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The Spectrum Of Cutaneous Deep Fungal Infections in HIV Positive Patients at Chris Hani Baragwanath Academic Hospital

(August 2013- July 2016)

Ayanda Motau



A research report submitted to the University of The Witwatersrand, Johannesburg in partial fulfilment for the requirements of the degree of Master of Dermatology 2018

DECLARATION

I, Ayanda Motau, declare that this research report is my own work, which is being submitted for the degree Master of Medicine in the branch of Dermatology at the University of the Witwatersrand, Johannesburg.

It has not been submitted before for any degree or examination at this or any other university.

Date:

Signature:

A.Motau

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ACKNOWLEDGEMENTS

God, you constantly amaze me.

To my loving family, especially my mother, husband and grandmother, thank you for your prayers and unwavering support. The assistance, knowledgeable guidance and supervision from Professor Karstaedt is greatly appreciated.

Thank you to Professor Deepak Modi for his expertise, supervision and mentoring throughout my registrar time. I am grateful to Mrs Helen Nxumalo for helping me trace biopsy results.

To Dr Reitumetse Tladi, thank you for walking this journey with me.

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ABSTRACT

Background

Cutaneous deep fungal infections may be classified as subcutaneous or systemic. Populations that are particularly at risk are patients with human immunodeficiency virus (HIV) or haematological malignancies, recipients of solid organ or hematopoietic stem cell transplants, and patients on immunosuppressants. Early diagnosis and prompt treatment are imperative in these potentially life-threatening infections where mortality rates for localised disease range from 4-10% and can be as high as 94% in patients with systemic infections. Unfortunately few studies have been published on this subject especially against the background of HIV infection. South Africa is at the epicentre of the HIV epidemic and the true spectrum of cutaneous deep fungal infections in HIV-infected patients in South Africa should ideally be demonstrated.

Objectives

To describe the prevalence and aetiological spectrum of cutaneous deep fungal infections in HIV positive adults at Chris Hani Baragwanath Academic Hospital.

Methods

This was a retrospective descriptive study of patients seen at Chris Hani Baragwanath Academic Hospital (CHBAH) from 1st August 2013 - 31st July 2016, who were HIV infected and had a skin biopsy done which proved a cutaneous deep fungal infection histologically. A list of subjects was obtained from the Department of Anatomical Pathology at CHBAH using the National Health Laboratory Services(NHLS) computer system. Participants' demographic information and HIV status were obtained from the histopathology report. Fungal culture

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results as well as other relevant results not found in the report were obtained using the NHLS Trakcare system.

Results

A total of 53 patients were diagnosed with a deep fungal infection on histopathology amongst 2576 adults who had skin biopsies giving a 2% prevalence rate. Only 20/53 (37%) cases had a definite diagnosis. The commonest infection was cryptococcosis with 9 cases, followed by emergomycosis (formerly known as emmonsiosis) with 7 cases and sporotrichosis and candidiasis with 2 cases each. Microbiology culture was positive in only 5 (17%) of 30 cases who had tissue cultures done and in only 9 (29%) of 31 who had blood culture performed. The other 33 cases did not have a definitive diagnosis. . However 22/53 cases were classified as having a possible diagnosis as suggested on histopathology and of these, 19/53 were classified as possible histoplasmosis/emergomycosis and 3/53 were possible sporotrichosis/cryptococcosis. The average CD4 count was 43 cell/mm^3 and 57% of patients were on antiretroviral treatment.

Conclusion

The prevalence of cutaneous deep fungal infections in adults with advanced HIV infection in South Africa who have skin biopsies performed is significant. There has been a striking emergence of emergomycosis, which was only recently recognised as a cause of disease in the HIV infected. Improved methods for definitive aetiological diagnosis are needed. We need to have a high index of suspicion in diagnosing and treating these potentially life threatening infections.

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ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
CD4	CD4 T cell
CNS	Central nervous system
CSF	Cerebrospinal fluid
GMS	Grocott's methamine stain
HIV	Human immunodeficiency virus
IRIS	Immune reconstitution inflammatory syndrome
IV	Intravenous
NICD	National institute for communicable diseases
PAS	Periodic acid-schiff
PCR	Polymerase chain reaction
SSKI	Saturated solution of potassium iodine

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CHAPTER 1- LITERATURE REVIEW

1.1 Introduction

Cutaneous deep fungal infections are fungal infections of the skin that are classified as subcutaneous or systemic. They are associated with significant morbidity and mortality especially against the background of immunosuppression [1].

Populations at risk of these infections are patients with acquired immunodeficiency syndrome (AIDS), recipients of solid organ or hematopoietic stem cell transplant, haematological malignancies and those on immunosuppressive therapy [2,3]. Mortality rates vary according to the infecting organism, the host's immune status and whether the infection is subcutaneous or systemic. Mortality ranges from 4% to 10% for localised infections and has been found to be as high as 83% to 94% in systemic disease [4]. Despite the seeming rarity of these infections, as they are likely underdiagnosed, the burden is still significant as they can be fatal unless diagnosed and treated early, especially in immunosuppressed patients [5].

1.2 Classification

1.2.1 Subcutaneous fungal infections

Subcutaneous deep fungal infections result from direct inoculation by organisms which are often saprophytic into skin via trauma. These organisms are directly implanted into the dermis or subcutaneous tissue. The infection can either spread to adjacent tissue or disseminate via lymphatics or rarely haematogenously [6] .Chromomycosis, sporotrichosis and mycetoma are the commonest examples of these infections while pheohyphomycosis, hyalohyphomycosis and lobomycosis are not as common [7] .

1.2.2 Systemic fungal infections

Systemic fungal infections can be further divided into opportunistic infections or true pathogens but some authors regard them as one entity. Cryptococcosis, systemic candidiasis, aspergillosis and mucormycosis are often considered opportunistic. While histoplasmosis, blastomycosis, coccidioimycosis and paracoccidioimycosis have been classically regarded as true pathogens [7] .The newly described emmonisiosis, now referred to as emergomycosis, caused by *Emergomyces africanus ,* was first described in South Africa in 2013 and is classified as a true pathogen [8] . The respiratory system is usually the first site to be involved, often leading to initial lung infection that can be self-limiting in an immunocompetent host. In the presence of marked immunosuppression there is dissemination of the infection via lymphatics or by haematogenous spread to involve other organs including skin [9,10].

1.3 Trends in Epidemiology

There has been a significant rise in the prevalence of deep fungal infections in the 20th century. This has been attributed to increasing numbers of immunosuppressed patients mostly due to human immunodeficiency virus (HIV) and the increased generalized usage of immunosuppressants in the treatment of a number of diseases and in transplantation[1,11]. There are very limited studies that have focused on the aetiological spectrum of cutaneous deep fungal infections in a single centre worldwide [1,4,5].

Table 1: **Selected studies of cutaneous deep fungal infections in different countries**

Study	**Year**	**Country**	**Period**	**No.of cases**	**References**
Kim et al	1999-2010	South Korea	12 years	41	(Kim, 2012)
Tsai et al	2001-2014	Taiwan	14 years	23	(Tsai, 2017)
Santiago et al	2003-2012	USA	10 years	33	(Santiago, 2014)

In resource limited centres, disease prevalence is not well understood due to late diagnosis and challenges in curbing HIV[12]. Unfortunately there is a paucity of data from African countries, with the available studies being conducted predominantly in the pre-HIV era[13].

1.3.1 Epidemiology in South Africa

In Johannesburg the most recent study conducted was by Klevansky in 1998. It included Hillbrow and Baragwanath Hospitals. A total number of 120 cases of deep fungal infections were found over a 10 year period. It was a retrospective study where only one patient was found to be HIV positive [13].

