Immunological and epidemiological factors affecting candidiasis in HIV patients beginning antiretroviral therapy in an Asian clinic

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Running Heading: Oropharyngeal candidiasis in HIV patients

Abstract

Objectives: Oropharyngeal candidiasis (OPC) is common in HIV patients beginning antiretroviral therapy (ART). Here we address the response to ART, and the roles of poor oral hygiene and defects in local innate immunity with a focus on salivary β-defensins, as they are implicated in control of candidiasis but have not been investigated in this context.

Design: ART naïve HIV-infected adults (n=82) with <200 $CD4^+$ T-cells/mm^3 attending clinics at at Cipto Mangunkusumo Hospital, Jakarta, were examined at the commencement of ART, and 73 were re-examined after 3 months. OPC was detected by clinical examination, and *candida* and fungal burdens were determined following culture on CHROMagar and saboroud-dextrose agar (resp). Salivary β-defensins (-2 and -3) were quantified by ELISA. Healthy control subjects (n=40) matched the patients by age and gender.

Results: OPC was evident in 47 patients before ART, and associated with greater fingal burdens. No OPC was detected in healthy controls and culture positivity was rare. ART decreased the prevalence of OPC to 8/73 HIV patients re-examined after 3 months, with reduced total fungal and *C. albicans* burdens. The incidence of OPC was independent of oral hygiene. Hyposalivation was more common in untreated HIV patients (16%) than after 3 months on ART and was rare in healthy controls. HIV patients were also more likely to have acidic saliva. Salivary β-defensin-2 was elevated in the presence of *C. albicans* pseudohyphae and OPC after 3 months on ART, but β-defensin-3 was not affected by OPC or ART.

Conclusions: ART reduces the prevalence of OPC, and the total fungal and *C. albicans* burden. Levels of salivary β-defensin-2 may associate with OPC in HIV patients responding to ART.

Introduction

Oropharyngeal candidiasis (OPC) accounts for about 50% of opportunistic infections among HIV patients, with higher rates in developing countries (Leao, Ribeiro, Carvalho, Frezzini, & Porter, 2009). However few studies have addressed this important condition in patients beginning ART administered under current WHO guidelines or evaluated factors affecting risk. Low $CD4^+$ T-cell counts and high plasma HIV RNA levels have been associated with oral disease in HIV patients. The low pH of saliva, reduced salivary flow rate and poor oral hygiene are risk factors for fungal infection in people without HIV (Muzurovic, Babajic, Masic, Smajic, & Selmanagic, 2012) and may be important in HIV patients. ART reduces mortality and morbidity, including the oral manifestations of HIV/AIDS (Gonc et al., 2013; Taiwo & Hassan, 2010). However pseudomembranous oral candidiasis can develop on ART (Espinosa, Ormsby, & Gonza, 2009).

Saliva contains antimicrobial proteins which inhibit microbial colonization (Alves et al., 2014; (Nittayananta, Kemapunmanus, Amornthatree, Talungchit, & Sriplung, 2014); Khan et al., 2013). Human β-defensins (hBD) are produced by epithelial cells in the buccal mucosa, gingiva, tongue, salivary glands and other parts of the mouth (Diamond & Ryan, 2012; Chung, Dommisch, Yin, & Dale, 2007; Abiko, Nishimura, & Kaku, 2003). hBD-2 and hBD-3 are known as inducible β defensins, which are found at low level under normal conditions and induced by microbial colonization and inflammation, for example in *Candida* infection (Diamond & Ryan, 2012; Hans & Hans, 2014). Production of hBD-2 increases in the presence of Candidiasis, oral lichen planus and leukoplakia in patients without HIV (Abiko et al., 2002). hBD-2 and hBD-3 are induced by TNF-α and interferon-γ with inhibition by IL-4 and IL-13 (Albanesi et al., 2007).

This study investigates factors which may play role in the occurrence of OPC and/or may influence the total fungal burden in HIV-infected patients commencing ART. These include T-cell recovery, oral hygiene, unstimulated salivary flow rate, salivary pH, smoking, alcohol consumption and salivary β-defensins. Smoking can promote many oral pathologies, including candidiasis (Soysa & Ellepola, 2005). Specifically smoking has been associated with the occurrence of pseudomembraneous oral candidiasis, while alcohol consumption was associated

with erythematous type of oral candidiasis (Gonc et al., 2013). Smoking and alcohol may affect saliva. Alcohol consumption can cause sialosis (infiltration of adipose into the salivary gland) and thus alter the salivary flow rate and its components (Carda et al., 2004). Smoking can reduce salivary flow rate and salivary pH. These changes may be accompanied by reduced salivary antimicrobial components (Soysa & Ellepola, 2005).

