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Candida Infection in Oral Lesions of Kaposi Sarcoma

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry

March, 2011

DECLARATION

I, Arshaad Sibda declare that this research report is my own work. It is being submitted for the degree of Master of Science in Dentistry to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

_____

_____ day of _____, 2011

DEDICATION

To my loving parents

Ahmed and Marriam Sibda

for their unwavering support

ABSTRACT

Background

Oral candidiasis is the most common infection of the oral mucosa of HIV-seropositive patients, although its frequency is rapidly decreasing with the advent of highly active antiretroviral therapy (HAART). Many questions regarding its complex pathogenesis remain unanswered. The diagnosis is usually established with non-invasive techniques such as mucosal smears. Oral lesions of HIV-associated Kaposi sarcoma (HIV-KS) are routinely biopsied and frequently show secondary infection with *Candida albicans* or other *Candida species*.

Aims and objectives

The aim of this investigation was to determine the frequency and histomorphology of secondary *Candidal* infection of the surface epithelium of oral HIV-associated KS lesions (HIV-KS), which are routinely biopsied in HIV infected patients.

Materials and methods

Haematoxylin and eosin (HE), and Periodic Acid-Schiff (PAS) stains of 133 cases of oral Kaposi sarcoma diagnosed between the period 2003 and 2007 within the Division of Oral Pathology were examined histologically for intensity and morphology of *Candidal* colonisation, depth of invasion, number of organisms, epithelial reactions and associated inflammatory response. The depth of *Candidal* invasion and severity of infection were correlated with the available CD4 T cell counts of HIV seropositive patients at the time of biopsy.

Results

Almost forty one percent (40.62%) of all oral HIV-KS cases were secondarily infected with *Candida species*. The intensity varied from an isolated single pseudohyphus to matted colonies of vegetative yeasts and psuedohyphae. Whilst in most cases the organisms did not invade beyond the parakeratin layer, pseudohyphae were noted extending into the stratum spinosum in 2 cases, and a single case showed a pseudohyphus within the lamina propria. A further 2 cases showed pseudohyphae growing in the pyogenic membrane. Neutrophilic permeation of the epithelium and Munro micro-abscess formation, features commonly associated with *Candidal* infection, were frequently present even in the absence of *Candidal* infection. *Candidal* organisms were often present in the absence of inflammation.

Conclusion

Oral lesions of HIV-KS are commonly secondarily infected with large numbers of *Candidal* organisms. The morphological characteristics of secondary *Candidal* infection within the surface epithelium of HIV-KS lesions suggest an altered pathogenetic pathway. Further studies are necessary in this regard.

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To my family, my parents Ahmed and Marriam Sibda who stood unwaveringly at my side through all obstacles and have continuously motivated me on a path of knowledge throughout my life. For this I am eternally grateful to them.

To God Almighty for giving me the knowledge, wisdom, strength and determination to conquer every hurdle.

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CHAPTER 1

1.0 INTRODUCTION

Our understanding of the epidemiology and pathology of human immunodeficiency virus (HIV) related oral disease has undergone significant change since the first description of oral lesions in HIV positive patients in 1982.[1] Since then it has been conclusively shown that oral lesions in HIV disease:

a. may present in individuals soon after sero-conversion as part of the acute infection syndrome[2]
b. may be indicative of the presence of HIV disease[3]
c. may predict progression of HIV disease[4]
d. can be related to a decline in CD4 counts and rising viral load[3,5]
e. can be used as indicators for initiation of anti HIV and anti-opportunistic infection prophylaxis and therapy in countries with poor resources while CD4 T cell counts are essential to monitor the response to treatment.[3,6]
f. are used in staging systems such as the World Health Organisation (WHO) clinical staging system[3]
g. may be indicative of successful highly active antiretroviral therapy (HAART) therapy, or the first sign of failure of such therapy,[7] or the emergence of the immune reconstitution inflammatory syndrome (IRIS)[8]

One of the most frequent oral lesions in HIV disease is candidiasis.[3] The pathogenesis of *Candida* infection is complex and although progress has been made in elucidating the factors affecting *Candidal* infection many questions remain unanswered. The surface epithelium in

oral lesions of HIV-KS frequently shows secondary infection by *C. albicans* with varying degrees of tissue invasion. The pathogenesis of such opportunistic fungal infections probably differs between oral lesions of HIV-KS, other examples of HIV related oral disease or even infection of normal oral mucosae in HIV seropositive patients. Such differences in pathogenetic pathways may manifest histologically as variations in the frequency or intensity of the infection or in the histomorphology of the tissue reactions. In order to investigate this hypothesis we decided to undertake a study designed to determine the histological variables in oral lesions of HIV-KS.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Introduction

An extensive review of the literature reveals a paucity of published material regarding *Candidal* secondary infection of oral HIV-KS lesions. The literature on HIV-KS and oral *Candida* infection per se is extensive, including several reviews and studies on the essential role of Human Herpes Virus 8 (HHV8) in the development of KS, the development of KS in HIV-seropositive patients and *Candida* infection in immunocompromised patients.[5,9,10]

2.2 Kaposi sarcoma

KS is a multifocal angioproliferative neoplasm characterised by inflammation, oedema, neoangiogenesis and spindle cell proliferation. The pathogenesis of HIV-associated KS (HIV-KS) is multifactorial. KS is a tumour of lymphatic endothelial origin that primarily affects mucocutaneous sites, but may also involve internal organs.[10] Five clinical presentations of KS are recognised and these include the classic, endemic (African), iatrogenic, immuno-suppression associated and HIV associated variants.[11]

2.2.1 Incidence of HIV-associated KS (HIV-KS)

HIV-KS is the most frequent oral neoplasm and the fourth most common dermatological neoplasm found in HIV-seropositive individuals and may occur at any level of CD4 T cell count during HIV infection, but usually affects HIV seropositive subjects with CD4 T cell counts of below 200 cells/µl.[12] According to Sitas *et al.*[13] KS was endemic in black South Africans even before the advent of HIV and the incidence has since risen threefold between

1988 and 1996 and continues to increase as the HIV pandemic grows.[13] Whilst they state that KS represents about 9% of all cancers in men in Sub-Saharan Africa, the authors do not comment on the exact prevalence of KS in South Africa.[13] According to the South African National Cancer Registry (1992-1996) the incidence of KS has doubled in men and has increased about seven-fold in women.[13] Thus the M:F ratio declined from 7:1 in 1988 to 2:1 in 2001.[13] Somdyala *et al.*[14] report that the incidence of Kaposi sarcoma was reported to be higher for males (1.6 per 100 000) than females (0.3 per 100 000).

2.2.2 Aetiopathogenesis

Human Herpes Virus 8 (HHV8) infection has been identified as the cause of KS in HIV-seropositive individuals.[10] HHV8 is a gamma-2 herpes virus and as with all herpes viruses, can exist in either a latent or a lytic state.[15]

Although HHV8 infection in HIV-seropositive individuals is common in Botswana and Gambia, with seroprevalence rates of 76–87% and 29–84% respectively, KS was rare in these areas prior to the HIV epidemic.[11] Only a single case of KS was documented in a population of approximately 45 000 Ethiopians living in Israel with an HHV8 seroprevalence rate of 39–57%, between 1982 and 1998.[9]

The seroprevalence of HHV8 has been reported to increase with age and is similar in men and women.[9] It is also found to decrease with increasing levels of education and is lower in whites than in blacks suggesting that poverty may be an important determinant in transmission.[13] The risk of KS increases with increasing antibody titres to HHV8 but for a given titre the risk is greater in HIV-seropositive compared with HIV-seronegative individuals.[13]

HHV8 infection alone is not sufficient however to initiate KS development. Many factors such as HIV tat protein, immune suppression and dysregulation of cytokines, a local inflammatory environment, HHV8 associated oncogenesis, angiogenesis, growth factors and adhesion molecule functions cooperate with HHV8 to drive the initiation and promotion of HIV-KS.[10]

Latent virus predominates in KS lesions, with a low percentage of cells exhibiting lytic replication.[15] Cell types identified as supporting lytic and/or latent gene expression include monocytes, endothelial/spindle cells of KS lesions, B cells, and epithelial cells.[15] It has been postulated that most HIV-KS lesions are monoclonal in origin, however it is not clear if HIV-KS is a monoclonal angioproliferative disorder or a malignant neoplasm.[10]

Sexual transmission of HHV8 may explain why KS is most common in homosexual and bisexual men especially those with a history of insertive oral and anal sexual contacts, this being the highest risk factor among a spectrum of sexual practices.[9,16-19] Another possible route of transmission of HHV8 is via saliva through non-sexual close personal contact.[9,12,18-20] Sitas *et al.*[13] have documented that mother to child transmission of HHV8 is also possible. In addition to HHV8 infection which dramatically increases the risk for KS,[13] the discrepancy between the geographical distribution of KS prior to and with the AIDS epidemic suggests the presence of an additional co-factor in the development of this malignancy that needs to be investigated and defined.[9]

HIV contributes to the pathogenesis of HIV-KS through various mechanisms: HIV Tat protein may directly promote HHV8 replication.[8] HIV-KS originates via induction of inflammatory T helper (Th)-1 cytokines which is associated with a marked impairment of

cellular immune responses brought about by HIV infection.[8] Inflammatory infiltrates in HIV-KS lesions comprise of CD8 T cells, monocytes, macrophages and dendritic cells.[8] These cells produce inflammatory cytokines that together with HHV8 gene products may sometimes activate endothelial cells and trigger the development of HIV-KS.[8]

2.2.3 Clinical features of oral HIV-KS

HIV-KS ranges from a mild slowly progressive disease to a rapidly progressive life threatening condition, the prognosis of which is poor if not treated.[10] HIV-KS occurs as single or more frequently as multiple lesions in the oral cavity either concomitantly with skin lesions or in isolation.[11] The trunk, arms, head and neck are the most commonly affected sites.[21] In 71% of HIV seropositive subjects with KS, the oral cavity will be affected at some time and is the initial site of involvement in approximately 25% of cases.[21] Subjects with oral HIV-KS have a higher mortality rate than subjects with exclusively cutaneous manifestations.[21]

Oral HIV-KS most frequently affects the hard palate, gingiva, dorsum of the tongue and in advanced cases the tumour may infiltrate into the underlying bone.[11] Symptoms in the oral cavity include pain, bleeding, mobility of teeth and functional interferences.[10] The lesions may be single or multifocal, initially presenting as macules that may progress to papulo-nodular lesions, which may eventually become confluent to form large exophytic masses.[21] The lesions are bluish-purple or red and do not blanch with pressure.[21] They may be indolent or locally aggressive.[21]

HIV-KS causes significant morbidity and mortality which is usually closely related to low CD4 T cell counts and high HIV and HHV8 viral loads.[12] However, in a series of 130 cases

of HIV seropositive patients with KS, only 75% of patients had CD4 T cell counts of less than 200/mm^3. The authors concluded that low CD4 T cell counts were not a prerequisite for the development of KS as 10% of patients in this series had CD4 T cell counts greater than 500/mm.[9,12,22] In advanced cases of HIV-KS, in the presence of other as yet unidentified co-factors co-operating with HHV8 oncogenes, a subset of the benign monoclonal cells evolve into a malignant clone that undergoes expansion.[10]

2.2.4 HIV infection, highly active antiretroviral therapy (HAART) and KS

In sub-Saharan Africa, the natural course of HIV-KS in the absence of HAART is characterised by rapid disease progression associated with a high HHV8 burden and short life expectancy.[23] Subjects with extensive exophytic oral lesions and tumour associated oedema have higher death rates than HIV seropositive subjects having exclusively cutaneous lesions.[23] Treatment of HIV-KS is focused on control of tumour growth and palliation. HAART should always be instituted in HAART naive HIV-seropositive subjects with KS, since it promotes regression of KS lesions.[23] KS may recrudesce early in HIV seropositive patients following the introduction of HAART, as an immune reconstitution inflammatory syndrome (IRIS).[23] IRIS can be defined as an exuberant immune system mediated inflammatory response to a pre-existing subclinical pathogen or tumour antigen after treatment has brought about an improvement in a host with a previously profoundly depressed immunity.[24] In HIV infected persons, HAART-induced IRIS has reportedly been described in relation to opportunistic infections including *Herpes simplex* virus, *Herpes zoster* virus, *Mycobacterium tuberculosis*,[23] *Mycobacterium avium* complex and *Cryptococcus neoformans*.[25] HAART-induced IRIS in HIV seropositive patients has also been described in relation to autoimmune thyroid disease.[23] HIV-IRIS is reported to occur despite a reduction in HIV load and an improvement in all HIV related immunological

parameters after the early introduction of HAART.[23] However, the exact immunopathogenetic mechanisms that bring about IRIS have not yet been clearly elucidated upon.[23]

In HIV-seropositive subjects who start HAART at an early stage of HIV infection, the number and function of CD4 T cells tend to return to normal. Subjects who start HAART when the HIV infection is moderately advanced (with CD4 T cell counts between 100×10^6/L and 300×10^6/L), will not show a similar recovery. However, even such a partial immune reconstitution results in a profound decline in HIV associated morbidity and mortality.[8]

All HIV-immune reconstitution inflammatory syndrome (HIV-IRIS) events occur in subjects who display as indicators of immune reconstitution, a decrease in HIV viral load and an increase in CD4 T cell count.[8] HIV seropositive subjects with IRIS episodes tend to be younger at the time of introduction of HAART and tend to have a lower median baseline CD4 T cell percentage than HIV seropositive subjects who do not experience IRIS.[8] The median time to onset of IRIS is reported to be 12 weeks in patients who display this response.[8]

2.2.5 Histological features of oral HIV-KS

HIV-KS evolves through three histopathological stages viz. the patch, plaque and nodular stages.[11] The patch stage is characterised by a proliferation of small vessels which results in an irregular, jagged, vascular network surrounding pre-existing vessels.[11] Lesional endothelial cells have a bland appearance and may be associated with scattered lymphocytes and plasma cells.[11] The plaque stage demonstrates further proliferation of these vascular

channels along with the development of a significant spindle cell component.[11] In the nodular stage, the spindle cell component increases to form a nodular tumour-like mass that may resemble a fibrosarcoma or other spindle cell sarcomas.[11] Numerous extravasated erythrocytes are present and slit-like vascular spaces may be discerned.[11] Even though secondary *Candidal* colonisation is often seen routinely within the superficial keratinous layers of the surface epithelium of HIV-KS lesional tissue during routine histological examination, secondary *Candidal* colonisation is not frequently reported in general histological descriptions of HIV-KS.