Few studies were conducted prior to this and they showed congruent findings with sporotrichosis, chromomycosis and blastomycosis being the commonest infections in the Gauteng region.

Table 2: **Studies from South Africa**

Study	**Year**	**Place**	**Period**	**Results**	**Reference**
Lurie	1947	Johannesburg	7 years	Chromomycosis - 41 Sporotrichosis – 34 Eumycetoma – 22	(Lurie, 1955)
Martin and Berson	1950	Johannesburg	21 years	Sporotrichosis - 688 Eumycetoma – 70 Chromomycosis – 64 Blastomycosis – 3	(Martin, 1973)
Klevansky	1987	Johannesburg	10 years	Sporotrichosis – 57 Chromomycosis – 16 Eumycetoma – 7 Blastomycosis – 5 Histoplasmosis – 0 Cryptococcosis – 1	(Klevansky, 1998)

The latest study to be conducted in South Africa was a prospective study of HIV-infected patients with CD4 counts < 100 cells/ml and widespread skin lesions present for < 6months,

where Schwartz described AIDS related endemic mycoses. The study was conducted in the Western Cape. There were 25 patients proven to have cutaneous systemic mycoses on histopathology over a 14 month period in 2013 and, of these, 20 had a definite diagnosis [14].

Table 3: **Results from Cape Town study** [14]

Emergomycosis	14 cases
Histoplasmosis	3 cases
Sporotrichosis	3 cases
Undifferentiated	5 cases

1.4 Clinical Features of Deep Fungal Infections

Clinical presentation is variable, with patients presenting with a wide spectrum of skin lesions which may be localised or generalised. Lesions range from erythematous plaques which may be crusted, ulcerated or scaly as well as nodules or papules that may be umbilicated or acneiform. Less common presentations include abscesses, cellulitis, mucocutaneous ulcers and varicelliform eruption. This often represents a diagnostic challenge therefore a skin biopsy should always be performed on any suspicious lesion. These biopsies should ideally be done on new lesions and multiple biopsies are recommended to yield better results[15].The commonest cutaneous lesions are crusted plaques with erythema followed by papules, nodules, patches and ulcers

1.4.1 Clinical characteristics of infections occurring in South Africa

The geographic pathogenic differences worldwide have been well described and therefore this literature survey focuses mostly on the infections that have been reported in South Africa, particularly those found to be associated with HIV such as histoplasmosis, cryptococcosis, emmonsiosis, sporotrichosis and candidiasis [12,14,16].

1.4.1.1 Subcutaneous Infections

SPOROTRICHOSIS

Sporotrichosis is a subacute or chronic infection caused by a dimorphic fungus *Sporothrix schenckii*. It is found worldwide but is common in South America, some parts of Asia, Europe and South Africa especially in gold miners. The fungus is a saprophyte and is found in vegetation, plants, flowers and mine timbers. Transmission is usually by traumatic inoculation by thorn, cat scratch, splinter or other penetrating injury [17,18].

It presents in three clinical forms:

Lymphocutaneous sporotricosis also known as lymphangitic sporotrichosis - this is the commonest type, seen in 75% of cases. It presents with a firm nodule that often ulcerates along draining lymphatics. A lymphangitis with secondary nodules along the line of the lymphatic drainage occurs. This pattern is common on limbs but can also occur on the face [7].

Fixed cutaneous sporotrichosis is seen in 20% of cases and clinically starts as a solitary ulcer, plaque or crateriform nodule with or without regional lymphangitis [6] .

Disseminated sporotrichosis is the least common and is seen in less than 5% of cases. Common risk factors include AIDS, haematologic malignancies, diabetes and immunosuppressive drugs. Systemic invasion may produce cutaneous, pulmonary, GIT, articular and brain lesions. Skin lesions present as tender nodules that suppurate and ulcerate [17].

CHROMOMYCOSIS

Chromomycosis, also known as chromoblastomycosis is a chronic infection caused by dematiaceous fungi. The common species are *Fonsecea pedrosi, Fonsecea compacta*, *Cladosporium carrionii, Phialophora verrucosa* and *Rhinocladilla aquaspersa*. It occurs worldwide but is common in South Africa, other parts of Africa and Asia. It is usually seen in barefooted farm workers following trauma from wood or vegetation [6].

Clinically it presents on lower limbs as a papule that progressively becomes a verrucous nodule. The infection usually remains localised but over time there is autoinoculation secondary to scratching with resultant satellite lesions and scarring. Regional lymphadenopathy may occur with secondary bacterial infection. It can be complicated by lymphedema that may progress to elephantiasis and squamous cell carcinoma may occur long term [9].

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EUMYCETOMA

Mycetoma or Madura foot is a chronic, granulomatous subcutaneous infection that can be caused by filamentous bacteria or true fungi. Therefore the term eumycetoma is designated for infections caused only by true fungi. The common infective organisms are *Madurella mycetomatis, Madurella grisea, Pseudallescheria boydii* and *Leptosphaeria senegalensi*. The disease is acquired from soil via direct inoculation into skin and it is common in Africa especially in Senegal, Sudan and Somalia [6,9] .

Mycetoma is characterised by the classic triad of draining sinuses, presence of grain (which represent macroscopic colonies of organisms) and oedema. Lesions are common on the feet and initially presents as a painless nodule that progresses slowly to produce papules and draining sinuses that discharge fluid and contain grains. Long term complications include bone involvement and deformities [7] .

1.4.1.2 Systemic Infections

HISTOPLASMOSIS

Histoplasmosis is a systemic infection caused by the dimorphic fungus *Histoplasma capsulatum* with a Central and West African variant named *Histoplasma capsulatum var duboisii*. It is endemic in Africa , North America and South America [17] . The primary site of infection is the respiratory system via inhalation of the organisms from bird, chicken and bat droppings with significant reservoirs being areas they live in e.g. caves, unoccupied buildings and chicken coops. High risk patients include those with underlying immunosuppression especially AIDS patients in whom cutaneous involvement is seen in up to 25% of cases [19] .

It can present in three clinical forms:

- Primary pulmonary histoplasmosis is usually benign and in 95% of cases it results in spontaneous resolution without any clinical signs and cavers are particularly at risk. In a few cases patients may present with acute symptoms ranging from mild flu-like symptoms to a serious pneumonitis. The acute forms may progress to chronic disease [7] .

- Progressive disseminated histoplasmosis typically affects patients with AIDS but can be seen in patients on immunosuppressive treatment, those with haematological malignancies and renal transplant patients. The prognosis is poor as it can be fatal if treatment is not initiated early. Cutaneous involvement can be seen in up to 80% of patients [19]. Two thirds of cases present with mucosal involvement such as ulceration and granulomas of the oropharynx [19]. Clinical lesions may be papules, pustules, nodules, crusted plaques, abscesses as well as purpura. Other system involvement include the reticuloendothelial, gastrointestinal (GIT), central nervous system (CNS), cardiovascular as well as genitourinary systems [15].

- Primary cutaneous histoplasmosis is rare and is caused by direct inoculation and presents like a chancre [7, 19]

The African variant predominantly presents with cutaneous lesions and osteomyelitis. The commonest skin lesions are superficial granulomas and subcutaneous granulomas in addition to the ones described above [10,19] .

CRYPTOCCOCOSIS

Cryptococcosis is a systemic infection caused by an encapsulated budding yeast, *Cryptococcus neoformans*. It has a worldwide distribution and the portal of entry is the lung via inhalation. It is found in pigeon droppings, soil, dust and typically on window ledges in large cities. High risk patients for acquiring the disease are patients with AIDS, haematological malignancies, renal failure, liver disease and severe diabetes mellitus and those on long term immunosuppressive drugs. In AIDS patients risk of dissemination is seen in up to 50% of patients and it is one of the common opportunistic infection seen in these patients [17] .