Materials and Methods

Subjects and oral health examinations

ART-naïve HIV-infected adult patients (n=82) with <200 $CD4^+$ T cells/ml were examined at the HIV clinic of an inner city tertiary hospital (Cipto Mangunkusumo Hospital, Jakarta) and 73 were re-examined 3 months after commencing triple therapy including lamivudine, zidovudine, nevirapine, stavudine, efavirens or tenofovir. Nine patients couldn't be re-examined at three months as they had died from conditions related to AIDS (5 patients), refused to continue (2 patients), experienced a severe drug-induced allergy requiring discontinuation of ART (1 patient) or were lost to follow up (1 patient). Hepatitis B and C were diagnosed serologically and pulmonary tuberculosis by chest x-ray and sputum microscopy. $CD4^+$ T cells and plasma HIV RNA, oral total fungal burden and oral health were assessed. Forty healthy controls were included, matched to the patients by age and gender. Ethical approval for this study was obtained from Health Research Ethic Committee Faculty of Medicine Universitas Indonesia & Cipto Mangunkusumo Hospital (no: 26/H2.F1/ETIK/2013).

All oral examinations were performed by one oral medicine specialist (EW), so no inter-examiner calibration was needed. Oral hygiene was assessed using Oral Hygiene Index-Simplified [OHI-S; (Greene & Vermillion, 1964)], based on the presence of debris and calculus. Gingival health was assessed using the Gingival Index (Benamghar, Penaud, Kaminsky, & Abt, 1982). Unstimulated salivary flow rate was determined from the volume of saliva collected over 5 minutes of spitting into a plastic tube. Hyposalivation was defined as a flow rate of <0.1 ml/minute (Humphrey & Williamson, 2001). Salivary pH was assessed immediately using pH-indicator strips (range 0-14). At least 1ml whole saliva was then collected by spitting into a

plastic tube, centrifuged (5 mins, 3500 rpm), supplemented with 0.5 M Phenylmethylsulfonyl Fluoride [PMSF] (1:10) and stored at -80ºC for salivary protein analyses.

Diagnosis of oropharyngeal candidiasis and detection of fungal colonization.

Diagnoses of pseudomembranous or erythematous OPC were based on a clinical appearance of removable white plaques or atrophic erythematous areas of the oral mucosa (Muzurovic et al., 2012). Clinical diagnostic of OPC was supported by the presence of pseudohyphae as the pathogenic form of *Candida*). After the examination and sampling described above, an oral rinse was performed using 10 ml saline and patients were asked to spit into a sterile container for mycology screening. These samples were streaked onto glass slides to visualise *Candida* yeast and pseudohyphae and onto Sabouraud Dextrose Agar (Oxoid; Basingstoke, UK; incubated at 34ºC) to assess total fungal burden and CHROMagar™ (Paris, France; incubated at 37ºC) to assess *Candida albicans.* Colony-forming units (CFU) were counted manually after 48 hours. Total fungal and *Candida albicans* burden was classified as mild-to-moderate (<50 CFU/ml) or strong (≥50 CFU/ml) (Torres, Peixoto & Caldas, 2002).

Salivary β defensins

96-well plates were coated with 0.83 µg/ml β-defensin coating antibody (Abcam; Cambridge, MA, USA) overnight in 4ºC, washed using PBS 0.05% Tween 20, and blocked using 5% bovine serum albumin (BSA) in PBS at room temperature (1 hour). Samples were diluted in PBS with 2% BSA and added to the plates (2 hours), followed by 0.83 µg/ml β-defensin biotinylated antibody (1 hour) and streptavidin-horse radish peroxidise (1 hour). TMB substrate was applied for 20 minutes, reactions were stopped with H_2SO_4 and plates were read at 450 nm. Data were read from standard curves prepared using aliquots of a pooled sample assigned a value of 1000 arbitrary units (AU)/ml.

Statistics

Controls and HIV^+ groups were compared using non-parametric Mann-Whitney tests, while longitudinal data were assessed using Wilcoxon matched pair tests. Categorical data were assessed by Chi^2 tests. P-values <0.05 were accepted as indicating a significant difference and p<0.10 is noted as suggesting a trend.