2.3 *Candidal* infection in HIV-seropositive patients

2.3.1 Incidence, epidemiology and aetiology of candidiasis

Oral candidiasis due to *Candida albicans* infection is the most common oral opportunistic infection in patients with HIV infection.[26,27] *C. albicans* is the most common fungal pathogen of humans accounting for 60% of all fungal infections.[28,29] *Candida albicans* is an imperfect diploid dimorphic fungal organism that normally exists as a commensal of the oral mucosa and the gastrointestinal tract.[28,30] *C. albicans* consists of oval budding yeast cells in its non-vegetative form and are 2 to 4 microns in size while in its vegetative form it consists of long septate pseudohyphae with large oval refractile chlamydospores at the septate junctions or free ends of the pseudohyphae.[31]

Factors that may influence the carriage of *Candida*, include immunosuppression particularly HIV infection and antimycotic or antibiotic treatment.[27] Several studies have shown that the asymptomatic carriage of oral *Candida species* varies geographically in healthy and compromised individuals.[27] Reports vary in different regions of South Africa.[27] The rate of asymptomatic carriage is reported to be higher in the Western Cape, with 68% of HIV-

seronegative subjects and 75% of HIV seropositive patients carrying yeasts.[32] The authors did not however document the population group of their sample.[32] Seventy per cent of the yeasts identified by Patel *et al.*[27] were *C. albicans* and approximately 30% were non-*albicans* species in HIV-seropositive subjects. *Candida* carrier rates are reported to be higher in the black South African population than elsewhere in Africa.[27] HIV-seropositive patients carry more as well as a greater variety of yeasts than HIV-seronegative subjects.[27]

The most common species of yeast isolated from patients with oral candidiasis is *C. albicans.*[19] The non-*albicans* yeasts isolated from the oral cavity in immunocompromised patients include *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Candida guilliermondii*, *Candida dubliniensis* and *saccharomyces cerevisiae*.[33] Studies have shown that non-*albicans* species may become pathogenic in HIV-seronegative and HIV-seropositive patients.[27] For example, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *S. cerevisiae* have been isolated from infections in neonates, secondary sepsis in bone marrow transplant patients, and fungaemia.[27] This may change the perception of non-*albicans* yeasts from opportunistic to exogenous infective agents that can be transmitted readily from person to person and be easily acquired by immunocompromised people.[27]

Several factors may contribute to the high carrier rate of *Candida albicans* yeasts. The importance of diet was highlighted in recent studies that showed malnourished HIV-seropositive children in Nigeria carried more yeasts than a similar population in the United States,[34] and from Turkey where different population and age groups with varying diets showed varying carriage rates.[35] Patel *et al.*[27] demonstrated in their sample of black South African adults, whose diet is usually high in carbohydrates, that diet enhanced the proliferation of *Candida*, its adhesion to epithelial cells and the production of acidic products.

Oral yeast colonisation among AIDS household contacts is reportedly high, which suggests that transfer and colonisation of strains can occur readily.[27] This transfer may not be related per se to HIV status but to exposure to AIDS patients with high *Candida albicans* counts.[27] The HIV-seropositive and HIV-seronegative patients were from the same low socioeconomic densely populated area, where several families live in one house and general hygiene was poor.[27]

The hallmark of HIV infection is immune suppression which *Candida* takes maximum advantage of.[28] Candidiasis can also develop in patients with lymphoma, those undergoing steroid therapy and in transplant patients, all of whom are immunocompromised.[15,28] However, the incidence of oral candidiasis in HIV infection has been significantly reduced since the introduction of HAART.[7]

2.3.2 Clinical features

HIV-associated oral candidiasis usually affects the hard and soft palate, tongue, buccal mucosa and the floor of the mouth.[36] It presents as erythematous patches, or creamy white curd-like lesions classified as the pseudomembranous type.[36]

The pseudomembranous form of HIV-associated oral candidiasis is characterised by the presence of multifocal smooth white papular lesions comprising the pseudohyphae, that can usually be rubbed away leaving a red surface.[28] The erythematous form of oral candidiasis typically presents as diffuse and multiple foci of macular erythema involving the palate, oropharynx, buccal mucosa, and dorsal tongue, but pseudohyphae are frequently absent.[28] In HIV infection, oral candidiasis can be complicated by oropharyngeal candidiasis, which may

limit food consumption and lead to weight loss, threatening the general health and well-being of HIV infected patients.[28]

Oral candidiasis is usually an infection with superficial levels of tissue invasion.[37] The pseudomembranous and erythematous variants of oral candidiasis are the most common clinical presentations of mucosal candidiasis associated with HIV infection.[36] Further clinical variants include angular cheilitis, exfoliative cheilitis, and *Candida* associated palatal papillary hyperplasia.[36] Diagnosis of these specific forms of oral candidiasis in HIV infected patients is obtained by clinical diagnostic criteria. Symptoms may include burning pain, altered taste sensation, and difficulty swallowing liquids and solids.[28]

The pseudomembranous form can be easily diagnosed by demonstrating the presence of *Candida* yeasts and pseudohyphae on wet mounts or Periodic Acid Schiff (PAS) or Grocott stained smears of material obtained by swabbing the lesions and may further be confirmed by isolation of the *Candida species* on culture.[28,36] In the erythematous form however, the sparse presence of *Candida* at the mucosal surface frequently requires a biopsy and Periodic Acid Schiff or Grocott staining to establish a formal diagnosis.[28,36]

Clinically, oral candidiasis is most common in HIV-seropositive individuals when the CD4 count drops below 200 cells/ul (lower limit).[15,22,28] Blood CD4 T cell numbers were historically the primary prognosticator for the development of oral candidiasis.[38] It has however been demonstrated in a study that evaluated the predictive role of HIV viral load against CD4 T cell counts, that viral load is a more reliable predictor for oral candidiasis.[39] It was also concluded that it should not be inferred that high viral load is causative for oral candidiasis.[10,12,28,39] It is suggested that other risk factors also play a role in the direct

development of oral candidiasis including alcohol or drug abuse and possible high risk sexual behaviour.[15,22,28]

2.3.3 Pathogenesis of *Candidal* infection

The development of oral candidiasis in HIV seropositive patients is mainly due to a complex interplay of factors such as an altered innate immunity, altered acquired humoral and cell mediated host immune responses and *C. albicans* virulence factors.[28]

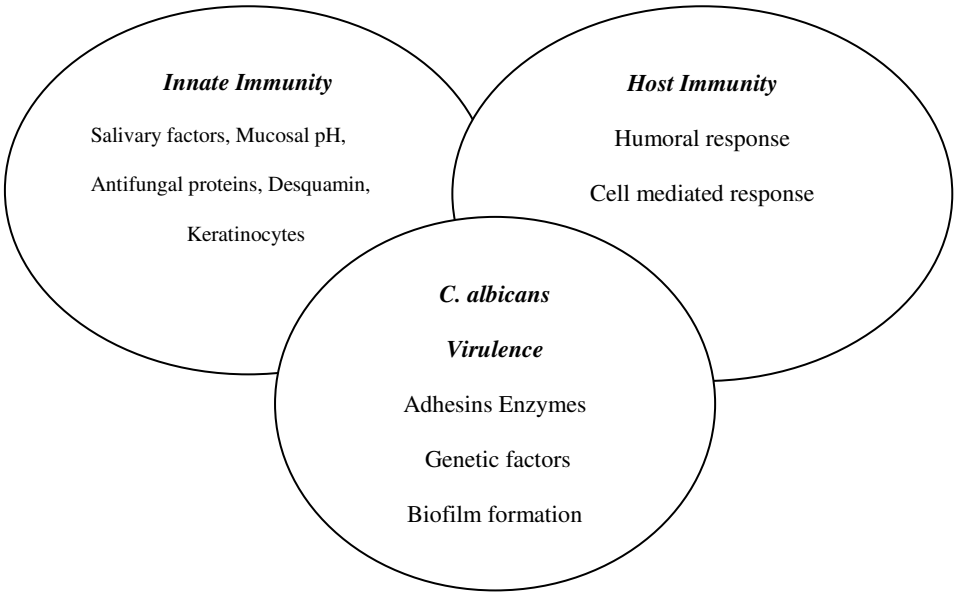


Fig.1 Diagrammatic representation of the interplay between altered innate immunity, altered host immunity and *Candidal* virulence factors in the pathogenesis of *Candidal* infection in HIV-seropositive patients

2.3.3.1 Alterations in oral innate resistance to *C. albicans* in HIV infection

Saliva establishes a dynamic equilibrium between *C. albicans* and other oral commensal microbiota, preventing oral candidiasis in normal hosts with an intact immunity.[28] A correlation was found between a 40% reduction in salivary flow rate in HIV-seropositive patients and enhanced recovery of *Candida* from the saliva of these patients.[40] The occurrence of oral candidiasis is increased in patients with acidic saliva.[41] A low pH has

been shown to increase the adherence of *C. albicans* to epithelial surfaces in vitro.[42] Glucose presence in saliva augments the growth rate of *C. albicans* in vitro and the resulting acidic pH promotes the increased activity of *Candida* secreted aspartyl proteinases (SAPs).[28] Increased *Candida* SAPs expression enhances *Candidal* virulence by degrading a host substrate such as mucin, which plays an important role in lubrication of epithelial surfaces and host defence.[28]

Lactoferrin, histatins, calprotectin and antileukoprotease inhibit the growth of *C. albicans* and its attachment to the oral epithelium.[28] Lysozyme and lactoferrin are two major non-immunological antimicrobial proteins in saliva which possess concentration, time and strain dependent fungicidal activity against *C. albicans* in vitro.[43] The concentration of lysozyme is reported to be increased in HIV-seropositive patients while the anti-*Candidal* activity of saliva is decreased, the contribution of salivary lysozyme to limiting the proliferation of *C. albicans* in the oral cavity of these patients is doubtful.[28] Salivary concentrations of lactoferrin in patients with HIV infection have been variously reported to be increased,[44] unchanged,[45] or decreased.[40] The predisposition to oral candidiasis in HIV-seropositive patients is therefore not definitively associated with a defective production of lactoferrin.[28]

Histatins are reported to have fungicidal activity against pathogenic fungi such as *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus,* and are present in the saliva of healthy adults.[28] The concentration of histatins in the saliva of HIV infected patients has been determined to be increased,[44] unchanged,[40] or decreased.[46] These varying results may have been caused by the different stages of HIV infection among patients in the studies as well as by the analytical methods employed.[28,44,40,46] Decreased concentrations of histatins correlate to an increased tendency for the development of oral candidiasis in HIV infected patients.[47] This suggests that decreased histatin concentrations and/or an inability of these

proteins in saliva to interact with *C. albicans* may contribute to the defective salivary anti-*Candidal* activity seen in HIV seropositive patients.[28,47]

Calprotectin is produced by PMNs, monocytes, macrophages and mucosal keratinocytes.[48] Salivary calprotectin concentrations and oral keratinocyte expression of calprotectin are augmented in response to oral candidiasis, in both HIV-seropositive and HIV-seronegative patients.[28] Salivary concentrations of calprotectin are reported to be deficient in HIV-seropositive patients with oral candidiasis or high salivary *Candidal* counts compared to those in HIV-seropositive patients without oral candidiasis or with low salivary *Candidal* counts.[49] Diminution of calprotectin may predispose to oral candidiasis in HIV infection.[28] A study of the oral mucosa of HIV infected patients with oral candidiasis demonstrated *Candidal* pseudohyphae penetrating through the epithelial parakeratin layer but not extending beyond the spinous layer zone of keratinocyte calprotectin expression.[50]

Antileukoprotease produced by keratinocytes is involved in non-immunological defence against *C. albicans* at mucosal sites.[28] In addition to its inhibition of leukocyte-derived proteinases, antileukoprotease has fungicidal activity against *C.albicans* and it may thus play an important role in innate mucosal defence.[51] Antileukoprotease also exhibits anti-HIV-1 activity in vitro and may contribute to the antiviral activity of saliva associated with the infrequent oral transmission of HIV-1.[51]

Interferon-gamma (IFN-γ) promotes expression of the glycoprotein desquamin, a cell adhesion molecule in the stratum corneum of the human epidermis which possesses lectin-like properties for amino sugars, as well as trypsin-like serine proteinase and RNase activity.[28] Desquamin thus plays a crucial role in desquamation and shedding of *Candida*

from the superficial portion of the epithelium. In addition to these indirect mechanisms, keratinocytes possess several potential antimicrobial mechanisms which may directly contribute to host defense against *Candida*: (i) oral keratinocytes express inducible nitric oxide synthase activity[28] and NO has been associated with anti-*Candidal* activity and resistance to mucosal candidiasis;[52] (ii) oral keratinocytes produce numerous antimicrobial peptides, including defensins 1 to 3, cathelicidins, adrenomedullin, calprotectin,[50] and bactericidal permeability increasing protein (BPI), which as natural antibiotics contribute to the innate immunity of the epithelium.[53]

Keratinocytes express on their cell membranes BPI, which is also an abundant constituent of polymorphonucleocytes (PMNs).[53] The role of BPI in limiting *C. albicans* colonization or infection of the oral mucosa remains to be determined.[53] Oral keratinocytes directly inhibit the growth of blastoconidia and/or pseudohyphae of *Candida* species in vitro, with an essential requirement for cell contact.[54] Direct growth inhibition of *Candida* by oral keratinocytes appears to occur through a novel and distinct mechanism, complementary to other components of the antimicrobial armamentarium of oral keratinocytes.[28] The role of keratinocytes in host protection against *Candida* at mucosal surfaces appears likely, since *C. albicans* pseudohyphae are restricted to the upper layers of the oral epithelium in candidiasis and are some distance away from lymphocytes and Langerhan's cells located in deeper layers.[28]

2.3.3.2 *C. albicans* virulence factors

The ability of *C. albicans* to colonize and penetrate human oral epithelium depends on imbalances between *Candidal* virulence attributes and specific defects in host immunity.[28] *C.albicans* possesses a multiplicity of properties, including adhesins, dimorphism, phenotypic

switching, biofilm formation, molecular mimicry of mammalian integrins and secretion of hydrolytic enzymes, each with a low propensity for enhancing fungal infection and none of these properties being necessarily dominant.[28] All of the virulence attributes, even in combination, are unable to fully overcome intact host defences.[28] An important virulence trait that promotes disease establishment and progression is a morphological transition from a yeast form to a pseudohyphal growth form which is considered to be the most invasive.[55]

Mucosal pH regulates expression of *C. albicans* virulence genes *PHR1* and *PHR2* and is an important environmental signal in determining the virulence capacity of *Candida* and/or alteration of the host defences.[56]

Hydrolytic enzymes are probable virulence factors in pathogenic *Candida* species.[57] Among these, *C. albicans* SAPs, under the control of a multigene family (*SAP1* to *SAP10*) expressing distinct isoenzymes that are regulated differentially at the mRNA level in vitro, are implicated in the breakdown of several host substrates.[57] Phospholipase B also contributes to the pathogenesis of candidiasis by the degradation of host tissues.[58] *Candidal* SAPs antigens have been detected on the surface of blastoconidia and pseudohyphae adhering to human oral mucosa.[59] *C. albicans* isolates from HIV-seropositive patients with candidiasis not only exhibit enhanced adherence to buccal epithelial cells but also produce higher SAPs levels in vitro than do strains isolated from HIV-seronegative control groups.[28]

