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The Relationship between Intestinal Parasitic Infection and CD₄⁺ Level among HIV Patients in DR. Sardjito Central Hospital, Yogyakarta

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Abstract. Intestine parasites were one of the causes behind the occurrence of intestinal disorder, such as diarrhea. The risk of being infected by the intestine parasites increased among the HIV patients due to the weakening immunity. As a result, the intestinal parasites would easily infect the patients. This study was conducted to determine the prevalence, to identify the relationship between the body immunity level and the intestinal parasitic infection among HIV patients in Dr. Sardjito Central Hospital Yogyakarta. Out of 75 samples of faeces HIV patients collected from July 2017 until January 2018. Intestinal parasites were identified by using Ziehl Neelsen painting and Ritchie concentration method. The data were analyzed by using chi-square test with p-value that had been higher than 0.05. The intestine parasites that had been found in the research were namely *Cryptosporidium parvum*, *Isospora belli*, *Entamoeba histolytica*, *Entamoeba coli*, *Balantidium coli*, *Iodamoeba butschlii*, *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm, and *Hymenolepis nana*. There had not been significant relationship between the intestinal parasitic infection and the immunity status of HIV patients. The prevalence of intestinal protozoa among the HIV patients was 52 (69.3%), while the prevalence of intestinal worm was 6 (8%).

INTRODUCTION

Infection by Human Immunodeficiency Virus (HIV) was one of the diseases that had caused the weakening immunity system within human's body. Based on the latest report, in 2017, there were around 42.9 million people who had been suffering from HIV throughout the world [1]. In relation to the statement, the number of HIV patients in Indonesia had also been increasing; until December 2016, in Indonesia itself there were 232,323 HIV patients [2]. With regards to the finding, the number of HIV cases in Yogyakarta showed that there had been 3,688 people who suffered from HIV from 1993 until 2016. HIV weakened the immunity system of human's body; individuals who had been infected by HIV would be more vulnerable to other diseases, such as intestinal parasite infection. Infection by intestine parasites was significant health problem and there were 3.5 million people who had been recorded to suffer from intestinal parasite infection both the protozoa-cause one and the worm-caused one [3]. Returning to the case of Indonesia, the prevalence of Soil Transmitted Helminth (STH) reached 28.12% in 2013 [4].

Individuals who had been infected by HIV would suffer from weakening immunity system and, consequently, they would be vulnerable from the attacks of other diseases, especially the diseases caused by both the bacteria or the parasite [5]. The gastrointestinal infection was the disease that had mostly been found among HIV/AIDS patients and it was expected that 50-93% HIV patients displayed gastrointestinal infection symptoms. One of the clinical

manifestations from opportunistic infection that still caused the high morbidity and mortality rate among HIV/AIDS patients was diarrhoea. One of the causes behind the occurrence of diarrhoea among the HIV patients was the intestinal parasite infection [6].

The opportunistic intestinal parasites were namely *C. parvum*, *Cyclospora cayetanensis*, *I. belli*, and *Microsporidia*. On the other hand, the non-opportunistic intestinal parasites were namely *E. histolytica* and *Giardia lamblia* that usually caused heavier symptoms in addition to several types of worms namely *T. trichura*, *A. lumbricoides*, *Strongyloides stercoralis*, *Necator americanus*, and *Ancylostoma duodenale* [5]. With regards to this case, in Yogyakarta, especially in Dr.Sardjito Central Hospital, there had not been any research about intestinal parasites among the HIV patients. Therefore, through the research, the types and the prevalence of intestinal parasites would be analyzed among the HIV patients who pursued their medication in Dr.Sardjito Central Hospital and the types and the prevalence would be linked to the status of immunity among these patients.

EXPERIMENTAL DETAILS

This research implemented the analytical descriptive observational method with cross-sectional design. The research was conducted in Parasitology Laboratory of Faculty of Medicine, Community Health, and Nursery Gadjah Mada University. The samples of the study were the HIV patients who pursued their medication in Dr.Sardjito Central Hospital. The samples were selected based on the inclusion criteria, namely the faeces should be from the ≥ 18 years old patients whose $CD4^+ \leq 700$ cell/ μ l based on the medical reports and the faeces should be collected under the researcher's instruction. Then, the criteria of exclusions were the samples had not been sufficient for further examination. The faeces examination was conducted by implementing two methods, namely Ritchie method, and ZiehlNeelsen method. After the data had been gathered, the data were described and were analyzed using SPSS Windows Version program with Chi Square and rate of significance (α) 5% to identify the relationship between the dependent and the independent variables [7].