Results

Patient demographics

HIV patients and control groups had more male subjects (67.5% for healthy controls, 68.3% for HIV patients, p=1.00). Median (range) ages were 30 (18-46) years for healthy controls and 31(19-49) years for HIV patients (p=0.40). $CD4^+$ T cell counts rose on ART and plasma HIV RNA levels decreased so that only 8.2% of patients had >10,000 copies HIV-1 RNA/ml after 3 months. 29% of patients had hepatitis (B and/or C), and 52% were infected with *Mycobacterium tuberculosis*. 57% of HIV patients were current or past smokers, compared with 50% of healthy controls. 27% reported moderate alcohol consumption, most declaring that they had ceased in the last 12 months. This is not significantly greater than controls (12.5%, data not shown). Oral hygiene was similar in the patients and healthy controls [see Table 1]. Unstimulated salivary flow rates similar in controls and untreated patients, but increased on ART (p=0.001). Compared to healthy controls, more HIV patients had saliva pH below 7 at baseline and after 3 months on ART (p=0.002).

Clinically apparent OPC often resolves on ART, but the total fungal and Candida albicans burdens remain high

OPC was diagnosed in 57% of ART-naive HIV patients. The prevalence decreased by 3 months on ART but OPC remained in 11% of subjects [Table 1]. No healthy control subjects had OPC. Compared to healthy controls, a high total fungal burden was more common in HIV patients before and on ART. Strong total fungal burdens were less common after 3 months on ART (p=0.04). Apart from *Candida albicans*, we identified *C. krusei, C. tropikalis, C. glabrata, C. parapsilosis* and *C. dubliniensis* - mostly in patients with a low fungal burden. *C. albicans* remained more common in patients than controls, but the incidence decreased on ART.

OPC is seen in patients with a high fungal burden and unrelated co-infections

A diagnosis of OPC was clearly associated with the total fungal burden assessed at baseline. Among patients with OPC, 100% had a total fungal burden ≥50 CFU/ml and 98% had a *Candida albicans* burden ≥50 CFU/ml. We observed a a weak association between OPC and high HIV RNA [Table 2], but this did not achieve statistical significance. Tuberculosis co-infection among

ART-naïve HIV patients associated significantly with the presence of OPC (p=0.05), and more subjects with hepatitis B or C co-infection had OPC (p=0.02) [Table 3].

The presence of OPC was not associated with salivary flow rate or salivary pH [Table 2] or with the daily frequency of tooth brushing, antiseptic mouthwash gargling, smoking or alcohol consumption (p>0.05). Smoking and alcohol consumption were not associated with OPC (p=0.53, p=0.8, resp.) [Table 3].

Salivary β defensin-2 may be affected by HIV or OPC

Salivary hBD-2 levels were similar in healthy controls and treated patients, and marginally higher (p=0.09) in untreated patients [Table 1]. Further analyses showed a small decline in salivary hBD-2 levels on ART in patients without OPC (p=0.08) or with a low burden of *Candida* (p=0.05) or total fungi (p=0.01) [Table 4]. Hence levels of hBD-2 were increased by the presence of OPC, *candida* pseudohyphae, total fungi and *Candida albicans* after 3 months on ART (p=0.002-0.06).

Salivary hBD-3 levels were not affected by HIV or ART [Table 1], but were higher in untreated patients with a low fungal burden than in equivalent healthy controls (p=0.03) and declined marginally on ART in this group (p=0.10) [Table 5]. This trend was not evident when patients were stratified by the presence of *Candida* or OPC.

Discussion

We present a longitudinal study of immunodeficient HIV patients in an Asian setting examining salivary, behavioural and HIV-associated factors affecting OPC. A high frequency of oral infections was observed, consistent with advanced immunodeficiency (<200 $CD4^+$ T-cells/μl) before the patients began ART (Bravo et al., 2006). OPC was not significantly linked with smoking even though 50-60% of participants smoked at some time. Many subjects reported that they did not drink alcohol, as expected in a Muslim country, and alcohol consumption did not affect OPC.