HIV infected individuals, compared with healthy controls have an increased incidence of symptom free oral *C. albicans* carriage and a heightened frequency of oral candidiasis.[38,60] The reasons for this may be the heightened bio-film forming ability of *Candida* colonising the oral cavity in HIV-seropositive individuals and the compromised immune system which

may lead to possible alterations in the function of host mucosal cells which offer variable receptivity and avidity to *Candida*.[38,39,60] In the initial adhesion phase of *Candidal* biofilm formation (adhesion phase), there are no complex structures, only a few layers of adherent cells in the budding-yeast phase of growth.[62] With biofilm development and maturation, the *Candidal* organisms differentiate to varying degrees,[63,64] comprising of complex intertwining layers of yeast, pseudohyphae and hyphae embedded in the extracellular matrix.[65] *Candidal* biofilms have a complex architecture.[66] A structural feature of *Candidal* biofilms is the presence of water channels, which are thought to develop as a result of the detachment of individual microcolonies from the biofilm matrix.[66] Water channels permit waste disposal and nutrient influx into biofilms, so that even the deeply embedded yeast cells have access to nutrients and oxygen.[39,66]

Furthermore, clinical and in vitro resistance to antifungal azoles frequently occurs in oral candidiasis when CD4 T cell counts fall to <200 cells/mm^3, either by selection or acquisition of resistant strains of *Candida albicans* or by infection with inherently resistant species of *Candida* other than *C. albicans*.[28] The full potential impact of antiretroviral therapy on the ability to reconstitute immunity and therefore to reduce the incidence of oral candidiasis and oesophageal candidiasis has been hampered by suboptimal adherence, toxicity, and resistance to antiretrovirals, as well as the limited availability of these treatments in developing countries where most HIV infected patients reside.[28]

2.3.3.3 Altered mucosal immunity against *C. albicans* in HIV infected patients

2.3.3.3.1 Humoral immune response

Analysis of *Candida* specific antibodies in the saliva of HIV-seropositive patients reveal that despite changes in total immunoglobulin levels, when levels of *Candida* specific antibodies

are normalized to total protein or total immunoglobulin levels of the corresponding isotype, no distinct differences in IgA or sIgA are seen, regardless of CD4 T cell count.[28] De Repentigny *et al.*[28] concluded that there is no evidence of appreciable changes in levels of *Candida* specific IgA in saliva that would account for the prevalence of candidiasis among HIV-seropositive patients.

2.3.3.3.2 Cellular immune response

HIV infection of oral mucosal cells is central to the pathogenesis of oral mucosal candidiasis in HIV-seropositive patients.[67] Because oral epithelial keratinocytes from HIV infected patients contain integrated HIV proviral DNA and HIV Tat/Rev RNA, possibly acquired through contact with submucosal HIV positive lymphocytes and/or Langerhan's cells, the anti-*Candidal* properties of these cells could be potentially impaired.[67] Although HIV-seropositive patients with oral candidiasis have a significant decrease in oral epithelial cell mediated growth inhibition of *C. albicans* in vitro compared to those without oral candidiasis, there is no difference in epithelial cell activity between HIV-seronegative and HIV-seropositive subjects without candidiasis.[54]

Despite being infected by HIV, oral mucosal Langerhan's cells are challenged to perform important tasks which include the uptake of *Candida* antigens in the mucosa and their presentation to CD4 T cells in draining lymph nodes.[28] Multiple defects of oral Langerhan's cells probably contribute to a progressive loss of protective acquired cell mediated immune responses to *C. albicans* antigens in HIV infection.[28] Numbers of both oral and oesophageal Langerhan's cells are depleted in HIV infection.[68] It is uncertain whether decreased dendritic cell (DC) populations result from cytopathic changes caused by productive HIV infection, altered cytotoxic T-cell responses resulting in lysis of targeted DCs, migration to lymph

nodes where active viral replication occurs, or down-regulation of DC surface markers[69]. Increased expression of CD40 and CD86 co-stimulatory molecules has however been observed in blood DCs from HIV-seropositive patients.[28]

Progressive diminution of CD8 T cells in HIV infection results from apoptosis mediated by macrophages through interaction of HIV gp120 with the chemokine receptor CXCR4.[70] Despite progressive diminution in absolute numbers, the remainingCD8 T cells successfully accumulate in the basal epithelial layer of the oral mucosa in HIV-seropositive patients with oral candidiasis, demonstrating that these cells can be actively recruited to the mucosa in response to candidiasis.[28,70]

CD4 T cell numbers are markedly reduced in oral mucosae of HIV-seropositive patients with or without oral candidiasis.[28] *C. albicans* specific peripheral CD4 T cells are depleted with HIV disease progression and concurrent oral candidiasis,[71] but these findings have not yet been demonstrated for CD4 T cells isolated from cervical lymph nodes draining the oral mucosal surface.[28] HIV-seropositive patients have a Th2 cytokine profile in saliva consistent with the well documented switch from Th1 to Th2 in HIV infection, which correlates with a loss of lymphocyte proliferation in response to *Candida* antigens.[72]

NK cells are cytotoxic in vitro to certain tumor cell lines and to virally infected cells and have direct antimicrobial activity against *Cryptococcus neoformans* but little or no effect against *C. albicans* pseudohyphae in vitro.[73]

Recruitment of phagocytes to the oral epithelium has not been demonstrated to be altered by HIV infection.[28] Their anti-*Candidal* attributes could be impaired either directly by HIV

infection or by altered stimulation by cytokines.[28] In several investigations yielding conflicting results, phagocytosis of *C. albicans* by blood monocyte derived macrophages from HIV-seropositive patients has been found to be either normal or reduced.[74]

Oral keratinocytes are of importance in the pathogenesis of candidiasis since they constitute a physical barrier after *C. albicans* adheres to the oral epithelial surface.[28] Oral keratinocytes also function as fixed immunocytes and are capable of producing a number of soluble factors, and express receptors that are involved in up or down regulation of immune responses.[28] Furthermore oral keratinocytes produce several growth factors including basic fibroblast growth factor, platelet-derived growth factors, transforming growth factors and tumour necrosis factor-alpha (TNF-α).[75] Keratinocytes also produce several cytokines including interleukin (IL)-1, IL-3, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15,IL-18, and IL-20, and a number of colony stimulating factors such as GM-CSF,G-CSF, and M-CSF .[75] mRNA expression of IL-1γ, IL-1, IL-8, GM-CSF, and TNF-α is up regulated in experimental cutaneous *C. albicans* infection with reconstituted human epidermis, demonstrating that *Candida albicans* induces a rapid cytokine response by host keratinocytes.[75] *C. albicans* also triggers the production of IL-1γ and TNF-α, as well as GM-CSF, by primary oral epithelial cells and oral epithelial cell lines in vitro.[75] The anti-*Candidal* activity of PMNs from healthy subjects and HIV-seropositive patients is impaired by the Th2 cytokines IL-4 and IL-10, suggesting a role in mediating a greater predisposition to oral candidiasis in HIV infection.[28]

In conclusion, it is not known which of the many factors reviewed above determines the change in nature of the *Candidal* organism from its yeast state to the penetrative or vegetative state that is characterised by pseudohyphae in association with chlamydospores. Chlamydospores cannot be seen on Haematoxylin and Eosin (H&E) or Periodic Acid Schiff

(PAS) stained sections but only under phase contrast microscopy. Any change in innate immunity, acquired host immunity or *Candidal* virulence factors (Fig. 1) will promote opportunistic organisms like *C. albicans* to switch from a normal commensal of the oral mucosae to a pathogenic organism.

2.3.4 Histological features of *Candida* infection

The *Candidal* pseudohyphae usually penetrate the superficial keratinous layers of the epithelium perpendicular to the surface.[50] The pseudohyphae are more readily identified and confirmed with the Periodic Acid Schiff (PAS) stain which is absorbed by the carbohydrates, contained in abundance by fungal cell walls; the organisms being easily identified by the bright magenta colour imparted by the stain.[36] The pseudohyphae are approximately 2µm in diameter, vary in length and may show branching. These pseudohyphae may be accompanied by a variable number of vegetative yeasts or chlamydospores (visible using phase contrast microscopy), and often by intraepithelial neutrophilic abscesses (Munro abscesses).[36] The histological pattern may vary slightly depending on the clinical form of oral candidiasis.[36] Common features include an increased thickness of parakeratin on the surface of the lesion in conjunction with elongation of the epithelial rete ridges.[36] Typically, a chronic inflammatory cell infiltrate can be seen in the connective tissue immediately subjacent to the infected epithelium and small collections of neutrophilic microabscesses (Munro abscesses) are often identified in the parakeratin layer with the organisms present in the superficial spinous layer.[36] *Candida* pseudohyphae are embedded in the parakeratin layer and rarely penetrate into the viable cell layers of the epithelium unless the patient is severely immune compromised.[36]

2.3.5 Diagnosis and treatment of oral candidiasis

The introduction in 1996 of HAART including protease inhibitors dramatically reduced the prevalence of oral candidiasis and oesophageal candidiasis in HIV infected patients.[7] It has been documented that over an average period of 12 months following antiretroviral treatment including a protease inhibitor there were significant decreases in the prevalence of oral candidiasis, the number of relapses of oral candidiasis, the rate of asymptomatic oral carriage of *C. albicans*, and overall oral *Candida* burdens.[28] A comparable decline in the incidence of oesophageal candidiasis has been observed in HIV infected children since the introduction of HAART.[28]

Some patients carry large numbers of yeasts that are resistant to several antifungal treatment regimens and do not respond to treatment.[33] Yeast counts decline during treatment but are restored to the original level once therapy is discontinued. Resistance to antifungal agents which may be acquired or intrinsic has been reported in *C. krusei* and *C. glabrata* with *C. krusei* being universally resistant to fluconazole.[33] For this reason, all oral lesions with a presumptive diagnosis of candidiasis should be subjected to microscopy and culture.[33] Isolated cultures should be identified to species level, antifungal sensitivity should be determined and primary resistance should be kept in mind during treatment.[33] Typing and sub-typing of yeasts are probably not critical to the clinical management of candidiasis caused by *Candida albicans* and non-*albicans* strains, including *C. dubliniensis,* because these are responsive to antifungal therapy. *C. glabrata* is probably the only exception.[27,33,39] The presence of oral thrush in infants younger than 6 months of age is associated with an increased postnatal transmission risk of HIV infection.[33] Thus, perinatal anti-retroviral therapy should be combined with the treatment of oral thrush to prevent the postnatal acquisition of HIV.[27,33,39]

CHAPTER 3

3.0 OBJECTIVES AND AIMS

The objective of the study was to determine the frequency and morphology of *Candidal* secondary infection of the surface epithelium of oral HIV-KS lesions.

More specifically the study aimed to determine:

a. the demographics of the patients showing oral HIV-KS lesions with secondary *C.albicans* infection in the surface epithelium
b. the frequency of biopsied oral HIV-KS secondarily infected with *Candida* and the density and depth of the secondary *Candidal* infection
c. the histomorphology of *Candidal* pseudohyphal invasion of the oral epithelium and the cellular reactions that occur
d. correlation of the presence, intensity and depth of invasion of *C. albicans* with CD4 T cell counts at the time of biopsy

CHAPTER 4

4.0 MATERIALS AND METHODS

4.1 Biopsy material and data collection

Oral lesions of KS are routinely biopsied in the course of diagnosis and the slides and wax blocks are stored in the Division of Oral Pathology. All cases diagnosed as oral KS were retrieved from the departmental archives for the period 2003 to 2007. All of the tissue specimens were originally fixed in 10% neutral buffered formalin (18-48 hours) and routinely processed and embedded in paraffin wax. Data regarding the patient's age, sex, race, site of the lesion, HIV status and CD4 count were obtained from the clinical information recorded on the histopathology reports and from the patients computerised hospital records. This was done following ethics approval (M00/08/29; M08.03.25). Each case was given a study number in order to maintain complete patient anonymity.

4.2 Study sample

One hundred and thirty three cases which had been histologically diagnosed as oral KS during the study period 2003 to 2007 including the glass slides and wax blocks were retrieved from the departmental archives. Twelve cases were excluded as the available material was inadequate for histopathological assessment. The age, gender, site, HIV status and CD4 T cell count were recorded for all cases where available. The 121 cases included in the study were then divided into two groups based on the HIV status of the patient. The first group was the HIV-seropositive group which consisted of 32 cases. In the second group of 89 cases, no information was available on the HIV status of the patient hence these cases were regarded as HIV status unknown and not analysed any further. For the seropositive group new 4µ

sections were cut and stained with the Periodic Acid Schiff (PAS) technique and Haematoxylin and Eosin (H&E).

4.3 Histological analysis

The H&E stained sections were reviewed and the diagnosis confirmed by the investigator in conjunction with a specialist pathologist using a double headed Nikon Eclipse 80i microscope. The PAS stained sections were examined histologically using conventional light microscopy and the following parameters were recorded:

a. presence or absence of secondary *Candidal* infection in the surface epithelium or pyogenic membrane (a layer of non-autolysed inflammatory cells lining an epithelial surface or an abscess cavity) of the oral HIV-KS lesion.

b. specific site of infection:
   epithelium only, epithelium and pyogenic membrane, epithelium and connective tissue or pyogenic membrane only

c. whether only one locus or many loci of *Candidal* infection could be found in the biopsy specimen

d. intensity of the *Candida* infection at each locus which was subjectively assessed as being mild, moderate or severe based on the number of pseudohyphae and yeasts

e. depth of *Candida* invasion/penetration graded as being present in:
   superficial parakeratin only, parakeratin and reaching the spinous layer, parakeratin and penetration into spinous layer reaching the lamina propria and involvement of the pyogenic membrane in ulcerated cases

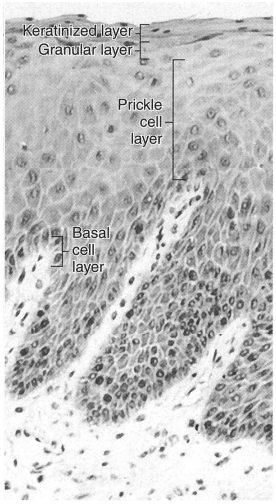


Fig. 2 Histological section of parakeratinised oral epithelium showing from most superficial to deep: the superficial keratinised layer, the darker staining granular cell layer, the prickle/spinous cell layer, the basal cell layer and the underlying lamina propria.

f. presence of pseudohyphae only or pseudohyphae with yeasts

g. association with neutrophilic microabscesses (Munro abscesses) in the epithelium

h. presence of epithelial hyperplasia or pseudoepitheliomatous hyperplasia

i. presence and type of inflammatory cells in the lamina propria

4.4 Data analysis

The clinical data obtained will be presented in table and graphic format. The histological data is descriptive in nature and could not be standardised or statistically verified. It was evaluated subjectively and will be presented as a series of histophotomicrographs without statistical comparison. Tests for statistical significance were deemed not to be necessary as this is primarily a histomorphological study.