RESULTS AND DISCUSSION

The research involved 75 samples of faeces that had been gathered from the HIV patients who pursued their medication in Dr.Sardjito Central Hospital. The research had been approved by the Board of Ethics Faculty of Medicine, Community Health, and Nursery Gadjah Mada University under the letter KE/FK/07/10/EC/2017.

Characteristics of HIV Patients as Research Subjects

The characteristics of HIV patients who became the subject in the research might be consulted briefly in Table 1. The number of male patients was 55 (73.3%) people, the number of female patients was 16 (21.3%) people, and the number of transsexual patients was 4 (5.3%) people. The number of HIV patients who had been around 18-35 years old was 41 (54.7%) people and the number of HIV patients who had been 35 years old and above was 34 (45.3%) people. The number of HIV patients who suffered from diarrhoea was 16 (21.3%) people and the number of HIV patients who did not suffered from diarrhoea was 59 (78.4%) people. Eventually, the number of HIV patients with the amount of $T CD4^+ \leq 200$ cell/ μ l was 39 (52%) people and the number of HIV patients with the amount of $T CD4^+ \geq 201$ cell/ μ l was 36 (48%) people. Based on the testing activities in the Parasitology Laboratory of Gadjah Mada University, there were 52 (69.3%) samples of positive intestinal parasite and 23 (30.7%) samples of negative intestinal parasite.

TABLE 1. Characteristics of HIV patients who pursued their medication in dr. Sardjito Central Hospital Yogyakarta who had been gathered from July 2017 until January 2018

Characteristics	Amount (%)
Gender	
Male	55 (73.3)
Female	16 (21.3)
Transsexual	4 (5.3)
Age	
18-35 years old	41 (54.7)
>35 years old	34 (45.3)
Clinical Symptoms	
Diarrhoea	16 (21.3)
Non-Diarrhoea	59 (78.7)
Amount of T CD ₄ ⁺ (cell/μl)	
≤ 200 cell/μL	39 (52)
≥ 201 cell/μL	36 (48)
Intestinal Protozoa Infection	
Negative	23 (30.7)
Positive	52 (69.3)
Intestinal Worm Infection	
Negative	68 (90.7)
Positive	7 (9.3)

From 52 patients with intestinal parasite infection, 69.3% patients were infected by intestinal protozoa and 9.3% patients were infected by intestinal worm. The mostly found intestine parasite was *C. parvum*; this intestine parasite was found in 47 samples (62.66%). The complete data with regards to the types of intestinal parasites that had been found among the HIV patients might be consulted in Fig. 1.

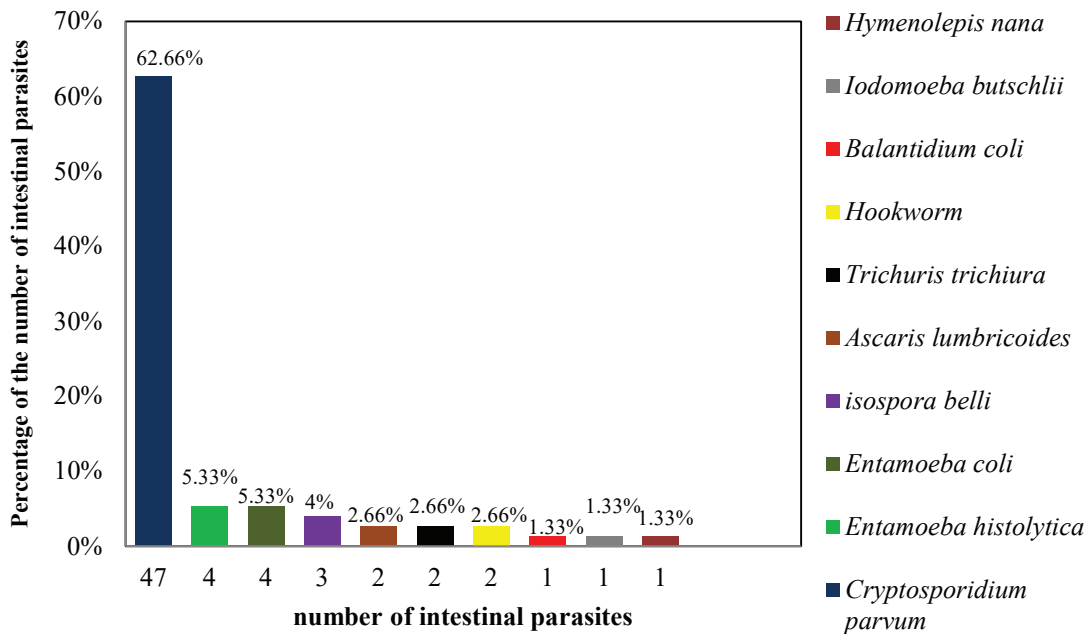


FIGURE 1. Prevalence and types of intestinal parasites among the HIV patients in dr. Sardjito Central Hospital Yogyakarta from July 2017 until January 2018

