PREVALENCE OF CRYPTOCOCCUS AMONG HIV-INFECTED PATIENTS
ATTENDING THE INTERMEDIATE HOSPITAL OSHAKATI, NAMIBIA

A THESIS SUBMITTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS
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BY
Tuyakula Nakale
200826417

Main Supervisor: Dr J. Sheehama
Co-supervisor: Dr L. Nelumbu
ABSTRACT

Cryptococcus is the most incriminated fungal pathogen that causes cryptococcal meningitis in HIV-infected patients and is known to constitute a major cause of mortality in AIDS patients. Previous studies, mostly from Africa have indicated that positive serum cryptococcus may precede the development of cryptococcal meningitis and cause early mortality among patients with advanced HIV infection. There is no published data on the burden of cryptococcal infections among HIV patients in Namibia; thus the magnitude of cryptococcal diseases associated with HIV is unknown. This study was done to determine the prevalence of cryptococcus among HIV-infected patients attending at Intermediate Hospital Oshakati and the level at which a patient’s CD4 count is significantly associated with cryptococcal antigenemia.

A descriptive cross-sectional study was conducted at the Intermediate Hospital Oshakati. The study included 384 HIV-infected patients (231 females and 153 males) whose blood samples were examined for cryptococcus by using IMMY CrAg test kit at the NIP laboratory. Baseline clinical data and demographic information were retrieved from the patient medical records and laboratory information system.

Among the 384 HIV-infected patients enrolled, 36 (9.38%) were positive for serum cryptococcal antigen. Among these 36 patients the CD4 count ranged from 2-301 cells/ul and median CD4 count was 72cells/ul. Of the 36 positive cryptococcus cases,
26 (72.22%) had CD4 counts below 100 cells/ul. When stratified by CD4 count, 72.22% of patients with ≤100 cells/ul had a positive cryptococcal antigen test as compared to 25.00% with CD4 counts between 101-200 cells/ul and 2.78% with CD4 counts >200 cells/ul.

This study demonstrates a high prevalence of cryptococcus among HIV-infected patients receiving their CD4 count measurements at the Communicable Disease Clinic, Intermediate Hospital Oshakati. Based on the study results, the cryptococcal antigen test and CD4 count levels Lower CD4 (≤100 cells/ul) were significantly associated with positivity for serum cryptococcal antigen.

The prevalence of cryptococcus among HIV-infected patients was high and as such it calls for drastic public health interventions spearheaded by the Ministry of Health and Social Services (MoHSS). It is recommended that the MoHSS should take the leading role in implementing a routine screening of cryptococcus neoformans antigen among HIV patients with CD4 count ≤100 cells/ul. This will improve the accurate early diagnosis and provide the surest way to reverse the deteriorating health status of the Namibian people.
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DEDICATION

This project is dedicated to my parents; Levi and Teopolina Nakale who gave me a firm foundation, supported me tirelessly and continuously inspire me to always follow my dreams.
DECLARATION

I, Tuyakula Nakale, hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree at any other institution of higher education.

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Tuyakula Nakale                        Date
ABBREVIATIONS AND ACRONYMS

AIDS Acquired Immune Deficiency Syndrome

ART Anti-Retroviral Therapy

ARVs Anti Retro Virals

BHIVA British Human Immune Virus Association

CRAG Cryptococcal Antigen

CDC Centre for Disease Control and prevention

CD4 Cluster Designate 4

CM Cryptococcal meningitis

CSF Cerebrospinal Fluid

DCS Data Collection Sheet

EDTA Ethylenediaminetetraacetic acid

FDA Food and Drug Association

FBC Full Blood Count

GDP Gross Domestic Product

HIV Human Immunodeficiency Virus

HAART Highly Active Antiretroviral Therapy

H20 Hydrogen oxide (Water)

IDSA Infectious Disease Society of America

IRIS Immune Reconstitution Inflammatory Syndrome

IHO Intermediate Hospital Oshakati
<table>
<thead>
<tr>
<th>Acronym</th>
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<tr>
<td>LIS</td>
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<td>MCS</td>
<td>Microscopy Culture and Sensitivity</td>
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<tr>
<td>MoHSS</td>
<td>Ministry of Health and Social Services</td>
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<tr>
<td>NIP</td>
<td>Namibia Institute of Pathology</td>
</tr>
<tr>
<td>SADC</td>
<td>Southern African Development Community</td>
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<tr>
<td>UNAIDS</td>
<td>The Joint United Nations Programme on HIV and AIDS</td>
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<tr>
<td>USFDA</td>
<td>United States Food and Drugs Association</td>
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<tr>
<td>USA</td>
<td>United States of America</td>
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<tr>
<td>USSR</td>
<td>Union of Soviet Socialist Republics</td>
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<td>WHO</td>
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CHAPTER 1

ORIENTATION AND BACKGROUND OF THE STUDY

1.1 INTRODUCTION

HIV has continued to pose significant social, economic and development challenges in the whole world. Sub-Saharan Africa remains the global epicentre of the HIV pandemic, and shows no evidence that the problem is on a decline. More than two-thirds (68%) of all infected people in the world, live in this region (UNAIDS, 2007). HIV prevalence in Namibia is among the highest in the world. The Ministry of Health and Social Services (MoHSS) conducts a national sentinel survey every two years to monitor the progression of the epidemic in the country. The 2012 National HIV survey indicated that the HIV prevalence rate has risen from 17.8% in 2008 to 18.2% in 2012, with Oshakati Intermediate Hospital recording 22.3% (MoHSS, 2012).

Serum cryptococcus (cryptococcal antigenemia) may precede the development of cryptococcal meningitis and earth mortality among patients with advanced HIV infection (CDC, 2014). Cryptococcal meningitis (CM) is a systemic fungal infection, which is caused by Cryptococcus species, known as \textit{Cryptococcal neoformans} and \textit{Cryptococcal gatti} (Kwon-Chung & Varma, 2006; Lin & Heitman, 2006). It is a common opportunistic infection among immunosuppressed patients with advanced human immunodeficiency virus (HIV) infection (Paul & Janet, 2010). \textit{Cryptococcus neoformans} is known to be the most incriminated fungal pathogen causing
meningitis in people living with HIV (Bicanic, Meintjis & Rebe et al., 2009). CM is not a contagious disease, so it cannot spread from one person to another. It specifically occurs when the *Cryptococcus* has spread from lungs to the brain (CDC, 2014).

Although cryptococcal meningitis has dramatically decreased in more affluent countries, it remains a public health problem in several Asian regions of the former USSR (Union of Soviet Socialist Republics), in parts of South and Southeast Asia, in Africa and in South America (CDC, 2014). The incidence of cryptococcal meningitis is increasing with the global emergence of AIDS and this now represents a major life-threatening fungal infection in HIV infected individuals (Warkentien & Crum-Cianflone, 2010). CM is now among the leading causes of death in HIV patients in Sub-Saharan Africa (Bekondi, Bernede & Passone, 2006) and contributes substantially to high mortality in other low-resource settings where healthcare and ART programs are limited (Warkentien & Crum-Cianflone, 2010).

According to the World Health Organisation (WHO, 2010), the burden of cryptococcal meningitis in developing countries is difficult to estimate. However, recent estimates suggest that there are about one million new cases of cryptococcal meningitis and at least 500,000 deaths annually worldwide due to HIV-associated cryptococcosis (Park, Wannemuehler and Marston et al., 2009). The majority of cases occur among patients in developing countries, particularly in Sub-Saharan Africa. In southern Africa CM accounts for between 33% and 63% of all adult meningitis (Bekondi, Bernede & Passone, 2006), and acute mortality ranges from 24% to 50% (Lessells, 2011). Recent studies have shown that the majority of CM
cases occur in patients who are already in care (Javis, Meintjies and Williams et al., 2010) and most of them occur at the time of initiation of ART (Jarvis, Meintjes & Harrison, 2010). There is no published data on cryptococcal meningitis in HIV-infected patients in Namibia.

HIV-associated cryptococcal meningitis is prevalent in developing countries due to many interrelated factors; these include inadequacy of current antifungal drugs and combinations, complications of raised intracranial pressure (Bicanic and Harrison, 2005). Furthermore, patients in low-resource settings tend to present late, and flucytosine treatment is often not available and there may not be facilities for inpatient intravenous therapy with amphotericin B. The results of this combination of factors are that in cohorts of HIV-infected patients from Sub-Saharan Africa, cryptococcal meningitis has accounted for 13-44% of all deaths (Osazuwa, Dirisu, Okuonghae and Ugbebor, 2012). The incidence of CM remains very high in impoverished areas and the emergence of multidrug resistance have made the situation worse. To compact and reduce the morbidity and mortality associated with Cryptococcal meningitis among HIV-infected patients, many preventative measures and strategies have been employed, the most important being early screening followed by vaccination.

In 2000, the Infectious Disease Society of America (IDSA) first published the “Practice Guidelines for the Management of Cryptococcal Disease” (IDSA, 2006). In the update version of guidelines, a group of medical mycology experts have explained the importance of drug therapy against cryptococcal meningitis. The goal
is to merge recent and established evidence-based clinical data together with the shared clinical opinions and insights to assist physicians in the management of infection with the *Cryptococcus* pathogen (Perfect et. al, 2010). Amphotericin B and flucytosine are antifungal medications that have proven to be more effective against cryptococcal meningitis and improve the survival rate in patients living with HIV. Although these medications are standard-of-care in developed countries, they are widely unavailable in Sub- Saharan Africa and some parts of Asia (CDC, 2014).

Even though there is limited data on the prevalence of CM among HIV-infected patients in Southern Africa, it is likely that the burden immense in view of lack of regular cryptococcal screening in hospitals and late presentation of the patients to the health facilities especially in the rural communities which comprise more than 50% of the population. The other factor that tends to augment the burden of CM is the HIV/AIDS pandemic in Southern Africa, which is the hardest hit region in the world. It is estimated that 22.4 million people are living with HIV in Southern Africa (UNAIDS, 2009). In the SADC (Southern African Development Community) region, Swaziland has a national prevalence estimated at 42%, Botswana 25%, Namibia 17.8% and Zimbabwe 15.8% (UNAIDS 2009).

Namibia has an area of 825214 square kilometres and is in Southern Africa. Its borders are Zimbabwe, Botswana, Zambia, Angola, South Africa, and the South Atlantic Ocean. The country has a good road network and communication infrastructure. The 2001 census indicated that 67.6% of the population lives in communal areas and 32.4% in commercial urban areas. The Namibian economy is
heavily dependent on the extraction and processing of minerals for export. Mining accounts for 20% of the gross domestic product (GDP) and agriculture accounts for 11.5% of GDP, industry 29.8%, and services 58.7% (countryfacts.com, 2003).

Intermediate Hospital Oshakati is a referral and teaching hospital in Oshana Region, situated in the northern part of Namibia. The hospital has a bed capacity of 700 and a Communicable Disease Clinic. This clinic provides anti-retroviral (ARV) treatment, routine counselling and management of HIV related investigations. Testing for HIV and CD4 count tests are offered by the Namibia Institute of Pathology (NIP) laboratory in the hospital.

In previous years, it has been noted by the Namibia Institute of Pathology (NIP), Laboratory Information System (LIS) that the requests for the cryptococcal test at Oshakati increased from 1000 in 2011-2012 to 3000 in 2013-2014. Most of the specimens tested positive for cryptococcal infections. It remains unclear whether northern Namibia is an endemic for cryptococcus especially among immunocompromised patients. According to NIP laboratory information system (2009), from 1 July 2008 to 15 June 2009 the Oshakati state hospital laboratory culture confirmed 68 cases of cryptococcal meningitis. To summarise, the prevalence of cryptococcus among HIV patients and population baseline CrAg titres in Namibia, specifically in the northern part, is unknown. This makes the regular cryptococcal screening virtually impossible.
1.2 STATEMENT OF THE PROBLEM

Cryptococcal meningitis (CM) is one of the leading causes of death in HIV-infected patients in Africa (CDC, 2014). Prevention strategies are therefore of great public health importance and opportunities exists for preventive interventions. The main problem in Namibia is that the prevalence of cryptococcus among HIV patients is unknown and justification of cryptococcal antigen screening among these patients has no supporting evidence. The second problem is the use of other preventative strategies, as fluconazole prophylaxis is very costly and clinical outcomes still fall short of cryptococcal antigen screening followed by treatment of those found positive for CM. The third problem is that, although initiation of ART has been associated with a marked decline of incidences of cryptococcal disease, unfortunately despite recent progress in expanding access to ART in Namibia, a substantial proportion of patients still present late with advanced immunodeficiency and high risk of new AIDS events and mortality (Lawn et al., 2008). In 2013, Oshana region recorded 248 deaths among the HIV infected inpatients (MoHSS, 2013). Thus, preventative interventions implemented immediately before or concomitantly with ART, could be an effective initial strategy in the treatment of patients with advanced HIV, allowing patients the best chance at long-term disease free survival. The preventative strategies need to be substantiated by the magnitude of CM. It is against this background that this study seeks to establish the prevalence of cryptococcus among HIV positive patients attending the Intermediate Hospital Oshakati, in order to use the findings as basis for lobbying policy makers to initiate cryptococcal antigen testing prior to initiation of ART.
1.3 PURPOSE AND OBJECTIVES OF THE STUDY

1.3.1 Purpose of the study

The purpose of the study is to establish the prevalence of the cryptococcus among HIV-infected patients attending Intermediate Hospital Oshakati.