4.5 Ethical considerations

Ethics clearance (M08.03.25) [Addendum] was granted by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand to view patient hospital files in

order to confirm the HIV status of the patient, the CD4 count and the viral load. Furthermore, the ethics code M00/08/29 used for this research project is a code granting permission for the use of archival tissue, obtained from human subjects, in histological or immunohistochemical research and allocated to the Division of Oral Pathology, Department of Anatomical Pathology.

CHAPTER 5

5.0 RESULTS

5.1 Initial sample (oral KS)

The initial study sample consisted of a total of 121 cases of oral KS. Patients in this initial sample ranged in age from 7 to 63 years (mean 35; median 35). The mean age for males and females was 36.71 years and 36.07 respectively. Most cases occurred in patients in the fourth decade. (Table 1) The male to female ratio of the patients with oral KS was 1:1.57 (47 males: 74 females).(Table 1) Virtually all of the patients in the initial sample of 121 cases were black with only 2 cases (2%) occurring in white patients.(Table 1) The two white patients were in their fourth decade of life.(Table 1) Forty four cases (36.36%) occurred in the palate followed by the gingiva in 24 cases (19.83%), tongue (22 cases; 18.18%), buccal mucosae (7 cases; 5.78%), labial mucosa (4 cases; 3.30%), retromolar area (3 cases; 2.47%) and 2 cases (1.65%) in the floor of the mouth. In 15 cases (12.39%), more than one area of the mouth was simultaneously affected. (Table 2) Patient records did not indicate whether patients in this group were receiving HAART or not. Dental records were also not available to confirm whether any of the patients in this group wore dentures or not.

Table 1. Age, gender and race distribution pattern in the initial sample of 121 cases of oral KS

Decade	Total	Female	Male	Black	White
0-9	1	1	0	1	0
10-19	2	2	0	2	0
20-29	32	27	5	32	0
30-39	60	34	26	58	2
40-49	16	5	11	16	0
50-59	6	2	4	6	0
60-69	4	3	1	4	0
	121	**74**	**47**	**119**	**2**

Table 2. Site distribution pattern of the initial 121 cases of oral KS

Site	n=Cases	%=n/121	Male	Female
Palate	44	36.36	19	25
Gingiva	24	19.83	9	15
Tongue	22	18.18	9	13
Labial mucosa	4	3.30	1	3
Floor of mouth	2	1.65	0	2
Retromolar area	3	2.47	1	2
Buccal mucosae	7	5.78	2	5
More than one concurrent site	15	12.39	6	9
	121	**100**	**47**	**74**

5.2 Study sample (HIV-seropositive patients with oral HIV-KS)

Only 32 cases of the initial sample of 121 cases (26%) were confirmed to be HIV-seropositive. In the remaining 89 cases (74%) the HIV status could not be confirmed from the available records. These cases were then excluded from further analysis.

Only 13 cases (40.65%) showed secondary *Candida* infection in the form of PAS positive pseudohyphae and yeast cells in the surface epithelium or pyogenic membrane.

Nineteen cases (59.37%) in the HIV seropositive sample showed no evidence of *Candidal* organisms in the surface epithelium or the pyogenic membrane of oral HIV-KS lesions.

5.3 Age, gender and site distribution

5.3.1 32 HIV-seropositive cases of oral HIV-KS

The ages ranged from 20 to 63 years (mean 41.5; median 41.5). The mean age for males and females in this group was 45.5 years and 41.5 years respectively (Table 3). Most cases occurred in the fourth decade and the group consisted of 12 males and 20 females. (Table 3; Fig. 3)The ages of the males was not statistically different from that of the females (P>0.5). The male to female ratio was 1:1.66 (12 males:20 females).(Table 3)

The site found to be most affected was the palate (12 cases; 37.5%) followed by the tongue (10 cases; 31.25).(Table 3; Fig. 5)

5.3.2 13 HIV-seropositive cases of oral HIV-KS secondarily infected with *Candida*

The ages in this group ranged from 21 to 63 years with a mean age of 42 years and a median age of 42 years. The mean age for males and females was 45.5 years and 42 years

respectively (Table 3). Most cases occurred in the fourth and third decades.(Table 3; Fig. 4) 6 cases (46.15%) occurred in males and 7 cases (53.84%) in females (male:female ratio of 1:1.16).(Table 3; Fig. 4)

The site found to be most affected in this group was the tongue (6 cases; 46.15%) followed by the palate in 4 cases (30.76%).(Table 3; Fig. 6)

5.3.3 19 HIV-seropositive cases of oral HIV-KS not secondarily infected with *Candida*

The age range in this group was from 20 to 63 years (mean 41.5; median 41.5). The mean age for males and females was 49.5 years and 40.5 years respectively.(Table 3) Most of these cases occurred in patients in the fourth decade followed by the third, fifth decades and seventh decades in both the males and the females. (Table 3) The male to female ratio was 1:2.16. (Table 3)

The site most commonly affected in this group was the palate in 42.10 % of cases (8 cases) followed by the tongue in 4 cases (10.52%). (Table 3)

Table 3. Table showing comparative demographic results in the HIV-seropositive group of 32 cases regardless of secondary *Candidal* infection, 19 cases of oral HIV-KS and in 13 cases of oral HIV-KS secondarily infected with *Candida*

	HIV-KS n=32				**HIV-KS and secondary *Candidal* infection n=13**				**HIV-KS without *Candida* n=19**			
	Male		**Female**		**Male**		**Female**		**Male**		**Female**	
Age & Gender												
Mean	45.5		41.5		45.5		42		49.5		40.5	
Mean (T)	**41.5**				**42**				**41.5**			
Median (T)	**41.5**				**42**				**41.5**			
0 - 9yrs	0		0		0		0		0		0	
10-19yrs	0		0		0		0		0		0	
20-29yrs	1		9		1		3		0		6	
30-39yrs	5		7		3		1		2		6	
40-49yrs	2		2		0		2		2		0	
50-59yrs	3		0		2		0		1		0	
60-69yrs	1		2		0		1		1		1	
Total	**12 (37.5%)**		**20 (62.5%)**		**6 (46.15%)**		**7 (53.84%)**		**6 (31.57%)**		**13 (68.42%)**	
M:F ratio	**1:1.66**				**1:1.16**				**1:2.16**			
Race	**B**	**W**	**B**	**W**	**B**	**W**	**B**	**W**	**B**	**W**	**B**	**W**
	11	1	20	0	6	0	7	0	5	1	13	0
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 mucosae	1	0	0	0	1	0
More than one concurrent site	1	2	0	1	1	1

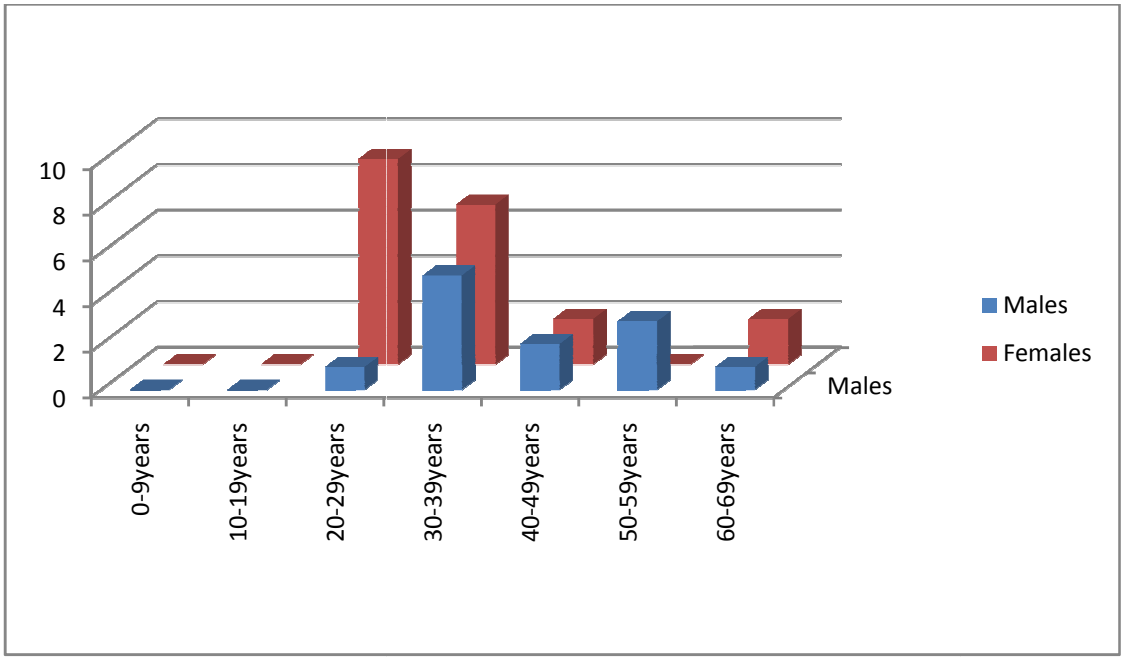


Fig. 3 Histogram showing the peak age occurrence of oral HIV-KS in 32 HIV-seropositive patients

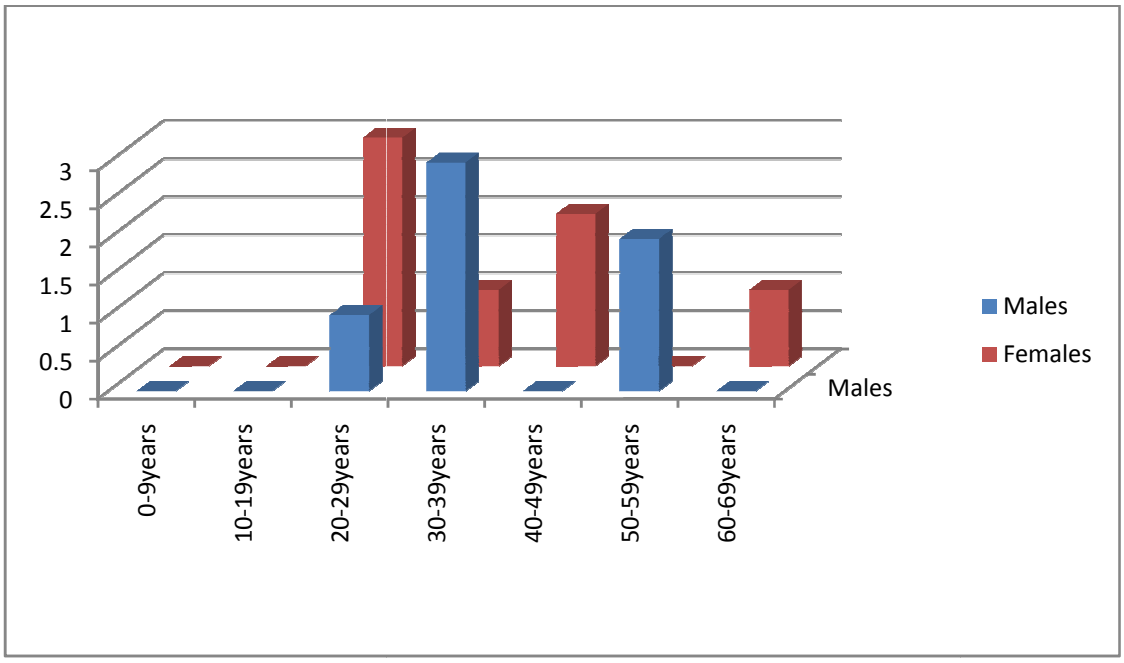


Fig. 4 Histogram showing the peak age occurrence of oral HIV-KS lesions secondarily infected by *Candida* (n=13)

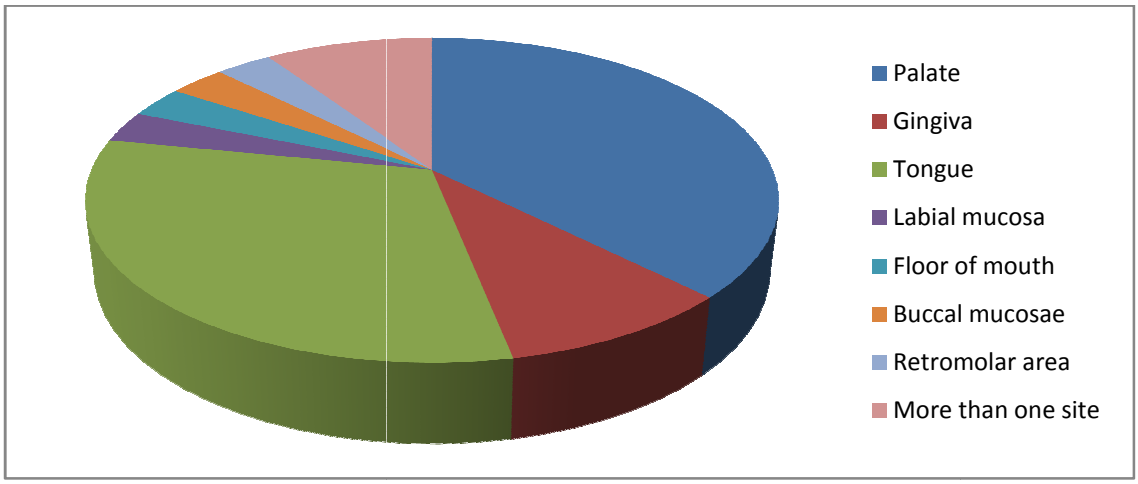


Fig. 5 Pie chart depicting palatally derived HIV-KS lesions as the most commonly affected site in 32 HIV-seropositive patients

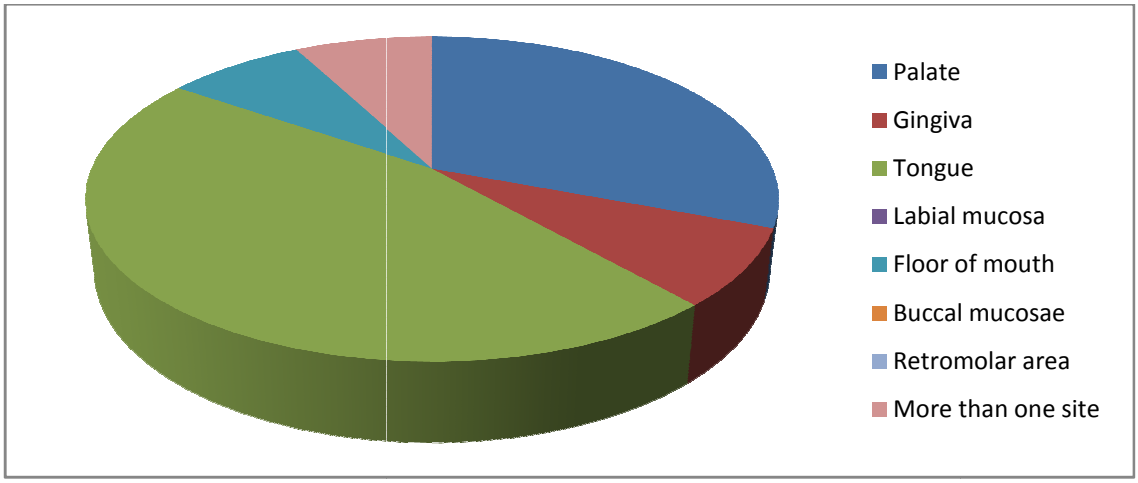


Fig. 6 Pie chart depicting tongue derived HIV-KS lesions as the most common site secondarily infected by *Candida* in HIV-seropositive patients (n=13)