The characteristics of HIV patients who had been infected by the intestinal parasites with regards to the amount of T CD₄⁺ ≤ 200 cell/πL might be consulted in Table 2. The amount of T CD₄⁺ cell was divided into two categories, namely T CD₄⁺ ≤ 200 cell/πL and T CD₄⁺ ≥ 201 cell/πL.

TABLE 2. Type and number of intestinal parasites based on the amount of T CD₄⁺ cell among the HIV patients in dr. Sardjito Central Hospital Yogyakarta from July 2017 until January 2018

T CD ₄ ⁺ Cell	Type of Intestinal Parasites	Positive	Negative	Total
≤200 cell/μL		27	12	39
	<i>Cryptosporidium sp</i>	25		
	<i>Entamoeba histolytica</i>	2		
	<i>Balantidium coli</i>	1		
	<i>Entamoeba coli</i>	1		
	<i>Isosporabelii</i>	3		
	<i>Iodomoebabutschlii</i>	1		
	<i>Ascaris lumbricoides</i>	2		
	<i>Trichuris trichiura</i>	1		
	<i>Hookworm</i>	1		
	<i>Hymenolepis nana</i>	1		
≥201 cell/μL		25	11	36
	<i>Cryptosporidium sp</i>	22		
	<i>Entamoeba histolytica</i>	2		
	<i>Entamoeba coli</i>	3		
	<i>Trichuris trichiura</i>	1		
	<i>Hookworm</i>	1		
Total		52	23	75

Based on the amount of T CD₄⁺ cell, there were several HIV patients who had suffered from single and multiple infections. The brief details on the single infection and the double infection might be consulted in Table 3.

TABLE 3. Number of single and double infection with regards to the amount of T CD₄⁺ cell among the HIV patients in dr. Sardjito Central Hospital Yogyakarta from July 2017 until January 2018.

Characteristics	Single Infection	Multiple Infection	Negative Intestinal Parasite
Amount of T CD ₄ ⁺ ≤200 cell/μL	19 (36.53%)	9 (17.3%)	
Amount of T CD ₄ ⁺ ≥201 cell/μL	21 (40.38%)	3 (5.76%)	
Total	40	12	23

The number of HIV patients with T CD₄⁺ ≤ 200 cell/πL who had been infected by single intestinal parasite infection was 19 people (36.53%), and who had been infected by multiple intestinal parasite infection was 9 people (17.3%). On the other hand, the number of HIV patients with T CD₄⁺ ≥ 201 cell/πL who had been infected by single intestinal parasite infection was 21 people (40.38%), and who had been infected by multiple intestinal parasite infection was 3 people (5.76%). The type of intestinal parasite that had mostly been found was *C. parvum*. Then, the HIV patients with T CD₄⁺ ≤ 200 cell/πL had higher risk to be infected by the intestinal parasite with 27 positive samples, while the HIV patients with T CD₄⁺ ≥ 201 cell/πL had lower risk to be infected by the intestinal parasite with 25 positive samples. The bivariate analysis on the intestinal protozoa with risk factors might be consulted on Table 4, and bivarit analysis on the intestinal worms on the Table 5.

TABLE 4. Differences on each risk factor with regards to intestinal protozoa infection among the HIV patients in dr. Sardjito Central Hospital from July 2017 until January 2018.

Variables	Intestinal Protozoa Infection		P (Ci = 95%)	Odds Ratio (OR)
	Positive	Negative		
Age	28			
18-35 years old	24	13	0.83*	1.114
> 35 years old		10		
Gender	43			
Male + Transsexual	9	16	0.165**	0.478
Female		7		
T CD₄⁺ (cell/μl)	27			
≤ 200 cell/μL	25	12	0.984*	1.01
≥ 201 cell/μL		11		
Clinical Symptoms	13			
Diarrhoea	39		0.197**	0.45
Non-diarrhoea				

Note: p values were significantly different on Chi-Square test (*) and Fisher test (**) with rate $\alpha > 0.05$.

TABLE 5. Differences on each risk factor with regards to intestinal worm infection among the HIV patients in Dr.Sardjito Central Hospital from July 2017 until January 2018.

Variable	Intestinal Worm Infection		P (Ci =95%)	Odds Ratio (OR)
	Positive	Negative		
Age				
18-35 years old	4	37	0.605**	0.895
> 35 years old	3	31		
Gender				
Male + Transsexual	5	54	0.465**	1.543
Female	2	14		
T CD₄⁺ (cell/μL)				
≤ 200 cell/μL	5	34	0.25**	0.4
≥ 201 cell/μL	2	34		
Clinical Symptoms				
Diarrhoea	1	15	0.535**	1.698
Non-diarrhoea	6	53		

Note: p values were significantly different on Chi-Square test (*) and Fisher test (**) with rate $\alpha > 0.05$.

Each of the variables that had been analyzed did not display different significant score on the intestinal worm infection. The multivariate analysis using logistic regression on intestinal parasite infection in relation to the given variables, such as gender and T CD₄⁺ cell, might be consulted on Table 6.

TABLE 6. Results of the most influential multivariate analysis on the HIV patients with intestinal parasite infection in dr. Sardjito Central Hospital Yogyakarta from July 2017 – January 2018

Variable	P Value	Exp ^(β) Value
Gender	0.165	2.039
Clinical symptoms	0.197	2.316
CD ₄ ⁺ T cell	0.984	0.939

Note: Exp^(β) value = The Odd Ratio value of risk factor on intestinal parasite infection

P value = P value was not significantly different on Chi Square test with rate $\alpha > 0.05$

Risk factors of gender, clinical symptoms, and CD₄⁺ T cell were insignificant, however, there had been influence on intestinal parasite infection with the highest Exp^(B) score on clinical symptoms namely, 2.316. The prevalence of intestinal parasite among the HIV patients that had been examined from Dr.Sardjito Central Hospital from July 2017 until January 2018 was 52 (69.33%) out of 75 samples. There were several intestinal parasites that had significant contribution in infecting the HIV patients; the highest contribution was provided by *C. parvum*, while the lowest contribution was provided by *B. coli*, *I. butchlii*, and *H. nana*. In the research [8] conducted in India ten types of intestinal parasites that infected the HIV patients had been found from the sample that had been collected from June 2010 until June 2013, and the ten types were *A. lumbricoides*, *Blastocystis hominis*, *C. parvum*, *Cyclospora cayetansis*, *E. histolytica*, *G. lamblia*, *A. duodenale*, *H. nana*, *S. stercoralis*, and *T. trichiura*. These types of intestinal parasite were collected from 334 patients with positive intestinal parasite infection.

More HIV patients with amount of T CD₄⁺ ≤ 200 cell/πl had suffered from multiple intestinal parasite infection. This situation caused the HIV patients to suffer from chronic diarrhoea with mal-absorption due to the lack of nutrition and body fluids. The patients who already suffered from chronic diarrhoea belonged to final stadium and thus they might suffer from fatal consequence if they had not been provided with immediate treatment. The intestinal parasite infection had mostly been caused by *C. parvum* among the HIV patients, namely 47 subjects.

The infection that had been caused by *C. parvum* was actually harmless for individuals with good body immunity because this type of protozoa might disappear by itself and sometimes this type of protozoa appeared without any symptoms. However, for the patients with body immunity-related problem, such as HIV patients, such infection should be handled with serious attention [9]. The contagion and the spread of the infection might occur through food, beverages, and waterpipes that the patients used.

There were several risk factors that had been related to the intestinal parasite infection from the HIV patients who pursued their medication in dr. Sardjito Central Hospital from July 2017 until January 2018. Due to the finding, the intestinal parasite infection should be investigated to decrease its infection and spread. Several risk factors that had been related to the intestinal parasite infection were age, gender, clinical symptoms, and T CD₄⁺ cell. The HIV patients whose T CD₄⁺ ≤ 200 cell/πL suffered from multiple intestinal parasite infection; consequently, these patients should be given attention to prevent the occurrence of more chronic infections.

