Disseminated Cryptococcal Infection in HIV-Infected Patients: A Retrospective Clinicopathological Review of 4 Autopsy Cases

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Moshawa Calvin Khaba[1], Mamokoma Becky Kgole[2], Lesedi Makgwethele Nevondo[1], Willem Johannes Van Aswegen[2], Tshegofatso Mabelane[3] and Ndivhuho Agnes Makhado[4]

[1]Department of Anatomical Pathology, Dr George Mukhari Academic Laboratory, National Health Laboratory Service, Sefako Makgatho Health Sciences University, Ga-Rankuwa, South Africa. [2]Department of Internal Medicine, Dr George Mukhari Academic Hospital, Sefako Makgatho Health Sciences University, Ga-Rankuwa, South Africa. [3]Department of Family Medicine, Job Shimankana Tabane Hospital, Sefako Makgatho Health Sciences University, Ga-Rankuwa, South Africa. [4]Department of Medical Microbiology, Dr George Mukhari Academic Laboratory, National Health Laboratory Services, Sefako Makgatho Health Sciences University, Ga-Rankuwa, South Africa.

ABSTRACT: Cryptococcosis is an opportunistic infection with high mortality if not diagnosed and treated in time. The objective of this study was to review the clinicopathological information of decendents with final autopsy diagnosis of disseminated cryptococcal infection. This study collected data from 4 decendents who presented to an academic hospital/laboratory between 1 January 2015 to 31 December 2018. Their clinical, radiological and pathological findings including treatment were reviewed. Two decendents presented with respiratory symptoms whilst the other 2 presented with meningeal symptoms. Three were confirmed HIV positive. One decendent was on ART, one had defaulted treatment and one was ART naïve. Two decendents were diagnosed with cryptococcal meningitis, one with bacterial pneumonia and one with pulmonary tuberculosis. Three decendents died in emergency unit and one in the ward whilst on antifungal therapy. The autopsy findings confirmed disseminated cryptococcal infection in all cases. A high index of suspicion should be maintained in the right clinical context. Multi-organ involvement should be suspected in all patients and be actively sought out.

KEYWORDS: Cryptococcosis, disseminated, human immunodeficiency virus, acquired immunodeficiency syndrome, autopsy

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CORRESPONDING AUTHOR: Moshawa Calvin Khaba, Department of Anatomical Pathology, Dr George Mukhari Academic Laboratory, National Health Laboratory Services, Sefako Makgatho Health Sciences University, Molotlegi Road, Ga-Rankuwa, Pretoria 0208, South Africa. Email: ckhaba@yahoo.co.uk

Introduction

Cryptococcal infection (CI) is an opportunistic infectious disease with high morbidity and mortality. The risk factor associated with this aggressive infection is immunodeficiency from variety of causes with Human immunodeficiency virus (HIV) as the commonest predisposing factors. However, it can also affect immunocompetent individuals.[1]

CI accounts for approximately 15% of AIDS-related diseases and is the second most common cause of death after tuberculosis.[2-6] CI is usually seen in patients with CD4+ count of less than 50 to 100 cells/mL.[7] CSF CrAg is the preferred method of diagnosis with a high sensitivity and specificity of 96% to 100% and 93% to 99% respectively.[5-8]

Despite extensive roll out of antiretroviral therapy (ART) in sub-Saharan Africa, cryptococcal-related mortality still remains high.[1]

Although clinical information/evidence is available on deaths related to cryptococcal infection, autopsy studies are still lacking which could be attributed to the declining number of autopsies performed globally. This decrease has contributed to lack of definitive diagnoses in cases with challenging antemortem diagnosis. Autopsy still remains the gold standard for assessing cause of death in challenging cases.[1]

In 2015, Venter et al.[9] reported that the number of autopsies had decreased from 308 in 1990 to 60 in 2010 in one academic centre in South Africa. This correlated with the global statistics.[10] In addition, a study done by Karat et al[11] revealed that only 4 cases had CI from a cohort of 34 cases in one South African Academic centre. Autopsy reports on acquired immunodeficiency syndrome (AIDS) defining infectious disease is still lacking, especially in South Africa where HIV infection is endemic.[12]

This study may add more information on the lesions of CI in HIV patients on other less emphasised organs.

Materials and Methods

This was a retrospective study of academic autopsies with final diagnosis of 'disseminated cryptococcal infection' from 1 January 2015 to 31 December 2018 in Academic Laboratory in South Africa. Departmental and hospital records of these decendents were accessed, where available, to record clinical details age (sex, race, HIV status, CD4+ T-lymphocyte count,

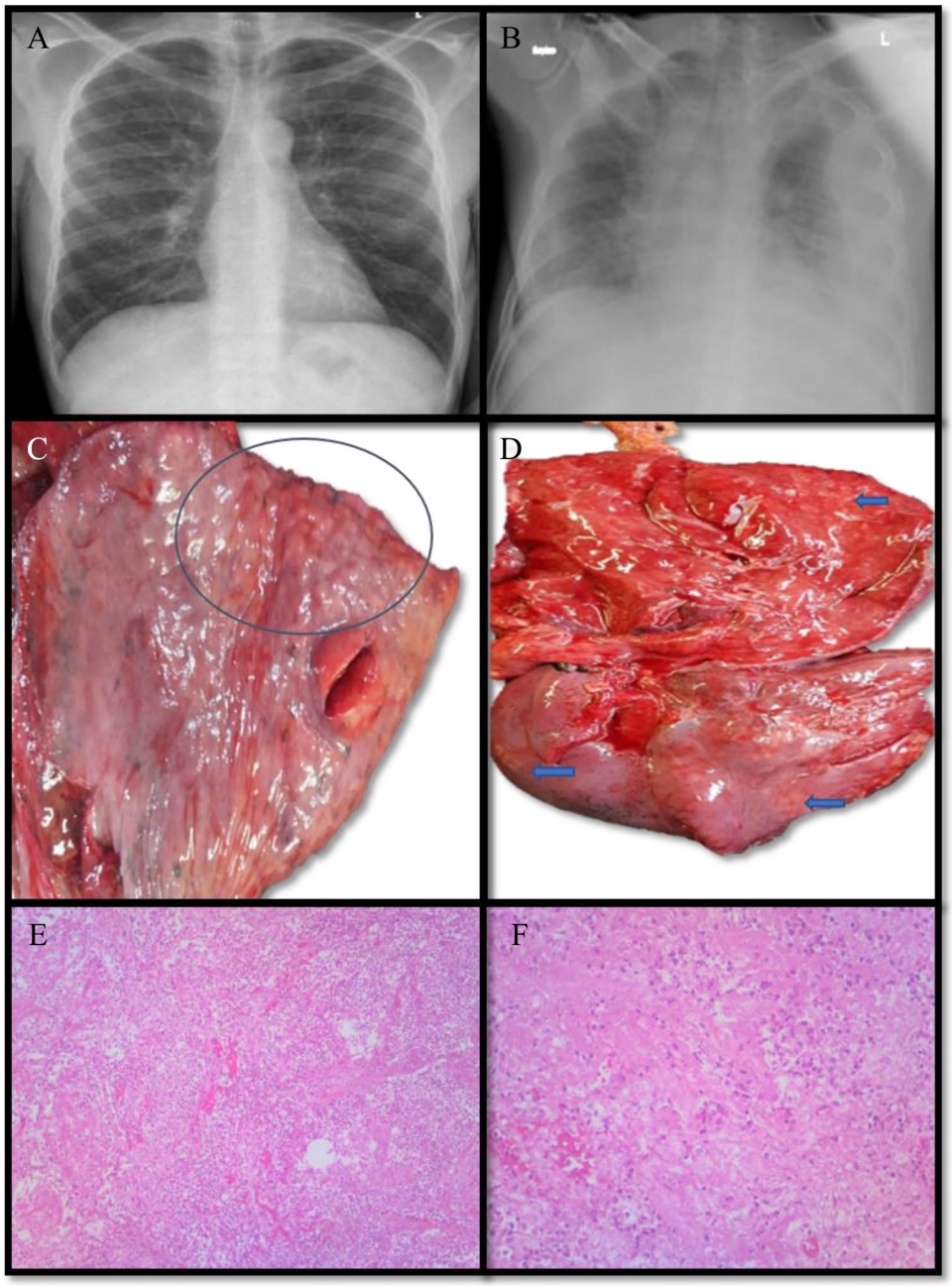

Figure 1. Chest X-ray: (A) widened mediastinum in keeping lymphadenopathy, (B) multiple reticulonodular infiltrates and left pleural effusion; Gross images, (C and D) lung with irregular and nodular surface (black circle and blue arrows) which is evident on cut surface (blue arrow); Microscopy: (E) acute suppurative inflammation of the lung and (F) necrosis.

ART, clinical presentation, CSF cryptococcal antigen, treatment). Extensive gross sampling of organs were performed which included lungs, liver, kidneys, spleen, heart, lymph nodes, bone marrow, thyroids and intestines. The brain was only sampled in 3 cases as consent was not given to remove the brain in one case. Stored haematoxylin and eosin, Periodic Acid Schiff (PAS) and Mucicarmine histochemical stained sections were reappraised.