5.4 Race distribution in the HIV-seropositive sample of 32 cases of oral HIV-KS

The majority of patients in this sample were black except for one white male in his fourth decade of life who did not present with secondary *Candidal* infection.(Table 3)

5.5 Histomorphology

The histomorphology of oral mucosal HIV-KS is well described and the lesions are easily recognisable. The lesional tissue in all cases of oral HIV-KS lesions showed a nodular growth pattern with fascicles of uniform spindle cells showing slit-like vascular spaces containing extravasated red blood cells and eosinophilic hyaline globules. Numerous mitotic figures were also present.(Fig. 7)

The *Candida* organisms were readily identified microscopically with the PAS stain which imparted a bright magenta colour to the organism.(Fig. 8) The non-septate pseudohyphae varied in length and exhibited simple branching patterns in some instances.(Fig. 8 and Fig. 10) These pseudohyphae were accompanied by a variable number of yeast cells in the majority of cases (56.25%). The *Candida* positive cases were sometimes characterised by a thick layer of parakeratin and hyperplastic elongated rete ridges of the surface epithelium compared to the non-infected cases.(Fig. 9)

Of the 13 cases which were positive for *Candida*, 6 cases (46.15%) showed pseudohyphae in the parakeratin only (Fig. 10 and Fig. 11), 1 cases (7.69%) showed pseudohyphae in the pyogenic membrane and the parakeratin simultaneously (Fig. 8), 1 case (7.69%) showed the penetration of the *Candidal* organisms into the superficial layers of the epithelium and in 2 cases (15.38%) organisms were found deep in the stratum spinosum.(Fig. 12) In 2 cases

(15.38%) organisms could only be found in the necrotic slough.(Fig. 13) A single case of severe *Candida* colonisation showed pseudohyphae to have penetrated the lamina propria as well as the HIV-KS lesional tissue.(Fig. 15)

The severity of the secondary *Candidal* infection (Figs. 16 and 17) was found to be severe in 6 cases (46.15%), moderate in 3 cases (23.07%) and mild in 4 cases (30.76%). In the mildly infected cases only single, isolated organisms could be detected. The grading of the severity of infection was subjective. The organisms were usually embedded in the superficial parakeratin layer which otherwise showed no significant changes.(Fig. 8) The moderately infected cases showed larger numbers of *Candida* pseudohyphae and yeast cells. The parakeratin layer was thicker with desquamation and the organisms were present mainly in the desquamated keratin but also in and amongst the superficial parakeratin layers.(Fig. 11) The severely infected cases showed matted colonies of pseudohyphae and yeast cells in both the desquamated keratin and in the parakeratin layer (Fig. 16) sometimes reaching into the stratum spinosum. The yeast cells always remained on the surface.(Fig. 16)

Neutrophilic microabscesses (Munro abscesses) were found located in the superficial layers of parakeratin (Figs.17 and 21) in 7 cases (53.84%) but in just under half of the cases (46.15%) no organisms were found in close association to the microabscesses.(Fig. 19) Conversely in over half of the cases (53.84%) pseudohyphae were found in the epithelium but not in close association to the microabscesses.(Fig. 18)

Inflammatory cells were frequently present beneath the *Candida* infected areas in the lamina propria.(Fig. 15) The infiltrate which was usually mixed in nature was severe in only 1 case (7.69%), moderate in 3 case (23.07%) and mild in 7 cases (53.84%) cases (Fig. 14). 2 cases

(15.38%) showed no evidence of inflammation.(Fig. 20) The grading of the severity of inflammation was also subjective. Acute inflammation was characterised by a predominance of neutrophils and chronic inflammation was characterised by few neutrophils, mainly lymphocytes, histiocytes and plasma cells.

Epithelial hyperplasia (Fig. 9) was noted in 8 cases (61.53%) of the infected cases usually when the infection was severe. Pseudoepitheliomatous hyperplasia occurred in only 1 case (7.69%) of severe infection.(Fig.22)

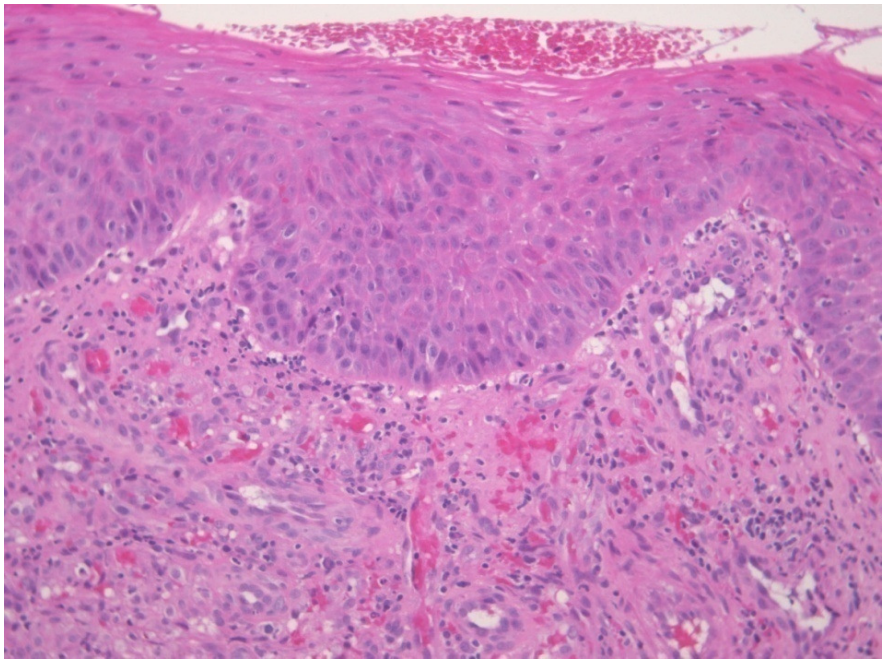


Fig. 7 Oral HIV-KS section. Note the hyperkeratotic oral epithelium with an underlying spindle cell component and prominent vascularity (H&E, original magnification x 20)

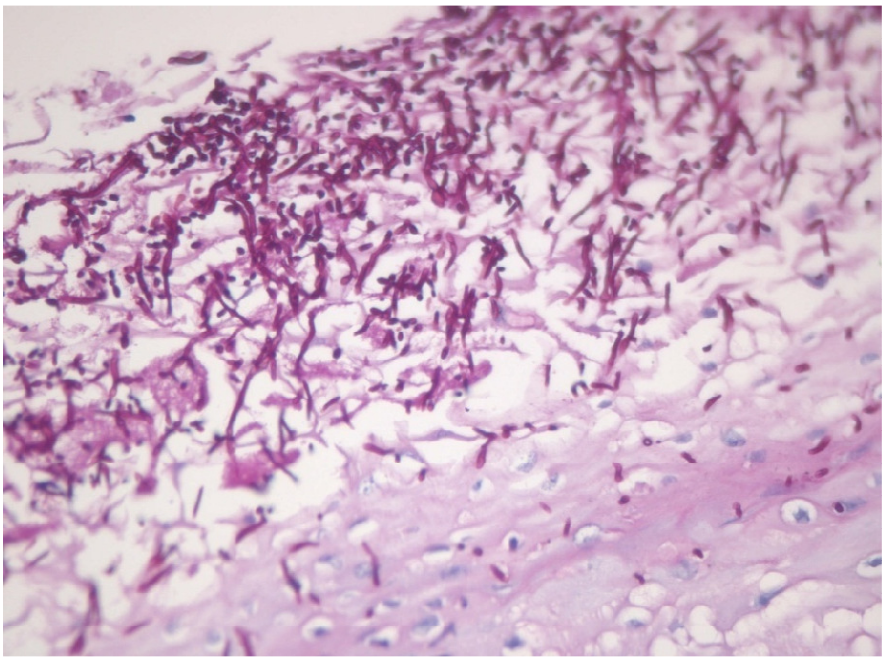


Fig. 8 High power view showing the preference of *Candidal* pseudohyphae and budding yeast cells for the desquamating surface keratin. Pseudohyphae do not penetrate the stratum spinosum (PAS, original magnification x 40)

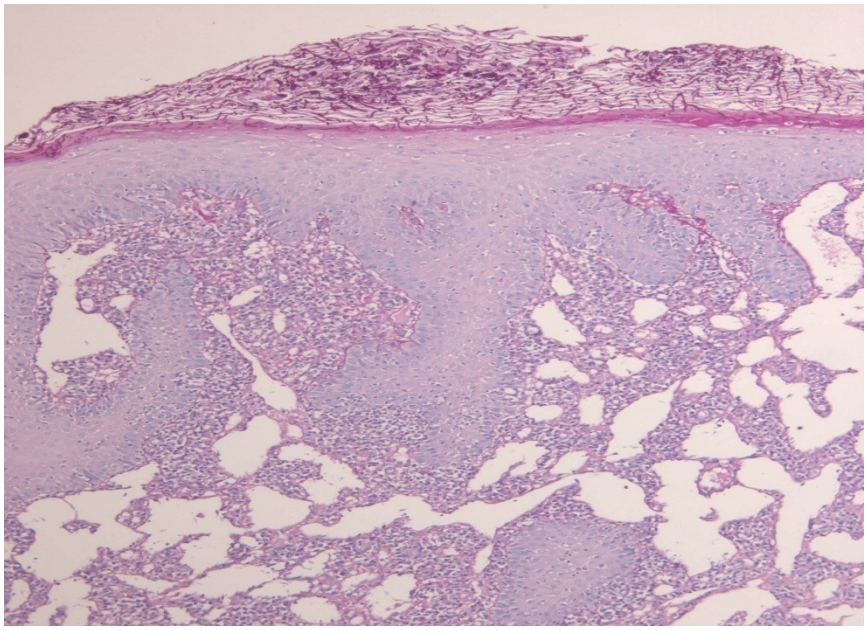


Fig. 9 Low power view of a telangiectatic variant of oral HIV-KS depicting numerous dilated telangiectatic spaces in the lesional tissue. The parakeratin is heavily infiltrated by *Candidal* pseudohyphae. Surface epithelial hyperplasia and pseudoepitheliomatous hyperplasia is also evident (PAS, original magnification x10)

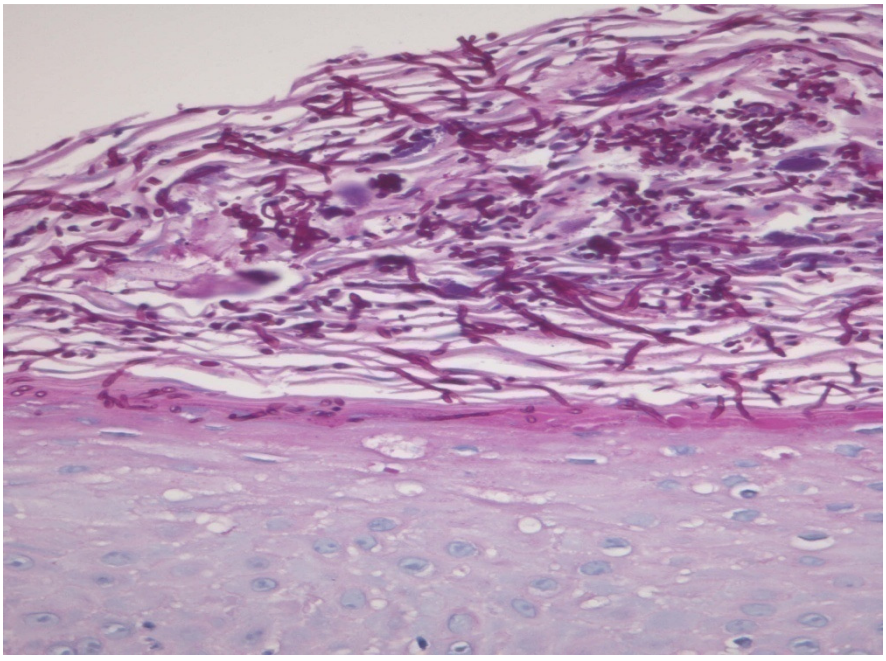


Fig. 10 High power view showing that although the infestation of yeast cells and pseudohyphae is heavy at the junction of the parakeratin layer and the stratum spinosum, penetration beyond the parakeratin does not occur (PAS, original magnification x 40)

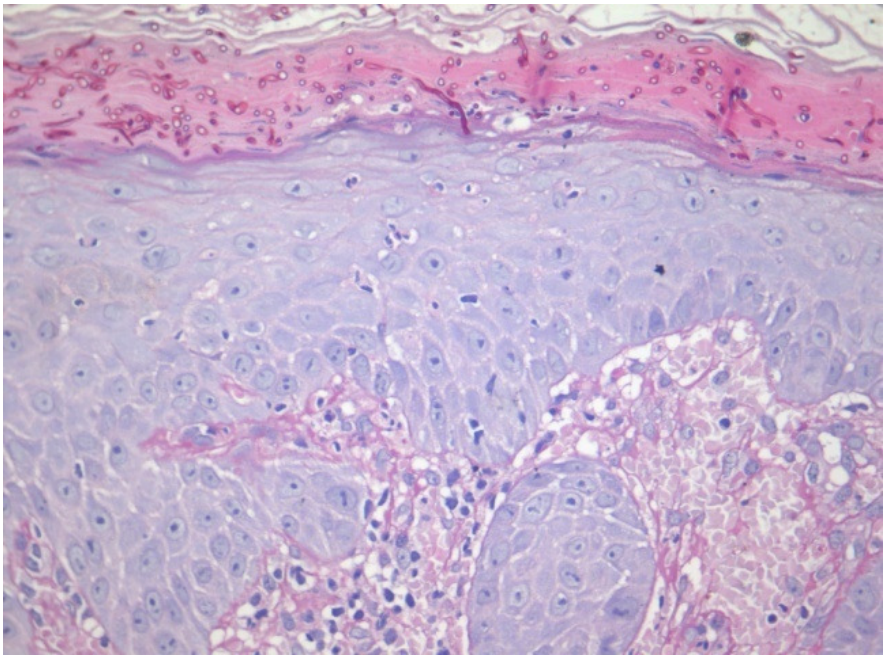


Fig. 11 High power view of *Candidal* infection restricted to the parakeratin layer (PAS, original magnification x 40)