The infection can present in three clinical forms:

- Primary pulmonary cryptococcosis seen in 90% of cases tends to be localised and asymptomatic [7].

- Disseminated cryptoccocosis is considered an AIDS defining disease and is the commonest clinical form. The organism has affinity for the CNS. Meningitis is seen in 60% - 70% of cases of disseminated cryptococcocal infection with CD4 counts usually below 100/mm^3. The skin is involved in 10% - 20% of patients and the presentation is variable. The commonest presentation is with dome - shaped papules with central umbilication and is found in 50% of cases in head and neck regions [7]. However the lesions may range from subcutaneous nodules to ulcers, papules, plaques and cellulitis. In a subset of patients skin manifestations may precede overt systemic disease by up to 8 months. Other sites that may be involved are the liver, skeletal system, spleen and lymph nodes [19] .

- Primary inoculation cryptococcosis is extremely rare and presents as a solitary skin lesion in the form of a whitlow. The risk factor for this type of infection is outdoor activities with exposure to bird droppings [7].

EMERGOMYCOSIS

Emergomycosis (formerly known as emmonsiosis) is a systemic opportunistic infection caused by the dimorphic fungus *Emergomyces africanus*. It was first described in 13 patients in Cape Town, South Africa in 2013 and has been proven to be one of the commonest deep fungal infections in HIV infected patients [8] . Since then cases have been described in all 9 provinces in South Africa and Lesotho [20]. In Johannesburg 3 cases were first described by Van Hougenhouck - Tulleken WG et al in 2014 and all the patients were HIV positive with CD4 counts < 5/mm^3 [21] .

Many aspects of this infection still remain unknown but it has been recently shown to be acquired via inhalation and to be found in soil suggesting that soil might be the reservoir [22] . The skin is commonly affected and patients present with widespread lesions. The morphology of lesions is variable and can range from erythematous papules and plaques, with or without scales, to ulcerations and crusting. The disease is commonly misdiagnosed as varicella, drug reactions and Kaposi's sarcoma. The other commonly affected system is the respiratory system with lower respiratory tract involvement seen in 88%. The haematological system may be involved. In the study conducted by Schwartz et al, 2013, anaemia was seen in 96% of the patients. Other systems less commonly involved are the GIT and CNS [20].

BLASTOMYCOSIS

Blastomycosis is caused by a dimorphic fungus *Blastomyces dermatitidis* and the natural habitat is not yet well established. It is endemic in North America and risk factors for acquiring the disease include immunosuppression especially diabetes mellitus. Infection occurs via inhalation and the lungs are the first to be involved [7] .

Clinical presentation of the pulmonary disease may be acute but can progress to a chronic disease. Dissemination is not common but when present, the skin is involved in 80% of cases. Cutaneous blastomycosis is secondary to a primary lung focus while primary inoculation to skin is rare. The skin lesions are chronic verrucous and granulomatous plaques with advancing edges that often have draining sinuses and pustules [10,19] .

CANDIDIASIS

Candidiasis is a systemic fungal infection caused by candida species and is considered an AIDS defining disease [3]. It is one of the commonest mucocutaneous manifestations affecting up to 70% of HIV patients [23]. Disseminated candidiasis, although not common, often originates from the GIT. There are at least 15 distinct candida species that cause human disease but >90% of invasive disease is caused by the 5 most common pathogens, *C. albicans*, C*. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*. *Candida albicans* is the commonest worldwide [24]. In Latin America *Candida Tropicalis* and *Candida parapsilosis* are the second commonest while *Candida glabrata* is the second commonest in the United States. The rate of non-albicans species has increased worldwide [(3).]

Cutaneous involvement is seen in 10 - 13% of affected individuals. They often present with asymptomatic papules or pustules on an erythematous base. Some may present with nodules with central necrosis with a few presenting with purpura. Systemic involvement includes the liver, spleen, kidney and heart [7].

1.5 Approach to diagnosis

Histopathological examination of skin tissue remains the primary method of early diagnosis as it is faster than culture and is more widely used. It identifies a probable deep fungal infection by displaying characteristic histological features seen with the infective organism. The predominant histopathological features are pseudoepitheliomatous hyperplasia, intraepidermal microabcesses and suppurative granulomatous inflammation. Less common histopathologic features may be perivascular and interstitial inflammation, small vessel vasculitis and necrosis. Fungal elements may be seen routinely but stains such as Groccot's methamine silver (GMS) and periodic acid – schiff stain (PAS) are often used to confirm the diagnosis by staining the cell walls, brown – to - black in living and dead fungi with GMS and staining magenta only in living organisms with PAS. This confirms the presence of fungus and helps in demonstrating whether the infecting organism is intracellular or extracellular, is in yeast form, and has a capsule and defines size [4] .

The histopathological morphological differences between these organisms may not be definitive and tissue fungal culture therefore still represents the diagnostic gold standard in current day practice [25] . Although direct correlation between these two diagnostic tests is expected, discrepancies between histopathology and culture can be found in up to

80% of cases and this may result in treatment delays associated with increased morbidity and mortality rates [4] .

Although histopathology may yield results faster than culture, this is still not rapid enough for the diagnosis of these potentially life-threatening infections. Faster and more accurate diagnostic tests are still required. Other non-culture methods, including antibody and antigen assays, metabolite detection assays and molecular methods using polymerase chain reaction (PCR) can add much needed value to the diagnostic process [26]. In situ hybridization utilizing oligonucleotide probes directed against fungal ribosome RNA, is a rapid and accurate assay for identification of dimorphic fungi in paraffin embedded sections . Although still not widely available it is a reliable tool in the prompt aetiological diagnosis of cutaneous deep fungal infections [25]. Fungal PCR is currently proving to be useful in the diagnosis of deep fungal infections on tissue biopsies especially when combined with culture and histopathology [27,28].

1.5.1 Different histopathological patterns

Table 4: **Histopathological Patterns in Subcutaneous Infections**

Sporotrichosis	Epidermis - psuedoepithelomatous hyperplasia associated with Intraepidermal neutrophilic microabcesses Dermis - suppurative granulomatous inflammation admixed with neutrophilic microabcesses [4,6] - fungal spores - round, oval or cigar shaped - rarely asteroid bodies may be found [29]
Chromomycosis	Epidermis - psuedoepitheliomatous hyperplasia, intraepidermal microabcesses not as common Dermis - diffuse granulomatous inflammation predominant finding - neutrophilic micro abscesses not as common [9,29] - fungal spores arranged in chains or clusters called Medlar bodies or copper pennies [6,9]
Eumycetoma	Epidermis - often non-specific - exudative crust can be seen Dermis - neutrophilic microabcesses with granulomatous inflammation is common - sclerotica - which are large colonies of fungi are found and brown to black in colour [9,29]

Table 5: **Histopathological findings in Systemic infections**

Histoplasmosis/ emergomycosis	Epidermis - often ulcerated Dermis - numerous minute yeasts that are Intracellular within histiocytes [17] - yeasts surrounded by halo- known as foamy histiocytes macrophages [19,21,29]

Cryptococcosis	Two main histopathologic patterns Gelatinous - diagnostic - prominent capsule around organism(stains with GMS and mucin stains) - budding yeasts numerous [17,19] Granulomatous - granulomatous inflammation often with psuedoepithelioamtous hyperplasia [29]
Blastomycosis	Epidermis – pseudoepitheliomatous Hyperplasia is marked - papillomatosis, neutrophilic microabcesses often seen Dermis - granulomatous inflammation - neutrophilic microabcesses - thick walled spores , broad based budding [19,29]
Candidiasis	Dermis – budding yeast and psuedohyphae - scanty inflammation [7,29]

1.6 Treatment

Following appropriate investigations, the diagnosis of a cutaneous deep fungal infection can either be possible , probable or proved. The treatment strategies can be prophylactic, empiric or specific [30] . Deciding on the best antifungal agent involves a balance of clinical factors of fungal susceptibility, potential drug toxicity as well as drug availability and cost. In severe and disseminated cases in patients with HIV infection admission for parenteral amphotericin B is usually indicated. In less severe cases, the recommended oral azole or other recommended drug can be used. The total cost of treating a patient with a deep fungal infection includes antifungal treatment, monitoring for drug toxicity as amphotericin B, the

cost of prolonged admission and the costs associated with managing any drug side effects [31] .

1.6.1 Summary of treatment

SPOROTRICHOSIS

For lymphocutaneous and localised cutaneous sporotrichosis, itraconazole 200mg orally daily is recommended for 3 - 6 months. Other treatment options are terbinafine or saturated solution of potassium iodide (SSKI). For disseminated or systemic sporotrichosis, amphotericin B followed by itraconazole 200mg twice daily for 12 months [7,18].

CHROMOMYCOSIS

Chromomycosis is challenging to treat and often combination therapy is required. For localized and early disease, itraconazole 200mg - 400mg daily for 6 - 12 months; terbinafine can be used as second line. For extensive and destructive disease a combination of surgery and systemic antifungal therapy is recommended. Other combination therapies that can be used are amphotericin B with itraconazole or cryotherapy with itraconazole and local hyperthermia [6,7].

EUMYCETOMA

For mild localized disease surgical excision or itraconazole 200 - 400mg /day is used. For severe disease a combination of surgical excision and itraconazole is recommended [6,7].

HISTOPLASMOSIS / ERMERGOMYCOSIS / BLASTOMYCOSIS

Mild early disease can be treated with itraconazole 200mg - 400mg for 3 - 6 months. Amphotericin B for 1 - 2 weeks, followed by oral itraconazole for a minimum of 12 months, is the recommended treatment for moderate to severe disseminated disease [10,20,31,32].

CRYPTOCOCCOSIS

Mild skin disease with CNS disease excluded, fluconazole at 200 - 400mg per day orally for 6 - 12 months is used. Amphotericin B per day intravenously (IV) plus flucytosine for 2 weeks, followed by fluconazole for a minimum of 8 weeks is recommended for disseminated disease. Lipid amphotericin B formulations IV for 2 weeks is reserved for patients predisposed to or with existing renal dysfunction [30,33].

CANDIDIASIS

For candidemia in non-neutropenic patients the recommendation is an echinocandin as initial therapy. Fluconazole IV or oral and amphotericin B can be used as alternatives [24,30].

1.7 Cutaneous deep fungal infections and HIV

The HIV epidemic now exists worldwide. South Africa is at the forefront of the epidemic with close to 6 million people living with HIV [34] . Cutaneous complications occur in over 90% of individuals with HIV infection and they can be debilitating, disfiguring and life threatening. Cutaneous fungal infections may often be the first manifestation of the HIV infection, or may be a manifestation of another underlying systemic disease [34].

With declining CD4 counts, HIV causes multiple defects in both the innate and adaptive immune systems and this leads to increased susceptibility to both mucosal and invasive fungal diseases [35] . Some deep fungal infections such as histoplasmosis and cryptoccocosis form part of the World Health Organisation (WHO) Clinical Staging System and are a sign of stage 4 disease requiring immediate initiation of antiretroviral therapy.

As more patients are being treated with antiretroviral therapy , the incidence of systemic fungal infections should decline, especially with the immediate antiretroviral treatment initiation at higher CD counts. It is however important for clinicians to always keep in mind the possibility of these infections appearing as part of the immune reconstitution inflammatory syndrome or with immunological treatment failure [23].

To date there are very few studies that have focused on cutaneous deep fungal infections in a single centre setting or regional or countrywide, with the unfortunate result

of a paucity of data particularly from African countries [5]. With the likely marked underdiagnoses of AIDS associated opportunistic mycoses and the known risk of mortality especially when left untreated, there is a need for enhanced surveillance and further prospective studies [16] .

The atypical presentation of most cutaneous lesions and their polymorphic nature in HIV infection leads to a diagnostic challenge. Under conditions of severe immunosuppression, a diverse group of fungi are potential pathogens and there is the potential for emergence of new organisms that have not been recognized as with the 'discovery' of emmonsia species in Cape Town. It is therefore imperative for clinicians to always have a high index of suspicion in order to make the diagnosis of fungal infections in these hosts [26] .

The true spectrum of cutaneous deep fungal infections in HIV is difficult to demonstrate as biopsy for histopathology and microbiology, at a minimum, are needed for diagnosis, unless there is access to screening tests like urine Histoplasma antigen.

This spectrum should be ideally demonstrated in South Africa, a country with the highest prevalence of HIV infection, and at Chris Hani Baragwanath Academic Hospital Dermatology Department, which sees the largest number of patients in the country, with a majority of the patients being HIV positive.

1.8 Study Objectives

1. To determine the number of cutaneous deep fungal infections in HIV positive adult patients amongst all the skin biopsies taken at Chris Hani Baragwanath Hospital.

2. To describe the aetiological spectrum of cutaneous deep fungal infections in HIV positive patients at Chris Hani Baragwanath Academic Hospital.

3. To describe the clinical presentation of skin lesions in these patients with deep fungal infections described in the histopathology request form.

4. To determine the utility of culture against histopathology in the diagnosis of cutaneous deep fungal infections.

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CHAPTER 2: PATIENTS AND METHODOLOGY

2.1 Study Design

This was a retrospective descriptive study of adult patients seen at Chris Hani Academic

Baragwanath Hospital who were HIV positive and had a skin biopsy done and those who were proven to have cutaneous deep fungal infection histologically from 1st August 2013 up to 31st July 2016.

The histopathology report from skin biopsy was used to obtain study information. On the histology request form, the dermatologists detail patient data. This includes demographic data (age, gender), HIV serostatus, CD4 count if HIV infected and a clinical description of the skin lesions. Clinical details such as suspected diagnosis, differential diagnosis and brief clinical history are important. This information is transcribed onto the histopathology report and is followed by a description of the histological findings and a final diagnosis.

When a patient is suspected of having a deep fungal infection, two 3mm punch biopsies are taken - one is sent for histopathology and one for culture. The pathology specimen is sent in formalin and the volume should be 10 - 20 times the volume of the specimen. In the laboratory the specimen is fixed in formalin and remains in this fixation medium for at least six hours. The aim of fixation is to preserve the skin specimen indefinitely in a 'life like' state. Tissue processing then follows with the purpose of removing the extractable water from the skin and thus provide a supporting matrix (paraffin) so that the tissue can be cut with minimal distortion. After fixation, specimens are processed in an automatic processor. The specimens then pass through ethanol for dehydration, followed by xylene for lipid extraction and clearing of alcohol. Then finally the tissue is melted with hot paraffin so that the specimen

can be easily cut. They are usually sectioned using a microtome into sections ranging between 3 - 5 microns. Routine staining with H&E is done. With this staining method, nuclei stain blue (basophilic) and collagen, muscles nerves stain red (eosinophilic). Special stains such as PAS and Grocott are further used. PAS stains fungi red while Groccot stains fungal cell walls black. The stained slide is then processed again through water, alcohol and xylene. A resin is applied to glue a cover slip and the slide is ready to be examined by the pathologist.

The final aetiological diagnosis was divided into 3 categories namely: definite, possible or undifferentiated diagnosis.