1.3.2 Objectives of the study

- To determine the magnitude of cryptococcus among HIV-infected individuals
- To determine the level at which CD4 count is associated with positive cryptococcal antigen.

1.4 HYPOTHESIS

For this study the following hypotheses were formulated

- Alternative hypothesis: There is a high number of cryptococcus cases among HIV-infected patients
- Null hypothesis: There is no high number of cryptococcus cases among HIV-infected patients
1.5 SIGNIFICANCE OF THE STUDY

The study will determine the prevalence of cryptococcus in HIV-infected patients. This will therefore contribute to the national and international knowledge bank. The prevalence of cryptococcus will also help in resource mobilization, prioritization and channelling to patients for the prevention of early death due to cryptococcal meningitis. The study might provide guidance to policy makers in coming up with proper management guidelines for HIV infected patients co-infected with cryptococcal infections. Information on the correlation between CD4 counts and cryptococcal antigen test will be established from this study. This information will be used to review the current laboratory diagnostic procedures and make timely interventions. Policy on vaccination against cryptococcal meningitis in HIV-infected patients may be promulgated from these findings.

1.6 LIMITATIONS OF THE STUDY

Limitations of the study are normally in the design, population, sample or data collection instrument. In this study, there were three main limitations. This is a cross-sectional study and therefore it did not allow the determination of risk factors for cryptococcal meningitis. Since this study was only based in one hospital, it may not be representative of the whole population of HIV infected patients in Namibia. There may be cross-reaction of CrAg test with other diseases or interferences that might have caused false positive results.
1.7 DEFINITIONS OF OPERATIONAL CONCEPTS

Prevalence
The number of existing cases of a disease or health condition in a population at some designated time. In this study, it refers to the number of all positive cryptococcal cases during the study period.

Meningitis
The inflammation of the membranes covering the brain and spinal cord called meninges. In this study, cryptococcal meningitis refers to the inflammation of the meninges by the Cryptococcus pathogens.

Titre
The concentration of a solution as determined by titration and in this study; it refers to the Cryptococcus antibody level in patient serum.

CrAg (cryptococcal antigen)
A foreign protein or particle capable of eliciting an immune response. In this study, it refers to the indicator of the cryptococcal infection.

Cryptococcus
A type of fungus that is found in the soil and is associated with bird droppings. In this study, Cryptococcus refers to the fungal pathogen that is causing cryptococcal meningitis.
1.9 SUMMARY

This chapter covers the global picture of cryptococcal infections among HIV-infected patients and the burden associated with it. It also addresses the continental as well as regional distribution and burden of the disease. An analysis is presented of Namibia’s infrastructure and shortfalls that may predispose the country to cryptococcosis. The research objectives and hypothesis were also discussed in this chapter. The significance of the study and three limitations of the study were also presented in this chapter.
CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Cryptococcal meningitis is an extremely infectious condition and is one of the leading causes of morbidity and mortality among HIV-negative and HIV-positive people living in much of the developing world (Walkers, Prendergast, Mugyenyi, Munderi, Hakim, Kekitiinwa et al., 2012; Lawn, Harries & Wood, 2010). Cryptococcus was first isolated from peach juice samples in 1984 (Tang-Bao, David & Chaoyang, 2012). Cryptococcus neoformans causes the most common fungal infection of blood and central nervous system in immunosuppressed populations with high morbidity and mortality (Cheersbrough, 2005).

After the introduction of antiretroviral therapy, there has been a decline in the incidence of cryptococcal meningitis across the developed world (Mirza, Phelan & RimLand, 2005). The disease however remains a major public health in developing countries, particularly in Africa and Southeast Asia (Jarvis and Harrison, 2007). For example, it is the most common cause of meningitis in central and Southern Africa (Bekondi, Bernede & Passone, 2006), accounting for 40% of cases in a recent study from Malawi (Scarborough, Gordon, Whitty et al., 2006). It is therefore difficult to obtain reliable data to estimate the burden of disease in developing areas because the access to healthcare is limited (CDC, 2014).
Cryptococcus specie that causes Cryptococcal meningitis remains an important pathogen, particularly among HIV patients (Bovers, Hagen and Boekhout, 2008). As stated above, cryptococcosis remains an important public health problem in developing countries with an estimated annual incidence of 720 000 cases (CDC, 2014). In 2009, it was estimated that the global annual burden of cryptococcal meningitis is 957 900 cases resulting in estimated 624 700 deaths within three months of cryptococcal infection (Park et al., 2009). Sub-Saharan has the highest burden with a median incidence of 3.2% among HIV-infected patients resulting in 720 000 cases with high mortality accounting between 20-50% (Kisenge, Hawkins Maro et al., 2007; Kambugu, Meya, Rhein et al., 2008). Even with the introduction and availability of ART, cryptococcal meningitis mortality remains high (Bicanic, Meintjes, Rebe et al., 2009). Therefore WHO recommends regular screening of cryptococcal meningitis among HIV-infected people especially in high-risk areas as a potential short-to-intermediate term control strategy.

Despite the current limitations of available epidemiologic data and published articles, a number of recent trends in cryptococcal disease have emerged in African, Asian and American regions. In Sub-Saharan Africa, which is an HIV epidemic region, the burden of cryptococcal disease is the least well characterised. Hospital based studies indicate that serotypes of cryptococcus particularly C. neoformans greatly outnumber C. gatti as a cause of cryptococcal meningitis among HIV infected people (Warkentien and Crum-Cianflone, 2010). Nonetheless, cases of cryptococcal meningitis cases are frequently reported from some areas of Sub-Saharan Africa with a large number of patients presenting late to the health facilities. It was also the
leading cause of meningitis among African patients living with HIV (Cohen, Zijlstra, Mukaka, et al., 2010). Important questions regarding epidemiology of cryptococcal disease in the region is however not explored (Warkentien and Crum-Cianflone, 2010).

In Asia, the disease burden estimates have normally relied on clinically diagnosed cases of cryptococcal meningitis compiled by governments and hospitals, usually with uncertain denominators (Banerjee, 2005). The three successive studies conducted in three Asian countries over the period of 12 years (1992-2004) had revealed that parallel to increase in the number of HIV cases, HIV cryptococcosis co-infection increased from 20% in 1992-96 to 30% in 1996-200 and 49% in 2000-04 (Banerjee, 2005). Furthermore, a large population-based prospective study that used standardized surveillance methods estimated cryptococcal meningitis incidence in China, India, Indonesia, Pakistan and Vietnam. This was done to consider the treatment policy (Roy and Chiller, 2011).

So what tests are available since the disease has dire consequences? The Crag Lateral Flow Assay is an immunochromatographic tests system for detection of the capsular polysaccharide antigens of Cryptococcus species (IMMY, 2014). The new dipstick test for detecting cryptococcal antigen is easy and simple to use on a small sample of serum. The test accurately detects both early and advanced cryptococcal disease more than 95% of the time. Furthermore, the test is inexpensive, and the results ready in just 10 minutes (CDC, 2014)
2.2 GLOBAL BURDEN OF THE DISEASE

Cryptococcal meningitis, a fungal infection caused by *Cryptococcus* species is one of the most important opportunistic infections in HIV-infected individuals. In countries with high prevalence of HIV, *Cryptococcus* is one of the most common causes of meningitis overall, more frequent than *Streptococcus pneumonia* or *Nisseria meningitidis* (Bekondi, Bernede and Passone et al., 2006; Helbok, Pongpakdees and Yenjun et al., 2006). Cryptococcal Meningitis in HIV positive individuals is a global health problem but its real impact is difficult to estimate because there is a limited number of available studies in the literature. Provider-based cohort studies may not be representative of the whole region and population-based may be limited due to loss to follow-up or case-ascertainment (McCathy, Morgan and Wannemuehler et al., 2006). Since many developing countries do not have the resources and standard laboratories to test for cryptococcal, this results in many cases not diagnosed (CDC, 2014).

Based on the review of the available epidemiological data, a substantial global burden was estimated, both in terms of numbers of infections and associated deaths. The data indicated that there are at least 957,900 (range, 371,000 - 1.54 million) cases of cryptococcal meningitis every year with 62,472 (range, 124,956 – 1.1 million) deaths. The region with the highest number of estimated cases was the Sub-Saharan Africa (720,000) followed by the South-east Asia (120,000). Oceania, Western and Central Europe, North Africa and Middle East and North America were the regions with the fewest (Park, Wannemuehler and Marson et al., 2009).
In the USA, the incidence of cryptococcosis was less than one case per million persons per year and that was before the AIDS epidemic. In the 1980s, cryptococcosis emerged as an important infection among people with HIV, occurring in 5-10% of HIV patients in the USA, Europe and Australia. With increasing use of fluconazole for treating candidiasis and advent of highly active ART in the 1990s, the annual incidence of cryptococcal meningitis decreased markedly in developed countries; in Atlanta, USA it declined from 66 cases per 1000 patients, in 1993 to 7 cases per 1000 in 2000 (Mirza, Phelan and Rimland, 2005).

In South Asia and Africa, cryptococcal meningitis appears to be relatively more common as an HIV related infection than ever was in Europe or North America. In Thailand cryptococcal meninges accounted for 19% of HIV-defining illness between 1994 to 1998 (Chariyalertsak, Sirisanthana, Saengwonloey and Nelson, 2001). In Uganda, the incidence of cryptococcal disease in patients with CD4 counts less than 200 cells/uL was estimated at 10.3 cases per 100 person years of follow-up (French, Gray and Watrea et al, 2002). It seems most likely that the high incidence of cryptococcal meningitis in parts of Africa and Asia reflects differences in exposure rather than susceptibility or strain virulence. Because of the increase in HIV-associated cryptococcal meningitis, there is a great shift in the epidemiology of meningitis, and cryptococcal meningitis is now the leading cause of community-acquired meningitis, accounting for 20-45% of laboratory cases of meningitis in South Africa (Hakim, Gangaidzo and Heyderman et al. 2005).
Patel et al. (2013) cohort study indicated a relatively high prevalence of cryptococcal meningitis among HIV-infected patients. There was a 5% prevalence of cryptococcal antigenemia in newly diagnosed patients with CD4 counts of less than 100 cells/uL. In the same study, most of CRAG positive patients were diagnosed with HIV at the time of presentation with cryptococcal meningitis. Late HIV diagnoses are not exclusive to resource-limited countries: In 2010, 28% of new UK HIV diagnoses had CD4 counts of less than 200 cells/uL (HPA, 2011) and in North America in 2008, 33% of newly HIV-diagnosed patients developed AIDS together with cryptococcal meningitis (Chen, Rhodes and Hall et al., 2012).

Ganiem et al. (2013) study revealed that patients with advanced HIV infection but without clinical signs of cryptococcal meningitis had a positive serum cryptococcal antigen test. Those who tested positive had a much higher chance of developing cryptococcal meningitis as compared to those who had a negative result. One in every 10 patients with a positive cryptococcal antigen test was diagnosed with Cryptococcal meningitis during follow-up. In fact, the number could have been much higher as early loss to follow-up was very high and significantly associated with cryptococcal antigenemia.

The worldwide number of morbidity and mortality due to cryptococcal meningitis among persons living with HIV appear similar to those infections that have received greater public health attention (WHO, 2008). Understanding the burden of the disease is important for public health specialists to adequately plan and make priorities for needed resources in order to prevent and control the public health problem.
2.3 AFRICAN BURDEN OF THE DISEASE

Cryptococcal meningitis is one of the common opportunistic infections and major contributor of early mortality, accounting for between 13% and 44% of deaths in HIV-infected patients living in resource-limited countries particularly Sub-Saharan Africa (Lessells et al, 2008). Increasing access to antiretroviral treatment has transformed the prognosis of HIV-infected patients in resource-limited settings. However, the treatment coverage in the developing countries remains relatively low and there is a late diagnosis of HIV. As a result, many people continue to die due to HIV-related cryptococcal meningitis. This disease remains to be the leading cause of death among HIV positive people (Hakim, Gangaidzo and Heyderman et al., 2014).

The overwhelming burden of cryptococcal meningitis is in Sub-Saharan Africa, where there are an estimated 720,000 cases every year with the annual mortality exceeding that of tuberculosis. In most Sub-Saharan African countries with high prevalence, cryptococcal meningitis is the leading cause of meninges accounting for 63% of cases in South Africa, 45% of cases in Zimbabwe, 26% of cases in Malawi (Jarvis, Meintjes and Williams, 2010). In other studies, the prevalence rates of cryptococcal meningitis with 21% in Cambodia, 12.9% in Bangkok, 13% in South Africa and 5.8% in Uganda among patients with a CD4 count of less than 100 cells/uL (Micol, Lortholary and Sar et al., 2007; Jarvis, Lawn and Vogt et al., 2009; Liechty, Solberg and Were et al., 2007; Pongsai, Atamasirikul and Sungkanuparp, 2010). The representative prospective Sub-Saharan African cohort indicated that initial CrAg screening prior to starting ART in patients with CD4 counts of less than
100 cells/µL can prevent disease and death in 8% of patients started on ART (Meya, Manabe & Castelnuovo, 2011)

Jacinta et al., 2012 reported a high prevalence (19%) of cryptococcal antigenemia in an urban setting of Uganda among HIV-infected persons with severe immunosuppression while in the rural setting, a prevalence of only 5.3% was reported. The high prevalence from the study encourages the need to do routine screening and early diagnosis of cryptococcal infection in HIV patients with severe immunosuppression prior to initiation of antiretroviral treatment. The same study stated that cryptococcal meningitis in HIV-infected patients is due to the delay in presentation with the diagnosis only possible when the disease is advanced and treatment is likely to be less effective.