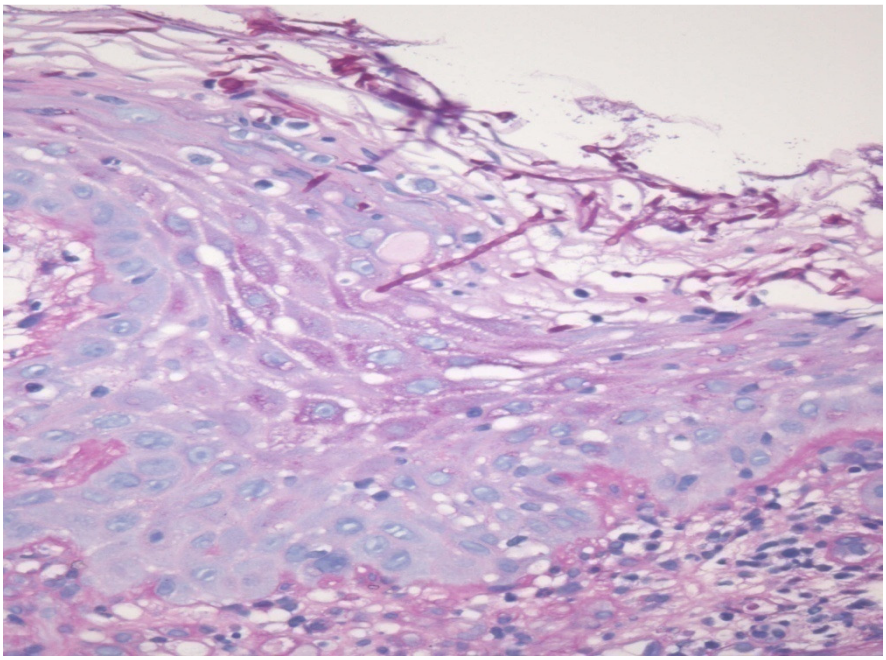


Fig. 12 High power view showing a moderate infiltration of pseudohyphae. Most of the pseudohyphae penetrate the superficial parakeratin layer and a single pseudohyphus has penetrated the superficial spinous layer (PAS, original magnification x 40)

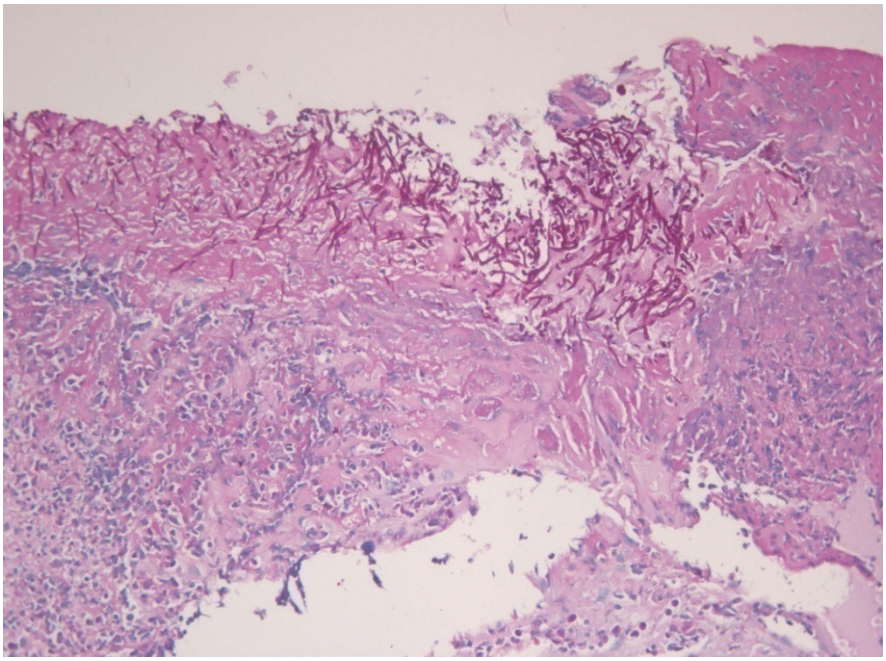


Fig. 13 An unusual case of organisms growing in the pyogenic membrane (PAS, original magnification x 20)

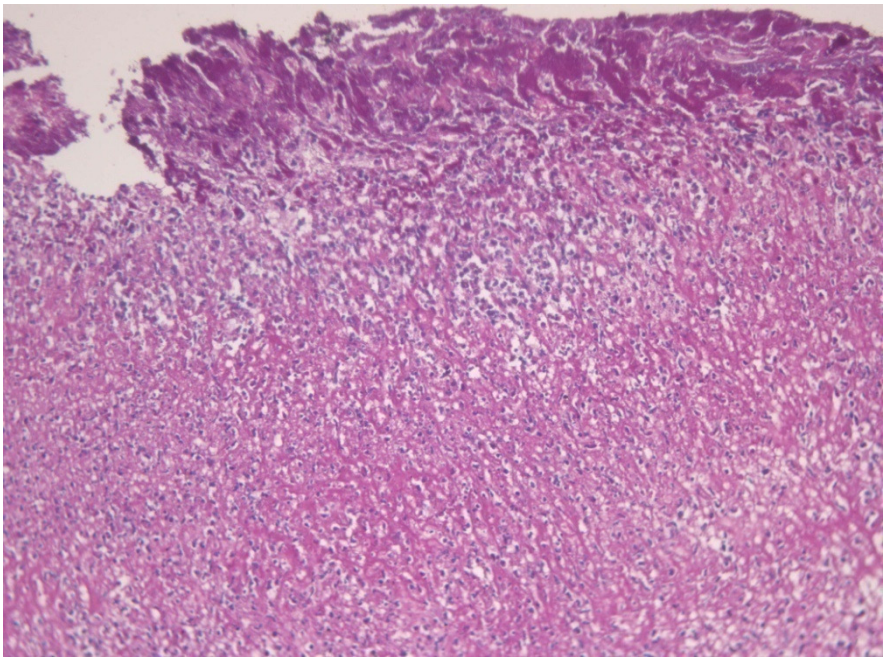


Fig. 14 High power view showing the fibrinous nature of the pyogenic membrane (H&E, original magnification x 20)

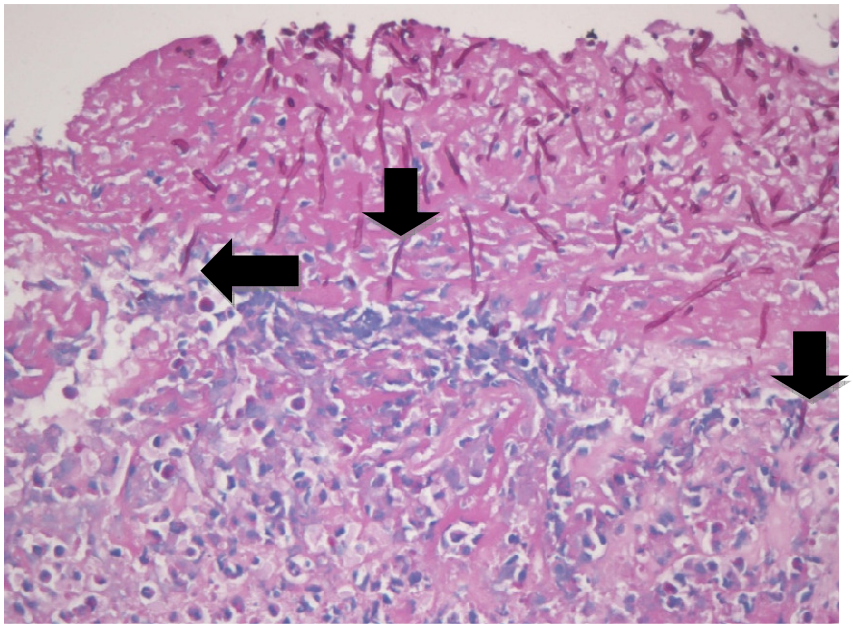


Fig. 15 High power view showing the most severe case of deep pseudohyphal penetration into the connective and ulcerated lesional tissue (arrowed). An inflammatory infiltrate is present in the lamina propria (PAS, original magnification x 40)

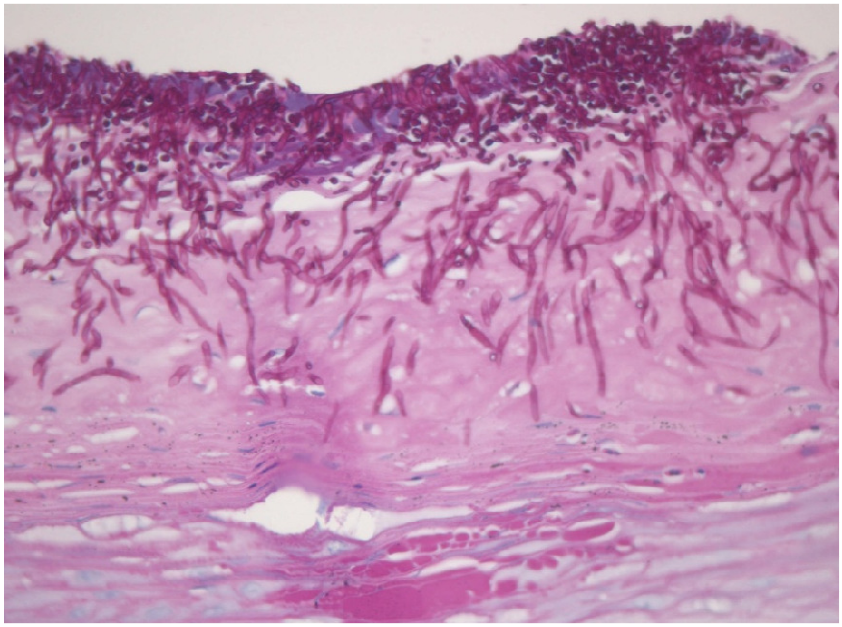


Fig. 16 High power view showing a severe and heavy infection on the surface by yeast cells, and by pseudohyphae which have a higher penetrative capacity (PAS, original magnification x 40)

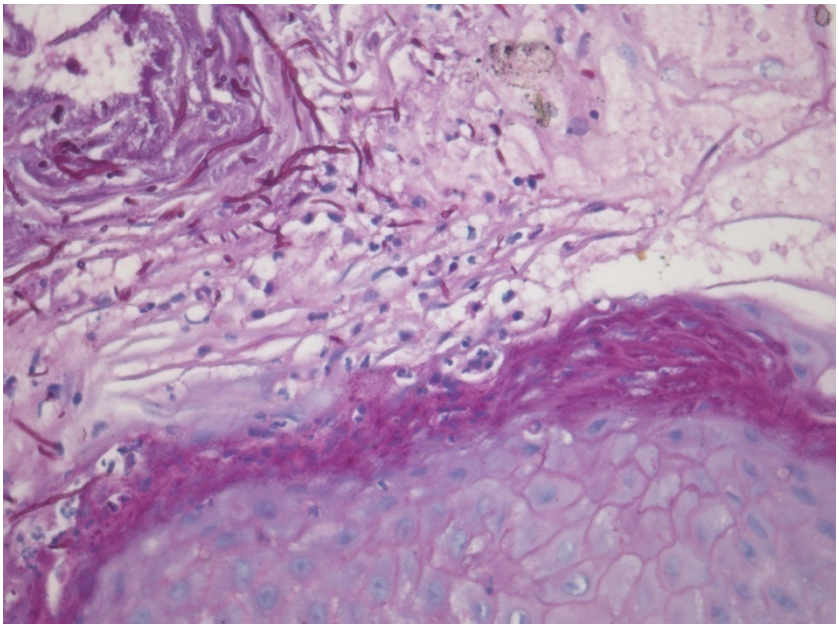


Fig. 17 High power view showing a moderate number of pseudohyphae, associated microabscesses and a bright pink band of glycogen (PAS, original magnification x 40)

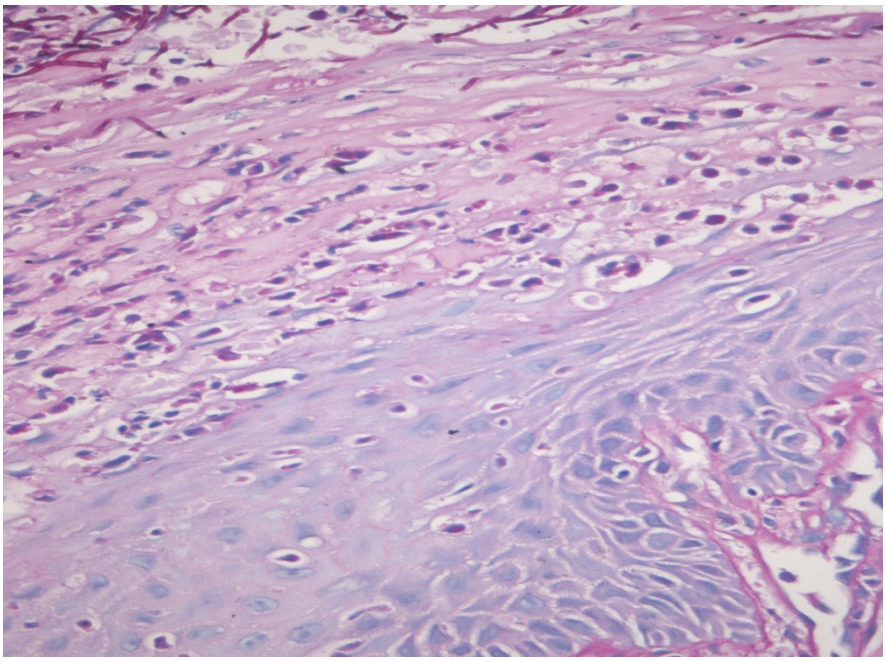


Fig. 18 High power view showing exocytosis of neutrophils from blood vessels forming Munro abscesses which are associated with the presence of pseudohyphae (PAS, original magnification x 40)

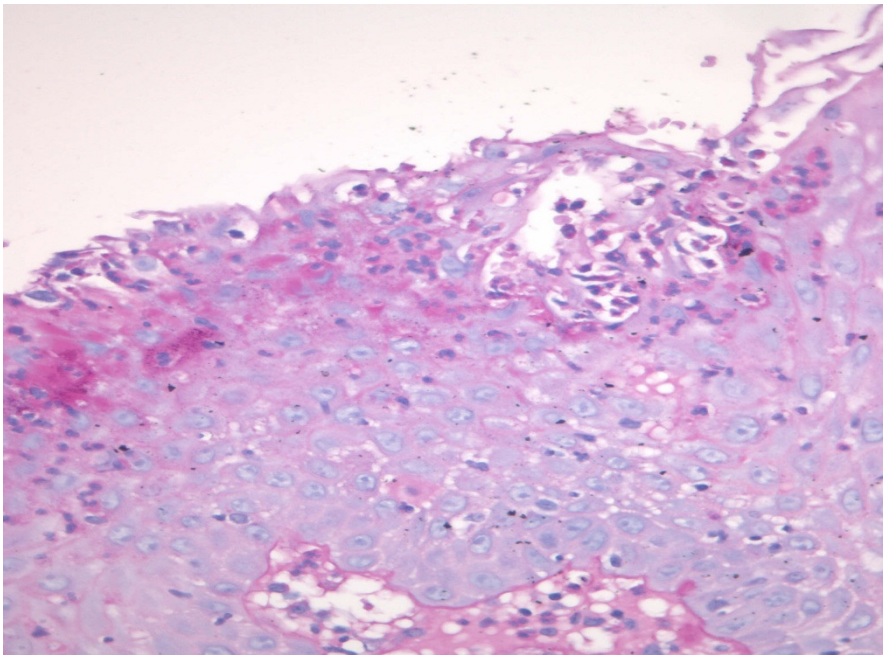


Fig. 19 High power view showing neutrophilic exocytosis from blood vessels and microabscesses without associated pseudohyphae. (PAS, original magnification x 40)