Definite diagnosis was based on histopathologic diagnosis of a deep fungal infection with identification of the fungal organism on microbiological culture of skin tissue, blood, bone marrow or CSF. In cryptococcosis however the histopathologic features of gelatinous pattern or the presence of encapsulated yeasts was enough to make a definitive diagnosis even without the presence of microbiology.

Possible aetiological diagnosis included the cases where histopathology confirmed a deep fungal infection based on characteristic patterns which suggested that specific organisms should be considered but there was no microbiological confirmation.

Undifferentiated diagnosis included the deep fungal infections where no possible aetiology was suggested based on the histopathologic pattern and microbiology was not done or was negative.

A list of adult patients with a diagnosis of deep cutaneous fungal infection based on histology was obtained from the Department of Anatomical Pathology, National Health Laboratory Services (NHLS), at Chris Hani Baragwanath Academic Hospital. The Trakcare system was used

to obtain the list and results were accessed using the Snomed coding system using key words e.g. Systemic fungal infection affecting skin, histoplasmosis etc. The total number of biopsies done was obtained from the Central Data Warehouse.

We accessed the Trakcare system to check whether fungal cultures had been submitted for patients and to get the results thereof . Fungal culture specimens were transported in normal saline and once they reached the laboratory, were placed directly on dextrose agar and chloramphenicol medium. Specimens were checked daily until day 5 - 7, then at 2 - 3weeks. If there was growth, they were identified and were sometimes sent to the National Institute for Communicable Diseases (NICD) for confirmatory identification of the organism.

We used Trakcare to check the results of HIV tests, CD4 counts and HIV viral load, if done, for subjects for whom these were not recorded in the histopathology form. In patients on ART where the viral load was done, we were able to divide them into those who were virologically suppressed (viral load <400copies/ml) and those who had virological failure (viral load >1000 copies/ml) despite having been on treatment for a substantial duration of time. Immune reconstitution inflammatory syndrome (IRIS) was considered in patients who were on ART and was based on the history of the duration of skin lesions in relation to the initiation of ARTas recorded in the histopathology form and on a low viral load when available. A paradoxical IRIS was one where the skin lesions worsened following ART while unmasking IRIS was considered when the skin lesions developed while already on ART.

INFORMATION OBTAINED FROM THE HISTOPATHOLOGY FORM and TRAKCARE

Demographic data: Age, race, gender

Clinical characteristics: HIV status, CD4 count, viral load, morphology of skin lesions, whether polymorphic or not, distribution (localised or generalised)

Histology results: Pattern, presence of spores, stains used (PAS or GMS)

Microbiology: Tissue culture, blood culture or other

Final diagnosis: Suggested on histopathology or confirmed on microbiology

2.2 Study Population

2.2.1 Inclusion criteria

All HIV infected adult patients (>18 years of age), both inpatients and outpatients, with confirmed deep fungal infection on histopathology, from 1st July 2013 till 31st July 2016 were studied.

2.2.2 Exclusion criteria

Patients with proven cutaneous deep fungal infections whose HIV status was negative or unknown and patients younger than 18 years of age were excluded.

2.3 Site of study

Chris Hani Baragwanath Hospital, is a 2700 bed, public sector university hospital in Soweto, South Africa. Serving primarily the population of Soweto.

2.4 Data Analysis

Descriptive analysis of the demographic variables – age, gender and race.

Objective 1 - Calculated effect measures (prevalence rates) of deep fungal infections in HIV positive patients.

Objective 2 - Described the aetiological spectrum of deep fungal infections using frequency tables.

Objective 3 - Used frequency tables to describe clinical skin lesions of the patients with deep fungal infections.

Objective 4 - Determined the utility of culture results compared to histopathology results by using frequency tables.

2.5 Ethics

Permission was obtained from the CEO / Medical Superintendent of CHBAH (Chris Hani Baragwanath Academic Hospital) as well as the Head of Department of Dermatology. The study proposal was approved by the Human Research Ethics Committee of the University of Witwatersrand. To protect participant's confidentiality, the data was sent in an anonymised format, making patient identification unlikely.

CHAPTER 3: RESULTS

3.1 Prevalence of deep fungal skin infections

A total of 53 HIV infected patients were identified with cutaneous deep fungal infections diagnosed on histopathology. This was amongst 2576 skin biopsies done in adults, irrespective of HIV serostatus, at Chris Hani Baragwanath Academic Hospital between the 1st of August 2013 and the 31st July 2016. The prevalence was therefore 2% of all skin biopsies.

Demographics

The mean age was 37 years and the median age was 36 years from the sample of 53 patients. The youngest patient was 19 years old and the oldest was 59 years of age. The median age for females was 35 years and for males was 38 years. The total sample of patients was black in ethnicity. There was a slight male predominance with 28 males (53%) and 25 females (47%).

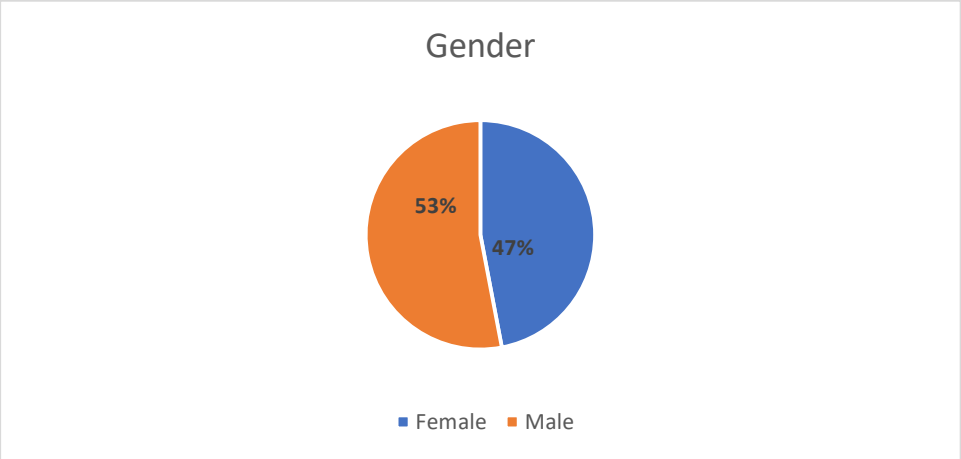


Fig 1: Gender of 53 patients

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3.2 Clinical characteristics

3.2.1 CD4 count and ART

CD4 count results were available for 46 patients while 7 patients had no recorded information. The average CD4 count was 43 cells/mm^3 and the median CD4 count was 21 cells/mm^3. The lowest recorded CD4 count was 1 cell/mm^3 and the highest was 321 cells/mm^3. CD4 counts <50cells/mm^3 were found in 33 (72%) of the 46 patients with results (Table 1). The results shown in table 1 below also reflect the findings of the 44 patients with information on antiretroviral therapy (ART): 30 patients (14 males and 16 females) were on ART, 12 were not on ART and 2 male patients were defaulters. The 30 patients on ART comprised 68% of the 44 with documentation on ART use and 57% of the total cohort. The duration on ART was not well documented.

Table 6: CD4 count and ART

CD4 count (cells/mm^3)	Frequency (n)	Percentage
<50	33	62%
50-200	11	21%
>200	2	4%
Total	46	87%
No record	7	13%
ARV treatment	**(n)**	**%**
Yes	30	57%
No	12	23%
Defaulter	2	4%
Total	44	83%
No record	9	17%

Data on viral loads was available only for 19 patients and 34 patients had no viral load results. Of the patients on ART who had viral loads done, 6 (32%) patients had virological failure and 13 (68%) were virologically suppressed. Of the patients that were virologically suppressed, 3/13 (23%) were diagnosed with immune reconstitution inflammatory syndrome (IRIS). This was based on a history of skin lesions starting 2 weeks after initiation of ART in 2 cases and lesions worsening after ART initiation in 1 case.

Table 7: Patients on ART: Viral load

Viral load	Frequency(n)	Percentage
Virological failure	6	11%
Virologically suppressed	13	25%
Total	19	36%
No record	34	64%
TOTAL	53	100%

3.2.2 Cutaneous Findings

The clinical morphology of lesions was recorded from the history section of the histopathology report. The results showed that 16 (30%) patients had one type of lesion documented, 31 (59%) patients had two types of lesions and 6 (11%) patients had three types of lesions (Figure 2). The commonest clinical lesions were plaques found in 40 patients (76%) followed by papules 33 (62%) (Table8).

Table 8: Morphology of skin lesions

Skin lesions	Number of responses	Percentage of cases
Plaques	40	75.5%
Papules	33	62.0%
Patches	9	17.0%
Ulcers	6	11.0%
Nodules	4	7.5%
Pustules	4	7.5%

The results shown in figure 2 below reflect the percentage of polymorphic skin lesions and monomorphic skin lesions in patients.

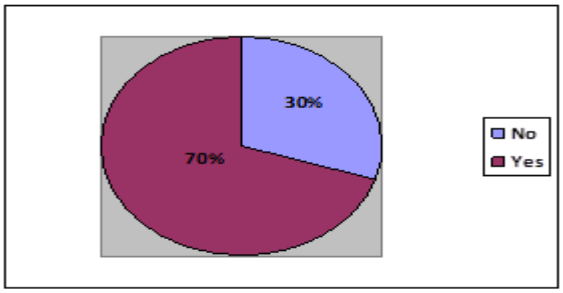


Fig 2: Polymorphic results from 53 patients

The results showed that 12 (23%) of the patients had localized skin lesions and 41 patients (77%) had generalized lesions. The most common combination of sites involved were the face, trunk, upper limbs and lower limbs. The head and neck region was the commonest site of involvement overall, followed by limbs combined. Of the cases involving the head and neck region, the face was involved in 41 patients (84%) with 3 patients (6.1%) presenting with lesions on the nose and 2(4.1%) on the scalp.

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Table 9: Distribution of skin lesions

Body site involved	Number of responses	Percentage of cases
Head and neck	46	87%
Trunk	27	55%
Upper and lower limbs	20	41%
Upper limbs	14	29%
Lower limbs	10	20%

3.3 Histopathological Findings

All cases had fungal elements that were either identified on routine hematoxylin and eosin (H&E) stainor with the specific fungal stains periodic acid Schiff (PAS) and/or Grocott methamine silver (GMS) stains.

The results showed that 37 (70%) patients had one histologic pattern, 14 (26%) had two histologic patterns and 2 (4%) had three histologic patterns. The most common combination was the gelatinous pattern with encapsulated fungal spores found in 4 (7.5%) patients, which is diagnostic for cryptococcosis. The commonest histopathologic findings were suppurative granulomas seen in 15 (28%) patients and this was mostly seen with the undifferentiated diagnosis, followed by histiocytes with small intracytoplasmic spores in 12 (23%) cases and the presence of encapsulated fungal spores in 8 (15%) as shown in table 10

Table 10: Histopathological patterns

Histology results	Responses	Percent of Cases
Suppurative granulomas	15	28%
Histiocytes with small intracytoplasmic spores	12	23%
Encapsulated fungal spores	8	15%
Gelatinous pattern	7	13%
Small minute fungal spores	7	13%
Neutrophilic microabscesses	5	9%
Lymphohistiocytic infiltrate	5	9%
Necrosis	4	8%
Mixed dermal infiltrate	3	6%
Foamy histiocytes	3	6%
Pseudoepitheliomatous hyperplasia	2	4%
Small spherical eosinophilic bodies	1	2%

Fungal spores

Fungal spores were found in 51/53 patients on H&E staining while 1 case was negative. This case was positive for both PAS and GMS staining. The remaining 1 case was not reported. With PAS staining, 52/53 patients had a positive result and 1 patient had a negative result. In the negative case, H&E and GMS stains were positive. GMS stain results revealed 45/53 patients as positive. Three cases had negative GMS stain with positive H&E and PAS results. The remaining 5 cases were not reported.

Table 11: Stains: H&E, PAS, GMS

H&E	Frequency(n)	Percent
Yes	51	96%
No	1	2%
Total	52	98%
No record	1	2%
PAS		
Positive	52	98%
Negative	1	2%
Total	53	100%
GMS		
Positive	45	85%
Negative	3	6%
Total	48	91%
No Record	5	9%

The results in table 12 reflected that 29 /53 cases had intracellular spores and 29/53 had extracellular spores. Amongst these cases, 9 (17%) patients had both intracellular and extracellular spores.

Table 12: Site of spores

Intracellular	Frequency(n)	Percent
Yes	29	55%
No	20	38%
Total	49	93%
No record	4	8%
Extracellular		
Yes	29	55%
No	19	36%
Total	48	91%
No record	5	9%

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3.4 Microbiology

There was a total of 67 microbiology tests performed (Table 13). Only 30 (57%) patients had tissue culture sent, while 20 (38%) patients had both tissue culture and blood culture taken and 3 (6%) patients had both blood culture and cerebrospinal fluid (CSF) mycology taken.

Table 13: Microbiology results

Microbiology tests	Test done	Percentage
Tissue culture	30	57%
Blood culture	31	58%
Bone Marrow	1	2%
CSF mycology	5	9%

TISSUE CULTURE RESULTS

The results for the 30 patients with a tissue culture were as follows; 2 (7%) patients cultured *Emmonsia* species, 1 (3%) patient had *Candida albicans*, 1 (3%) patient had *Candida parapsilosis* and 1 (3%) patient had *Sporothrix schenkii.* Twenty five (83%) patients had no growth.

BLOOD CULTURE RESULTS

The results for the 31 patients with a blood culture taken were as follows; 5 (16%) patients cultured *Emmonsia* species, 3 (10%) patients had *Cryptococcus neoformans* and 1 (3%) patient had *Sporothrix schenkii.* Twenty two (71%) patients had no growth.

OTHER MICROBIOLOGY RESULTS

One patient had a bone marrow culture test and 5 had CSF mycology cultures. All 6 patients cultured *Cryptococcus neoformans.*

Of all the 53 cases with histologically proven deep fungal infections only 17 patients had positive microbiology results. In 3/17 cases, an etiology was not suggested on the histopathology form (concluded as undifferentiated) however in the remaining 14 cases an etiological diagnosis was suggested. Only 3/14 (21%) cases were misdiagnosed - the 2 sporotrichosis cases were classified as histoplasmosis/emmonsia and 1 candidiasis case (*candida parapsilosis*) was misdiagnosed as histoplasmosis/emmonsia. This means 11/14 (79%) were correctly diagnosed on histopathology.

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Table 14: Comparison of microbiology studies

Cultured Species	Tissue Culture	Blood Culture	Other Culture
Emmonsia			
Case 1	Positive	Not done	Not done
Case 2	Positive	Not done	Not done
Case 3	Negative	Positive	Not done
Case 4	Negative	Positive	Not done
Case 5	Not done	Positive	Not done
Case 6	Not done	Positive	Not done
Case 7	Not done	Positive	Not done
Cryptococcus neoformans			
Case 1	Negative	Negative	CSF Positive
Case 2	Negative	Positive	CSF Positive
Case 3	Not done	Positive	CSF Positive
Case 4	Not done	Not done	CSF Positive
Case 5	Not done	Not done	CSF Positive
Case 6	Not done	Not done	Bone marrow Positive
Sporotrix Schenkii			
Case 1	Positive	Negative	Not done
Case 2	Negative	Positive	Not done
Candida albicans			
Case 1	Positive	Not done	Not done
Candida parapsilosis			
Case 1	Positive	Not done	Not done

Table 14 demonstrates the frequency with which the same organisms were cultured using different specimens. Of the 6/17 (35%) patients who had more than one culture done, in only 2/6 cases were both positive and these were cryptococcosis in blood and CSF mycology.