Another study done on high prevalence of cryptococcal antigenemia among HIV-infected patients receiving antiretroviral therapy in Ethiopia, (Abere et al., 2013) revealed a high prevalence of cryptococcal meningitis among HIV-infected patients attending ART clinics in Addis Ababa. The inclusion criteria was CD4 counts of less than 200 cells/µl. It demonstrated that the overall prevalence was 8.4% and in patients with CD4 counts less than 100 cells/µl, the prevalence exceeded 11%. The study adds to the mounting evidence for the public health importance of routine screening for cryptococcal meningitis in HIV-infected individuals. The current WHO guidelines also recommend screening in high prevalence resource low settings among those with low counts of CD4 (WHO, 2011).
In a study done in Cameroon, Luma et al (2013) stated that cryptococcal meningitis was found in 11.2% of HIV patients admitted in the medical ward, Douala General Hospital. In the same study, the confirmatory diagnosis of cryptococcal meningitis was made when *Cryptococcus* was identified in CSF by Indian ink stain and the diagnosis by antigen test and culture was not a common practice during study period. This might have led to an underestimation of the prevalence of cryptococcal meningitis in their study population and therefore explaining the difference in prevalence in settings with similar burden as to Cameroon.

The prevalence of cryptococcal meningitis among HIV adult patients admitted in a Tanzanian hospital may be decreasing with earlier HIV diagnosis and increasing ART use (Wajanga et al, 2011). A lower prevalence of cryptococcal meningitis (4.5%) than prior studies was reported. Despite the low prevalence of cryptococcal meningitis among HIV adults inpatients in the same study and the high rates of ART use, cryptococcal meningitis still accounted for 26% of all in-hospital HIV deaths (Wajanga et al., 2011).

Several studies evaluating the prevalence of serum cryptococcal antigenemia in AIDS patients have reported a consistently higher prevalence of serum CrAg in patients with lower CD4 cell counts (Kisenge, Hawkins and Maro et al., 2007). In a study done in Nigeria, Osazuwa et al (2012) stated that the distribution of cryptococcal antigenemia was highly varying with CD4 cell levels. Patients with CD4 counts less 50 cells/uL had the highest prevalence of serum CrAg, followed by patients with a CD4 counts less than 100 cells/uL.
In their study Su et al., (2011) reported that most of the laboratory findings (CSF glucose, CrAg titre, CSF glucose, CSF cell count and Cryptococcus culture in CSF) done on HIV positive and HIV negative patients had no statistical significance. However, there was a higher positive rate of cryptococcal meningitis in the blood of patients with HIV than those without HIV (45.5% vs. 11.1%, respectively), showing a high burden of cryptococcal meningitis in patients infected by HIV.

In many developing countries in Sub-Saharan Africa, the capacity to perform the complicated management of severe cryptococcal meningitis is limited (Hamill, 2006). An important action in reducing the impact of the cryptococcal meningitis is the marked expansion of the ART access for HIV, as a major risk of the disease is substantially reduced among people receiving the treatment. Despite access to treatment, the number of people with HIV and cryptococcal diseases remains high and therefore specific public health efforts are needed.

Joseph et al., (2009) documented that in most African countries the burden of cryptococcal meningitis in HIV patients is still unknown mainly because credible measurements of the disease prevalence, which inherently require confirmed diagnosis based on blood or cerebrospinal fluid is almost non-existent in endemic countries where laboratory capacity is often limited. Prevalence of cryptococcal meningitis and population baseline CrAg titres in Namibia and specifically Oshakati Intermediate Hospital is also unknown. This makes the interpretation of the CrAg test virtually impossible. For CrAg test to be of clinical relevance it is very important to establish titres as a baseline for interpretation of the results.
2.4 PATHOGENESIS AND HOST DEFENCE

Infection of cryptococcal meningitis is acquired by the inhalation of the small yeast cells or possibly basidiopores. The primary infection is frequently asymptomatic and may be eradicated or contained within granulomata. However depending on host factors, inoculum and isolate virulence, the organism may disseminate either acutely or after a period of latency to extrapulmonary sites, with a particular predilection for the brain.

Direct evidence for the latent infection was provided by autopsy studies demonstrating cryptococcal cells within pulmonary granulomata in individuals dying of unrelated causes (19). The viable cryptococcal cells remain for at least 18 months in interstitial granuloma within macrophages and epithelial cells. Work done by Dromer et al (2005) who typed isolates from the patients diagnosed with cryptococcosis in France, some of whom were from Africa but lived in France for long, suggested that reactivation of such latent infection may be important in HIV-associated cryptococcosis. There was a significant clustering suggesting that patients had acquired their isolates before the development of clinical disease.

Much has been learnt about the immune response of cryptococcal infection from the study of animal models and from the in vitro experimentation. In common with a number of other chronic fungal and bacterial infections, protection is associated with an active granulomatous inflammatory response, and depends on cell mediated immunity involving both CD4 and CD8 cells.
2.5 DIAGNOSIS OF CRYPTOCOCCAL MENINGITIS

The World Health Organisation recently released guidelines for diagnosis and treatment of cryptococcal in HIV-infected people (WHO, 2011). These guidelines are based on the principle that early diagnosis and treatment are central to reducing mortality from cryptococcal meningitis among HIV patients. In countries with ready access to and no contraindication for a lumber puncture, CSF for CrAg is recommended. If there is no access to lumber puncture, the WHO Rapid Advice guidelines recommend testing of serum or plasma for CrAg using lateral flow immunoassay (LFA). Furthermore, the WHO guidelines emphasized that the CrAg LFA had several advantages over latex agglutination including lower cost, rapid turnaround time, little training required as well as minimal laboratory infrastructure.

Diagnosis is rarely a problem in HIV-associated cryptococcal infection, since the high organism load means Indian ink preparations of CSF are usually positive and cryptococcal antigen testing of either CSF or serum has a high sensitivity and specificity. While the gold standard for the diagnosis of cryptococcal disease is culture from bodily fluids, cryptococcal antigen (CrAg) is used to presumptively diagnose cryptococcal disease with sensitivity and specificity near 100% in Sub-Saharan Africa in an HIV-infected population (Pongsai, Atamasirikul and Sungkanuparph, 2010). Notably, detectable CrAg in peripheral blood precedes symptoms of cryptococcal meningitis by an average of 22 days (French, Gray and Wateria et al., 2002), and approximately 11% of people will have antigen present greater than 100 days prior to disease onset.
Historically, cryptococcal has been detected by latex agglutination or enzyme immunoassay. However, these processes require heat inactivation, refrigeration of reagents, lab infrastructure for agglutination testing (e.g. rotator), lab expertise and labour. Given that the majority of the cryptococcal disease occurs in resource-constrained settings, these facilities are often unavailable for diagnosis outside of urban settings of referral centres. In July 2011, the lateral flow immunoassay (LFA) was improved by the U.S. FDA for detection of cryptococcal antigen. The CrAg LFA is an ideal point of care test, as it can be performed by persons with minimal training or any additional laboratory equipment.

### 2.5.1 Signs and symptoms

Cryptococcal meningitis is characterized by a number of signs and symptoms, namely:

- Headache
- Fever
- Malaise
- Altered mental status
- Papilloedema
- Cranial nerve palsies
- Depressed conscious level
2.5.1 Complication of cryptococcal meningitis

- Raised intracranial pressure
- Visual/hearing loss
- Cognitive impairment
- Gait ataxia (WHO, 2011)

2.5.3 Investigations

A diagnostic workout for cryptococcal fever includes the following:

- Blood
- Blood culture
- Microscopy of cerebrospinal fluid

2.5.4 How the CrAg LFA works

Imunoassay for CrAg is an antigen-capture test that detects free capsular antigen that has been released by the yeast into body fluids such as blood, CSF or urine. The ideal immunoassay for diagnosis of cryptococcal meningitis must meet two criteria, first, the test should be able to detect cryptococcal antigen of all serotypes. Second, the test needs to meet the needs for diagnosis of disease in countries or regions with both advanced and limited infrastructure. The majority of the cryptococcal cases occur in countries with very limited infrastructure and as a consequence, a test that addresses the needs of resource-limited countries must meet the WHO ASSURED criteria for diagnostic test.
In 2011, the IMMY CrAg LFA was designed to be the test that addresses the global spectrum of the species complex of pathogenic *Cryptococcus*. It uses the lateral flow immunochromatographic assay platform commonly referred to as a dipstick assay. The LFA detects the same antigen that is detected by the widely accepted CrAg latex agglutination and ELISA assays and will therefore have the same specificity. The initial assessment of serotype sensitivity of CrAg LFA (Kozel and Bauman, 2011), shows a sensitivity that is greater than that of the commercially available latex agglutination and EIA assays for CrAg of serotypes (Kozel, Thorkildson and Pereival, 2011).

A WHO Rapid Advice report recently recommended the CrAg LFA for diagnosis of suspected cryptococcal meningitis (WHO, 2011). In a retrospective study of culture-confirmed cryptococcal meningitis in South Africa, the CrAg LFA had a sensitivity of 100% when used with serum/plasma and 98% with Urine (Jarvis, Percival, Bauman et al., 2011). A preliminary report from a study done in Uganda was recently presented at the 2012 Conference on Retroviruses and Opportunistic Infections found that the CrAg LFA was highly sensitive and specific relative to both culture and CrAg agglutination (Rolfes, Butler, Hohenberg, et al., 2012).
2.6 MANAGEMENT AND TREATMENT

In 2000, the Infectious Diseases Society of America (IDSA) first published the “Practice Guidelines for the Management of Cryptococcal Disease” (Saag, Graybill, Larsen, et al., 2005). In this updated version of the guidelines, the experts in medical mycology have approached cryptococcal management using the framework of key diagnostic clinical questions. The goal was to merge recent and established evidence-based clinical data along with shared expert clinical opinions and insights to assist clinicians in the management of cryptococcal meningitis in HIV patients. However, the basis for the successful management of cryptococcal meningitis was carefully detailed in the previous IDSA guidelines published in 2000. In fact, by following specific parts of the guidelines for management of cryptococcal meningitis, a good improvement in outcome has been validated in some retrospective studies (Shoham, Cover and Donegan, et al., 2005; Dromer, Bernede-Bauduin, Guillemot, et al., 2008). However, over the past decade a series of new clinical issues and host risk groups have risen, and it is necessary that these guidelines be revised to assist practicing clinicians in management of cryptococcosis.

*Cryptococcus neoformans* and *Cryptococcus gattii* have now been divided into different species, although most clinical laboratories in developing countries will not routinely identify *Cryptococcus* to the species level. *C. gattii* has recently been reported responsible for an ongoing outbreak of cryptococcosis in immunocompetent humans on Vancouver Island and areas surrounding Canada and the northwest of the
United States, and the management cryptococcal infections caused by *C. gatti* in immunocompetent hosts needs to be addressed (Kidd, Hagen, Tscharke, et al., 2006). Similarly, the HIV pandemic continues, and cryptococcosis is a major opportunistic pathogen in HIV-infected patients, but its management entirely depends on the medical resources available to clinicians in specific regions. In the era of highly active antiretroviral therapy (HAART), the management of cryptococcosis disease has become a blend of established antifungal regimens together with aggressive treatment of the underlying disease (Kidd, Hagen, Tscharke, et al., 2006).

Antifungal drug regimens for management of cryptococcosis are some of the best-characterized for invasive fungal diseases (Chayakulkeeree and Perfect, 2006). However, they remain poorly studied issues, many of which revolve around the host. For example, controlling host immunodeficiency and immune reconstitution can become a complex clinical scenario during management of cryptococcal meningitis. Furthermore, certain complications, for instance; increased intra-cranial pressure, immune reconstitution inflammatory syndrome (IRIS) and cryptococcomas may require special approaches for their successful management in cryptococcal disease.

Since the last IDSA guidelines in 2000, only the extended-spectrum azoles (posaconazole and voriconazole) and the echinocandins (micafungin, anidulafungin and caspofungin) become available as new antifungal drugs. The former drugs have been studied clinically in salvage situations (Pitisuttithum, Negroni, Graybill, et al.,
2005), and the latter have no in vivo activity versus *Cryptococcus* species. Pathobiologically, although recent studies from the cryptococcosis outbreak in Vancouver support the observation that a recombinant strain in nature became more virulent than its parent, there are few other clinical data to suggest that cryptococcal strains have become more virulent or drug resistant over the past decade (Fraser, Giles, Wenink, et al., 2005). According to the updated treatment guidelines, host immunity, site of infection, antifungal drug toxicity and the underlying disease still remain to be the most critical factors for successful management of cryptococcosis.

