J Med Sci
Volume 46, No. 4, December 2014: 153-160

Low CD4$^+$ T cell counts are not risk factor for Malassezia species infection in HIV/AIDS patients

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ABSTRACT

Human immunodefiiency virus (HIV) infection and aquired immunodeficiency syndrome (AIDS) cause a progressive depletion of CD4$^+$ T cell populations accompanied by progressive impairment of cellular immunity and increasing susceptibility to opportunistic infections. Seborrheic dermatitis is one of the most common skin opportunistic infections on HIV/AIDS patients. Malassezia species is bilieved as the causative of seborrheic dermatitis. The aim of the study was to evaluate low CD4$^+$ T cell counts as risk factor for Malassezia sp. infection in HIV/AIDS patients. This was an observational study with cross-sectional design conducted on HIV/AIDS patients who attended in Department of Dermatology and Venereology, Faculty of Medicine Universitas Gadjah Mada/ Dr Sardjito General Hospital, Yogyakarta and met the inclusion and exclusion criteria. Culture of Malassezia sp. was conducted in Department of Microbiology and classified as high ($\geq$100 CFU/ tape) and low ($<$100 CFU/tape) density colonies. CD4$^+$ T cell counts were measured in Department of Clinical Pathology and classified as high ($\geq$200 cells/mm^3) and low ($<$200 cells/mm^3) CD4$^+$ T cell counts. A total of 83 subjects with HIV/AIDS comprising 54 (65.1%) males and 29 (34.9%) females aged 20 - $>$60 years were involved in the study. The number of Malassezia sp. colony on subjects with high and low CD4$^+$ T cell counts were 31.55 $\pm$ 26.21 and 25.2 $\pm$ 33.89 CFU/ tape, respectively. No significantly relationship between between CD4$^+$ T cell count and Malassezia sp. colony number was observed in the study ($p=0.607$; 95%CI=0.04-5.19; RP=0.452). In conclusion, low CD4$^+$ T cell counts is not risk factor for Malassezia sp. infection in HIV/AIDS patients.

ABSTRAK

Infeksi *human immunodefiiency virus* (HIV) dan *aquired immunodeficiency syndrome* (AIDS) menyebabkan penurunan progresif jumlah sel T CD4$^+$ yang diikuti dengan gagguan imunitas seluler dan meningkatnya risiko infeksi oportunitis. Dermatitis seboroik merupakan salah satu infeksi oportunitis kulit yang umum terjadi pada penderita HIV/AIDS. Malassezia sp. dipercaya penyebab dermatitis seboroik. Tujuan penelitian ini untuk mengkaji kadar sel T CD4$^+$ yang rendah sebagai risiko terjadinya infeksi Malassezia sp. pada penderita HIV/AIDS. Penelitian ini merupakan penelitian observasional dengan rancangan potong lintang yang dilakukan terhadap penderita HIV/AIDS yang berkunjung ke Bagian Kulit dan Kelamin, Fakultas Kedokteran, Universitas Gadjah Mada/RSUP Dr. Sardjito, Yogyakarta dan memenuhi kriteria pemasukan dan pengeluaran. Kultur Malassezia sp. dilakukan di Bagian Mikrobiologi dan dikelompokkan sebagai koloni densitas tinggi (($\geq$100 CFU/tape) dan rendah ($<$100 CFU/tape). Kadar sel T CD4+ ditetapkan di Bagian Patologi Klinik dan dikelompokkan sebagai sel T CD4$^+$ tinggi ($\geq$200 sel/mm^3) dan rendah ($<$200 sel/mm^3). Total 83 penderita HIV/AIDS terdiri 54 (65.7%) pria dan 29 (34.9%) wanita berumur 20 - $>$60 tahun terlibat dalam penelitian. Jumlah koloni Malassezia sp. pada penderita dengan kadar sel T CD4$^+$ tinggi dan rendah masing-masing adalah 31,55 $\pm$ 26,21 dan 25,2 $\pm$ 33,89

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CFU/tape. Tidak ada hubungan bermakna adalah kadar sel T CD4+ dengan jumlah koloni Malassezia sp dalam penelitian ini (p = 0,607; 95%CI = 0,04-5,19; RP = 0,452). Dapat disimpulkan, kadar sel T CD4+ yang rendah bukan merupakan faktor terjadinya infeksi Malassezia sp. pada penderita HIV/AIDS.

