RELATIONSHIP BETWEEN CD4 LEVELS, VIRAL LOAD, AND THE NUMBER OF OPPORTUNISTIC INFECTIONS AMONG PATIENTS WITH HIV INFECTION AT SANGLAH GENERAL HOSPITAL

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Abstract

Human Immunodeficiency Virus (HIV) is a virus that attacks cells with CD4 antigens. Overtime, it causes opportunistic infection (OI). Opportunistic infection is the most common complication of HIV infection and causes major morbidity and mortality in people with HIV infection. A cross-sectional design conducted at VCT Polyclinic of Sanglah General Hospital, Denpasar. Sampling was carried out by total sampling from secondary data on medical records of patients with first visit to polyclinic from January 2018 - December 2019 containing identity, OI, CD4 levels, and viral load of the subjects. Spearman correlation test was used to determine the bivariate relationship. In this study, from 527 subjects, OI were detected in 320 samples (60.7%), with candidiasis as the most prevalent OI (58.8%). The highest prevalence of OI was in the group with CD4 level < 200 cells/μL and viral load > 50 copies/ml. In bivariate analysis, CD4 level had a significant negative correlation with OI numbers (r = -0.63), viral load had a significant positive correlation with the number of OIs (r = 0.52), and viral load had a significant negative correlation with CD4 level (r = -0.46). Low CD4 and high viral load levels were associated with higher number of OIs in people with HIV infection.

Keywords: HIV, Opportunistic Infection, CD4, Viral Load

Introduction

Human Immunodeficiency Virus (HIV) is classified as a retrovirus that has RNA genetic material that is able to infect CD4 lymphocyte, by making changes according to the host DNA. The HIV virus tends to attack specific cell types that have CD4 antigens, especially T4 lymphocytes which play an important role in regulating and maintaining the immune system (1). Acquired immune deficiency syndrome (AIDS) is a collection of symptoms or diseases caused by decreased immunity due to infection by HIV. AIDS is the end result of HIV infection. Opportunistic infection (OI) is the most common complication of HIV infection and causes major morbidity and mortality in people with HIV infection (90%) (2, 3). OI is defined as infection that occurs more frequently and more severe in immunocompromised people (4). Identification of causative pathogens of OI is very important in managing HIV infection (2, 4).

United Nations Program on HIV/AIDS (UNAIDS) reported the number of people living with HIV/AIDS worldwide in 2018 was 37.9 million, of which 79% of them acknowledged their HIV status. There was a decrease in the number of mortalities related to HIV/AIDS by 770 thousand in 2018 from the previous 1.7 million in 2004 and 1.2 million in 2010 where the major aetiology of HIV-related deaths was still accounted by tuberculosis (TB), which was one third of deaths. The number of new cases of HIV infection also decreased by 40%, from 2.9 million people in 1997 to 1.7 million in 2018 (5). The HIV/AIDS epidemic is a problem in Indonesia, which is the fifth country at risk of HIV/AIDS in Asia (6). In 2017 in Indonesia, the number of people living with HIV was 48,300 people and AIDS patients were 9,280 people, Bali was in the sixth position with 2,441 people with HIV and fifth for AIDS (746 people) (6).

Antiretroviral (ARV) therapy has reduced the incidence of OI in HIV-infected individuals, but the efficacy of ARVs depends on patient adherence to the drug regimen, stage at which treatment was started, drug resistance, and other factors. Currently, initiation of prophylactic therapy against opportunistic pathogens is based on absolute CD4 cell count which is generally accepted as the best

indicator of immunological competence of patients with HIV infection (2). CD4 is a 55 kDa glycoprotein and is found mainly on helper T lymphocytes and is also expressed on monocytes, macrophages, and dendritic or Langerhans cells. When the CD4 count drops to <200 cells/µL, a person becomes vulnerable to opportunistic infections (OI). HIV infection causes a low CD4 count which makes the body more susceptible to OI. This results in increased patient morbidity and mortality, which is actually due to OI rather than HIV itself (7).