Patients who are infected by HIV and cryptococcal meningitis account for more than 80% of the patients with cryptococcosis. Many specialists now recommend an initial aggressive treatment course. Initially, administer amphotericin B at 0.7-1 mg/kg/d for 2 weeks, with or without 2 weeks of flucytosine at 100 mg/kg/d in 4 divided doses, followed by fluconazole at 400 mg/d for a minimum of 8-10 weeks. The addition of flucytosine to amphotericin B results in quicker clearance of viable yeast from the cerebrospinal fluid (CSF) than is seen with amphotericin B alone or amphotericin B plus fluconazole. However, patients may be treated successfully without the addition of flucytosine and its potential toxicity. The potential toxicity of flucytosine increases in patients who have renal dysfunction from any cause.

Alternative initial therapies include lipid formulations of amphotericin B to be administered in doses of 4-6 mg/kg per day for 3 weeks. Doses of Fluconazole ranging from 400-800 mg per day plus flucytosine is another option in patients unable to tolerate amphotericin B. However, fluconazole combined with flucytosine has been regarded as clinically inferior to amphotericin B–based therapy. Initial
therapy should be considered successful only after CSF culture is negative for cryptococcal organisms and the patient has had significant clinical improvement.

Guidelines from 2005 recommended that initial therapy be followed with maintenance therapy using fluconazole at 200 mg/d for life (Saag, Graybill, Larsen, et al., 2005). A study of patients in the maintenance phase of treatment, itraconazole was found inferior to fluconazole. The same study indicated that no clear benefits were evident when flucytosine was added to the 2-week initial course of amphotericin B. Treatment guidelines published in 2002 support the discontinuation of suppressive therapy for cryptococcal disease if CD4 counts remain greater than 200 cells/µL but reinstitution if the CD4 counts fall to fewer than 200 cells/µL (Kaplan, Masur and Holmes 2002).

Although the two newer drugs (posaconazole, triazoles and voriconazole) show in vitro activity against C neoformans, clinical data remain limited. Inpatients who require life-long suppressive therapy, oral fluconazole was found superior to therapy with weekly intravenous amphotericin B given as 1 mg/kg 1 to 3 times per week.

CSF pressure should be monitored during the initial phase of therapy and reduced by therapeutic CSF removal when the opening pressure exceeds 250 mm H$_2$O. Following removal of CSF, the closing pressure should be less than 200 mm H$_2$O or at least 50% of the elevated opening pressure. Lumber puncture repeat was once
recommended in all patients 2 weeks after the initiation of therapy to ensure that CSF cultures were not positive. However, further spinal taps in patients who have normal neurologic function and no evidence of ineffectively treated cryptococcal infection is now considered acceptable by some specialists.

Recently alternative initial therapy of fluconazole together with flucytosine for 6 weeks, followed fluconazole maintenance therapy has been anticipated. However, pilot studies done have indicated that initial therapy with fluconazole and flucytosine is not as reliably effective as therapy that includes amphotericin B during the initial phase (Pappas, Perfect, Larsen, et al. 2008). Furthermore, the same study indicated that the combination of flucytosine and fluconazole has a significant toxicity.

HIV-infected patients who are not already on antiretroviral therapy; starting treatment for cryptococcal meningitis prior to starting antiretroviral therapy can reduce the risk of immune reconstitution inflammatory syndrome (Lortholary, Fontanet and Mémain, et al., 2005). Once cryptococcal antigen has been significantly decreased, ART can be initiated while the treatment for cryptococcal infection continues. However, newer available data demonstrated an improved clinical outcome when highly active antiretroviral therapy (HAART) is started within 6 months of the diagnosis of cryptococcal meningitis (Sungkanuparph, Filler and Chetchotisakd, et al., 2009).

A study of 27 HIV-infected adult patients with cryptococcal meningitis done in Nigeria found no significant difference in the rate of clearance of fungus from the CSF whether antiretroviral therapy was initiated at 7 or 28 days after the start of
amphotericin B treatment. However, the risk of IRIS in patients with cryptococcal meningitis was significantly higher in the early antiretroviral therapy group. Seven of 13 subjects (54%) in the 7-day arm had IRIS, as compared to 0 of 14 in the delayed-intervention arm (Rassel, 2009).

An emerging concept is that of screening ART-naïve patients with low CD4 counts for serum or plasma CrAg in regions where there is a high prevalence of cryptococcal antigenemia (WHO, 2011). Such screening would identify patients at high risk of cryptococcal disease and allow for pre-emptive treatment with antifungal agents to prevent cryptococcal disease. This approach is based on the findings that CrAg is detectable in the serum of the patients infected with HIV.

### 2.7 SUMMARY

This chapter covered an extensive review of literature in the areas of global and regional burden of cryptococcal meningitis among HIV-infected patients, as well as comparison of studies done on the prevalence of cryptococcal infections in HIV-infected patients. The chapter also looked at the pathogenesis of the disease, signs & symptoms, treatment, and complications of cryptococcal disease. The issue of diagnosis using in particular the cryptococcal antigen lateral flow immunoassay (CrAg LFA), was extensively covered.
CHAPTER 3

RESEARCH METHODOLOGY

3.1 INTRODUCTION

This chapter provides an outline on how the research process was carried out. It presents an in-depth analysis of both the design and methodology of the research. The main purpose of the study is to establish the prevalence of cryptococcus among HIV patients attending Intermediate Oshakati Hospital (IHO). The focus was on the study population, sampling techniques, sample size, data collection methods and the control measures to ensure variability and confidentiality.

3.2 RESEARCH DESIGN

A research design seeks answers to the research question (Polit & Hungler, 2010). The design is a general plan or blueprint that describes how the research will be conducted. It focuses on the kind of study proposed and its desired result. It begins with a problem, or question, and in the context of the logic of the research, determines what kind of evidence will address the research question adequately (Mouton, 2002).
It is a plan according to which the researcher obtains research participants (subjects) and collects information from them. In it the researcher describes what activities were done in the field with a view to reaching conclusions about the research problem (Welman, Kruger and Mitchell, 2009). McGivern (2006) views a research design as a two-tier process. In the first level, a research design is about the logic of the research, its framework and structure. In view of what is known about the problem to be researched and the sort of research enquiry (exploratory, descriptive or explanatory) which that demands, decisions about structure of the research are made at the first level. The cited author further stresses that the structure may comprise a cross-sectional, a longitudinal or an experimental design, or a case study. Decisions on units of analysis are also made in the first level of research design and include the ‘who’ or ‘what’ to question or to observe.

The secondary level of research design is about the research process: what type of data (primary or secondary, qualitative or quantitative or a combination), what method of data collection, what sampling strategy, and so on (McGivern 2006). In conclusion McGivern (2006) eludes that the first level is about designing the overall structure of the research so that it can deliver the sort of evidence one needs to answer the research problem, while the second level concerns decisions about how to collect that evidence. Welman, Kruger & Mitchell (2009) stated that in research design researchers have to specify:
i. The number of groups that should be used (necessary to decide which statistical technique to use).

ii. Whether these groups are to be drawn randomly from the populations involved and whether they should be assigned randomly to groups.

iii. What exactly should be done with them in case of experimental research?

In view of the above, a descriptive, cross-sectional study was conducted utilising quantitative methodology. This study was a laboratory and hospital-based whereby the samples were withdrawn from HIV patients attending at Communicable Disease Clinic and were tested at NIP laboratory.

### 3.2.1 Descriptive Design

A great deal of epidemiologic research is about description, as well as exploration, used to find answers to questions such as: Who; What; Where; When; How; and How Many? The purpose of descriptive research is to answer research questions. Descriptive research aims to build a set of experiences…it aims to identify, describe, and in some cases count things (Babbie & Monton, 2007). It can be used to examine some of the key issues facing marketers and policy-makers (McGivern, 2006). The researcher defined the descriptive components of a quantitative study of prevalence of cryptococcal meningitis among HIV-infected patients attending the Intermediate Hospital Oshakati.
3.2.2 Cross-sectional design

A cross-sectional design is a study design in which the exposure and disease status are assessed simultaneously among individuals in a well-defined population. There can be a specific time window, such as a given calendar year during which a community wide survey/study is conducted or a fixed point in the course of the event that varies in real time from person to person. Thus, cross-sectional studies provide information about the frequency and characteristics of a disease by furnishing a snapshot of the health experience of the population at a specified time. Such data can be of great value to public health administrators in assessing the health status and health care needs of a population (Hennekens, 2006).

Cross-sectional studies can also be used to provide information on the prevalence disease or other health outcomes in certain occupations. In one special circumstance, a cross-sectional design can be considered as a type of analytic study and used to test epidemiologic hypotheses. This can only occur when the current values of the exposure variables are unalterable over time, representing the value present at the initiation of the disease. Such variables include factors present at birth, such as eye colour or blood group. However, in most cross-sectional studies, the risk factors may be subject to alteration subsequent to the development of disease. In these instances, the data can be used to describe characteristics of individuals with the disease and formulate a hypothesis (Hennekens, 2006).
3.2.3 Quantitative research

Quantitative methodology relies upon measurements to analyse different variables by using various scales. This formal, objective and systematic process intends to analyse, compare and describe different variables in the study. The quantitative research approach was considered suitable for estimating the prevalence of cryptococcal meningitis among HIV-infected patients attending the Intermediate Hospital Oshakati.

3.3 RESEARCH METHODS

The research methodology focuses on the research process and the tools and procedures utilised. Beginning with the tasks it must accomplish, namely, data collection and sampling, it focuses on individual steps in the research process, trying to employ objective (unbiased) procedures (Mouton, 2002). Quantitative data is information that can be numerically measured and analysed. This involves computer analysis of statistical data to test their significance. Quantitative research methodology facilitates easy comparison of data and reproduction of results (Brink, 2006). In this study quantitative descriptive statistical methods were used. Graphs and tables were used to present the data and the Epi Info version 7 was used to analyse the data.
3.3.1 Population

A population is any defined group that is selected as a subject for research. If a population can be defined, from oxygen molecules in the universe to supercomputers in the world, then it can be subjected to study and analysis (Melville & Goddard, 2006). A study population includes all the members, or units, of a group that can be clearly defined in terms of its distinguishing criteria, whether they are people, objects or events (Uys & Basson, 2008).

In a research context, a population refers to the universe of enquiry or, put another way, to the people, organisations, events or items that are relevant to the research problem (McGivern, 2006). It is important to define the population of interest as precisely as possible. Any flaws in definition of the population will mean flaws in the sample drawn from it (McGivern, 2006). On the other hand, Welman, Kruger and Mitchell (2009) define population as the study object that consists of individuals, groups, organizations, human products and events, or conditions to which they are exposed. A research problem relates to a specific population and the population encompasses the total collection of all units of analyses about which the researcher wishes to make specific conclusions (Welman, Kruger & Mitchell, 2009).
The way in which a study population is defined depends on the issues the research aims to address. For example, if a study of the health and social welfare needs of older people has been commissioned to help develop policy in relation to community health activities the researcher may decide that those in residential care, nursing homes or hospitals are not part of the relevant population (McGivern, 2006).

McGivern (2006) further categorizes population as target and survey population, respectively. Moser and Kalton (cited in McGivern 2006) make a distinction between these two populations. The target population is the one from which the results are required and the survey population is that actually covered by the research. The two populations should ideally be the same but for practical reasons they may not be.

The population for this study consisted of all HIV-infected patients (adults and children of all age groups and gender) attending at Communicable Disease Clinic, Intermediate Hospital Oshakati (IHO) from June to July 2015. The clinic in IHO has private and confidential bleeding rooms which allowed the researcher to draw blood samples in privacy. The researcher collected 4.5 ml of blood in EDTA tubes by venipuncture. The 4.5 ml of blood was collected once per patient as this will suffice the test requirements in terms of quantity and frequency.
3.1.1.1 Inclusion criteria

The general inclusion criteria of the study included the following:

- All HIV-infected patients who attend follow-ups at Communicable Disease Clinic, the Intermediate Hospital Oshakati during the study period.
- HIV-infected patients who signed the consent or have given assent for participation.

3.1.1.2 Exclusion criteria

- All HIV-infected patients who do not attend follow-ups at Communicable Disease Clinic, the Intermediate Hospital Oshakati during the study period.
- HIV-infected patients who did not agree to participate in the study.

3.4 SAMPLING AND SAMPLE

Sampling is described as a process of selecting a smaller group of people from the research population as a representative of that population (Melville & Goddard, 2006). Sampling is less costly and time consuming for the researcher than including the entire population.

A sample is a group of people or elements that form part of a study population. Results from a study sample allow general observations to be made about the entire population (Melville & Goddard, 2006). De Vos (2002) defines a sample as a small
portion of the total set of the population; together they comprise the subject of the study. Sampling is the most feasible way of studying large populations when there are resources, time and financial limitations.

Once the population is clearly defined a researcher must decide whether to collect data from every member or element of that population (usually defined as a census) or from a representative subset or sample of it (McGivern, 2006). In most health and social research, the population of interest is often too large for census to be practicable either in terms of time it would involve or cost. The argument for using a well-designed sample rather than a census rests on two issues: on the practical issue of the time and cost involved in administering it, and on the methodological issue of the ability of a sample to be representative of the population (to deliver external validity).