Candida albicans yeast cell must adhere to host cells and tissue, or co-aggregate with the oral microbiota, in order to colonize and infect (Kanaguchi, Narisawa, Ito, Kinoshita & Kusumoto, 2012). In a previous study 83% of otherwise healthy individuals from Eastern Europe with oral *Candida* had poor oral hygiene and more dental plaque (Muzurovic et al., 2012). However we found no association between poor oral hygiene and OPC, despite a rigorous investigation of oral health and a detailed questionnaire related to tooth brushing and gargling. It may be relevant that the previous study was performed in healthy subjects without advanced immune suppression, whilst here all subjects were in advanced immune suppression, so even subjects with good oral hygiene may be at high risk of having oral candidiasis. Similarly Campisi (2002) that found no evidence that oral hygiene affect oral yeast carriage and dental plaque was not important *Candida* oral reservoir in non HIV-infected subject (Campisi, Pizzo, Milici, Mancuso & Margiotta, 2002)

HIV patients were also more likely than controls to have acidic saliva (55 *vs* 22.5%). *Candida albicans* growth and burden have been associated with low salivary pH, which may increase yeast adherence to mucosal epithelial surfaces (Bikandi, Moragues & Polonelli, 2000). In acidic saliva (pH <6), *Candida albicans* may grow predominantly in yeast form, while hyphal growth will be induced when pH >7 (Mayer, Wilson & Hube, 2013). Here salivary acidity did not affect yeast or pseudohyphal counts in patients (data not shown). However the development of hyphae is not solely pH-dependent, temperatures above 37^{o}C also have a role (Lu, Su, & Liu, 2014). This could affected our result, but was not assesed. Elevated temperatures may induce heat shock protein (hsp) 90 to inhibit hyphal development (Lu et al., 2014).

Reduced salivary flow rate can promote OPC by altering the oral microbiota and reducing the cleansing action and antimicrobial components of saliva. These changes promote growth of *Candida* (Torres et al., 2002). Here the reduced salivary flow rate in HIV patients is in accord with previous studies (Mahajan, Bagul & Desai, 2015). These authors considered it plausible that decreased salivary flow rate may increase *Candida albicans* adherence to buccal epithelial cells and hence the risk of OPC, but causation was not established. Accordingly we found no link between hyposalivation and OPC [Table 2].

The presence of *Candida albicans* elevated salivary β defensin-2 levels after 3-months on ART, with no clear differences in salivary β defensin-3 levels [Tables 1, 4 and 5]. Previous studies reported that the presence of *Candida albicans* hyphae up-regulates hBD-2 and hBD-3 in epithelial cells (Feng et al., 2005; Cheng, Joosten, Kullberg & Netea, 2012). Here levels of salivary hBD-2 were marginally higher in untreated patients than controls or patients on ART, and increases triggered by candida and the fungal burden were clear after 3 months on ART. The small differences in salivary hBD-2 levels and lack of any difference with salivary hBD-3 remained following adjustment for salivary total protein concentrations (data not shown). This warrants confirmation in larger studies as poor induction of defensins may have broader effects on the immune system. β-defensins can promote chemotaxis of dendritic cells, mast cells, neutrophils and T lymphocytes, as well as complement activation (Feng et al., 2005); (Ślebioda, Szponar & Kowalska, 2013).

Overall the high burden of candidiasis observed here cannot be attributed solely to defects in the induction of β-defensins. A role for systemic defects in T-cell immunity has been argued as there are clear correlations with a Th1 environment (Zhang et al, 2015). This would be consistent with the parallel between susceptibility to *Candida* and tuberculosis, and by the resolution of most cases of candidiasis on ART.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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Table 1. HIV disease and ART affect salivation and candidiasis

	HEALTHY CONTROLS (N= 40)	**HIV PATIENTS**		***p*-values[b]**		
		ART-naïve (N=82)	**3 months ART (N=73)**			
	a	***b***	***c***	***a vs b***	***a vs c***	***b vs c***
$CD4^+$ T-cells/μl	-	62 (2-199)	189 (7-601)	-	-	**<0.001**
Log_{10} HIV RNA	-	5.1 (2.6-6.5)	1.8 (1.3-5.2)	-	-	**<0.001**
Oral Hygiene (%)						
Good	15[a]	16	18			
Moderate	52.5	45	45	0.73	0.76	0.97
Poor	32.5	39	37			
Unstimulated salivary flow rate (USSFR)						
Rate (ml/min)	0.3 (0.06 - 0.9)	0.2 (0 - 2.1)	0.4 (0 - 0.9)	0.64	0.65	**0.001**
USSFR category (%)						
Hyposalivation	2.5	16	4.1			
Below Normal	32	43	26	**0.04**	0.88	**<0.001**
Normal	40	22	44			
Above Normal	25	20	26			
Saliva pH (%)						
5	0	8.5	5.5			
6	22.5	51	51	**<0.001**	**0.002**	0.72
7	77.5	40	44			
Oropharyngeal candidiasis (%)						
Absent	100	43	89	**<0.001**	**0.03**	**<0.001**
Present	0	57	11			
Candida - yeast form (%)						
Absent	85	26	41	**<0.001**	**<0.001**	**0.04**
Present	15	74	59			
Candida – pseudohyphae (%)						
Absent	95	52	81	**<0.001**	**0.04**	**<0.001**
Present	5	48	19			
Total fungal burden by culture (%)						
< 50 CFU/ml	70	13	27	**<0.001**	**<0.001**	**0.04**
≥ 50 CFU/ml	30	87	73			
Candida albicans burden by culture (%)						
< 50 CFU/ml	75	20	34	**<0.001**	**<0.001**	**0.04**
≥ 50 CFU/ml	25	80	66			
Salivary β defensins (AU/ml)						
β defensin-2	46 (2.4-1394)	70 (0-4286)	53 (0-469)	0.24	0.76	0.09
β defensin-3	36 (0-804)	34 (0-502)	37 (0-324)	0.99	0.88	0.56

[a] Percentage of individuals in each category

[b] Continuous data were analysed by non-parametric Mann Whitney tests and categorical data by χ^2

Table 2. Fungal burden, *Candida* yeast and pseudohyphae associate with OPC before ART

	Before ART			***After 3 months on ART***		
	No OPC N=35	OPC N=47	*p*-value[b]	No OPC N=65	OPC N= 8	*p*-value[b]
CD4⁺ T-Cells (%)						
<100 cells/μl	57[a]	83	0.22	14	25	0.17
100-200 cells/μl	43	17		35	50	
>200 cells/μl	0	0		51	25	
Plasma HIV RNA (%)						
Undetected	0	0	0.22	38	12	0.10
<10,000 copies/ml	8.6	19		55	62	
>10,000 copies/ml	91	81		6.2	25	
Gingival Inflammation (%)						
No Inflammation	8.6	0	0.14	1.5	0	0.37
Mild Inflammation	69	81		86	100	
Moderate Inflammation	23	19		12	0	
Oral Hygiene (%)						
Good	20	13	0.42	18	12	0.19
Moderate	49	43		42	75	
Poor	31	45		40	12	
USSFR (ml/min)						
	0.2 (0.02-1.2)	0.2 (0-2.1)	0.84	0.3 (0-0.9)	0.4 (0.1-0.6)	0.72
Salivary pH (%)						
5	5.7	11	0.71	4.6	12	0.18
6	51	51		52	38	
7	43	38		43	50	
Total Fungal burden (%)						
< 50 CFU/ml	31	0	**<0.0001**	29	12	0.32
≥ 50 CFU/ml	69	100		71	88	
Candida albicans burden (%)						
< 50 CFU/ml	43	2.1	**<0.0001**	37	12	0.17
≥ 50 CFU/ml	57	98		63	88	
***Candida* Yeast (%)**						
Absent	49	8.5	**<0.0001**	43	25	0.33
Present	51	91.5		57	75	
***Candida* Pseudohyphae (%)**						
Absent	74	36	**0.006**	81.5	75	0.12
Present	26	64		18.5	25	

[a] Percentage of individuals in each category

[b] Continuous data were analysed by non-parametric Mann Whitney tests and categorical data by χ^2 tests

Table 3. Systemic co-infections associate with OPC, with no clear effect of smoking or oral hygiene

	No OPC (N=35)	**OPC (N=47)**	***p*-value[a]**
Tuberculosis Co-infection (%)			
Absent	60	38	**0.05**
Present	40	62	
Hepatitis Co-infection (%)			
No Hepatitis co-infection	76	68	**0.02**
Hepatitis B co-infection	6.1	6.4	
Hepatitis C co-infection	6.1	25	
Hepatitis B + C co-infection	12	0	
Smoking habit (%)			
Still smoking	29	32	0.53
Stop <12 months	23	30	
Stop >12 months	8.6	2.1	
Never smoked	40	36	
Alcohol consumption (%)			
Still drinking alcohol	5.7	2.2	0.88
Stop <12 months	20	26	
Stop >12 months	17	17	
Never drank alcohol	57	54	
Frequency of tooth brushing (%)			
1 time per day	11	8.5	0.42
2 times per day	74	85	
3 times per day	14	6.4	
Antiseptic mouthwash gargling habit (%)			
No	77	77	0.95
Yes	23	23	

[a] Categorical data were analysed by χ^2 tests

Table 4. The fungal burden and OPC affect levels of **s**alivary β defensin-2 on ART

	HEALTHY CONTROLS (N= 40)	**HIV PATIENTS**		**p-values**		
		ART-naïve (N=82)	**3 months ART (N= 73)**			
	a	**b**	**c**	**a *vs* b**	**a *vs* c**	**b *vs* c**
Oropharyngeal candidiasis						
Absent	46 (2.4-1394)[a]	82 (0-1794)	45 (0-469)	0.76	0.72	**0.03**
Present	No cases	66 (3.7-4287)	159 (1.2-386)	-	-	0.84
		p= 0.30	*p*= 0.06			
***Candida* Pseudohyphae**						
Absent	43 (2.4-1394)	66 (0-1794)	42 (0-299)	0.37	0.82	0.10
Present	528 (182-874)[b]	72 (0.5-4289)	90 (0-469)	-	-	0.86
	-	*p*= 0.67	***p*= 0.04**			
Total fungal burden						
< 50 CFU/ml	43 (2.4-463)	100 (16-234)	13 (0-126)	0.07	0.06	**0.01**
≥ 50 CFU/ml	49 (3.8-1394)	66 (0-4287)	71 (0-469)	0.75	0.87	0.26
	p= 0.37	*p*= 0.82	***p*= 0.002**			
***Candida albicans* burden**						
< 50 CFU/ml	43 (2.4-463)	49 (0-338)	18 (0-202)	0.82	0.35	**0.05**
≥ 50 CFU/ml	115 (4.1-1394)	77 (0-4287)	62 (0-469)	0.63	0.38	0.20
	p= 0.19	*p*= 0.26	***p*= 0.04**			

[a] Levels of **s**alivary β defensin-2 presented in AU/ml

[b] Positive only in 2 subjects, statistical analysis cannot be performed

Table 5. Levels of **s**alivary β defensin-3 were not associated with OPC, fungal burden, or *Candida* pseudohyphae

	HEALTHY CONTROLS (N=40)	**HIV PATIENTS**		***p*-values**		
		ART-naïve (N=82)	**3 months ART (N=73)**			
	a	**b**	**c**	**a *vs* b**	**a *vs* c**	**b *vs* c**
Oropharyngeal candidiasis						
Absent	36 (0-804)[a]	46 (0-300.4)	37 (0-324)	0.86	0.84	0.25
Present	No cases	33 (0-502.2)	40 (0-131)	-	-	1.00
		$p = 0.17$	$p = 0.79$			
***Candida* Pseudohyphae**						
Absent	35 (0-624)	43 (0-300)	32 (0-287)	0.55	0.87	0.36
Present	447 (90-804)[b]	33 (0-502)	53 (0-324)	-	-	0.79
	-	$p = 0.69$	$p = 0.18$			
Total fungal burden						
< 50 CFU/ml	32 (0-624)	54 (9.6-126)	31 (0-287)	**0.03**	0.78	0.10
≥ 50 CFU/ml	60 (0-804)	32 (0-502)	47 (0-324)	0.15	0.34	0.60
	$p=0.07$	$p = 0.06$	$p = 0.33$			
***Candida albicans* burden**						
< 50 CFU/ml	34 (0-624)	33 (0-126)	38 (0-287)	0.48	0.83	0.24
≥ 50 CFU/ml	64 (0-804)	37 (0-502)	34 (0-324)	0.31	0.32	0.60
	$p=0.14$	$p = 0.67$	$p = 0.92$			

[a] Levels of **s**alivary β defensin-3 presented in AU/ml

[b] Positive only in 2 subjects, statistical analysis cannot be performed

Highlights

Oropharyngeal candidiasis (OPC) is common in HIV patients beginning ART

We present a study of 82 ART naïve HIV-infected adults beginning ART with <200 $CD4^+$ T-cells/μl at Cipto Mangunkusumo Hospital, Jakarta

At baseline, OPC was detected in 47 patients and linked with a high burden of culturable candida species. No OPC was detected in healthy controls and culture positivity was rare. ART decreased the prevalence of OPC to 8/73 HIV patients re-assessed after 3 months, with reduced total fungal and *C. albicans* burdens.

Salivary β-defensin-2 was elevated in the presence of *C. albicans* hyphae and OPC after 3 months on ART, but β-defensin-3 was not affected by OPC or ART.