Keywords: Malassezia species - colony - CD4+ T cell – risk factor – opportunitic infection

INTRODUCTION

The number of people infected with human immunodeficiency virus (HIV) continues to rise significantly in most parts of the world in the last decade. It was estimated that between 30 and 36 million people in the world are living with HIV in 2012. Approximately 1.8 to 2.3 million of them develop to be acquired immunodeficiency syndrome (AIDS).[1] In Indonesia, approximately 150.200 cases of HIV and 55.700 cases of AIDS with 9.700 deaths from AIDS have been reported in 2014. In Yogyakarta Special Region, 2.611 cases of HIV and 916 cases of AIDS have been reported in 2014.[2]

Human immunodefiiency virus infection causes a progressive depletion of CD4+ T cell populations accompanied by progressive impairment of cellular immunity and increasing susceptibility to opportunistic infections.[3] Seborrheic dermatitis is one of the most common skin opportunistic infections on HIV/AIDS patients. Seborrheic dermatitis is a chronic inflammatory skin disorder that particularly affects the sebaceous-land-rich areas of skin. In general population, the prevalence of seborrheic dermatitis varies between 3 and 5%, while in patients with HIV its prevalence increases between 20 and 30% and up to 80% in patients with AIDS.[1,4,5]

The etiology of seborrheic dermatitis has not been clearly understood. Malassezia sp. is bilieved as the causative of seborrheic dermatitis. Malassezia sp. is part of the normal skin flora. They are found in almost all over the body with varied density depending on age, body site, geographic area, and the presence of normal or diseased skin.[6] The highest density is found in the seborrhoic areas, namely the scalp, face, chest and upper trunk, whereas the lowest density is found on the hands.[7] The pathogenesis of Malassezia sp in seborrheic dermatitis is multifactorial involving external factors such as climate, drug use, lifestyle and internal factors such as immune deficiency, amount of sebum, hyperhidrosis, pregnancy.[8]

The role of Malassezia sp. in seborrheic dermatitis in patients of HIV/AIDS has been investigated by some authors with varied results. The associtation of seborrheic dermatitis and AIDS first reported by Eisenstat in 1984.[9] Further studies showed tha the prevalence of seborrheic dermatitis in patients with HIV differs according to various authors.[10-12] It appears that the subtype of Malassezia sp. and level of CD4+ T cell are found to be risk factors of seborrheic dermatitis in patients of HIV/AIDS. This study was conducted to evaluate level of CD4+ T-cell in HIV/AIDS patients in correlation with density of Malassezia sp. colony.

MATERIALS AND METHODS

Subjects

This was an analytical observational study with cross-sectional design conducted in Department of Dermatology and Venereology, Faculty of Medicine Universitas Gadjah Mada/ Dr Sardjito General Hospital, Yogyakarta. Patients with HIV/AIDS who attended the Dr.

Sardjito General Hospital between June and September 2012 and fulfulled the inclusion and exclusion criteria were involved in this study. The inclusion criteria were male or female HIV/AIDS patients aged over 18 years and agreed to participate in this study by signed an informed consent. The exclusion criteria were pregnant women, currently use of oral contraceptives, use of topical or systemic antifungal, corticosteroids, antibiotics and immunosupresive agents during two last months, and patients with hyperhidrosis. Ethical approval for the study was obtained from the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

Protocol of study

Subjects who welling to participate in the study were given a questionnaire to be filled. The questionnaire consisted of interview-administrative questions regarding age, education, occupation, marrital status and duration of illness. Body weight and body height of subjects were then measured using digital weight completed with microtoise statur meter.

Culture of Malassezia sp. was conducted in Department of Microbiology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. Sample was taken from forehead skin of subjects. The Malassezia sp. were then isolated from the skin with contact plates containing a modified Leeming-Notman's agar medium. The culture plates were incubated at 32 ^{o}C for 14 days. The relative humidity in the incubator was 85% and all culture plates were incubated in plastic bags. Followed after 14 days incubation, the Malassezia sp. were identified and the number of colonies was counted, giving a semi-quantitative number of organisms. The number of colonies was then classified into two groups i.e. subjects with high colonies density (≥ 100 CFU/tape) and those with low colonies density (<100 CFU/tape).

Blood sample for $CD4^+$ T cell measuremnet was taken from veins within fossa cubiti. The measurement of $CD4^+$ T cell was conducted in Department of Clinical Pathology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. $CD4^+$ T cell counts were then classified into two groups i.e. subjects with high $CD4^+$ T cell counts (≥ 200 cells/mm^3) and those with low $CD4^+$ T cell counts (<200 cells/mm^3).

Statistical analysis

Descriptive statistics were used to analyze the characteristics of subjects. Data were presented as percentage. Chi Square test was used to evaluate the correlation between the $CD4^+$ T cell count and the density of Malassezia sp. colonies. p value <0.05 was considered significant.