Welman, Kruger and Mitchell (2009) define a sample as a miniature image or likeness of the population. The aspect of generalising is extremely important. It is only when the results can be generalised from a sample to a population that the results of research have meaning beyond the limited setting in which they were originally obtained. A sample must therefore be a true representative of the population: the sample must have the exact properties in the exact same proportions as the population from which it was drawn but in smaller numbers (Welman, Kruger and Mitchell 2009).
The sample for this study included 384 HIV-infected patients attending at Communicable Disease Clinic in IHO, utilising a systematic random sampling and it was calculated based on the estimation of proportions by using Stat Cal in Epi Info.

3.4.1 Sample size criteria

In addition to the purpose of the study and population size, three criteria usually need to be specified to determine the appropriate sample size. The level of precision; the level of confidence or risk; and the degree of variability in the attributes being measured should be clearly specified (Williams, Kennedy and Miaoulis, 2007). The level of precision is also known as sampling error and is discussed in the following section.

3.4.1.1 The level of precision

This level, which is sometimes called sampling error, is the range in which the true value of the population is estimated to be. This range is often expressed in percentage points (for example, ±5 percent), in the same way that results for political campaign polls are reported by the media. Thus, if a researcher finds that 60% of farmers in the sample have adopted a recommended practice with a precision rate of ±5%, then the researcher can conclude that between 55% and 65% of farmers in the
population have adopted the practice. In this study the level of precision was set at 5% which is the maximum precision point (Williams, Kennedy and Miaoulis, 2007).

3.4.1.2 The confidence level

The confidence or risk level is based on ideas encompassed under the central limit theorem. The key idea encompassed in this theorem is that when a population is repeatedly sampled, the average value of the attribute obtained by those samples is equal to the true population value. Furthermore, the values obtained by these samples are distributed normally about the true value, with some samples having a higher value and some obtaining a lower score than the true population value. In a normal distribution, approximately 95% of the sample values are within two standard deviations of the true population value (the mean value). In other words if a 95% confidence level is selected, 95 out of 100 samples will have the true population value within the range of precision specified earlier. There is always a chance that the obtained sample does not represent the true population value. Such samples with extreme values are represented by the 5% (2.5% on each side) area under the normal distribution curve. This risk is reduced for 99% confidence levels and increased for 90% (or lower) confidence levels (Williams, Kennedy and Miaoulis, 2007).

3.4.1.3 Degree of variability

The degree of variability in the attributes being measured refers to the distribution of attributes in the population. The more heterogeneous a population, the larger the
sample size required to obtain a given level of precision. The smaller the sample size 
the less variable the population is. Note that a proportion of 50% indicates a greater 
level of variability than either 20% or 80%. This is because 20% and 80% indicate 
that a large majority do not or do, respectively, have the attribute of interest. Since a 
proportion of 0.5 indicates the maximum variability in a population it is often used in 
determining a more conservative sample size: the sample size may be larger than if 
the true variability of the population attribute were used (Williams, Kennedy and 
Miaoulis, 2007).

Since in this study there is a large population, and little is known about the 
prevalence of cryptococcal meningitis among HIV-infected patients, an assumption 
was made for a maximum variability, p=0.5 (maximum variability). Furthermore, a 
95% confidence level and ±5% precision was desired in this study.

The resulting sample size is demonstrated in the equation below:

\[ n_0 = Z^2pq = (1.96)^2(0.5)(0.5) = 384 \text{ blood samples} \]

Which is valid where \( n_0 \) is the sample size, \( Z^2 \) is the abscissa of the normal curve that 
cuts off an area at the tails (1 - equals the desired confidence level, for example, 
95%), \( e \) is the desired level of precision, \( p \) is the estimated proportion of an attribute
that is present in the population, and \( q \) is \( 1-p \). The value for \( Z \) is found in statistical tables which contain the area under the normal curve. From the above equation it follows then that at least 384 blood samples were needed in this study. This sample size accorded the researcher the ability to outrightly reject outliers and also make inferences for the population (Williams, Kennedy and Miaoulis, 2007).

### 3.4.2 Sample Collection

The researcher identified every second HIV positive patient coming in the CDC for consent and assent where applicable, to participate in the study. Where consent and assent were given, the researcher withdrew 4.5 ml of blood in an EDTA tube using the venipuncture procedure. Three hundred and eighty four (\( n=384 \)) clotted blood samples were collected over a period of two months, namely June to July 2015. The researcher tested all the blood samples collected for cryptococcal antigen and CD4 Count. The test results as well as patient demographic were recorded on a data collection sheet.
3.5 TESTING PROCEDURES

Whole blood samples were spun at 3000 r.p.m for 10 minutes in automatic centrifuge. The plasma was used to perform the crAg test using IMMY CrAg test kit.

The rapid tube screening test (qualitative) was carried out first, followed by semi-quantitative titration procedure according to the manufacturer’s specification. The Glycine buffered saline is the specimen diluent which contains blocking agents and preservative to enhance the reading of the rapid tests.

3.5.1 Quantitative Procedure

The rapid tube technique was used to screen samples for cryptococcal meningitis and entailed a specific procedure, namely:

- The samples and reagents were brought to room temperature
- One drop of the LF specimen diluent and 40ul of each control (positive/ and negative) were placed into separate test tubes
- One drop of the LF specimen diluent and 40ul of the sample to be tested were placed into a test tube.
• The white end of the cryptococcal Antigen Lateral Flow Test strip was submerged into the specimen, and left to stand for ten minutes.

• The results were read and recorded.

3.5.2 Reading and Interpretation

The reading and interpretation of the CrAg test depends on the visual interpretation of the line(s) on the strip. The procedure was as follows:

• The strips were examined macroscopically for the presence or absence of visible line(s) on the strip.

• The presence of two lines (test and control) on the test strip, regardless of the intensity of the test line, indicated a positive result.

• A single control line indicated a negative result.

• If a control line does not appear, the results were considered invalid and the test was repeated.
3.5.3 Semi-quantitative procedure

The positive results from the qualitative procedure were confirmed and titrated by the rapid test using the following technique:

- Dilutions were prepared starting with an initial dilution of 1:20 by 1 in 2 serial dilution to 1:2560
- Ten tubes were labelled as set out in Table 3.1 below. Additional dilution was necessary if the specimen was positive at 1:2560
- Four drops of LF sample diluent were added to the tube labelled number 1
- Two drops of LF titration diluent were added to each of the tubes labelled 2 - 10
- Forty (40) ul of the specimen is added to the 1st tube and mixed well.
- Eighty (80) ul of the specimen in tube number 1 was transferred to tube 2 and proceeded by serial dilution through a tube labelled 10
- The white end of the cryptococcal Antigen Lateral Flow Test strip was submerged into the specimen, and left to stand for ten minutes
- The results were read and recorded

3.5.4 Reading the test

- For Semi-Quantitative titration procedure, the patient’s titre was reported as the highest dilution that yields a positive result.
Table 3.1 Preparation of dilution for the semi-quantitative procedure

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Specimen diluent (ul)</th>
<th>Titration diluent (ul)</th>
<th>Serum (ul)</th>
<th>Dilution Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 drops = 160 ul</td>
<td>40 ul</td>
<td>40 ul</td>
<td>1:20</td>
</tr>
<tr>
<td>2</td>
<td>2 drops = 80 ul</td>
<td></td>
<td>80 ul</td>
<td>1:40</td>
</tr>
<tr>
<td>3</td>
<td>2 drops</td>
<td></td>
<td>Serial dilution</td>
<td>1:80</td>
</tr>
<tr>
<td>4</td>
<td>2 drops</td>
<td></td>
<td></td>
<td>1:160</td>
</tr>
<tr>
<td>5</td>
<td>2 drops</td>
<td></td>
<td></td>
<td>1:320</td>
</tr>
<tr>
<td>6</td>
<td>2 drops</td>
<td></td>
<td></td>
<td>1:640</td>
</tr>
<tr>
<td>7</td>
<td>2 drops</td>
<td></td>
<td></td>
<td>1:1280</td>
</tr>
<tr>
<td>8</td>
<td>2 drops</td>
<td></td>
<td></td>
<td>1:2560</td>
</tr>
<tr>
<td>9</td>
<td>2 drops</td>
<td></td>
<td></td>
<td>1:5120</td>
</tr>
<tr>
<td>10</td>
<td>2 drops</td>
<td></td>
<td></td>
<td>1:10240</td>
</tr>
</tbody>
</table>

Titres less than 20 are negative for cryptococcal antigenemia, while any titre greater than 20 is positive for cryptococcal antigenemia.
3.6 DATA COLLECTION

The blood samples were collected at the Communicable Disease Clinic in Intermediate Hospital Oshakati. The researcher indicated with a survey code on the blood samples of patients who agreed to participate. The blood samples were withdrawn and taken to the laboratory, where they were tested for cryptococcal antigen. The demographic data of patients and treatment information were extracted from the medical files at Communicable Disease Clinic. Data on cryptococcus and CrAg titres were generated from the laboratory CrAg test done on the blood samples collected for the study. The data on CD4 count were also generated from the laboratory automated instruments. These results were all entered on the designed data collection sheets (DCS).

3.7 DATA ANALYSIS

Data analysis includes categorizing, ordering, manipulating and summarizing the data so that they can be described in meaningful terms (McGivern, 2006). In this study, data collected were analysed using Microsoft Excel and Epi-Info 7 computer program for further statistical analysis. Descriptive statistics and tables, graphs and charts were used to summarize and display the data in excel. These data were also used for comparative analysis of data according to age, gender, CD4 count, CrAg titres and other variables.
3.8 RESEARCH ETHICS

Conducting research implies the acceptance of certain responsibilities. A researcher should be responsible to fellow researchers, respondents, society as a whole and most importantly, to her/himself (Melville & Goddard, 2006). A high professional standard regarding confidentiality was strictly maintained. Ethics in research refer to moral principles that call for respect and protection of the rights of research participants by researchers (Nengomasha, 2010). Human ethics rest on four basic principles that are considered the foundation of all regulations or guidelines governing research ethics: principle of respect for people, principle of beneficence/non-maleficence and the principle of justice.

The right to privacy encompasses both the right to respect for the dignity of the patient, namely his/her physical privacy, and the right to respect for the patient’s secrets, namely confidentiality (Pera & Van Tonder, 2005). Information regarding voluntary participation in the study as well as the right to refuse before the sample was withdrawn was explained. The objectives of the study were explained to the research participants after which informed written consent was obtained from the HIV patient who had agreed to participate. All the participants signed the consent form (Annexure A) for permission to withdraw blood samples and their participation in the study. In the case of minor patients, their assent were sought through verbal/non-verbal reactions and the parents/guardians signed the consent on their behalf.
3.8.1 Permission

Ethical clearance and permission to conduct the study was sought and obtained from the University of Namibia Post-Graduate Committee (Annexure B and C) and the Ministry of Health and Social Services (Annexure D). The written proposal was reviewed by the committee to ensure that it conformed to ethical standards of scientific research. Permission to utilise the facilities (for testing) of NIP laboratory was sought from management of NIP.

3.8.2 Confidentiality and Anonymity

All patient information on specimens and laboratory information system was treated with the strictest confidentiality. The data obtained were used for the stated purpose of the research and no other person had access to patient specimen details. Specimens which met the inclusion criteria were given a survey code without patient names and laboratory or hospital numbers. Study samples were not traceable to the submitting patient.

3.8.3 Anticipated risks and benefits

The anticipated risks included infections for both the researcher and participants. The researcher was at risk of being infected when withdrawing the blood samples. The patient was also at risk of contracting infections if there was no sterile procedure used. The risk was minimized by using universal safety precautions for handling
sharps and needles. Safety procedures including disinfecting alcohol and protective equipments were used when withdrawing blood. There was also the possibility of trauma (shock) for the minors when pricking them with needles and this was minimized by reassuring the patients. Another risk was the development of allergic reactions to the patients who are allergic to disinfecting alcohol and was minimized by excluding the patient who had allergic reactions before.

On the anticipated benefits, the study gathered data that can be used for improving health care of HIV positive patients, and which will improve the follow up for the participants. Moreover, the participants will be able to receive patient specific treatment and management according to the outcome of the analysis. The study will also strengthen scientific, professional and academic knowledge of the researcher.

3.9 SUMMARY

This chapter discussed the research design and research methods concentrating on descriptive and cross-sectional designs. It also looked at the population and sampling methods. Data collection and analysis methods were also discussed in this chapter. Ethical issues of confidentiality, anonymity and permission were also discussed.
CHAPTER 4

RESULTS OF THE STUDY

4.1 INTRODUCTION

This chapter presents the analysis and findings of the study. The main findings of the study are on the prevalence of cryptococcal antigenemia among HIV-infected patients attending the Intermediate Hospital Oshakati, in Oshana region. The results are presented as descriptive statistics in tables, pie charts and graphs in terms of the objectives of the study.

4.1.1 Characteristics of study patients

A total of 384 HIV-infected patients were enrolled in the study. The majority of the participants were female (60.2%, 231/384) and the remaining were male (39.8%, 153/384). The median age of the patients was 35 years and it ranged from a minimum of two years to a maximum of >70 years. Of the total of 384 HIV infected patients, 36 (9.38%) were diagnosed with positive cryptococcus. Of these, almost two-thirds (72.22%, 26/36) of the study patients had a CD4 count of less than 100 cells/ul. The majority had cryptococcal antigen titres of either 1:80 or greater.
4.1.2 Overview of CrAg test

The Cryptococcal antigen test, when used together with the baseline CrAg titres normal to the population, may suffice. Physicians usually elect to treat a patient rather than wait for culture results. The latter can take between five to eight days to be available from the laboratory. Although there may be some merit in this approach, particularly in some areas where culture facilities are either poor or non-existent, the CrAg test can be used to raise the suspicion index and help to give an early treatment to the patient.

4.2 TESTING PROCESS

The cryptococcal antigenemia among the blood samples taken from the HIV infected patients was determined on separated serum by standard tube antibody-antigen reaction tests; appropriate controls were included in each procedure. The CrAg titres were also done in every positive sample. The distribution of positive cryptococcal antigen was analysed by age, gender, CD4 count and CrAg titres and those results are presented in the subsequent sections below.
4.3 PREVALENCE OF CRYPTOCCUS AMONG HIV-INFECTED PATIENTS (n=384)

Of the 384 (100%) HIV-infected patients tested for cryptococcus, 36 (9.38%) tested positive for cryptococcal antigen test and 348(90.62%) tested negative for cryptococcal antigen as shown in Figure 4.1 and Table 4.1

Figure 4.1 Prevalence of serum cryptococcus among 384 HIV-infected patients attending the Intermediate Hospital Oshakati, July-September 2015
Table 4.1: Prevalence of Serum CrAg among HIV-infected patients attending the Intermediate Hospital Oshakati, July-September 2015 (n=384)

<table>
<thead>
<tr>
<th>Crag test results</th>
<th>Frequency</th>
<th>Percent (%)</th>
<th>Cum. Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>348</td>
<td>90.62</td>
<td>90.62</td>
</tr>
<tr>
<td>Positive</td>
<td>36</td>
<td>9.38</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

A total of 384 HIV-infected patients attending the Communicable Disease Clinic were enrolled into the study. The prevalence (95% CI) of Cryptococcus among the HIV-infected patients attending the Intermediate Hospital Oshakati, in Oshana Region, is estimated by 9.38% (6.46% to 12.30%).

4.3.1 Prevalence of cryptococcus among HIV-infected patients by age groups

The proportions of HIV infected patients with cryptococcal infections were analyzed by age groups and the results are presented in Table 4.2
Table 4.2 Age based Prevalence of Cryptococcal antigenemia among HIV-infected patients attending the Intermediate Hospital Oshakati, July-September 2015 (n=384)

<table>
<thead>
<tr>
<th>Age groups Years</th>
<th>No. Negative (%)</th>
<th>No. Positive (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10</td>
<td>9(2.59)</td>
<td>0 (0.00)</td>
<td>9(2.34)</td>
</tr>
<tr>
<td>11-20</td>
<td>17(4.89)</td>
<td>4 (11.11)</td>
<td>21(5.47)</td>
</tr>
<tr>
<td>21-30</td>
<td>90(25.86)</td>
<td>10 (27.78)</td>
<td>100(26.04)</td>
</tr>
<tr>
<td>31-40</td>
<td>92(26.44)</td>
<td>10 (27.78)</td>
<td>102(26.56)</td>
</tr>
<tr>
<td>41-50</td>
<td>74(21.26)</td>
<td>5(13.89)</td>
<td>79(20.57)</td>
</tr>
<tr>
<td>51-60</td>
<td>54(15.52)</td>
<td>4(11.11)</td>
<td>58(15.10)</td>
</tr>
<tr>
<td>61-70</td>
<td>9(2.59)</td>
<td>2(5.56)</td>
<td>11(2.89)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>3(0.85)</td>
<td>1(2.78)</td>
<td>4(1.04)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>348 (100.00)</strong></td>
<td><strong>36(100.00)</strong></td>
<td><strong>384(100.00)</strong></td>
</tr>
</tbody>
</table>

Pearson Chi^2 =6.7472  df=7  P. value =0.456

The majority of the cryptococcus cases (27.78%) among HIV-infected patients were in the age group of 21-30 and 31-40 years. Adults over the age of 70 years had the least number of cases (2.78%) of cryptococcal antigenemia while children under the age of 10 had no cases of positive cryptococcal antigen test. A Chi-square test showed no association between age and cryptococcus positivity (Chi^2 =6.7472, df=7, p=0.456), implying that there were no significant differences in the prevalence of Cryptococcus across the different age groups.
4.3.2 Prevalence of Cryptococcus by gender among 384 HIV-infected patients

The prevalence of cryptococcal antigenemia was analyzed by gender and the results are presented in Table 4.3.

Table 4.3 Sex-based prevalence of cryptococcal/cryptococcal antigenemia among HIV-infected patients attending the Intermediate Hospital Oshakati, July-September 2015

<table>
<thead>
<tr>
<th>Sex</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>214 (61.49%)</td>
<td>17 (47.22%)</td>
<td>321 (60.16)</td>
</tr>
<tr>
<td>Male</td>
<td>134 (38.51)</td>
<td>19 (52.78)</td>
<td>153 (39.84)</td>
</tr>
<tr>
<td>Total</td>
<td>348 (100.00)</td>
<td>36 (100.00)</td>
<td>384 (100.00)</td>
</tr>
</tbody>
</table>

Pearson $\chi^2=2.7726$ df=1  P value=0.092

Table 4.3 above presents the cryptococcal antigen positive cases by gender among the 384 HIV-infected patients. Out of all the laboratory confirmed cases, 19 (52.78%) were male and 17 (47.22%) were female. The Chi-square test showed no significant association between gender and Cryptococcus positivity ($\chi^2=2.7726$, df=1, p=0.092) meaning that there were no significant differences in the prevalence of cryptococcus between males and females.
4.3.3 Prevalence of Cryptococcus by residential area

Figure 4.2 Number of positive cryptococcal antigen cases by residential area among 384 HIV infected patients attending the Intermediate Hospital Oshakati, July-September 2015

Figure 4.2 presents the HIV infected patients with positive cryptococcal antigen by residential area. Most of the HIV patients (89%) were residing in rural areas and only few (11%) were living in urban areas. The Chi-square test showed no significant
association between residential area and positive cryptococcal antigen test ($\chi^2 = 8.9483 \ df=2 \ p=0.9022$) implying that there were no significant differences in cryptococcal antigen positivity between patients coming from rural and urban areas.

### 4.3.3 Patients and anti-retroviral therapy (ART) use

Table 4.4 and Table 4.5 below presents the results of cryptococcal antigen positive among HIV infected patients by ART use and duration of treatment.

### Table 4.4 Treatment status of all HIV-infected patients screened for cryptococcus the Intermediate Hospital Oshakati, July-September 2015

<table>
<thead>
<tr>
<th>Treatment status</th>
<th>Negative (%)</th>
<th>No. positive (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>On ART</td>
<td>325 (93.39)</td>
<td>35 (97.22)</td>
<td>360(93.75)</td>
</tr>
<tr>
<td>Not on ART</td>
<td>23 (6.61)</td>
<td>1 (2.78)</td>
<td>24(6.25)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>348 (100.00)</strong></td>
<td><strong>36 (100.00)</strong></td>
<td><strong>384(100.00)</strong></td>
</tr>
</tbody>
</table>

Pearson $\chi^2 = 0.8174 \ df = 1 \ p = 0.366$

As shown in Table 4.4, the majority of patients attending the Intermediate Hospital Oshakati enrolled into the study were being prescribed anti-retroviral therapy (ART). Out of the 384 HIV patients in our study, 360 (94%) were already on treatment of
which 35 (97.22%) had a positive cryptococcal antigen test. Among the 24 (6.25%) patients not on treatment, only 1 (2.78%) was diagnosed with cryptococcus. The Pearson Chi-square test revealed that there was no significant difference in the prevalence of cryptococcus among HIV infected patients on ART or those not on ART ($\chi^2_{1}=0.174, \ df=1, \ p=0.366$).

Table 4.5: Prevalence of cryptococcus among HIV-infected Patients by duration of treatment, Intermediate Hospital Oshakati, July-September 2015

<table>
<thead>
<tr>
<th>Duration of Treatment</th>
<th>Negative (%)</th>
<th>Positive (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not on treatment</td>
<td>18 (5.19%)</td>
<td>1 (2.78%)</td>
<td>19 (4.95%)</td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>58 (16.67%)</td>
<td>29 (80.56%)</td>
<td>87 (22.66%)</td>
</tr>
<tr>
<td>1-2 years</td>
<td>66 (18.97%)</td>
<td>5 (13.89%)</td>
<td>71 (18.49%)</td>
</tr>
<tr>
<td>3-4 years</td>
<td>62 (17.87%)</td>
<td>0 (0.00%)</td>
<td>62 (16.15%)</td>
</tr>
<tr>
<td>≥5 years</td>
<td>144 (41.38%)</td>
<td>1 (2.78%)</td>
<td>145 (37.76%)</td>
</tr>
<tr>
<td>Total</td>
<td>348 (100.00%)</td>
<td>36 (100.00%)</td>
<td>384 (100.00%)</td>
</tr>
</tbody>
</table>

$\chi^2 = 78.8987, \ df = 4, \ p < 0.001$

A Chi-square test showed a significant association between duration of treatment and Cryptococcus positivity ($\chi^2 = 78.8987, \ df = 4, \ p < 0.001$). As it can be seen from Table 4.5 and Figure 4.5, the majority (80.56%, 29/36) had treatment duration of less than 1 year.
As shown in Table 4.4, the majority of patients with positive cryptococcal antigen test (94%) were receiving ART with mean duration of 37 months. In Table 4.5 and Figure 4.3, the duration of treatment was categorized as not on ART, <1 year, 1-2 years, 3-4 years and ≥ 5 years. The majority of the cases were patients who were on treatment for less than 1 year (80.56%) followed by 1-2 years (13.89%) of treatment. There was one case (2.78%) in the category of “not on ART” and duration of ≥ 5 years.
Table 4.6 Prevalence of Cryptococcus by age and duration of treatment in the most affected age groups (21-30 and 31-40 years)

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>Positive cryptococcal antigen cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group of 21-30 years</strong></td>
<td></td>
</tr>
<tr>
<td>Not on Treatment</td>
<td>1 (10.00)</td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>7 (70.00)</td>
</tr>
<tr>
<td>1-2 years</td>
<td>2 (20.00)</td>
</tr>
<tr>
<td>3-4 years</td>
<td>0</td>
</tr>
<tr>
<td>≥ 5 years</td>
<td>0</td>
</tr>
<tr>
<td><strong>Age group of 31-40</strong></td>
<td></td>
</tr>
<tr>
<td>Not on treatment</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>9 (90.00)</td>
</tr>
<tr>
<td>1-2 years</td>
<td>1 (10.00)</td>
</tr>
<tr>
<td>3-4 years</td>
<td>0</td>
</tr>
<tr>
<td>≥ 5 years</td>
<td>0</td>
</tr>
</tbody>
</table>

The majority of positive cryptococcal antigenemia were in the age group of (21-30 and 31-40 years) and constituting patients who had been on treatment for less than one year. In the age group of 21-30 years the duration of treatment less than 1 year had 9 (70%) cryptococcus cases while in 31-40 years was 9 contributing 90%.
4.4 CD4 COUNT LEVELS AND CRYPTOCOCCAL ANTIGEN TEST

Table 4.7 Prevalence of serum cryptococcus by CD4 count among HIV-infected patients attending the Intermediate Hospital Oshakati, July-September 2015

<table>
<thead>
<tr>
<th>CD4 count(cells/ul)</th>
<th>No. Negative (%)</th>
<th>No. positive (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤100</td>
<td>44 (12.6)</td>
<td>26 (72.22)</td>
<td>70 (18.23)</td>
</tr>
<tr>
<td>101-200</td>
<td>30 (8.62)</td>
<td>9 (25.00)</td>
<td>39 (10.16)</td>
</tr>
<tr>
<td>201-300</td>
<td>54 (15.52)</td>
<td>0</td>
<td>54 (14.06)</td>
</tr>
<tr>
<td>301-400</td>
<td>51 (14.66)</td>
<td>1 (2.78)</td>
<td>52 (13.54)</td>
</tr>
<tr>
<td>401-500</td>
<td>35 (10.06)</td>
<td>0</td>
<td>35 (9.11)</td>
</tr>
<tr>
<td>501-600</td>
<td>40 (11.49)</td>
<td>0</td>
<td>40 (10.4)</td>
</tr>
<tr>
<td>&gt;600</td>
<td>94 (27.01)</td>
<td>0</td>
<td>94 (24.48)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>384 (100.00)</strong></td>
<td><strong>36 (100.00)</strong></td>
<td><strong>384 (100.00)</strong></td>
</tr>
</tbody>
</table>

$\chi^2=98.6135$  df=6  
p=<0.001

Out of the 384 HIV-infected patients enrolled for this study, 36 (9.38%) had a positive serum cryptococcal antigen. Among these 36 patients the CD4 counts ranged from 2-301 cells/ul and median CD4 count was 72 cells/ul. Of the 36 positive cryptococcus cases, 26 (72.22%) had CD4 counts below 100 cells/ul. When stratified by CD4 count, 72.32% of patients with ≤100 cells/ul had a positive cryptococcal antigen test as compared to 25.00% with CD4 count levels between 101-200 cells/ul and 2.78% with CD4 >200 cells/ul (Table 4.7). There was a significant association between the CD4 count levels and the Cryptococcus serum positivity ($\chi^2=98.6135$, df=6, p=<0.001).
df=6, p<0.001), implying that significantly more HIV-infected patients who tested positive for Cryptococcus had lower levels of CD4 count (35/36 or 97.2% of the Cryptococcus positive patients had CD4 < 200 cells/ul).

Figure 4.4 Cryptococcal antigen test titres among HIV-infected patients attending the Intermediate Hospital Oshakati, July-September 2015

The cryptococcal antigen test titres ranged from 1:20 to 1:320. Among the 36 (100.00%) who had a positive cryptococcal antigen test, the majority of the HIV-infected patients (55.56%) had titres of 1:160 followed by 1:80 (27.78%).
Table 4.8 Titre levels of patients with positive cryptococcal antigen to CD4 count ≤100 cells/ul

<table>
<thead>
<tr>
<th>Titre levels</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:20</td>
<td>1 (3.85)</td>
</tr>
<tr>
<td>1:40</td>
<td>3 (11.54)</td>
</tr>
<tr>
<td>1:80</td>
<td>5 (19.23)</td>
</tr>
<tr>
<td>1:160</td>
<td>15 (57.69)</td>
</tr>
<tr>
<td>1:320</td>
<td>2 (7.69)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (100)</td>
</tr>
</tbody>
</table>

As shown in Table 4.8 above, the highest number of HIV patients (57.69%) who had a positive cryptococcal antigen test and CD4 counts less than or equal to 100 cells/ul had a cryptococcal antigen titer of 1:160

Table 4.9 Titre levels of patients with a positive cryptococcal antigen test to CD4 count between 101-200 cells/ul

<table>
<thead>
<tr>
<th>Titre levels</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:80</td>
<td>1 (33.33)</td>
</tr>
<tr>
<td>1:160</td>
<td>2 (66.67)</td>
</tr>
<tr>
<td>Total</td>
<td>3 (100.00)</td>
</tr>
</tbody>
</table>

Among patients with CD4 counts between 101-200 cells/ul, titers were 1: 80 or greater (table 4.9)
Table 4.10 Effects of treatment on the cryptococcal antigen titres among HIV patients attending the Intermediate Hospital Oshakati, July-September 2015

<table>
<thead>
<tr>
<th>Duration of treatment (ART)</th>
<th>Cryptococcus titres (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not on ART</td>
<td>1:160 (100%)</td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>1:160 (87.6%)</td>
</tr>
<tr>
<td>1-2 years</td>
<td>1:80 (95.5%)</td>
</tr>
<tr>
<td>3-4 years</td>
<td>-</td>
</tr>
<tr>
<td>≥ 5 years</td>
<td>1: 20 (100%)</td>
</tr>
</tbody>
</table>

As shown in Table 4.10, the cryptococcal antigen titers decreased with the duration of treatment. The subjects not on ART or who had been on treatment for less than 1 year had higher titres (1:160) compared to the subjects with long duration of ART use (1-2 years: 95.5% of the titres were 1:80 and ≥ 5 years: all the titres were 1:20).
As shown in Figure 4.5, the majority of patients with positive cryptococcal antigen were the ones with CD4 counts less than 100 cells/ul (62%) followed by CD4 counts between 101-200 cells/ul (9%). The ART use was well distributed among HIV patients of all categories and only few were not on treatment yet (CD4 counts greater than 500 cells/ul). In this study the Pearson Chi-Square statistics indicated that the p value on the association between CD4 count levels and cryptococcal antigenemia was 0.00054 (p value <0.05).
Table 4.11 presents the results on cryptococcal titer levels within age groups. The majority of the cases had titers of 1:160 in both age groups. Bivariate analysis produced a p-value of 0.56 between titers and age group (P value > 0.05).

<table>
<thead>
<tr>
<th>Cryptococcal antigen titre levels</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group: 21-30 years</strong></td>
<td></td>
</tr>
<tr>
<td>1:20</td>
<td>0</td>
</tr>
<tr>
<td>1:40</td>
<td>0</td>
</tr>
<tr>
<td>1:80</td>
<td>2 (20.00)</td>
</tr>
<tr>
<td>1:160</td>
<td>8 (80.00)</td>
</tr>
<tr>
<td>1:320</td>
<td>0</td>
</tr>
<tr>
<td><strong>Age group: 31-40 years</strong></td>
<td></td>
</tr>
<tr>
<td>1:20</td>
<td>0</td>
</tr>
<tr>
<td>1:40</td>
<td>0</td>
</tr>
<tr>
<td>1:80</td>
<td>2 (20.00)</td>
</tr>
<tr>
<td>1:160</td>
<td>7 (70.00)</td>
</tr>
<tr>
<td>1:320</td>
<td>1 (10.00)</td>
</tr>
</tbody>
</table>
4.5 SUMMARY

The findings/ results of the study are presented in this chapter. The presentation places particular emphasis on the prevalence of cryptococcus among HIV-infected patients attending the Intermediate Hospital Oshakati (n=384). Also presented are the levels at which CD4 counts could be attributed to the positive cryptococcal antigen test. The next chapter discusses the relationship between this current study and other published studies.
CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 INTRODUCTION

This chapter will focus on the interpretation of the main findings of the study. The interpretation of the findings will be presented on the relationship between this current study and studies previously done in other countries. In this chapter, research findings are summarised and conclusions are drawn in the context of the purpose and stated objectives of the study. From these conclusions, a number of recommendations are formulated and presented with acknowledgement of the limitations of the study. These limitations are highlighted and placed in context.

5.2 DISCUSSION OF THE FINDINGS

5.2.1 Prevalence of cryptococcus among HIV patients

In this study, we determined the prevalence of cryptococcus among HIV infected patients attending the Intermediate Hospital Oshakati. To our knowledge, this is the first study to assess the prevalence of cryptococcus (cryptococcal antigenemia) among a cohort of HIV-infected patients in the Northern regions. This study demonstrates a high prevalence (9.38%) of cryptococcus among HIV-infected patients receiving their CD4 count measurements at the Communicable Disease Clinic, Intermediate Hospital Oshakati.
The findings of this study concur with the high prevalence of cryptococcal antigenemia of 9.2% in Thailand (Pongsai, Atamasirikul and Sungkanuparph, 2010). Furthermore, a higher prevalence (21%) has been reported in Cambodia (Micol, Lortholary and Sar et al, 2007), 12.2% in Congo, 13.5% in Kampala and 12.9% in Bangkok (Liechy, Solberg and Were, et al, 2007 and Meya, Manabe and Castelnuovo et al, 2010). The prevalence report of this study is relatively higher than prevalence rates of other countries; 7% was reported in a retrospective study on anti-retroviral therapy naïve patients in South Africa (Jarvis, Lawn and Vogt et al, 2009) and 6.8% in Botswana (Lingsta, 2012).

In contrast with this current study, a study done in a rural community clinic in Tororo, Uganda, reported a low prevalence (4.5%) of cryptococcal antigenemia among HIV-infected patients. The low prevalence was most likely because only patients who had no symptoms participated in the study (Liechy, Solberg and Were, et al, 2007). In another study carried out in Tanzania, a prevalence of 4.5% was reported. Despite the low prevalence of cryptococcal antigenemia reported and high rates of ART use in their study, cryptococcal infections still accounted for 26% of all in-hospital AIDS deaths (Wajanga, Kalluvya and Downs et al, 2011). At two hospitals in Southwest London comprising an international population, there was a 5% prevalence of cryptococcus in newly diagnosed HIV-infected patients. All these prevalence of cryptococcal infections were lower than the prevalence of this current study.
The high positivity rate of cryptococcus in this study represents the burden of cryptococcal infection among HIV-infected patients attending the Intermediate Hospital Oshakati. An ongoing study evaluating the prevalence of fungal opportunistic infections in HIV patients in the same hospital at NIP laboratory, using culture and microscopy already revealed a 10.2% prevalence of *Cryptococcus neoformans* (NIP, 2015). The study further goes to confirm the burden of cryptococcal infections in HIV-infected patients at that hospital. Thus, questions may arise regarding the routine screening of cryptococcal antigenemia among HIV-infected patients.

The findings of this study add to the mounting evidence for the public health importance of screening for cryptococcal antigenemia among HIV-infected patients in Namibia, particularly the Intermediate Hospital Oshakati. The overall prevalence of cryptococcus (9.38%) is in line with the results from South Africa (13%), Uganda (5-9%) and Kenya (6%) and this reaffirms that in Sub-Saharan Africa (SSA) the high positivity rates of cryptococcal infections are usually when looked for. However, compared to tuberculosis prevalence, there are limited data on the magnitude of cryptococcal disease in SSA even though both cryptococcal and tuberculosis have been estimated to be responsible for similar death rates in HIV-infected patients (Park, Wannemuehler and Marston et al, 2009).
Having a large number of people living with HIV in Namibia, the potential enormity of cryptococcal disease based on our findings is significant and urges for rapid scale-up of cryptococcal antigen screening among HIV-infected patients. The high prevalence of cryptococcal subclinical infection highlights the need for the implementation of routine cryptococcus screening in Namibia. Current WHO guidelines strongly emphasize and recommend consideration of cryptococcus screening in high prevalence resource-limited settings among HIV-infected patients.

In this study, the distribution of cryptococcal antigenemia among female and male HIV patients was almost the same. Male patients slightly had a higher seropositivity (52.78%) compared to their female (47.22%) counterparts. In the United States, more men compared to women have been reported to carry a higher burden of cryptococcal infections (Currie and Casadevall, 2009). The bivariate analysis revealed that there was no significant difference in the prevalence of cryptococcus in men and women among HIV-infected patients attending the Intermediate Hospital Oshakati. In contrast with our findings, a study done in Benin City, Nigeria, found out that female patients had a higher prevalence compared to male patients (Osazuwa,Dirisu, Okuonhgae and Ugbebor, 2012).

The majority of the cryptocococcus positive cases (27.78%) among HIV patients were in the age group of 21-30 and 31-40 years, but age did not play a significant role in
the serum cryptococcal antigen positivity in these patients. These findings are comparable with one study done in Benin City, Nigeria.

In this study, 94% of the patients enrolled into the study were already receiving anti-retroviral therapy (ART) the Intermediate Hospital Oshakati. Of the 36 cases of cryptococcal antigenemia in our study, 27 (80.56%) occurred in patients who had been on ART for the duration of less than 1 year and qualify as “ART-associated cryptococcosis” according to the new consensus definitions (Haddow, Colebunders and Meintjies, 2010). A Chi-square test showed a significant association between the duration of treatment and Cryptococcus positivity. This indicates that the risk of having positive Cryptococcal antigen is higher among patients having shorter duration of treatment compared to those having longer duration of ART. It was suspected that some if not all of these cryptococcal cases represent unmasking cryptococcal infection, but cannot make that diagnosis due to the absence of baseline cryptococcal investigations among HIV patients in this study. Similar to the same study conducted in Tanzania, 88% of the cases were reported in adults who had been on treatment for less than three months (Wajanga, Kulluvya and Downs, 2011).

In this current study, one case of positive cryptococcus was not receiving treatment yet because the CD4 count was initially more 500 cells/ul and therefore did not meet the requirement to be put on treatment. The treatment is only started when the CD4 count drops below 500 cells/ul (MoHSS, 2012).
A recent study conducted in Addis Ababa, Ethiopia, reported a prevalence of cryptococcal antigen of 10% among Ethiopians; however in contrast with the current study, 74% of the cohort was receiving ART for an average of three years (Alemu, Kempker and Tenna, et al, 2013). This may reflect a survival bias as the unmasking of cryptococcosis, and death typically occurs within the first four months of ART for those pre-ART CrAg positive with high titers (Meya, Manabe and Castelnuovo, et al, 2010; Jarvis, Lawn and Vogt et al, 2009).

**5.2.2 CD4 count levels and cryptococcal antigen test**

The distribution of cryptococcal antigenemia among patients in this study was highly varying according to CD4 count levels. HIV-infected patients with CD4 count ≤100 cells/ul had the highest prevalence which were closely followed by patients with CD4 count between 100-200 cells/ul and then >200 cells/ul. In bivariate analysis, the study found that CD4 count ≤100 cells/ul was significantly associated with a positive cryptococcal antigen test (p<0.05). The majority of patients (72.22%) with positive cryptococcus had a CD4 count less than or equal to 100 cells/ul. Several studies previously done in other countries have reported a consistently higher prevalence of cryptococcal antigenemia in patients with lower CD4 cell counts (Kisenge, Hawkins and Maro et al, 2007).
Our findings are comparable to the results of a study done in Uganda. The study indicated that patients with CD4 counts ≤100 cells/ul were more likely to have cryptococcal antigenemia (78%) compared to patients with CD4 counts >100 cells/ul. This result is also similar to that reported by Micol et al, (2007). The association between the two variables could be explained by the fact that low CD4 counts predispose HIV-infected patients to cryptococcal infections because of the weak and dysfunctional immune system. In resource-limited settings, screening of patients with a CD4 count less than 100 cells/ul for cryptococcus may even be more clinically relevant as CD4 tests become more available.

The results of higher prevalence of cryptococcus among HIV-infected patients with CD4 count ≤100 cells/ul are consistent with studies from Sub-Saharan Africa and Southeastern part of Asia. Micol et al, (2007) reported that in terms of potential use of cryptococcal antigen screening for patients starting ART, the majority of patients (90%) with positive cryptococcal antigen had a CD4 count ≤100 cells/ul (finding replicated in Cambodia). The reason for high prevalence among patients with CD4 counts ≤100 cells could be due to late diagnosis of HIV. A substantial proportion of patients are still presenting late with advanced immunodeficiency and this poses a high risk of opportunistic infections such as cryptococcal diseases.
For those diagnosed at a late stage for HIV, the question remains whether cryptococcal antigen screening at HIV diagnosis should be routinely recommended. To be 100% effective, screening of cryptococcus needs to be done prior to asymptomatic presentation the antigenic period, which ranges from a few weeks to months (French, Gray and Watera, et al., 2005). Current BHIVA guidelines recommend excluding cryptococcal infection in patients with <200 cells/ul but do not advocate routine screening or fluconazole prophylaxis (Asboe, Aitken and Booth et al., 2012).

This study also found positive cryptococcal antigenemia in patients (25.00%) with a CD4 count of between 101 - 200 cells/ul. High prevalence of cryptococcal antigenemia among patients with CD4 >100 cells/ul in this study could be due to a large number of patients who are already on ART. These patients could have developed the infection at lower CD4 counts prior to ART initiation and remained antigenemic as their CD4 count improved on ART. Another study done in Ethiopia reported that the majority of their positive cases (58%) were patients who had CD4 counts between 100 - 200 cells/ul (Alemu, Kempker and Tenna et al, 2013).

Serum cryptococcal antigen titres were measured among all 36 patients who tested positive for cryptococcal antigen test. The titres of most patients were 1:80 and 1:160 showing an association between low CD4 counts and very higher titres. The analysis revealed that the cryptococcal antigen titres were indirectly proportional to the duration of treatment. When the duration of treatment increases, the titre decreases.
This means that when patients are on treatment for a longer period, they are likely to be protected against cryptococcal infections. These findings are consistent with another study done in South Africa. The patients who were on treatment for more than three years did not test positive for cryptococcal antigenemia (Javis, Lawn and Vogt, et al., 2009).
5.3 CONCLUSION

In conclusion, it is worth noting that there is no routine screening of cryptococcal infections done by the MoHSS in Namibia, and as such established prevalence of cryptococcus found in this study are a true reflection of cryptococcal infections among HIV-infected patients attending the Intermediate Hospital Oshakati. Overall, 9.38% of all HIV-infected patients enrolled into this study tested positive for serum cryptococcal antigen test. This result indicates a high prevalence of cryptococcal antigenemia among HIV-infected patients attending the Intermediate Hospital Oshakati. Despite increased access and use of ART among these patients, the burden of cryptococcal infections among HIV-infected patients remains high.

Based on the study results there is a significant association (p<0.05) between cryptococcal antigen test and lower CD4 count levels. Prevalence of cryptococcus was significantly associated with CD4 counts ≤100 cells/ul. The HIV-infected patients with CD4 counts ≤100 cells had the highest prevalence (72.22%). Therefore the level of CD4 count below or equal to 100 cells/ul is highly associated with positive cryptococcal antigen test. However, there were cases of positive cryptococcus among HIV patients with CD4 count ≥100 cells/ul.
This study suffers from several limitations as described below:

- The study was done in a single referral hospital by retrospective review of patient medical records and testing patients’ blood samples and thus the number of enrolled patients was small. These facts may limit generalization of findings of the current study to the entire Namibian population.

- Limited statistical analysis did not allow the measurement of the magnitude of the association between cryptococcus and some other covariates.

- Fluconazole use among HIV-infected patients (for other purposes) was not recorded. Fluconazole prescribed to patients presenting with advanced HIV infection may have prevented the identification of cryptococcus in the patient serum. As such, the study may actually have underestimated the importance of cryptococcal antigenemia in this setting.
5.4 RECOMMENDATIONS

From the above presented conclusions in section 5.3 various recommendations are presented in accordance with the objectives and findings of the study.

Prevalence of cryptococcus among HIV-infected patients is high and as such it calls for drastic public health interventions spearheaded by the Ministry of Health and Social Services (MoHSS). It is recommended that MoHSS should take the leading role in implementing a routine screening of cryptococcus neoformans antigen among HIV patients with CD4 count ≤100 cells/ul. The HIV population should be informed about the importance of cryptococcal screening and the disease in terms of: what it is, its symptoms and community about cryptococcus, as this could lessen the burden on an already strained health care delivery system.

Antigen screening of patients with CD4 count ≤100 cells/ul could allow a targeted preemptive treatment strategy, reducing costs and related deaths. However important questions remain to be answered. What should be done when an HIV-infected patient has a positive cryptococcal antigen results? Many medical experts recommend lumber puncture to rule out cryptococcal meningitis. However, this would entail lumber punctures in a sustained number of HIV-infected patients and this would place an additional workload on overstretched ART programs and may not be acceptable to all HIV patients. There is limited evidence that fluconazole alone is
enough to prevent clinical disease. It is therefore recommended that below a certain cryptococcal antigen titer, daily fluconazole treatment may be adequate so that lumber puncture can be avoided. In a primary prophylaxis strategy, as opposed to preemptive treatment based on antigen screening, the patients would receive intermittent fluconazole. This study recommends that prospective researches are urgently required to address these issues and to test the benefits of antigen-based screening.

The key to reducing cryptococcus cases among HIV-infected patients (which might lead to early mortality), is early diagnosis of cryptococcal antigen in HIV-infected patients prior to initiation of ART therapy and integration of both ART and primary prophylaxis with Fluconazole.

5.5 SUMMARY

This chapter presented the interpretation of the main findings of the study based on the specific objectives. The study revealed a high prevalence of cryptococcus among HIV-infected patients attending the Intermediate Hospital Oshakati. The levels of CD4 count that was significantly associated with positive cryptococcal antigen test was CD4 count ≤100 cells/ul. The high prevalence of cryptococcus the Intermediate Hospital Oshakati calls for routine cryptococcal screening among HIV patients. This chapter further discussed the conclusion, limitations as well as the recommendations.
REFERENCES


CDC. (2014). *Preventing deaths due to Cryptococcus with targeted screening*. Atlanta.


MoHSS. (2013). Distich Health Information System. Oshana Region.


ANNEXURE A: Patient consent form

Dear Participants

My name is Tuyakula Nakale, a final year Masters of Science (Field Epidemiology) student at the University of Namibia, Faculty of Health Sciences, under the supervision of Dr Sheehama and Dr Nelumbu. I am conducting a research on the following topic:

“Prevalence of cryptococcus among HIV patients attending the Intermediate Hospital Oshakati”

The study aims to determine the prevalence of cryptococcal antigenemia among HIV patients attending the Intermediate Hospital Oshakati.

You are being requested to participate in the study of the above-mentioned topic. I, as researcher with your permission, will withdraw your blood sample that will be tested for cryptococcal meningitis. Your participation in this research is voluntary; you may refuse to participate if you are not comfortable with it.

The results of the study may be published but your name will not be revealed and no individual identification information will be provided. Although there may be no direct or immediate benefit derived from the study, the result of the study may generate the burden of cryptococcal antigenemia among HIV patients attending the Intermediate Hospital Oshakati.

I thank you for your participation.
Consent

I have read the above-informed consent, the nature, demands and benefits of the study.

I understand that I may withdraw my consent and discontinue participation if I am not comfortable.

Signature of the Participant: .................................. Date..................................

Signature of parent/Guardian (Participants under 18 years)..........................

Date: ..........................

I certify that I have explained to the above participant the nature, purpose, and potential benefits and risks associated with participation in this study.

Signature of the researcher ................................. Date.................................
ANNEXURE B: UNAM letter of permission

Date: 17 June 2015

TO WHOM IT MAY CONCERN

RE: RESEARCH PERMISSION LETTER

1. This letter serves to inform that student T Nakale, (Student number: 200826417) is a registered student in the School of Public Health at the University of Namibia. His research proposal was reviewed and successfully met the University of Namibia requirements.

2. The purpose of this letter is to kindly notify you that the student has been granted permission to carry out postgraduate studies research. The School of Postgraduate Studies has approved the research to be carried out by the student for purposes of fulfilling the requirements of the degree being pursued.

3. The proposal adheres to ethical principles.

Thank you so much in advance and many regards.

Yours truly,

Name of Main Supervisor: Dr J Sheehama
Signed: [Signature]

Dr. C. N.S. Shamenanya
Signed: [Signature]

Director: School of Postgraduate Studies
Tel: 2063523
E-mail: cshamenanya@unam.na
ANNEXURE C: UNAM ethical clearance

STUDENT ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SONPH/23/2015 Date: 27 May 2015

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project: Prevalence of Cryptococcal Meningitis Among HIV Patients Attending At Intermediate Hospital Oshakati
Nature/Level of Project: Masters

Principal Researcher: W. Nakale
Student Number: 200826417
Host Department & Faculty: School of Nursing and Public Health
Main Supervisor: Dr. J. Sheehama (Main) Dr. S. Antara (Co)

Take note of the following:
(a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.
(b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.
(c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.
(d) The UREC retains the right to:
   (i). withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
   (ii). request for an ethical compliance report at any point during the course of the research.

UREC wishes you the best in your research.

Prof. T. Mapaure
UNAM Research Coordinator
ON BEHALF OF UREC
ANNEXURE D: MoHSS ethical clearance

REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198  Ministerial Building  Tel: 061 – 203 2510
Windhoek  Harvey Street  Fax: 061 – 222558
Namibia  Windhoek  E-mail: shaama@mhss.gov.na

OFFICE OF THE PERMANENT SECRETARY

Ref: 17/3/3
Enquiries: Ms. E.N. Shaama

Date: 29 September 2015

Ms. Tuyakula Nakale
P.O. Box 55399
Rocky crest
Windhoek

Dear Ms. Nakale

Re: Prevalence of Cryptococcus among HIV patients attending at Intermediate Hospital Oshakati,

1. Reference is made to your application to conduct the above-mentioned study.
2. The proposal has been evaluated and found to have merit.
3. Kindly be informed that permission to conduct the study has been granted under the following conditions:
   3.1. The data to be collected must only be used for completion of your Master of Science in Applied Epidemiology;
   3.2. No other data should be collected other than the data stated in the proposal;
   3.3. Stipulated ethical considerations in the protocol related to the protection of Human Subjects’ information should be observed and adhered to, any violation thereof will lead to termination of the study at any stage;
   3.4. A quarterly report to be submitted to the Ministry’s Research Unit:
3.5 Preliminary findings to be submitted upon completion of the study;
3.6 Final report to be submitted upon completion of the study;
3.7 Separate permission should be sought from the Ministry for the publication of the findings.

Yours sincerely,

[Signature]

Andrew Mlishihi (Mr)
Permanent Secretary

"Health for All"
OFFICE OF THE CHIEF OPERATIONS OFFICER

15 July 2015

Enquiries: Boniface Makumbi

Ms Tuyuluka Nakale
Polytechnic of Namibia
School of Health and Applied Sciences
Department of Biomedical Science

RE: REQUEST FOR RESEARCH “THE PREVALENCE OF CRYPTOCOCCAL MENINGITIS AMONG HIV PATIENTS ATTENDING AT INTERMEDIATE HOSPITAL OSHAKATI.”

The above mentioned research was reviewed by the Research Committee of NIP and was thus approved with the following conditions:

- You are granted an approval from the Ministry of Health and Social Services.
- That you will carry the cost of conducting the tests.
ANNEXURE F: Permission letter from Oshana Regional Directorate

TO WHOM IT MAY CONCERNED

Re: Permission to Conduct a Study on prevalence of Cryptococcus among HIV patients attending at Intermediate Hospital Oshakati.

Kindly be informed that Ms. Tuyakula Nakale has been granted permission to conduct the above mentioned study at Intermediate Hospital Oshakati.

Please render her the necessary assistance and support.

Yours Sincerely,

[Signature]

SARKARE TAPOPI
REGIONAL DIRECTOR
ANNEXURE J: Data collection sheet

1.1 Demographic data

<table>
<thead>
<tr>
<th>Variable</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Residential area (Urban/Rural)</td>
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</tbody>
</table>

1.2 Laboratory Data

<table>
<thead>
<tr>
<th>Variables</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute CD4 count (ul/L)</td>
<td></td>
</tr>
<tr>
<td>CrAg (Positive/Negative)</td>
<td></td>
</tr>
<tr>
<td>CrAg Titre</td>
<td></td>
</tr>
</tbody>
</table>

1.3 Clinical Data

<table>
<thead>
<tr>
<th>ART treatment (yes/no)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of treatment</td>
<td></td>
</tr>
</tbody>
</table>