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3.5 Diagnosis

The diagnosis was classified as definitive, possible or undifferentiated as described in the methodology section. Twenty (38%) patients had a definitive diagnosis, 22 (42%) had a possible diagnosis and 11 (21%) had an undifferentiated diagnosis in the study.

Definitive diagnosis

The results for the patients with a definitive diagnosis were as follows: 9 (17%) were diagnosed with cryptococcosis, 7 (13%) were diagnosed with emmonsiosis, 2 (4%) with sporotrichosis and 2 (4%) with candidiasis.

Possible diagnosis

The results for the patients with a diagnosis that was possible were as follows; 19 (35.8%) had histopathology suggestive of histoplasmosis/ emmonsia and 3 (5.7%) were suggestive of sporotrichosis/ cryptococcosis.

Table 15: Diagnosis

Diagnosis	Definitive	Probable	Total	Percentage
Cryptococcosis	9	3	12	17 - 23%
Emmonsiosis	7	19	26	13 - 49%
Sporotrichosis	2	3	5	4 - 9 %
Candidasis	2	0	2	4%
Histoplasmosis	0	19	19	0 – 23 %

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The characteristics of the patients with a definitive diagnosis were as follows:

Cryptococcosis

Cryptococcosis was diagnosed in 9 cases. Average age amongst the patients was 40 years and there were 4 females and 5 males. The average CD count was 60 cells/mm^3 with 5 of these patients being on ART, of whom only 2 were virally suppressed. The head and neck area was involved in 7/9 cases with umbilicated papules and plaques being the commonest presentation. These were localised to the face in 3/9 of our cases and generalised in the remaining 6/9. Cryptococcosis was diagnosed on histopathology by the presence of numerous encapsulated yeasts and this was seen in 8/9 of our cases with the one remaining cases documented to have only the diagnostic gelatinous pattern. The gelatinous pattern was noted in 6 other cases. In 6/9 patients, microbiology was confirmatory either on CSF mycology or blood culture or bone marrow. In the presence of suppurative granulomas and fungal spores that were predominantly extracellular, another 3 cases were suggestive of sporotrichosis / cryptococcosis. Since it was possible that all 3 cases could have been cryptococcosis the total number of cases could have been 12 (23%).

Emergomycosis (formerly known as Emmonsiosis)

The average age of the 7 cases with emergomycosis was 39 years and there were 3 females and 4 males. The average CD4 count was 19 cells/mm^3 in the 6 that were available. Only 4 were documented to be on ART: 3/4 had virologic failure and there was one that had defaulted treatment. Clinically 6/7 cases had generalised and polymorphic skin lesions. The commonest presentation was with plaques which were scaly or crusted followed by macules

which were vasculitic and then by acneiform papules. In one case there was an isolated necrotic plaque on the nose. Histologically the commonest patterns described were foamy histiocytes in 3/7 cases and histiocytes with small intracytoplasmic spores in 2 cases. All the cases had intracellular spores. These histological features are similar to histoplasmosis and hence in the possible cases they were suggested together. This means emergomycosis could have been found in up to 26 (49%) of cases.

Sporotrichosis

Only 2/53 cases were found with a further 3 possible sporotrichosis/cryptococcosis cases. One case was on ART, and was virally suppressed. The second case had a CD4 count of 2 and the patient was ARV naïve. Both cases presented with polymorphic lesions with widespread plaques, papules and macules. The histological pattern in both cases showed both intracellular and extracellular spores and the suggested diagnosis was probable histoplasmosis/emmonsiosis. These patients represented 2 of the 3 cases that were misdiagnosed on histopathology.

Candidiasis

The first case had *Candida parapsilosis* with necrotic macules and pustules that were generalised. The patient was on ART with a CD4 count of 110 and was virally suppressed. Histologically there were intracellular and extracellular spores, hyphae were not identified, and there were numerous of histiocytes. The second case was *Candida albicans* in a patient with generalised crusted papules and plaques. Histology showed neutrophilic microabscesses

with intracellular spores. Both these cases were suggestive of histoplasmosis/emmonsiosis on histopathology but both cultured *Candida* species on tissue.

Histoplasmosis/Emmonsiosis

There were no definitive cases of histoplasmosis but 19 possible cases (with a differential diagnosis of emmonsiosis). The average age was 47 years, and there were 8 females and 11 males. Only 16/19 had CD4 counts done and the average was 40 cells/mm^3. Twelve were on ART. Clinically polymorphic and generalised skin lesions were found in 18/19 patients with crusted, scaly and vasculitic lesions being the commonest presentation. Histologically, histiocytes with small intracytoplasmic spores were found in 9/19 cases followed by the presence of small minute spores in 5/19 cases. There were 3/19 of cases with lymphohistiocytic infiltrates and all the cases had intracellular spores with 7/19 having both intracellular and extracellular spores. Tissue culture was done on 14/19 (74%) and blood culture was done on 11/19 (58%) but all these were negative. Of these cases 9/19 (47%) had both blood and tissue cultures done.

CHAPTER 4: DISCUSSION

In this retrospective review of the spectrum of cutaneous deep fungal infections in HIV infected adults at Chris Hani Baragwanath Academic Hospital Soweto, a 2% prevalence rate of deep fungal infections among all adults was found. This was a high rate. In a study done in Southern Taiwan over a 14 year period, only 23 (0.06%) cases were identified amongst 38,000 skin biopsies [1]. Therefore we have reported a much higher rate of cutaneous deep fungal infections in a single centre. This may have been an underestimate of the prevalence in HIV infected adults as the denominator was all adult skin biopsies. Conversely, it may have been an underestimate of all deep fungal skin infections as patients who were HIV-uninfected were excluded as were those whose HIV serostatus was unknown. It is however important to highlight that our results could be a slight overestimation too as not all common HIV related skin diseases require a skin biopsy in our setting.

The mean age of our study population was 36 years and this was in keeping with the Western Cape study with a mean age of 35 years [14] and the age of the HIV population in general. It is however striking that the mean age was higher in international studies done by Tsai et al and Kim et al, where mean age was 47 years and 49 years respectively [1,5]. The most likely reason is that the underlying immunosuppression was due to malignancies, diabetes mellitus and use of immunosuppressant agents as these are more common in the older population. There was a slight male predominance in our study while in a Korean study [2], it was marked with 71% of the patients being male and in the Western Cape study males accounted for 75% of the cases [14].

Amongst the 46 cases whose CD4 counts were available, the average CD4 was 43 cells/mm^3 with 62% of patients having a CD4 count of less than 50 and 83% with CD4 counts of less than 200 cells/mm^3 . It has been well documented in both international and local literature that these infections occur with advanced immunosuppression. Although more than half of our patients were known to be on ART, only 25% of them were known to be virologically suppressed. Deep fungal infections usually occur in patients pre-ART, as part of IRIS and in patients with virologic failure and in defaulters .Of the patients on ART there was a slight female predominance, while the 2 defaulters were both male. Statistics in South Africa generally show that females use the public health services more than their male counterparts and therefore could be more likely to seek medical attention and to initiate ART. Of the patients on ART, only 36% had viral loads available. This data on ART use could be an indirect measure of the efficacy of the health care system and reflected possible suboptimal initiation of ART and monitoring of its efficacy. This may also mean that the dermatology department needs to institute proper guidelines on how to monitor these patients so that treatment failure can be addressed early. Ten percent (3/30) of all the patients on ART and 3/13 (23%) of those who were virally suppressed, were diagnosed with IRIS with skin lesions appearing or worsening following ART initiation. In the Western Cape, 40% of the cases had IRIS [14]. Since this was a retrospective study we relied on the history recorded on the histology report. Data on ART use and its efficacy was often missing or inadequate. More detailed information would have been recorded in the outpatient file that patients take home.