High virus replication rate reflects the viral load (HIV RNA). Viral load reflects the HIV virus count detected in serum, disease progression, and mortality risk. ARV treatment failure can be defined clinically by evaluating disease progression, immunologically with CD4 levels, and virologically by estimating the viral load (8). There were several studies regarding the relationship between CD4 levels and viral load with OI incidence, but very few studies about the relationship between CD4 levels with OI number by HIV patients. Study by Yogani et al. at Dr. Cipto Mangunkusumo Hospital obtained results that opportunistic infection number was not associated with an increase in CD4 (p = 0.480). Other studies relate CD4 levels with OI incidence developed by patients (9).

Although currently antiretroviral therapy has reduced hospitalizations and deaths in patients with HIV, OI remain a major cause of morbidity and mortality in HIV-infected person and an increasing socio-economic burden (10). Therefore, clinicians must have optimal strategies for prevention and management of OI in order to provide comprehensive care for patients (10, 11).

Several studies have examined the characteristics of OI in HIV patients worldwide. Previous study at Sanglah General Hospital Denpasar from June 2014 to October 2014 found that the most common OI was Candidiasis (28.3%), followed by wasting syndrome (24.2%), Pneumonia (15%), Pneumocystis carinii pneumonia (12%), tuberculosis (11.5%), diarrhea (4.6%), toxoplasmosis cerebri (3.8%), generalized lymphadenopathy (0.2%), herpes zoster (0.2%), and cryptococcosis (0.2%). Therefore, identification of the pathogens responsible for OI is very important in managing HIV-infected individuals. The spectrum of the OI of a given area must be known to prevent this infection by providing adequate prophylaxis (2).

Based on the above-mentioned background, therefore this study aims to know the latest data regarding the OI characteristics of HIV patients and examine relationship between CD4 levels, viral load, and OI numbers in HIV patients at Sanglah General Hospital Denpasar. The results of this study are expected to be able to provide knowledge for us that CD4 levels represent individual immune competence is a protective factor against OI numbers and viral load which reflects HIV virus replication associated with OI numbers in HIV patients.

Materials and Methods

Sample collection

This study used cross sectional design located at VCT Polyclinic Sanglah General Hospital by observing the medical record status of people with HIV. The population and sample of the study were medical record data of HIV patients with first visit to the VCT Polyclinic at Sanglah General Hospital from period January 2018 to December 2019 and all the subjects had not initiated on ARVs.

The sampling method was carried out by total sampling with a total of 527 subjects. The tool used in this study is secondary data by medical records containing the identity of the respondents, type of opportunistic infection, CD4 levels and viral load. The independent variables in this study were CD4 levels and viral load, meanwhile the dependent variable was the number of OI.

Data analysis

Data was analyzed using the Statistical Package Social Sciences (SPSS) 22.0 software. The Spearman Correlation Test was used to test the bivariate correlation between the independent and dependent variables. Confidence Interval (CI) used is 95% and p value < 0.05 is considered significant.

Results

Characteristics of research subjects

A total of 527 subjects were selected from in the VCT Polyclinic of Sanglah General Hospital. Mean age of the subjects was 35.48 + 9.2 years old with median 34 years old with the youngest age was 16 years old and the oldest was 67 years old. Based on the gender distribution, there were 178 females (33.8%) and 349 males (66.2%). Based on age group, the highest percentage of age patients with HIV/AIDS is age group 30-39 years old at 39.9%. Based on the risk factors for transmission, it was found the most common transmission was through heterosexuals (41.7%) and the lowest was through transfusion (0.2%). Based on the presence or absence of opportunistic infections, 320 subjects (60.7%) had OIs and 207 samples (39.3%) did not have OIs. For the number of OI, 182 (56.9%) subjects had 1 type of OI, 106 (33.1%) with 2 types of OI, 28 (8.8%) subjects with 3 types of OI, 3 (0.9%) with 4 types of OI, and 1 (0.3%) with 5 types of OI. Sample demographics and characteristics can be seen in Table 1.