RESULTS

Characteristics of subjects

A total of 83 subjects with HIV/AIDS comprising 54 (65.1%) males and 29 (34.9%) females aged 20 - >60 years who met the inclusion and exclusion criteria were involved in the study. The characteristics of subjects are presented in TABLE 1.

TABLE 1. Characteristics of subjects

Characteristics	Number (n/%)
Sex	
• Male	54 (65.1)
• Female	29 (34.9)
Age (year)	
• 20-29	26 (31.3)
• 30-39	40 (48.2)
• 40-49	11 (13.3)
• 50-59	5 (6.0)
• > 60	1 (1.2)
Education	
• Elementary school	6 (7.2)
• Secondary sschool	19 (22.9)
• High school	44 (53.0)
• University	14 (16.9)
Occupation	
• Jobless	14 (16.9)
• Labor	8 (9.6)
• Entrepreneur	4 (4.8)
• Private employees	55 (66.3)
• Goverment employees	2 (2.4)
Matrial Status	
• Divorced	11 (13.3)
• Single	28 (33.7)
• Married	44 (53.0)
Body mas index (BMI)	
• Obesity (> 25)	7 (8.4)
• Normal (18.5-25)	51 (61.4)
• Malnutrition (< 18.5)	25 (30.1)
Duration HIV infection (month)	
• 0-12	28 (34.0)
• 13-24	10 (12.0)
• 25-36	14 (17.0)
• 37-48	15 (18.0)
• 49-60	1 (1.0)
• 61-72	7 (8.0)
• 73-84	4 (5.0)
• 85-96	3 (4.0)
• >96	1 (1.0)

Risk factor of HIV infections/AIDS

Multi partner (64 or 77.1% subjects) was found to be risk factors of HIV infection, followed by intravenous drug users (36 or 43.4% subjects) and blood transfution (21 or 25.3% subjects). The risk factors of HIV infections/AIDS are presented in TABLE 2.

TABLE 2. Risk factor of HIV infections/AIDS

Risk factors	Number (n/%)
Multi partner	
• Yes	64 (77.1)
• No	19 (22.9)
Intravenous drug user	
• Yes	36 (43.4)
• No	47 (56.6)
Tranfusion	
• Yes	21 (25.3)
• No	62 (74.7)

Relationship between sex and $CD4^+$ T cell count

The relationship between sex and $CD4^+$ T cell count is presented in TABEL 3. The $CD4^+$ T cell count of male patients with HIV/AIDS (283.66 ± 274.08 cells/mm^3) was higher than female patients (258.81 ± 200.56 cells//mm^3). However, it was not significantly different (p=0.637).

TABLE 3. Relationship between sex and $CD4^+$T cell count

Variable	$CD4^+$ T cell count (cell/mm^3)		p
	≥ 200	< 200	
Sex			
• Male	29	25	0.637
• Female	14	15	

Relationship between sex, age, and Malassezia sp. colony

The relationship between sex and $CD4^+$ T cell count is presented in TABEL 4. Malassezia sp. growth was observed on 74 subjects out of 83 subjects involved in the study. The highest colony number was 165 CFU, whereas the lowest was 1 CFU. The average colony number observed on males subjects was 25.66 ± 32.59 CFU/tape, whereas on females subjects was 25.83 ± 32.90 CFU/tape. No significantly relationship between Malassezia sp. colony number and sex (p= 1.000) as well as age (p= 0.858) were observed in the study.

TABLE 4. Relationship between sex, age, and colony of Malassezia sp colony

Variables	Malassezia sp. colony		P
	High density (≥ 100 CFU/tape)	Low density (< 100 CFU/tape)	
Sex			
• Male	2	52	1.000
• Female	1	28	
Age (year)			
• 20-29	1	25	
• 30-39	1	39	
• 40-49	1	10	0.850
• 50-59	0	5	
• >60	0	1	

Relationship between CD4+ T cell count and Malassezia sp. colony

The relationship between CD4+ T cell count and Malassezia sp. is presented in TABEL 5. The number of Malassezia sp. colony on subjects with high and low CD4+ T cell counts were 31.55 ± 26.21 and 25.2 ± 33.89 CFU/tape, respectively. Low density colony (<100 CFU/tape) was observed on most subjects both who had CD4+ T cell count > 200 cells/mm^3 (42 or 97.9% subjects) and < 200 cells/mm^3 (38 or 95.0% subjects). High density colony was observed only on one subject on subjects who had CD4+ T cell count > 200 cells/mm^3 and two subjects on subjects who had CD4+ T cell count < 200 cells/mm^3. No significantly relationship between between CD4+ T cell count and Malassezia sp. colony number was observed in the study (p=0.607).

TABLE 5. Relationship between CD4+ T cell counts and Malassezia sp colony

Variable	High density (≥ 100 CFU/tape)	Low density (<100 CFU/tape)	Total	95% CI	p	RP
CD4+ T cell*						
• High [n (%)]	1 (2.3%)	42 (97.7%)	3 (3.6%)	0.04-5.19	0.607	0.452
• Low [n (%)]	2 (5.0%)	38 (95.0%)	80 (96.4%)			
Total	3 (.6%)	80 (96.4%)				

*High: CD4+ Tcell counts > 200 cells/mm^3; Low: CD4+ Tcell counts > 200 cells/mm^3

DISCUSSION

This study showed that the prevalence of HIV/AIDS on males was higher than on females. Moreover, the highest prevalence of HIV/AIDS was observed on patients aged between 30 and 39 years. These results were not in accordance with a report that released by The *Directorate General of Disease Control and Environmental* Health, Ministry of *Health* of the Republic of Indonesia which reported that the prevalence of HIV/AIDS on males and females was similar and the highest prevalence was observed on patients aged between 20 and 29 years.[13] However, the education and

occupational of HIV/AIDS patients in this study was similar to that reported by the *Directorate General of Disease Control and Environmental* Health.

Multi partner was found to be risk factor for HIV infection in this study. It was similar with the report that released by the *Directorate General of Disease Control and Environmental* Health. It was reported that unsafe sex (77%) was risk factor for HIV infection, followed by the use unsterile syringe by injecting drug users (8.5%), mother to child transmission (5.1%), male to male sex (2.7%).[13] This study proved that unsafe intercourse was the largest risk factor for HIV infection.

The $CD4^+$ T cell counts in female patients tended to be higher than in male patients in this study although it was not significantly different. A study conducted in Netherlands showed that the mean $CD4^+$ T cell counts were significantly higher in female HIV/AIDS patients than male patients.[14] Similar results were obtained in the studies that conducted in India and in Nigeria.[15,16] It has been suggested that a sex hormone effect could be one possible explanation for the reported higher in $CD4^+$ T cell counts in felamle HIV/AIDS patients.[17]

The number of Malassezia sp. colony in male patients was not signidicantly different than in female patients. A study conducte in Japan showed that the density of Malassezia sp. colony in male patients was higher than those females and the highest density was observed on patients aged from 30 to 40 years.[18] However, a study conducted in Canada showed that no significantly different in number of Malassezia sp. colony between male and female patients was observed.[19]

No significantly relationship between between $CD4^+$ T cell count and Malassezia sp. colony number in HIV/AIDS patients was observed in this study. Several studies to evalute the relationship between Malassezia sp. colonization and the immune status of HIV/AIDS patients have been performed with different results. Hakansson *et al*.[20] reported that there was no correlation between the number of *Pityrosporum ovale* (Malassezia sp.) and the immune status of the HIV-seropositive patients. Moreover, Munoz-Perez *et al*.[21] reported that Malassezia sp. was not assocoated with the onset of seborrheic dermatitis lesions formation on HIV/AIDS patients. In contrast, Farrokh *et al*.[22] demonstrated that low $CD4^+$ T cell counts was associated with the incidence of seborrheic dermatitis in HIV-positive patients. Furthermore, Schechtman *et al*.[23] demonstrated that there was a trend between numbers of Malassezia yeasts present on lesional skin, severity of seborrheic dermatitis and $CD4^+$ T cell counts in HIV-positive patients. Nnoruko *et al*.[24] showed that seborrheic dermatitis had occurred at $CD4^+$ T cell counts of > 200 cells/mm^3 in HIV/AIDS patients.

The variability of data obtained in several studies could be explained by the differences in sampling sampling techniques, media culture race or region. Several sampling techniques are available for Malassezia culture including adhesive tapes stripping, swabbing, agar contact plate and scrub method. The sampling techniques could influence the quality of sample obtained. Dixon media or Leeming and Notman agar are often used for Malassezia cultures. The media used for the cultures could influence Malassezia recovery from sample.[18,25]

CONCLUSIONS

In conclusion, low $CD4^+$ T cell counts are not risk factor for Malassezia sp. infections in HIV/AIDS patients. Further study with large number of subjects is needed to confirm the results of this study.

ACKNOWLEDGEMENTS

Authors would like to thank all subjek who have participated in the study.

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