Clinical presentation of skin lesions was polymorphic in 70% of cases. The commonest presentation was with plaques (75.5%) and these were documented as crusted in 30% of

patients. These plaques were often in combination with papules and less commonly with patches, ulcers , nodules and pustules. The clinical diversity of skin lesions of deep fungal infections is well described especially in HIV infected patients in whom skin lesions are often atypical. This may lead to misdiagnosis by both inexperienced clinicians and dermatologists as was documented in the Western Cape study where dermatologists had misdiagnosed 28% of cases as secondary syphilis, varicella, drug reactions, Kaposi sarcoma, seborrheic dermatitis, guttate psoriasis and pyoderma gangrenosum (1 patient each)[14]. This raises further concerns as some of these are skin diseases that are diagnosed clinically and often do not require histological confirmation. Therefore in HIV positive patients with low CD4 counts (<100 cells/mm^3) and with widespread skin lesions, there should be a high index of suspicion for these deep fungal infections and a low barrier for skin biopsy, either at the time first seen or when there is a poor response to empirical treatment.

The commonest histological findings were suppurative granulomas seen in 15/53 cases and this was in keeping with Gonzalez Santiago[4]. These were largely seen in the undifferentiated group which is possibly the reason the pathologist did not give a diagnosis as this is not diagnostic of any specific organism. Histiocytes with small intracytoplasmic spores were the second commonest and this pattern was commonly seen with either suspected histoplasmosis or emergomycosis. For cryptococcosis, encapsulated fungal spores (8/53) and the gelatinous pattern (7/53) were both diagnostic features.

Although histopathology is an important tool for the diagnosis of deep fungal infections, culture is still the gold standard. In our cohort only 30/53 (57%) patients had tissue cultures done which is very low for an academic institution. Only 5/30 (17%) had positive results, therefore 83% of cases had negative results. Blood cultures appeared to have a slightly higher yield than tissue cultures as 29% were positive compared to 17% on tissue. Of the 6 patients with a definitive diagnosis based on a positive tissue or blood culture and who had both cultures, none had congruent results. In other studies the yield has been significantly higher as reported in the Western Cape study in which 80% were positive and as found at the Mayo Clinic, Rochester, Minnesota, USA in which 76% were positive [4,14]. This low yield in our cohort could be one the reasons for the high percentage of patients not having skin tissue cultures done. Some of the dermatologists may have considered it futile especially as treatment is generally similar for emmonsia and histoplasmosis. Possible reasons for this low yield may have included technical and laboratory factors such as inadequate sample, incorrect fluid in the specimen bottle, delayed transport, non-viable fungal organisms in the specimen, delayed sub-culturing and specimens not kept and reviewed for long enough. The histopathology suspected diagnoses were accurate in 79% of the definitive cases. This is in keeping with Gonzalez Santiago et al [4] where 70% of their cases were correctly classified when compared to culture results [4].

There were 38% with a definitive diagnosis The 21% with an undifferentiated diagnosis is similar to the experience in the Western Cape and the USA [4,14]. Possible reasons for the low percentage of proven cases include the retrospective nature of the study , the fact that not all appropriate tests were done, especially cultures, and that some tests were not repeated if

previous results were negative. A similar study conducted in the Western Cape was a prospective cross-sectional observational study in patients with advanced HIV infection in which all patients had the designated tests. The aetiological spectrum in our study found that in definitive cases, cryptococcosis was the commonest, followed by emergomycosis (formerly known as emmonsiosis), and then sporotrichosis and candidiasis. In patients with a possible diagnosis, based on histopathological patterns, histoplasmosis / emergomycosis was commonest followed by sporotrichosis / cryptococcosis In the Western Cape, the findings were slightly different. In 25 cases with confirmed deep fungal infections, emergomycosis was the commonest with 14 cases, while there were 3 cases each of sporotrichosis and histoplasmosis and 5 were undifferentiated [14]. Definitive diagnosis was seen in 80% of their cases and this could be largely attributed to the study being prospective, all the tests being done and with patients followed up appropriately.

The geographical variation of deep fungal organisms have been well described including the organisms that have been associated with HIV. Our report displays that cryptococcosis and emergomycosis were important infections in patients with advanced HIV infection. It is noteworthy that in the Western study there were no cryptococcosis cases despite its strong association with HIV. The second commonest infection in our study was emergomycosis accounting for 13% of our cases while it accounted for 56% in the Western Cape [14]. Emergomycosis in the HIV infected was first reported n from Western Cape in 2013. It now seems to be the commonest cause for deep fungal skin infections in South Africa. Candidiasis is not an uncommon skin finding. In the Korean study conducted by Kim [5], candida was the commonest infection accounting for 39% of all the cases.

Sporotrichosis was the commonest fungal skin infection in the pre-HIV era in Johannesburg as reflected by Klevanksy and Lurie [13,36]. It was most commonly seen in miners. It is still an important differential especially since the clinical presentation is not the classic sporotrichoid pattern. There were no proven histoplasmosis cases in our cohort but there were 19 possible cases. This highlights the need for further investigations to be performed such as urine histoplasmosis antigen as well as molecular studies to diagnose these infections.

CHAPTER 5: LIMITATIONS

This was a retrospective study and as such there was data that was missing or not documented correctly. In our study we relied on the information from the histopathology report which meant that the history and clinical findings were only a summarized version of the information obtained from the patient provided to help guide the pathologist. Some patients may have been missed as we relied on the Snomed coding system to identify them.

If CD4 counts and viral loads were not recorded, we used the Trakcare system of the NHLS to try to find results. These may have been missed sometimes if names were spelt incorrectly or hospital numbers were incorrect. The clinical picture was a subjective assessment by other doctors which may not have been affected by the experience of the dermatologist. There was no standardised reporting format.

The histopathologic findings were also not consistent in the manner in which they were reported and in some cases stains were not done or the site of organisms was not described. We did not review the histological specimens but accepted the original report. The low number of cultures done contributed to the relatively low rate of definitive diagnoses. Other tests such as molecular studies and more specimens sent for culture could have improved the number of cases with definitive diagnoses.

There was no available follow up data on patients to assess whether they received treatment and their response to treatment.

CHAPTER 6: CONCLUSION

The prevalence of cutaneous deep fungal infections was 2% in HIV positive adults using a denominator of all adult skin biopsies done in our centre and this is a very high rate.

Advanced immunosuppression, specifically CD4 counts less than 50 cells/mm^3 despite more than 50% of cases being on ART, was a common finding. It is also important to note that some of these infections occurred as part of an IRIS phenomenon as well as in patients who were virally suppressed.

Histopathology still plays an important role in the prompt diagnosis of these infections and although culture is considered the gold standard , the use of molecular studies are proving to be important in accurate identification of the organisms and need to be done where the aetiology cannot be determined on histopathology.

The striking emergence of emergomycosis as an important cause of deep fungal infections in South Africa highlights how HIV continues to change the face of medicine despite increased access to ART. It is important for clinicians to have a high index of suspicion for deep fungal skin infections when dealing with widespread skin lesions in patients with advanced HIV infection including those who have recently started ART given the occurrence of the IRIS phenomenon. Prospective studies need to be done in South Africa in patients with HIV infection with the added utilisation of molecular studies to define the true aetiological spectrum of these infections.

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