From a total of 320 subjects with OIs who came to the VCT Polyclinic at Sanglah General Hospital, there were 495 numbers of OI found. Candidiasis was the most common OI found in 188 samples (58.8%) and the least common was Cryptococcosis in 1 sample (0.3%). The proportion of OIs can be seen in Table 2.

Table 1: Subjects characteristics and demographics

Demographic characteristics	Total (N=527)	%
Gender		
Male	349	66.2%
Female	178	33.8%
Age		
15-19 years old	8	1.5%
20-29 years old	147	27.9%
30-39 years old	210	39.9%
40-49 years old	118	22.4%
50-59 years old	36	6.8%
60 years old	8	1.5%
Transmission risk		
Heterosexuals	220	41.7%
Homosexuals	64	12.1%
Bisexual	20	3.8%
High risk partner	123	23.3%
Intravenous drug users	23	4.4%
Tattoo	40	7.6%
Transfusion	1	0.2%
Unknown	36	6.9%
Opportunistic infection		
Present	320	60.7%
Not present	207	39.3%
Opportunistic infection number(s)		
1	182	56.9%
2	106	33.1%
3	28	8.8%
4	3	0.9%
5	1	0.3%

Table 2: Opportunistic infections proportion in HIV patients at Sanglah General Hospital

Opportunistic infection type	Total (N=320)	%
Candidiasis	188	58.8%
Tuberculosis	111	34.7%
Pulmonary	90	28.1%
Extra pulmonary	21	6.6%
Pneumocystis carinii pneumonia	106	33.1%
Toxoplasmosis	51	15.9%
Syphilis	22	6.9%
Herpes Simplex Virus (HSV)	6	1.9%
Varicella-Zoster	5	1.6%
Cytomegalovirus (CMV) disease	3	0.9%
Histoplasmosis	2	0.6%
Cryptococcosis	1	0.3%

From 527 subjects, CD4 levels were obtained in 287 subjects. This is due to the availability of reagents in the laboratory when the subject comes to the polyclinic. The median value of CD4 was 86.00 (1-945) cells/µL. Of all opportunistic infections found in HIV patients, it was found that there was an increase in the prevalence of OI which was proportional to the decrease in CD4 levels in patients. The prevalence of OI such as candidiasis, tuberculosis, PCP, toxoplasmosis, syphilis, Herpes simplex, Varicella-Zoster, and CMV were also found to be higher at CD4 <200 cells/µL (Table 3 and 4).

Table 3: Group of patients with and without OI Based on CD4 levels at Sanglah Hospital

CD4 levels	With OI	Without OI	Total
0-49	109 (93.2%)	8 (6.8%)	117 (40.8%)
50-99	26 (81.2)	6 (18.8%)	32 (11.1%)
100-199	29 (67.4%)	14 (32.6%)	43 (14.9%)
200-299	9 (23.1%)	30 (76.9%)	39 (13.6%)
≥300	8 (14.3%)	48 (85.7%)	56 (19.5%)
Total	181 (63.1%)	106 (36.9%)	287 (100%)

Table 4: Types of OI based on CD4 levels at Sanglah General Hospital

OI type	0-49	50-99	100-199	200-299	>300	Total
Candidiasis	86	19	23	5	3	136 (44.0%)
Pulmonary Tb	32	9	8	1	2	52 (16.8%)
Extra pulmonary TB	6	2	4	1	0	13 (4.2%)
PCP	42	7	10	0	0	59 (19.1%)
Toxoplasmosis	24	4	3	1	0	32 (10.4%)
Syphilis	2	3	1	2	3	11 (3.6%)
HSV	1	1	1	0	0	3 (0.9%)
Varicella-Zoster	1	0	1	0	0	2 (0.6%)
CMV	1	0	0	0	0	1 (0.3%)
Histoplasmosis	0	0	0	0	0	0 (0.0%)
Cryptococcosis	0	0	0	0	0	0 (0.0%)
Total	195 (63.1%)	45 (14.6%)	51 (16.5%)	10 (3.2%)	8 (2.6%)	309 (100%)

Of the 527 subjects, viral load levels were obtained in 182 subjects. The median value of viral load was 93,000 (0-10,000,000) copies/mL. Of all the opportunistic infections found in HIV/AIDS patients, an increase in the prevalence of OI was found which was proportional to the increase in viral load levels in patients (Table 5).

Table 5: Groups of patients with and without OI based on viral load levels at Sanglah General Hospital

Viral Load level	With OI	Without OI	Total
0-49	2 (6.7%)	28 (93.3%)	30 (16.5%)
50-9.999	12 (50.0%)	12 (50.0%)	24 (13.2%)
10.000-99.999	27 (67.5%)	13 (32.5%)	40 (22.0%)
100.000-499.999	46 (86.8%)	7 (13.2%)	53 (29.1%)
≥500.000	34 (97.1%)	1 (2.9%)	35 (19.2%)
Total	121 (66.5%)	61 (33.5%)	182 (100%)

Relationship between CD4 levels and opportunistic infection numbers

Considering the distribution of data on the number of OI was not normal ($p<0.001$) in the Kolmogorov-Smirnov test, then Spearman correlation test (bivariate) was used to test the relationship between CD4 levels and opportunistic infections number. It was found that ($r = -0.63$, $p = 0.001$), which means that CD4 levels had significant negative correlation with OI numbers.

Relationship between viral load and opportunistic infection numbers

Spearman correlation test (bivariate) to examine the relationship between viral load and the number of opportunistic infections. It was found that ($r = 0.52$, $p = 0.001$), which means that viral load had significant positive correlation with OI numbers.

Relationship between viral load and CD4 levels

Among 287 subjects who had CD4 and 182 who had viral load, only 123 subjects had complete data on both. Considering that the distribution of CD4 levels data was not normal ($p < 0.001$) in the Kolmogorov-Smirnov test, the Spearman correlation test (bivariate) was used to test the relationship between viral load and CD4 levels. It was found ($r = -0.46$, $p = 0.001$), which means that viral load levels had significant negative correlation with CD4 levels.

Discussion

In this study, it was found that patients with HIV/AIDS consist of 349 male (66.2%) and 178 females (33.8%). This result is similar with data from the Ministry of Health of Republic Indonesia (Kemenkes) in 2017 where the proportion of male was 64% and female was 36% in Indonesia (6). Previous study in the inpatient wards of Sanglah General Hospital from July 2013 - June 2014 conducted by Saktina and Bagus (2017) also obtained similar results, which consist of 67.6% male and 32.4% female HIV/AIDS patients (12). This phenomenon can be caused by various factors, including the number of Injecting Drugs Users (IDUs) are dominated by male rather than female, in addition to sexual behavior among men (homosexuals) also plays a role in HIV transmission to men (13).

In this study, based on age group, data obtained from 527 samples, the highest percentage of patients with HIV/AIDS was age range 30-39 years old at 39.9%. This is in accordance with data from the Ministry of Health of Republic Indonesia in 2017 with the result age range of 25-49 years old was the age range with the highest number of HIV/AIDS patients compared to other age groups. The results of this study was also similar to research by Lubis (2011) at the Sulianti Saroso Hospital in Jakarta on 109 HIV/AIDS patients, this study found the highest percentage of AIDS patients was in the 30-39 years old age group, which was 45.9%, followed by the 20-29 years old age group by 39.4%, and the age group of 40-49 years old by 11.9% (14). The results of this study were also not much different from previous study in the inpatient wards of Sanglah General Hospital in July 2013 - June 2014 where from 179 medical records of HIV patients in the inpatient ward of Sanglah General Hospital, Denpasar, the highest percentage of HIV patients was in the 30-39 years old age group with total 71 people (39.7%), followed by the 40 - 49 years old age group with total 50 people (27.9%), and the 20-29 years old age group with total 39 people (21.8%). The results of these three studies support data from the Indonesian Ministry of Health in 2017 where the highest number of HIV/AIDS patients is in the productive age group. The main reason why the HIV infection rate is high among young adults is due to the influence of sexual activity which is still high at the productive age which is a period of discovery, feelings of freedom, and exploration of new relationships and behaviors, especially free sex, are the most important risk factors of HIV infection. Another possible reason is because some of them try to experiment with drugs abuse (12).

In this study, the most common risk factor for transmission was through heterosexuals contact (41.7%), followed by High risk partners (23.3%), and homosexuals (12.1%). These results were similar with the results of study conducted by Suyanto et al. (2016) with the most common transmission route was through heterosexuals (52.43%) (15). Another study at dr. Soetomo Surabaya Hospital also found that the pattern of transmission of HIV/AIDS in 2006 - 2010 was mostly through heterosexuals group, which was 57.9% (13). Based on a study by Haverkos et al. in the United States, patients classified as heterosexuals were defined as subjects who denied any other risk factors such as homosexuals, injection needle users, and blood recipients, and have had heterosexuals contact with partners who were infected or had a high risk of HIV/AIDS. Those defined as high-risk partners were homosexuals, bisexual men, injecting needle users, and blood recipients. Haverkos also stated that there was a shift in the epidemic from homosexuals and injecting needle users to heterosexuals contact. This may be due to an increase in primary transmission from high-risk groups to heterosexuals contact and also an increase in secondary and tertiary transmission from low-risk heterosexuals to other people that happen continuously (16).

A study by Laksana and Lestari in 2010 at Purwokerto concluded that the higher number of HIV/AIDS cases in the heterosexuals group was due to limited data on

the homosexuals group. This limitation is influenced by several factors such as the bad stigma of society towards homosexuals group, so that the risk factors for HIV/AIDS transmission in homosexuals group remain hidden. The higher cases of HIV/AIDS in the heterosexuals group in Purwokerto were also influenced by the habit of not wearing condoms when engaging in risky sexual activities. Compared with homosexual men, more heterosexual men have sex with commercial sex workers (CSW), and when they had sex with CSWs they did not use condoms (17).

Opportunistic infections are now less common than in the early days of HIV because better treatments reduce the number of HIV and maintain individual immune system. However, many people with HIV still developed OIs because they do not know they had HIV, were not on ARV therapy, or their therapy has failed. Therefore, it is important for people with HIV to know the most common OIs so that they can prevent and get treatment as early as possible (18).

In this study, of total of 320 subjects with OI who came to the VCT Polyclinic of Sanglah General Hospital, there were 495 types of OI where candidiasis was the most common OI found in 188 samples (58.8%) as shown in table 2. The results of this study were in accordance with the study at VCT Polyclinic Sanglah General Hospital on the period September 2015 - September 2016 that found candidiasis was the most common OI which was 35.6% (15).

The results of study by Lubis et al. in 2011 at RSPI Sulianti Saroso Jakarta found that the most common type of OI in HIV patients was tuberculosis (67.4%), followed by toxoplasmosis (22.8%), candidiasis (5.4%), chronic diarrhea (3.3%), and Hepatitis C (1.1%) (14). This indicates that in addition to OIs, not only it can vary from one country with others, it can also vary within a region within the same country. This is due to differences in the pattern of pathogenic microbes (2, 12). Study in China by Xiao et al. concluded that the frequency and types of OI occurred in HIV-infected patients depends on geographic location and varies from one region with another due to different climates and socioeconomic conditions (10).

Candidiasis was the most common OI found possibly because this infection was the most prominent clinical symptoms and easily recognized as an early sign of HIV infection (10). In addition, Candida organisms are common commensals on mucosal surfaces in healthy individuals and there are no specific measures available to reduce the risk of exposure to this fungus. The development of oropharyngeal or esophageal candidiasis has been recognized as an indicator of decreased immunity and was most common in patients with CD4 level <200 cells/µL. The diagnosis of esophageal candidiasis is often made clinically based on symptoms plus response to therapy, or visualization of the lesion plus a fungal smear without histopathological examination. Definitive diagnosis of esophageal candidiasis requires direct endoscopic visualization of the lesion with histopathological results of the typical yeast form of Candida in the tissue and confirmation by fungal culture (11).

Among the HIV patients in this study, the majority who developed OI had a CD4 cell levels below 200 cells/µL, which was 90.6%. This is in accordance with study by Kulkarni et al. which demonstrated that the majority of patients with OI have CD4 counts below 200 cells/µL (7). This study results also in accordance with another study by Jamil et al. (2014) in Aceh which concluded that a decrease in CD4 levels indicates an increase in the incidence of opportunistic infections in HIV patients, although patients with equal number of CD4 levels can still have different OIs (19).

From this study, it was found that in HIV patients, the increase in the prevalence of OI was proportional to the increasing levels of viral load (HIV RNA) in patients as shown in table 5. This is because at high viral load levels, immune deficiency will be more severe. CD4 levels and viral load are laboratory markers that are regularly used for the management of HIV/AIDS patients in addition to predicts the disease progression and treatment outcomes. The target of ARVs is to suppress plasma HIV RNA levels, thereby increasing CD4 levels and consequently reduce the risk of OI as well as drug resistance (20).

In the Spearman correlation test (bivariate) it was found that CD4 levels had a significant negative correlation with the OI numbers. Absolute CD4 level is generally accepted as the best indicator of the immunological competence of patients with HIV infection (2). HIV infection causes a low CD4 levels which makes the body more vulnerable to OI (9). This causes a person with HIV to experience several types of OI at once if the patient has low CD4 levels. Several studies have examined the relationship between CD4 levels and the incidence of OI, but studies on the relationship between CD4 levels and OI are still very few. Research by Yogani et al. in Dr. Cipto Mangunkusumo hospital found that the number of OIs had no relationship with an increase in CD4 cells (p=0.480). This was hypothesized because most of the patients presented with OI <2 came with mild manifestation of OI which was oral candidiasis. Other studies evaluate CD4 levels associated with the OI incidence experienced by patients (9). Research by Damtie et al. in Ethiopia in 2012 concluded that CD4 levels <200 cells/µL were 4.9 times more likely to develop OI compared with group of patients with CD4 levels >350 cells/µL (21).

In the Spearman correlation test (bivariate) it was found that viral load had a significant positive correlation with OI numbers. Clinical observations in HIV patients showed an increase in viral load during the course of OI. Research by Damtie et al. found that HIV RNA was more likely to be detected if people with HIV had an OI (14%) than when they did not have an OI (4%). This difference was statistically significant ($OR = 3.8$, $p < 0.001$). While HIV RNA is a predictor of an increased risk of OI, previous studies have shown an increase in viral load after OI among HIV patients due to infection of tissue macrophages by OI that can increase HIV viral production (21).

In the Spearman correlation test (bivariate) it was found that viral load had a significant negative correlation

with CD4 levels. This may cause by high viral load levels, therefore more severe immune deficiency progress. It was evidenced by a study conducted by Utama and Merati where there was a significant reverse correlation (p-value = 0.016) between viral load and CD4 which means the higher the viral load levels, then the lower the CD4 levels (8). Research by Vennet et al. also concluded that viral load was associated with CD4 levels. An increase in viral load contributes to CD4 cells damage, and this damage can cause defects to immune responses that regulates virus replication (22).

Limitation of this study was in diagnosis establishment on some candidiasis cases of which microbiology test was not conducted that might interferes with diagnostic accuracy.

Conclusion

In this study we found 527 samples patients with HIV/AIDS at VCT Polyclinic Sanglah General Hospital Denpasar with male proportion was 66.2% and female 33.8%. Majority of the samples in this study were in age range of 30-39 years old (39.9%). Of total 527 samples identified, 320 samples obtained had OIs with the most common type of OI was candidiasis (58.8%). The prevalence of OI more commonly found in low CD4 levels and high viral load. Low CD4 and high viral load levels were associated with higher number of OIs in people with HIV infection.

Competing Interests

The authors declare that they have no competing interests.

Ethical Clearance

We obtained approval from the Medical Research and Ethics Committee of Faculty of Medicine of Udayana University and Sanglah General Hospital (Reference No.: 247/UN14.2.2.VII.14/LT/2020)

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