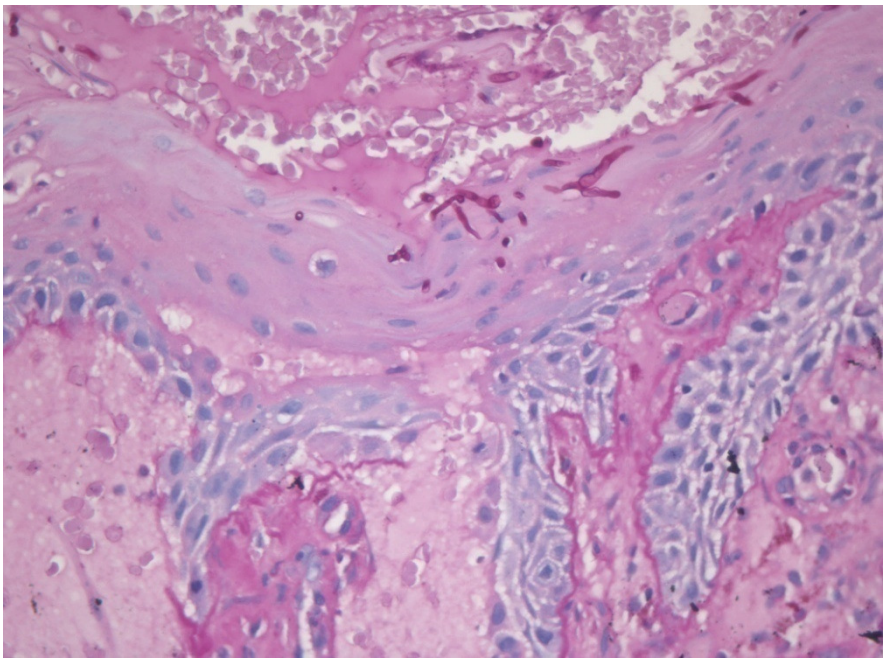


Fig. 20 High power view showing pseudohyphae without an associated inflammatory cell exocytosis (PAS, original magnification x 40)

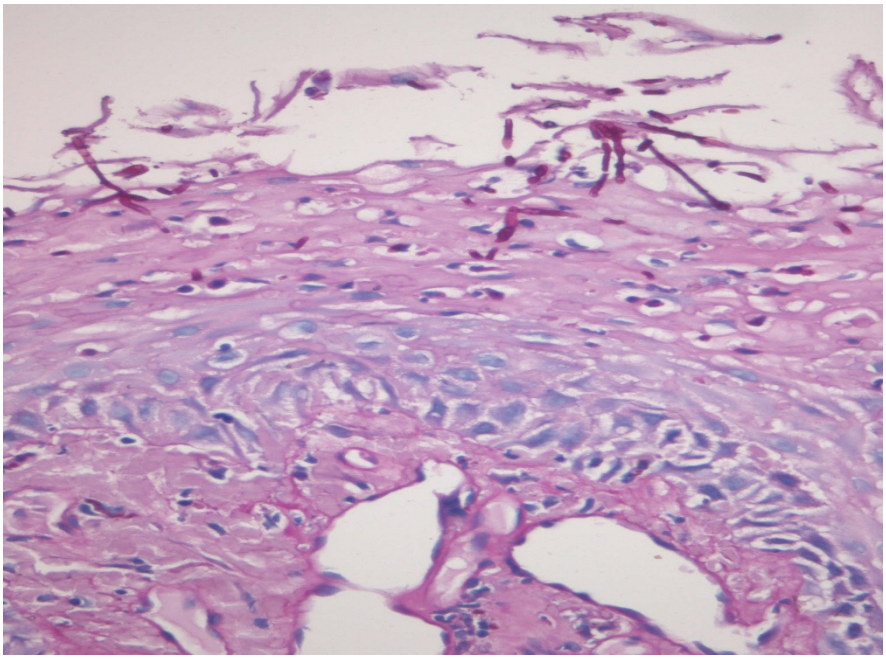


Fig. 21 High power view showing organisms in the parakeratin layer and the presence of an inflammatory exocytosis (PAS, original magnification x 40)

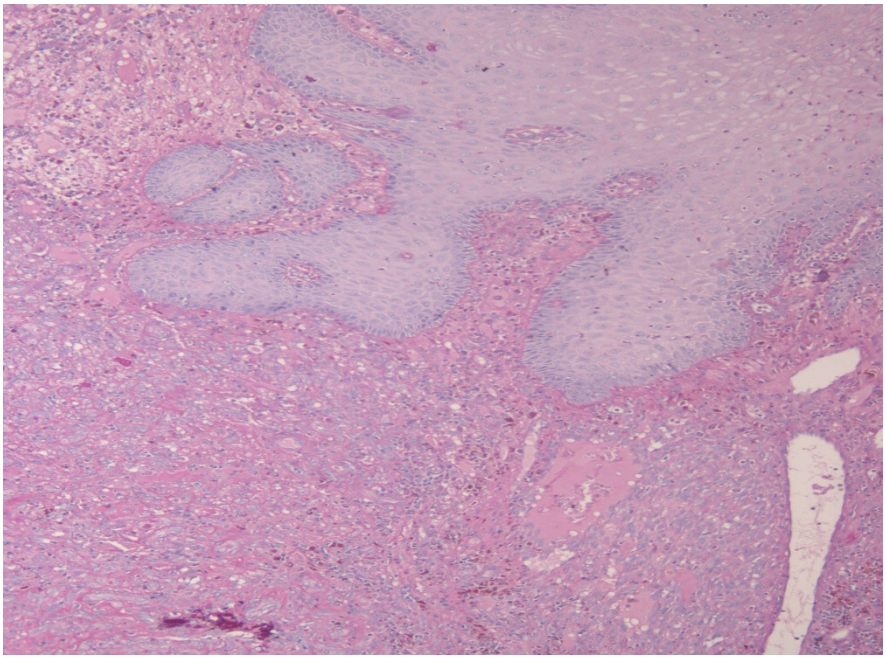


Fig. 22 Low power view showing pseudoepitheliomatous hyperplasia of the surface epithelium covering the HIV-KS lesional tissue area (PAS, original magnification x 10)

5.6 Correlation of CD4 T cell count with *Candidal* presence, intensity of infection and depth of invasion

The CD4 T cell count was known in 31 of the 32 cases of the oral HIV-KS group.(Fig. 23) Patient records did not indicate whether patients in this group were receiving HAART or not. All of the 31 patients whose CD4 T cell counts were recorded were HIV- seropositive. If the CD4 T cell count in the 13 cases of oral HIV-KS secondarily infected with *Candida* is plotted against the presence, intensity and depth of penetration of the fungal organism, it appears as if an inverse relationship exists between the CD4 T cell count and the intensity and depth of penetration.(Table 4) Cases with low CD4 T cell counts show a deeper penetration of *Candidal* pseudohyphae.(Table 4; Fig 24) This correlation must be regarded as pure speculation due to too few CD4 T cell records being available. In the group of 19 cases of oral HIV-KS where there was no *Candidal* growth, the CD4 T cell counts are equally as low as the CD4 T cell counts in the 13 cases of oral HIV-KS secondarily infected with *Candida*, with 9 cases recording counts lower than 100 cells/mm^3 .(Fig. 25) We therefore speculate that CD4 T cell count is not a determinant in *Candidal* secondary infection of the surface epithelium oral HIV-KS lesions but this correlation remains to be confirmed in a larger study sample.

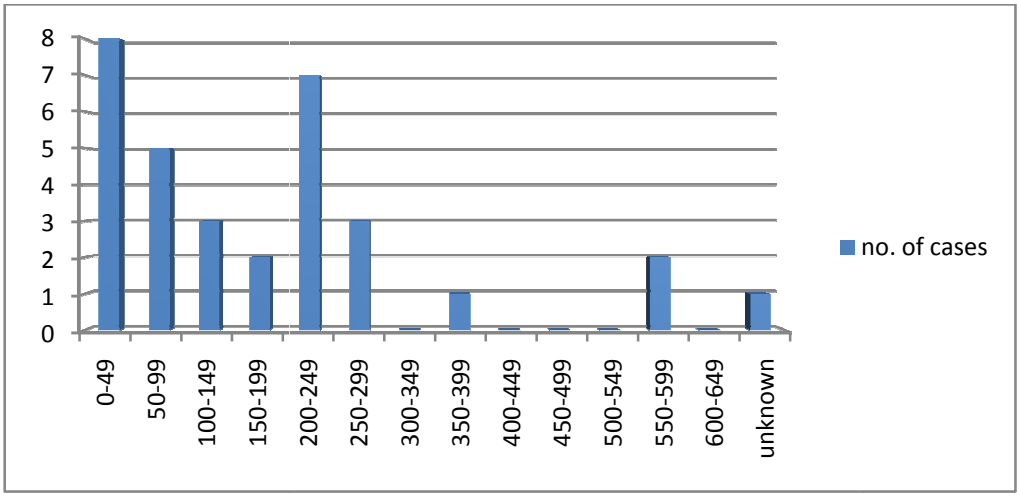


Fig. 23 Histogram showing CD4 T cell counts in 32 HIV-seropositive cases of oral HIV-KS

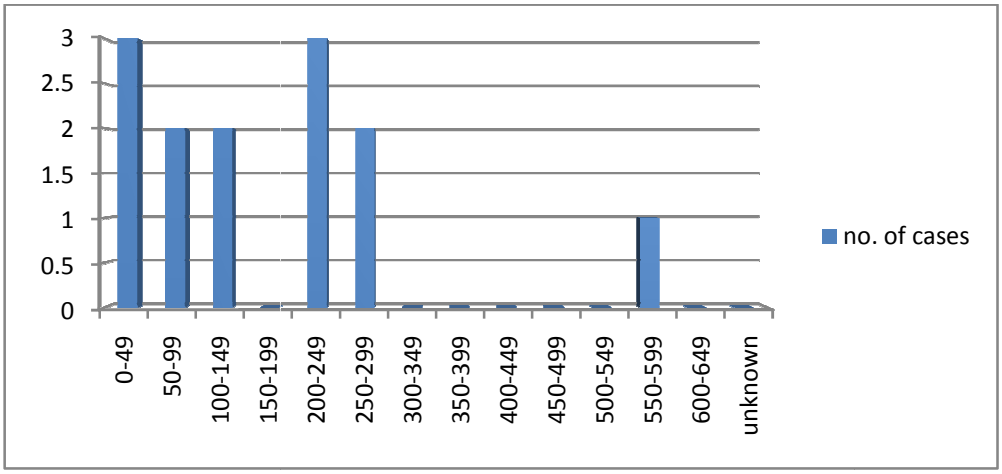


Fig. 24 Histogram showing CD4 T cell counts in 13 HIV-seropositive cases of oral HIV-KS secondarily infected with *Candida*

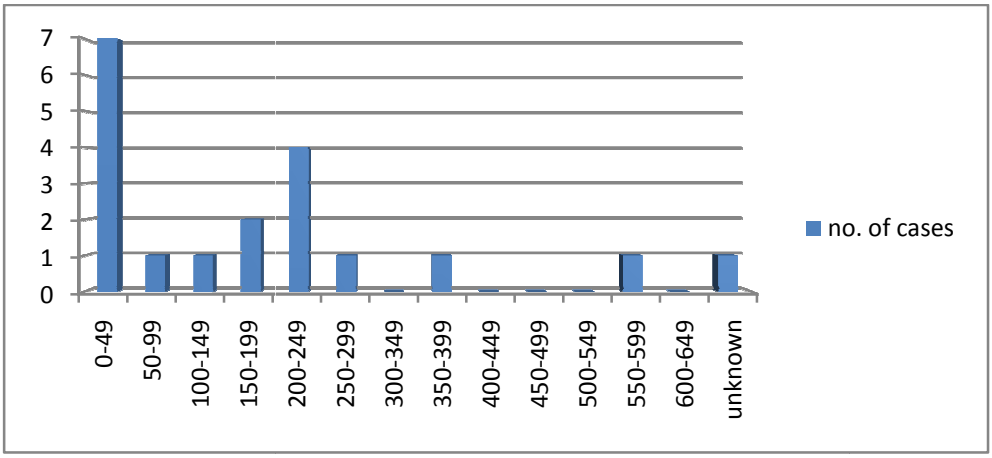


Fig. 25 Histogram showing CD4 T cell counts in 19 HIV-seropositive cases of oral HIV-KS without secondary *Candidal* infection

Table 4. Table showing histological features of the biopsy specimen/s at the time of haematological analysis

CD4 T cell count	Histological Features
599 – 500:	*Candida* present in the epithelium in all cases, multiple loci of infection, mild infection in all cases, mild epithelial hyperplasia, presence of pseudohyphae and yeast cells, presence of an acute to mild chronic inflammatory infiltrate
499 – 400:	No correlating data available for this subset
399 – 300:	No correlating data available for this subset
299 – 200:	*Candida* present in the epithelium in all cases, multiple loci of infection, mild infection in all cases, moderate epithelial hyperplasia, presence of pseudohyphae and yeast cells, presence of an acute to a mild chronic inflammatory infiltrate. One case presented with an infection that was graded as severe.
199 – 100:	No data available for this subset
99 – 0:	In 2 cases involvement of the spinous layer, multiple loci of infection, moderate epithelial hyperplasia, presence of pseudohyphae and yeast cells, presence of a mild chronic infiltrate, grading of infection which ranged between moderate (1 case) and severe infection (1 case).

CHAPTER 6

6.0 DISCUSSION

The frequency of HIV/AIDS has currently reached epidemic proportions in South Africa.[13] As could be expected the frequency of KS has also increased dramatically.[13] In 21% of HIV-seropositive people who develop KS, the initial presentation will be in the mouth, while in 71.5% of HIV-seropositive people who develop KS, the mouth will be affected at some time in the course of the disease.[76] The number of cases of oral KS seen in our department's biopsy service increased from 84 cases for the period 1973 to 2002,[77] to 133 new cases during the period 2003 to 2007.

6.1 HIV testing in South Africa

In South Africa HIV testing is not compulsory nor is AIDS a notifiable disease.[78] In fact performing an HIV test without informed consent or pre-testing and post-testing counselling is a criminal offence since the patients constitutional rights will have been violated.[78] Therefore, it was to be expected that hospital and pathology records would be lacking in essential information such as HIV status, CD4 T cell counts and viral loads making retrospective studies of this nature difficult. Thus in this study a potentially large initial study sample of 121 cases was depleted to 32 cases because we were unable to confirm the HIV status of the 89 patients.