One of the risk factors groups that had been analyzed by using univariate analysis from the HIV patients was gender. In the research, the HIV patients were dominated by male respondents, namely 55 people, whereas the female HIV patients were 16 people and the transsexual HIV patients were four people. Then, the results of bivariate analysis on the gender group with intestinal protozoa infection and intestinal worm infection had not statistically and significantly been different with p value of intestinal protozoa = 0.165 and OR = 0.478. This figure implied that there had not been any influence of gender on intestinal protozoa infection. On the other hand, the results of bivariate analysis on the gender group with intestinal worm infection had not also been statistically and significant different with p = 0.465 and OR = 1.543. This figure implied that there had not been any influence of gender on intestinal worm infection. However, clinically male and transsexual patients had higher risk of being infected by intestinal parasite in comparison to female patients. The contagion of intestinal parasite infection among homosexual and transsexual patients had higher risk in comparison to female patients due to their sexual behaviours, such as anal intercourse.

The age of HIV patients who had been infected with intestinal parasite in the study was ≥ 18 years old. The results of univariate analysis showed that there were 41 patients whose age had been 18-35 years old and there were 35 patients whose age had been > 35 years old. The age category of the patients who had been involved in the study was adult; for the adult patients, the body immunity had been well established because the primary and secondary lymphoid organ had been perfectly developed and the antibody had been perfectly developed as well due to the previous infection. In the children, the body immunity system had not been perfectly developed and, consequently, the cellular response, such as lymphocytes proliferation, cytokinin synthesis, and antibody responses, that had not been developed decreased. The results of bivariate analysis showed that there had not been any significant difference and there had not been any influence of intestinal parasite infection and age [10].

A similar research was conducted by [11] using 90 samples of faeces that had been gathered from HIV/AIDS patients. Based on the results of bivariate analysis, age, and gender did not have significant difference which implied that there had not been any influence of age and gender on intestinal parasite infection with p = 0.7132.

The body immunity system in the HIV patients might be identified by viewing the amount of T CD₄⁺ cell from the examination results. The results of bivariate analysis that compared the HIV patients based on the strong and weak body immunity system did not show significant difference; in other words, statistically there had not been any influence of intestinal parasite infection on T CD₄⁺ with p = 0.984 for intestinal protozoa and p = 0.25 for intestinal worms. Based on the results of the interview that had been conducted to the patients, the absence of influence by intestinal parasite infection might be caused by the hygiene aspect of food and beverages that had not been maintained

by the patients; these patients might consume raw vegetables that had not been cleansed, might not fully boiled the tap water, might not wash their hand, might consume raw fish that had probably been infected by oocyst, might use the waterpipes that had been infected by oocyst, and might swim in the area that had been infected by oocyst.

One of the body parts that might establish direct contact to the antigens and that contained numerous macrophage cells were the gastrointestinal channel. In the middle of gastrointestinal channels, there was central germinal area that contained B-lymphocytes cells, while in the intrafollicular area, there were T-lymphocytes cells. The immunoglobulin A (IgA) was also produced in the gastrointestinal channel; IgA came from local mucosa plasma cells in the form of dimer IgA and was secreted as body defense mechanism toward infection. This response would be stimulated if pathogen had entered the gastrointestinal channel through gastrointestinal lumen and the pathogen would be responded by the dendritic cells that expressed MHC II and would be presented to T-cells or that would be directly responded to B-cells. The lymphocyte cells would migrate to the lymph gland and would go into thoracic duct; the migration would end in the blood circulation and from the blood circulation the lymphocyte cells would return to the mucosa in the intestinal-part propria lamina. This response would go through the wall of epithelial cells to sustain and expel the pathogen along with the faeces. The HIV infection caused the weakening immunity system among the patients; as a result, the function of defense mechanism toward pathogen might not be fully exerted [12].

The results of the research on intestinal parasite infection among HIV patients in Dr.Sardjito Central Hospital from July 2017 until January 2018 showed that all risk factors, such as T CD₄⁺ cells, clinical symptoms, age, and gender factors did not have significant influence. As a consequence, another review should be conducted to identify other factors that caused the occurrence of intestinal parasite infection among the HIV patients so that fatal consequences due to the intestinal parasite infection might be prevented, and thus be decreased.

SUMMARY AND RECOMMENDATION

Summary

Based on the results of the research, it might be concluded that the prevalence of intestinal protozoa among the HIV patients in Dr.Sardjito Central Hospital Yogyakarta from July 2017 until January 2018 had been 52 (69.3%) while the prevalence of intestinal worms for the same subjects and the same period had been 6 (8%); both prevalence had been attained from 75 samples of faeces that had been examined. In addition, more types of intestinal parasite had been found among the HIV patients with CD₄⁺ ≤ 200 cell/μL.

Recommendation

Therefore, another research with other risk factors such as eating pattern, patients' hygiene level, and environment that might impact the spread of intestinal parasite infection among HIV patients should be reviewed.

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