribution of the sample was calculated based on sociodemographic characteristics including age, sex, and ART medication history.

**Table1. Subjects characteristics**

Characteristic	n	(%)
<b>Age</b>		
18 – 50year	37	94,9
> 50year	2	5,1
<b>Sex</b>		
Male	23	59
Female	16	41
<b>History of ART</b>		
ART (-)	23	59
ART (+)	16	41

Of the thirty nine subjects who met the criteria for inclusion, the most common subjects were in the age group of 18 - 49 years (94.9 percent), and by sex 23 were men (59 percent) and 16 were women (41 percent). Distribution of medical history of the subjects indicated that more subjects had never received antiretroviral drugs before (59 percent).

On CD4 cell counts of all thirty nine study subjects, the lowest value was 1 cell/ $\mu$ l and the highest value was 861 cells/ $\mu$ l, with an average of 44.49 cells/ $\mu$ l and standard deviation of 138.59.

On microscopic examination of fecal specimens, there were a number of intestinal protozoas found, both opportunistic and non-opportunistic. All the protozoas found were recorded, but only the subjects with opportunistic intestinal protozoas were called as opportunistic infected.

**Table2. Opportunistic Intestinal Protozoan Infections on Diarrhea Suffering HIV/AIDS Patients**

	N	%
Opportunistic intestinal protozoan infection (+)	11	28,2
Opportunistic intestinal protozoan infection (-)	28	71,8

**Tabel 3. Distribution of Protozoas Found in Subjects' Stool Specimens**

Protozoas Found	n	%
<i>Cryptosporidium parvum</i>	9	42,9%
<i>Cystoisospora belli</i>	2	9,5%
<i>Cyclospora cayetanensis</i>	1	4,8%
<i>Entamoeba histolytica</i> *	2	9,5%
<i>Entamoeba coli</i> *	6	28,6%
<i>Iodamoeba buetschlii</i> *	1	4,8%

\*) Non-opportunistic Protozoa

Of the thirty nine diarrhea suffering HIV patients, 28.2% had opportunistic intestinal protozoan infections. While on recording all the protozoas found, of all recorded intestinal protozoa, the most common opportunistic protozoan was *Cryptosporidium parvum* (42.9% of all protozoan infections), followed by *Cystoisospora belli* (9.5%). Of all study subjects, only one (4.8%) was infected with *Cyclospora cayetanensis*.

**Tabel 4. Corellation between CD4 level and Opportunistic Intestinal Protozoan Infectionsdengan Infeksi Protozoa Oportunistik Usus**

CD4 level	Opportunistic Protozoa		<i>p.</i>	OR	95% CI
	(+)	(-)			
≤ 50 sel/μl	8 (72,7%)	25 (89,3%)	0,197	0,32	0,05 – 1,91
> 50 sel/μl	3 (27,3%)	3 (10,7%)			

Table 4 is a bivariate analysis between CD4 levels and opportunistic protozoan infection. Analysis was carried out in a categorical scale and CD4 levels were divided into two groups with cut-off points of 50 cell/μl. Results of the analysis with Chi Square test, indicated no significant correlation between CD4 levels and opportunistic protozoan infection ( $p > 0.05$ ). The results of this analysis were also supported by 95% CI which included number 1, thus showing no significant correlation between the two variables.

## Discussion

In the era of HIV / AIDS today, there is an increase in opportunistic infections, and opportunistic infections in the gastrointestinal tract are one of the main causes of morbidity and mortality in HIV positive patients in the world. Among all opportunistic infections, coccidian protozoas often cause chronic diarrhea and can be a cause of death<sup>10</sup>.

This study tried to describe the characteristics of HIV/AIDS patients infected with opportunistic intestinal protozoa. This study found that men suffered from HIV/AIDS (59%) more than women, this was similar with Adamu's study (2009) which found 55% of HIV seropositive research subjects were men. The results of this study were also in line with the research of Amatya R et al (2011), who found 70% of the research subjects were men.

This study found that 59% of the subjects had never received antiretroviral drugs before. The results of this study differed from Adamu's study (2009) which found that 60% of participants had previously received ARTs. The results of this study were also different from those of Pavie et al (2012) who found 59% of all HIV positive study subjects had received antiretroviral drugs before. This difference evinced the low personal awareness of patients in this area, that they do not want to seek for treatment before the symptoms become severe.

In this study, it was found that the most common opportunistic protozoa was *Cryptosporidium parvum*. These results were in line with the study of Sapkota et al (2004) who found *Cryptosporidium parvum* as the most common opportunistic protozoa infecting HIV/AIDS patients. This research is also in line with the results of the study of Tarigan R (2009) who found *Cryptosporidium parvum* as the most common opportunistic protozoa found in HAM Center Hospital in Medan.

This study found that the subjects infected with opportunistic intestinal protozoas had an average CD4 level that was only slightly lower compared to non-infected subjects, and there was no significant correlation between the two. When being compared to the results of almost similar studies, such as Adamu and Petros (2009), it was found that more of HIV patients who had low CD4 levels were infected with opportunistic protozoa (*Cryptosporidium spp* and *Cycloisospora belli*) than those with HIV who had high CD4 levels.

Discrepancy between the results of these studies, was because Adamu and Presto included HIV patients who did not experience diarrhea on the inclusion criteria of their subjects, hence found more heterogeneous CD4 levels with a wider range. Whereas in this study, all study subjects suffered from diarrhea, resulting that all study subjects had low CD4 levels below 200 cells/μL.

A study with almost similar result was also carried out by Assefa et al (2009), of which that HIV patients who had low CD4 levels had higher rates of parasitic infections, especially opportunistic protozoa than those with HIV who had higher CD4 levels. In this study, it can also be seen that the researchers included all HIV patients both with or without diarrhea on their research inclusion criteria so that they were compared to differences in CD4 levels with more heterogeneous values and wider ranges.

From this study, it can be seen that in symptomatic HIV/AIDS patients, CD4 levels in their blood are all low beneath 200 cells/ $\mu$ L. In patients with low CD4 levels, it is no longer possible to see the correlation between CD4 levels and opportunistic infections. This indicates that all patients with low CD4 levels are already vulnerable to all opportunistic infections, both opportunistic intestinal protozoa and other infections.

In conclusion, the result of this study showed that in HIV/AIDS patients who had complained of gastrointestinal disorders, there is no significant correlation between CD4 levels with opportunistic intestinal protozoan infections.

Based on this study, we would recommend to record all drugs taken by patients, whether ARTs, antibiotics or other drugs that can affect the patient's clinical symptoms. We also recommend to record the data of medication span, regularity, and continuity. This is expected to assess the role of ARVs and reduce confounding factors from other drugs.

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