6.2 Oral HIV-KS and candidiasis

The results of this study showed a secondary *Candidal* infection rate of oral HIV-KS lesions of only 40.62%. Prior to this study an anecdotal and subjective view held by histopathologists was that the secondary *Candidal* infection rate of HIV-KS lesions would be very high. There are unfortunately no comparable studies in which the infection rate or the histomorphology of *Candidal* secondary infection of oral HIV-KS lesions have been studied to compare our results with.

In considering the possible reasons for the relatively low rate of infection it must be realised that the surface epithelium covering a lesion of oral KS or oral HIV-KS might very well be unique and might not be comparable to other HIV related lesions or indeed even to normal epithelium in an HIV-seropositive patient. The presence of an oral KS or oral HIV-KS lesion may well influence the surface epithelium by induction from a range of cytokines, prostaglandins and genetic influences, all of which influence the response of the oral epithelium to penetration by *C. albicans*. Oral KS or oral HIV-KS tumour cells may create a unique micro-milieu that cannot be reproduced in other situations. One cannot infer that the mechanism by which secondary *Candidal* infection of oral HIV-KS occurs is necessarily the same as that of primary penetration of *Candida* in HIV-seropositive and HIV-seronegative patients or in other situations of *Candidal* infection in the mouth and more importantly in other mucosal sites. For example oropharyngeal candidiasis (OPC) occurs very commonly in the oropharyngeal mucosa of HIV-seropositive women but is rarely seen in the vaginal mucosa of the same cohort.[28]

Surveys of the frequency of all forms of oral candidiasis in HIV-seropositive patients have shown that *Candidal* infection rates vary from a low of 38% in Tanzania to a high of 94% in Zaire.[30,79] In South Africa *Candidal* infection rates ranging from 37.8% to 63% have been reported.[30,79] The rate of secondary *Candidal* infection of oral HIV-KS lesions of 40.62% in our study seems to be consistent with these reported figures.

6.3 Oral HIV-KS

6.3.1 Oral HIV-KS and gender

Although KS has traditionally been regarded as a male predominant disease, in South Africa this lesion has shown an ever greater involvement of women in very recent times.[80] According to the South African National Cancer Registry (1992-1996) the incidence of KS has doubled in men and has increased about seven-fold in women resulting in the decline of M:F ratio from 7:1 in 1988 to 2:1 in 2001.[13] Somdyala *et al.*[14] also reported a higher incidence of KS in males (1.6 per 100 000) than females (0.3 per 100 000) whilst Mosam *et al.*[81] reported that HIV-KS in black South Africans has an equal gender predilection. In England and Wales the male to female ratio of KS was reported to be 2:1.[82]

In a previous study from our department in which 81 cases of oral KS were accessioned during the period 1997-2003 the male to female ratio was reported to be 1.31:1.[77] At that time we suggested that the male predominance might have been accounted for by differences in the mode of transmission of HIV being predominantly heterosexual in the South African context.[77]

The results of this study showed a marked female predominance in all sample groups analysed (Table 1; Table 3) which is consistent with the dramatic increase in HIV infection of

women in this population.[83] In all age groups in Africa there are more females infected with HIV than males which may explain the reversal of the male to female ratio in favour of females.[83] This gender imbalance of HIV infected persons in Africa is most marked amongst the youth where in the 15-24 age group there are 4 HIV infected females to every male.[83]

The decline in the male to female ratio to 1.1:1 in Uganda in cases of HIV-KS may be attributed to detection bias during earlier periods and an increased awareness of HIV-KS in women.[82] The decrease in the male to female ratio of HIV-KS in Africa being due to HAART is unlikely as the decline occurred in Uganda before HAART becoming available in 1998 and the incidence of HIV-KS has not changed significantly in the 15-49 year age group since the introduction of HAART.[82]

6.3.2 Oral HIV-KS and age

HIV-KS has been reported as occurring in all age groups even in patients as young as 2 years old with the majority of patients being in their third and fourth decade of life.[77] Our study has similarly reported a predominance of patients in their third and fourth decades with no difference in ages between those HIV-KS patients who were secondarily infected with *Candida* and those who were not.

Lager *et al.*[77] also reported that the mean age of the patients with oral KS in his sample was 34.7 years which is consistent with our findings of a mean age for oral KS of 35 years. Our study reported a mean age of 41.5 years for subjects with HIV-KS (32 cases) regardless of secondary infection (Table 3). The mean age for males and females in the HIV-KS group of 32 cases was 45.5 years and 41.5 years respectively confirming Mosam *et al's*.[81] findings that females afflicted by HIV-KS were younger than males (Table 3).

6.3.3 Oral HIV-KS and site

Oral KS most frequently affects the palate, gingiva and the dorsum of the tongue.[77] Our study has demonstrated a similar predilection of oral HIV-KS for the palate followed by the tongue in the study sample of 32 HIV seropositive patients regardless of secondary *Candidal* infection. This finding was similar in the 19 cases of oral HIV-KS without *Candidal* secondary infection, however in the 13 cases of oral HIV-KS secondarily infected with *Candida*, the site found to be most commonly affected was the tongue followed by the palate.

6.3.4 Oral HIV-KS and dendritic cells

In considering the reasons why only 40.62% of cases are secondarily infected with *Candida*, an associated study in our department has shown that there is a dramatic decrease in the numbers of intraepithelial dendritic cells in oral lesions of HIV-KS and that this decrease was most marked in the cases which were secondarily infected with *C. albicans*. Dendritic cells appear to play a significant role in oral HIV-KS tumourogenesis due to either qualitative or quantitative changes in these cells.[84]

Dendritic cells are targets of HIV infection. It is uncertain whether decreased dendritic cell populations result from cytopathic changes caused by productive HIV infection, altered cytotoxic T-cell responses resulting in lysis of targeted dendritic cells, phenotypic change with a decrease in HLA-DR phenotype, migration to lymph nodes where active viral replication occurs, or down or up-regulation of dendritic cell surface markers.[69,85] We can only speculate that the more severe and deeper *Candidal* secondary infections is owing to the decreased numbers of dendritic cells rather than to the inherent penetrative capacity of the organisms or its ability to grow under anaerobic conditions. Two kidney transplant patients are reported to have developed KS simultaneously with a reduction in dendritic cells after

being treated with cyclosporine.[86] Reduction of cyclosporine led to a return of normal levels of dendritic cells and complete regression of KS.[86]

6.4 CD4 T cell counts

It is well known that oral HIV-KS may develop at any stage of HIV infection and that it undoubtedly occurs more frequently when the CD4 T cell count falls below 200 cells/mm^3.[9,12,22] This fact was well illustrated in our study where analysis of the CD4 T cell counts confirmed that most cases of oral HIV-KS occurred at CD4 T cell counts below 100 cells/mm^3 but there were some patients whose CD4 T cell counts were in the order of 600 cells/mm^3. Of greater interest in our study was the fact that there appeared to be no difference in CD4 T cell counts between those cases which were and those which were not secondarily infected with *C. albicans*. The inevitable conclusion to be drawn from this result is that CD4 T cells are not a determining factor in the pathogenesis of secondary candidiasis in oral HIV-KS but this conclusion remains speculative due to the small sample size. In a similar study, 75% of 130 cases of HIV-KS lesions were reported to have CD4 T cell counts of less than 200 cells/mm^3 and the authors also concluded that low CD4 T cell counts were not a prerequisite for the development of HIV-KS.[9,12,22]

6.5 Histopathology

KS has a very broad histological spectrum although most cases can be readily identified as the lesion changes from patch to plaque and then on to a nodular growth phase. New histomorphological variants are reported regularly in the literature. A review of the various histological types of cutaneous KS has been provided by Grayson and Pantanowitz.[84] These variants are important for correct histological diagnosis but may also be of prognostic significance.[84] There are the usual variants of patch, plaque and nodular as well as the

variants reported in the older literature which include the anaplastic, lymphodeamotous, lymphangioma-like, lymphangiectatic, bullous, telangiectatic and lastly the contemporary variants which include the hyperkeartotic verrucous, keloidal, micro-nodular, pyogenic granuloma-like, ecchymotic, intravascular, glomeruloid, pigmented and myoid nodular KS.[84]

This study made no attempt to characterise the histological variants of oral HIV-KS, but was restricted to the surface epithelium or pyogenic membrane and the presence of secondary *C. albicans* infection. Histopathologists are aware of the presence of secondary infections or co-existing lesions in KS and HIV-KS.[87] AIDS associated cases may show evidence of opportunistic infections such as bacillary angiomatosis, cryptococcus or cytomegalovirus infections but these are usually found in the lesional tissue itself.[84,87]

We are not aware of any studies in which the frequency and morphology of secondary *Candidal* infection in oral lesions of HIV-KS have been studied and ours seems to be the first to establish this frequency and to describe the histomorphology. The relatively low frequency of secondary *Candidal* infection of oral HIV-KS was indeed of interest and requires further investigation as does the deeper penetration of *Candidal* pseudohyphae into the epithelium, the proliferation of the pseudohyphae in the pyogenic membrane and even into the connective tissue. Such growth characteristics are not usual for this organism.

The results of this study have shown that less than half (40.62%) of all biopsies of oral HIV-KS are secondarily infected with *C. albicans*. The *Candidal* pseudohyphae are generally present in the surface epithelium immediately above the oral HIV-KS lesional cells. We do not know whether the oral HIV-KS lesions with *Candida* infected epithelium could be distinguished clinically from the cases without secondary *Candidal* infection as we did not

have the opportunity to monitor the patients. Furthermore, we have shown that the severity of the infection caused by *C. albicans* varies considerably from single isolated pseudohyphae to matted colonies of fungal organisms. The morphology of the less severe infections closely mimics *Candidal* infections seen in immunocompetent hosts whereas in the severe infections, the organisms in the HIV-seropositive group penetrate deeper than do those in immunocompetent hosts and are also more numerous.

C. albicans pseudohyphae are usually restricted to the upper layers of the oral epithelium in candidiasis and are some distance away from lymphocytes and dendritic cells located in deeper layers.[28] Our study has further confirmed that generally *C. albicans*, regardless of the severity of the infection, rarely reaches the lamina propria by penetrating through the epithelium. In a single case of severe infection where the organism had penetrated the connective tissue and the KS lesional tissue it had penetrated through the necrotic ulcerated surface. In fact the pseudohyphae are usually found only in the superficial layers of parakeratin and very seldom in the stratum spinosum. The reasons for this are unclear but perhaps the more anaerobic conditions found deep in the epithelium limit the depth of infiltration of the organism. It did however appear to us that in the more severe infections the organisms did penetrate deeper reaching the upper third of the stratum spinosum.

The presence of neutrophilic permeation leading to the formation of small microabscesses within the superficial layers of the epithelium in *C. albicans* infected epithelium is well known to oral histopathologists.[36] Very often the presence of these abscesses is an indication of the presence of organisms in that area but we have seen organisms in the epithelium which was devoid of microabscesses and we have seen the presence of microabscesses but with no associated organisms. The significance of these inflammatory cells is therefore unknown.

Of further interest was the finding of pseudohyphae within the pyogenic membrane of the biopsy specimens. This is an unusual finding and we have not previously noted this phenomenon. The *C. albicans* organism is epitheliotrophic and depends on attachment and penetrative biological processes to infect host tissue.[28] We do not know whether those are inherent in either of or both the organism and host epithelium. Clearly invasion of the pyogenic membrane shows that epithelial factors are not absolutely essential to the growth of the organism but that under certain circumstances the organism is also capable of living in the fibrinopurulent exudate that forms the pyogenic membrane.

Although assessment of the histology was entirely subjective, we did agree that as far as tissue reactions are concerned, there were no differences between the histological sections studied in the HIV seropositive group and the HIV seronegative group. If we compare the histology of those of our oral HIV-KS cases which were secondarily infected with *Candida* from those which were not secondarily infected with *Candida*, the only discernable differences were the absence of a thick pseudomembrane of desquamated parakeratin, the presence of *Candidal* organisms and the absence of pseudoepitheliomatous hyperplasia.

This study has raised many further questions regarding the pathogenesis of tissue invasion and penetration, the effect of HIV in these processes and the very rare ability of the organism to grow in non-epithelial surface tissues.

6.6 Extent to which aims have been reached

The aims of this study were to establish the frequency of secondary *Candidal* infection of the surface epithelium of oral lesions of HIV-KS and to describe the histomorphology of this infection and the cellular reactions that occur. We have largely succeeded in this regard.

6.7 Strengths and weaknesses of the study

The main difficulty in this study stemmed from the fact that we were not able to definitively establish the HIV status of 89 cases. We were therefore faced with the choice of either assuming that they were seropositive or eliminating them from the initial sample. We chose the latter course of action thus reducing a potentially large sample to one that may be too small from which to draw any meaningful conclusions.

6.8 Directions for future research

Histomorphological studies very often open up new directions of research as investigators seek answers to explain particular morphological trends or features of disease. So was the case in this study when the pathogenesis of oral candidiasis came under scrutiny, to explain features such as the increased intensity of *Candidal* infection, deeper penetration of *Candidal* pseudohyphae, the change from yeast to vegetative to pseudohyphal form and the growth of the organism in non-epithelial tissues such as the pyogenic membrane.

CHAPTER 7

7.0 CONCLUSION

In conclusion,

- 40.62% of oral HIV-KS lesions were secondarily infected with *Candida*. We expected this figure to be much higher. One of the reasons for this is that many cases of oral HIV-KS are covered by a pyogenic membrane rather than by epithelium.
- a significant finding was the striking predominance of females in the initial sample of oral KS lesions and the oral HIV-KS sample compared to other studies in the literature reporting a male predominance.
- an unusual finding was that *Candidal* pseudohyphae and yeasts were very rarely found in the fibrinopurulent exudate overlying the oral HIV-KS lesional tissue. *Candida* is strongly epitheliotrophic and its growth in exudate is most unusual.
- in a few cases of severe infection pseudohyphae were shown to invade beyond the parakeratin into the upper third of the stratum spinosum.
- one case showed the organism penetrating into the connective tissue and oral HIV-KS lesional tissue.
- inflammatory exocytosis and Munro micro-abscess formation was frequently present, but this did not invariably indicate proximity of the organisms. Organisms were often present in the absence of inflammation in the epithelium.
- although it appeared as if an inverse relationship existed between CD4 count and the the intensity and depth of infection, this data could not be confirmed.

- CD4 T cell count does not play a role in *Candidal* secondary infection of oral HIV-KS lesions, however this conclusion must be regarded with caution because of the small study sample.

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9.0 ADDENDUM

9.1 Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Sibda

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080307**
PROJECT	Candida Infection in Oral Lesions of Kaposi's Sarcoma
INVESTIGATORS	Dr A Sibda
DEPARTMENT	Oral Pathology
DATE CONSIDERED	08.03.25
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.04.07 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof M Altini

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES