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Pathology of *Candida* infection in oral HIV-associated Kaposi sarcoma: a descriptive study.

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ABSTRACT

Aims and objectives: To determine the frequency and histomorphology of secondary *Candida* infection in oral HIV-associated Kaposi sarcoma (HIV-KS) and to describe the demographics of patients with oral HIV-KS with and without secondary *Candida* infection.

Materials and methods: Haematoxylin and eosin, and periodic acid-Schiff stains of 32 oral HIV- KS were examined histologically for intensity and morphology of *Candida* colonisation, depth of invasion, number of organisms, epithelial reactions and inflammatory response. Depth of *Candida* invasion and severity of infection were correlated with CD4 T-cell counts of HIV-positive patients.

Results: Forty-one percent of oral HIV-KS were secondarily infected with *Candida* (*n*=13). Intensity varied from an isolated single pseudohyphus to matted colonies. Whilst in most cases, organisms did not invade beyond the parakeratin layer, pseudohyphae extended into stratum spinosum in two cases, and a single case showed a pseudohyphus within the lamina propria. Two cases showed pseudohyphae in the pyogenic membrane. Neutrophilic permeation of epithelium, commonly associated with *Candida* infection was frequently present even in absence of *Candida* infection.

Conclusion: Oral HIV-KS is commonly secondarily infected with large numbers of *Candida* organisms. Morphological characteristics of secondary *Candida* infection in surface epithelium of HIV-KS suggest an altered pathogenetic pathway. Further studies are indicated.

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INTRODUCTION

The surface epithelium in oral human immunodeficiency virus-associated Kaposi sarcoma (HIV-KS) frequently shows secondary *Candida* infection with varying degrees of tissue invasion. The pathogenesis of such opportunistic fungal infections probably differs in oral HIV-KS, other HIV-related oral disease, and infection of normal oral mucosa in HIV-positive patients.[1] Differences in pathogenetic pathways may vary in histomorphology, frequency or intensity of infection. The aim was to determine the frequency and histomorphology of secondary *Candida* infection of surface epithelium in oral HIV-KS, and to highlight patient demographics in oral HIV-KS with and without secondary *Candida* infection of overlying epithelium.

MATERIALS AND METHODS

Thirty-two oral HIV-KS diagnosed in the Department of Oral Pathology, University of the Witwatersrand, South

Table 1: Criteria for which Oral HIV-KS were histologically examined

Frequency and presence of secondary *Candida* infection	• surface epithelium • pyogenic membrane (fibrinopurulent exudate over ulcer)
Site of infection	• epithelium only • epithelium and pyogenic membrane • epithelium and connective tissue • pyogenic membrane only
Foci and intensity of *Candida* infection	• mild, moderate or severe • number of pseudohyphae and yeasts
Depth of *Candida* penetration	• superficial parakeratin only • parakeratin and superficial spinous layer • parakeratin, deep spinous layer and lamina propria • pyogenic membrane in ulcerated areas
Presence of *Candida* organisms	• pseudohyphae only • pseudohyphae with yeasts • association with neutrophilic micro-abscesses
Presence of epithelial hyperplasia	
Presence and type of inflammatory cells in lamina propria	
Correlation of presence, intensity and depth of invasion of *Candida* infection with CD4 counts at time of biopsy	

Table 2: Comparative clinico-pathologic demographic data in HIV-seropositive group regardless of secondary Candida infection, and specifically in oral HIV-KS with Candida and oral HIV-KS without Candida

		HIV-KS *n*=32		HIV-KS 2° Candida + *n*=13		HIV-KS 2° Candida – *n*=19	
		M	F	M	F	M	F
Age and Gender	Mean	45.5	41.5	45.5	42	49.5	40.5
	Mean (total)	41.5		42		41.5	
	Median (total)	41.5		42		41.5	
	Total	12 (37.5%)	20 (62.5%)	6 (46.15%)	7 (53.84%)	6 (31.57%)	13 (68.42%)
	M:F ratio	1:1.7		1:1.2		1:2.2	
Site	Palate	5	7	3	1	2	6
	Tongue	4	6	2	4	2	2
	Gingiva	1	2	0	1	1	1
	Labial mucosa	0	1	0	0	0	1
	Floor of mouth	0	1	0	1	0	0
	Retromolar area	0	1	0	0	0	1
	Buccal mucosa	1	0	0	0	1	0
	>1 site	1	2	0	1	1	1
Inflammation: intensity				*n*	%		
Acute	Mild			11	22.9		
	Moderate			6	12.5		
	Severe			1	2.1		
Chronic	Mild			9	18.8		
	Moderate			0	0		
	Severe			0	0		
Acute and chronic	Mild			11	22.9		
	Moderate			2	4.2		
	Severe			0	0		
No inflammation				8	16.7		

Africa over a five-year period were analysed. Data included patient age, gender, site of lesion, HIV status and CD4 counts. Ethics clearance was granted by the Human Research Ethics Committee (Medical), University of the Witwatersrand, Johannesburg (M00/08/29; M08.03.25).

Haematoxylin and eosin (H&E) and periodic acid Schiff (PAS) stained 4µ sections of HIV-seropositive oral KS (HIV-KS) were histologically examined for the criteria listed in Table 1. This is primarily a descriptive histomorphologic study with data being of a descriptive nature without statistical comparison.

RESULTS

The 32 patients who presented with oral HIV-KS ranged in age from 20 to 63 years and were mainly in the fourth decade (M:F=1:1.7). The age of males was not statistically different from females (p>0.5). The site most affected was the palate (37.5%) followed by the tongue (31.5%) (Table 2).

Only 40.6% of oral HIV-KS showed secondary *Candida* infection with PAS positive pseudohyphae and yeast cells in the surface epithelium or pyogenic membrane. The remaining 59.4% showed no *Candida* infection. The age of patients with oral HIV-KS secondarily infected with *Candida* ranged from 21-63 years; with most cases in the fourth and third decades (M:F=1:1.2). The site most affected was the tongue (46.2%) followed by the palate (30.8%) (Table 2). The age range of the 19 HIV-positive patients with oral HIV-KS not secondarily infected with *Candida* was 20-63 years. Most cases occurred in the fourth decade, followed by the third, fifth and seventh decades in both males and females, with a M:F ratio of 1:2.2. The most common site was the palate (42.1%) followed by the tongue (10.5%) (Table 2).

Of the 13 oral HIV-KS positive for *Candida*, pseudohyphae penetrated the parakeratin layer only (46.2%) (Figures 1a,b,c), both the pyogenic membrane and parakeratin layer simultaneously (7.7%), the superficial epithelium (7.7%) and deep stratum spinosum (15.4%) (Figures 2a,b). In 15.4% of cases, *Candida* pseudohyphae were noted in the necrotic slough only (Figure 1d). There was a single exceptional case of severe *Candida* colonisation with pseudohyphae penetrating the lamina propria and HIV-KS tissue (Figure 2c).

Secondary *Candida* infection ranged from severe (46.2%) to moderate (23.1%) to mild (30.8%) (Figures 1c,d). Mild infection showed penetration into tissues by only single, isolated *Candida* organisms, usually in the superficial parakeratin layer (Figure 1e). Moderately infected cases showed greater numbers of *Candida* pseudohyphae and yeasts. The parakeratin layer was thicker, with desquamation, and organisms mainly in the desquamated keratin and superficial parakeratin layers (Figures 1b,c).

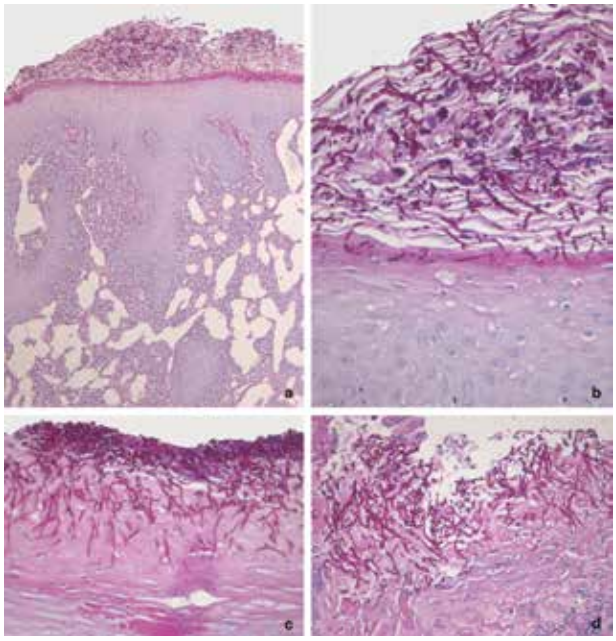

Figure 1: (a) Oral HIV-KS: parakeratin overlying epithelium showing pseudoepitheliomatous hyperplasia and heavy infiltration by *Candida* pseudohyphae (PAS; x10) **(b).** Despite heavy colonisation by *Candida* yeasts and pseudohyphae at the junction of parakeratin and stratum spinosum, penetration beyond parakeratin does not usually occur (PAS, x40) **(c).** Severe surface *Candida* infection by yeasts and pseudohyphae, with the latter showing a higher penetrative capacity (PAS, x40) **(d).** This is an unusual finding of *Candida* organisms growing in the pyogenic membrane (PAS, x20).

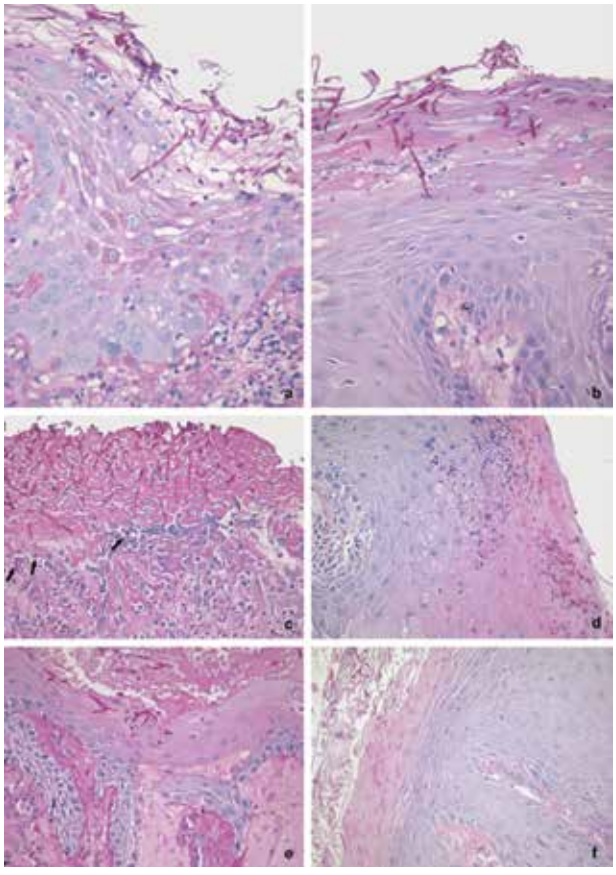

Figure 2: (a,b) *Candida* infiltration restricted to parakeratin with only single pseudohyphus penetrating the spinous cell layer, and chronic inflammation in the lamina propria underlying the *Candida* invasion (PAS, x40). **(c)** Deep penetration by *Candida* into ulcerated oral HIV-KS, connective tissue and lesional tissue (arrowed); intense inflammation in the lamina propria (PAS, x40). **(d)** *Candida* restricted to parakeratin with neutrophilic micro-abscesses (PAS, x40). **(e, f)** *Candida* in parakeratin without inflammation in the epithelium or connective tissue (PAS, x40).

Severe infection showed matted colonies of *Candida* pseudohyphae and yeasts in both the desquamated keratin and parakeratin layers (Figure 1c), sometimes reaching the stratum spinosum (Figures 2a,b). The yeasts always remained on the surface.

Neutrophilic micro-abscesses in superficial parakeratin were present in 53.8% of cases, however no *Candida* organisms were seen in close association to the micro-abscesses in 46.2% of cases. Surprisingly, *Candida* pseudohyphae were present without micro-abscesses in 53.8% of cases (Figures 2d,e,f).

Inflammation beneath *Candida* infected areas in the lamina propria ranged from severe (7.7%) to moderate (23.1%) to mild (53.8%), with areas having no inflammation (15.4%) (Figure 2). Epithelial hyperplasia (Figure 1a) was noted in 61.5% of infected cases, usually with severe infection. *Candida* positive cases were characterised by hyperparakeratosis and hyperplasia of surface epithelium compared to non-infected cases. Pseudoepitheliomatous hyperplasia was present in 7.7% of cases with severe infection.

An inverse relationship was noted between the CD4 count in the 13 oral HIV-KS secondarily infected with *Candida* and the presence, intensity and depth of penetration of *Candida* infection (Table 3). Cases with low CD4 counts showed deeper penetration of *Candida* pseudohyphae (Table 3). The 19 oral HIV-KS with no *Candida* growth showed equally low CD4 counts as the 13 oral HIV-KS secondarily infected with *Candida*; 9 cases had CD4 counts of < 100 cells/mm^3.

DISCUSSION

HIV/AIDS has reached epidemic proportions in South Africa, with a dramatic increase in KS frequency.[2-4] In 21% of HIV-positive people with KS, the initial presentation is in the mouth, while in 71.5% the mouth is affected at some time during the course of the disease. The number of oral KS cases in the Department of Oral Pathology increased from 84 cases over a 29-year period (1973-2002) to 133 new cases during the five-year study period (2003-2007).[5] There has however been a 70-80% reduction in the risk of developing KS in South Africa, where up to 48% of HIV-infected adults are positive for HHV8.[6]

This study showed a secondary *Candida* infection rate of oral HIV-KS of only 40.6%. Many histopathologists anecdotally believe that the secondary *Candida* infection rate of HIV-KS is much higher. There are no comparable studies of infection rate or histomorphology of *Candida* secondary infection of oral HIV-KS to which the results of this study can be compared.

When considering reasons for the relatively low infection rate, it must be realised that the surface epithelium covering oral KS or oral HIV-KS may be unique and not comparable with other HIV-related lesions or even with normal epithelium in an HIV-positive patient. Oral HIV-KS may well influence the surface epithelium by induction from cytokines, prostaglandins and genetic influences, all of which influence the oral epithelial response to *C. albicans* penetration.[7,8] Oral HIV-KS cells may create a unique micro-milieu not reproducible in other situations.

Table 3: Histological features of tissue biopsy specimens at time of serological analysis

CD4 T cell count (cells/mm^3)	Histological Features
599 – 500	- *Candida* pseudohyphae and yeast cells in epithelium in all cases - multiple loci of infection - mild acute and/or chronic infection in all cases - mild epithelial hyperplasia
499 – 400	No correlating data available for this subset
399 – 300	No correlating data available for this subset
299 – 200	- *Candida* pseudohyphae and yeast cells in epithelium in all cases - multiple loci of infection - mild acute and/or chronic infection in all cases - a single case of severe infection - moderate epithelial hyperplasia
199 – 100	No correlating data available for this subset
99 – 0	- *Candida* pseudohyphae and yeast cells in epithelium in all cases - 2 cases with *Candida* invasion into spinous epithelial layer - multiple loci of infection, - mild acute and/or chronic infection in all cases - a single case each of moderate and severe infection - moderate epithelial hyperplasia

One cannot infer that mechanisms by which secondary *Candida* infection of oral HIV-KS occur are the same as those of primary *Candida* penetration in HIV-positive and HIV-negative patients or in other situations of oral *Candida* infection, and more importantly in other mucosal sites. For example, whilst oropharyngeal candidiasis occurs commonly in HIV-positive women *Candida* infection is rarely seen in vaginal mucosa of the same cohort.[7]

Oral *Candida* infection rates in HIV-positive patients vary from 26.3% (India)[9] and 38% (Tanzania) to 94% (Zaire), and range from 37.8% to 63% in South Africa.[10] Our findings of secondary *Candida* infection rate in oral HIV-KS (40.6%) are consistent with previous reports.

In South Africa, though KS was regarded as occurring predominantly in males, greater female involvement is recorded.[2] The KS incidence has doubled in men and increased 7-fold in women resulting in a M:F ratio decline from 7:1 (1988) to 2:1 (2001).[2] A previous study in our Department showed M:F ratio of 1.3:1 in 81 oral HIV-KS during 1997-2003.[5] This was suggested to be due to differences in mode of HIV transmission, which is predominantly heterosexual in South Africa.[5] The current study showed a prominent female predominance in oral HIV-KS, consistent with the dramatic increase in HIV infection of women in this population.[11] In Africa across all ages, more females than males are infected with HIV, which may explain the M:F ratio reversal in favour of females. This gender imbalance of HIV-infected persons in Africa is most marked amongst the 15-24 age group, where M:F ratio is 1:4.[11] The decrease in M:F ratio of HIV-KS in Africa due to ART is unlikely as the decline occurred before ART availability and HIV-KS incidence has not changed significantly since ART introduction.[11]

HIV-KS is reported in all ages; mainly in the third and fourth decades of life.[5] Our study reported a predominance in third and fourth decades with no difference in age between HIV-KS patients secondarily infected with *Candida* and those who were not. Oral KS most frequently affects the palate, gingiva and dorsum of tongue.[5] Our study demonstrated a similar predilection of oral HIV-KS for the palate followed by the tongue in HIV-positive patients regardless of secondary *Candida* infection.

Oral HIV-KS may develop at any stage of HIV infection and especially when CD4 T-cell counts fall below 200 cells/mm.[12] Our study confirmed that most oral HIV-KS occurred at CD4 counts <100 cells/mm^3. There was no difference in CD4 counts between lesions infected and those not secondarily infected with *C. albicans*. This implies that CD4 cells are not a determining factor in the pathogenesis of secondary candidiasis in oral HIV-KS. This remains to be confirmed with a larger study sample. A similar study showed 75% of 130 HIV-KS had CD4 counts of <200 cells/mm3 confirming that low CD4 counts are not a prerequisite for HIV-KS development.[13] Previous reports show that CD4 T-cell numbers are markedly reduced in the oral mucosa of HIV-positive patients with or without candidiasis, and that *C. albicans* specific peripheral CD4 T-cells are depleted with HIV disease progression and concurrent oral candidiasis.[14]

This study is the first to establish the frequency of secondary *Candida* infection in oral HIV-KS and to describe the histomorphology. *Candida* pseudohyphae were present in epithelium overlying oral HIV-KS. Severity of *Candida* infection varied from single isolated pseudohyphae to matted colonies of fungal organisms. Morphology of less severe *Candida* infections closely mimicked that of immune competent hosts whereas severe *Candida* infections in oral HIV-KS showed more numerous and deeper penetration of *Candida* organisms than that in immune competent hosts.

Our study confirmed that *Candida* infection regardless of severity, rarely reaches the lamina propria by penetration through epithelium. In the single case where the organism penetrated connective tissue and KS tissue, it entered through the necrotic ulcerated surface, and not through intact epithelium. The reasons for this are unclear but perhaps the more anaerobic conditions found deep in the epithelium limit the depth of infiltration by organisms.

Further, *Candida* is epitheliotrophic and depends on attachment and penetrative biological processes to infect host tissue.[7] It is uncertain whether these characteristics are inherent in either or both the organism and the host epithelium. Invasion of pyogenic membrane shows that epithelial factors are not absolutely essential for organism growth but that under exceptional circumstances organisms are capable of living in the fibrinopurulent exudate.

CONCLUSION

Only less than half of the cases of oral HIV-KS were secondarily infected with *Candida*. This may be due to the fact that many oral HIV-KS are covered by a pyogenic membrane. Furthermore, *Candida* is strongly epitheliotrophic and *Candida* infection in the fibrinopurulent

exudate overlying oral HIV-KS is unusual. The relatively low frequency of secondary *Candida* infection of oral HIV-KS, its deep tissue penetration, and its presence in the absence of inflammation requires further investigation.

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