Results

Within the study period, a total of 184 autopsies were performed, which comprised of 36 HIV infected decendents. There was a total of 4 cases with disseminated cryptococcal infection which consisted of 2 females and 2 males patients with a mean age of 40.3 years. Three decendents were HIV positive. Of the 3 patients, 2 had CD4 count with average of 5.5 cells/μL. One decendent was on antiretroviral therapy (ART), one defaulted treatment and one was ART naïve. Two decendents presented with respiratory symptoms whilst the other two presented with meningeal irritation. Of the 2 confirmed cryptococcal meningitis, only 1 received antifungal therapy for 2 weeks. Decendents who presented with respiratory symptoms were investigated for bacterial pneumonia and pulmonary tuberculosis. The chest X-ray of the latter and former showed mediastinal lymphadenopathy and bilateral infiltrates and pleural effusion, respectively (Figure 1A and B). The 3 decendents died in emergency units

Table 1. Clinicopathological features.

FEATURES	CASE 1	CASE 2	CASE 3	CASE 4
Clinical				
Age (y)	49	40	37	36
Sex	Male	Male	Female	Female
HIV status	Unknown	+	+	+
CD4+ count (cells/μL)	n/a	5	Unknown	6
ART	n/a	Defaulted	Yes	Naive
Chest X-ray	Mediastinal lymphadenopathy	Not done	Bilateral infiltrates and pleural effusion	Not done
CRP (mg/L)	Elevated	Elevated	Elevated	Elevated
Cryptococcal antigen (CrAg)	Not done	+	Not done	+
Clinical diagnosis	Pulmonary tuberculosis	Cryptococcal meningitis	Community Acquired pneumonia	Cyptococcal meningitis
Treatment	Antibiotics	Amphotericin B and Fluconazole	Died before started	Died before started
Gross				
Lung	Oedematous, pale and firm. Surface and parenchymal nodules	Oedematous and friable. Surface and parenchymal nodules	Oedematous, pale, congested and friable	Oedematous, congested, alternating firm and friable areas
Brain	Dusky surface. Features of raised intracranial pressure with tonsillar herniation	Dusky surface. Multiple cystic spaces in the basal ganglia and dentate nucleus. Necrosis of the anterior commissure	Not sampled	Dusky surface. Multiple cysts in the thalamus. Necrosis of anterior commissure
Others	Increased weight and size	Increased weight and size	Increased weight and size	Increased weight and size
Microscopy				
Organs involved	Brain, meninges, lungs, kidneys, liver, spleen, thyroid, lymph nodes	Brain, meninges, lungs, kidneys, liver, oesophagus	Lungs, liver and heart	Brain, meninges, Lungs, kidneys
Inflammatory response	Lungs	Lungs	Lungs	Lungs

and 1 died in the ward. The clinicopathological features of the 4 cases are presented in Table 1.

Pathological Findings

Three full and one limited autopsies were performed were performed under strict biosafety rules.

Grossly, the visceral organs evaluated showed increase in both size and weight. There was generalised lymphadenopathy.

All the cases had bilateral pleural effusion, oedematous and pale lungs with alternating firm and friable areas. Two cases showed surface and parenchymal nodules measuring 2to 3 mm (Figure 1C and D).

The brains from cases 1, 2 and 4 were dusky surface and non-suppurative. Features of raised intracranial pressure with tonsillar herniation were only evident in case 1 (Figure 2A). On cut section, case 2 and 4 showed multiple cystic spaces measuring 2 to 5 mm in diameter in the basal ganglia, thalamus and dentate nucleus (Figure 2B). Necrosis of the anterior commissure was also noted (Figure 2C).

Microscopically, the yeast infection affected multiple organs in all the cases evaluated which included the following organs: heart, lung, bone marrow, thyroid, lymph nodes, brain and spleen (Figure 3A-G; Table 1). Except for the lungs, none of these organs showed inflammatory response, however, they showed soap bubble appearance. The fungal yeasts were pleomorphic (4-10 μm), encapsulated with narrow-neck budding. They were highlighted by PAS and mucicarmine stained the capsule (Figure 3H and I).

The lungs from all 4 cases showed oedema, interstitial haemorrhage and chronic inflammation. Furthermore, in case 3 there was multinucleated giant cells while in case 4 there was necrosis and micro-abscess. Granulomatous inflammation was not seen.

None of the organs examined showed other opportunistic infections.

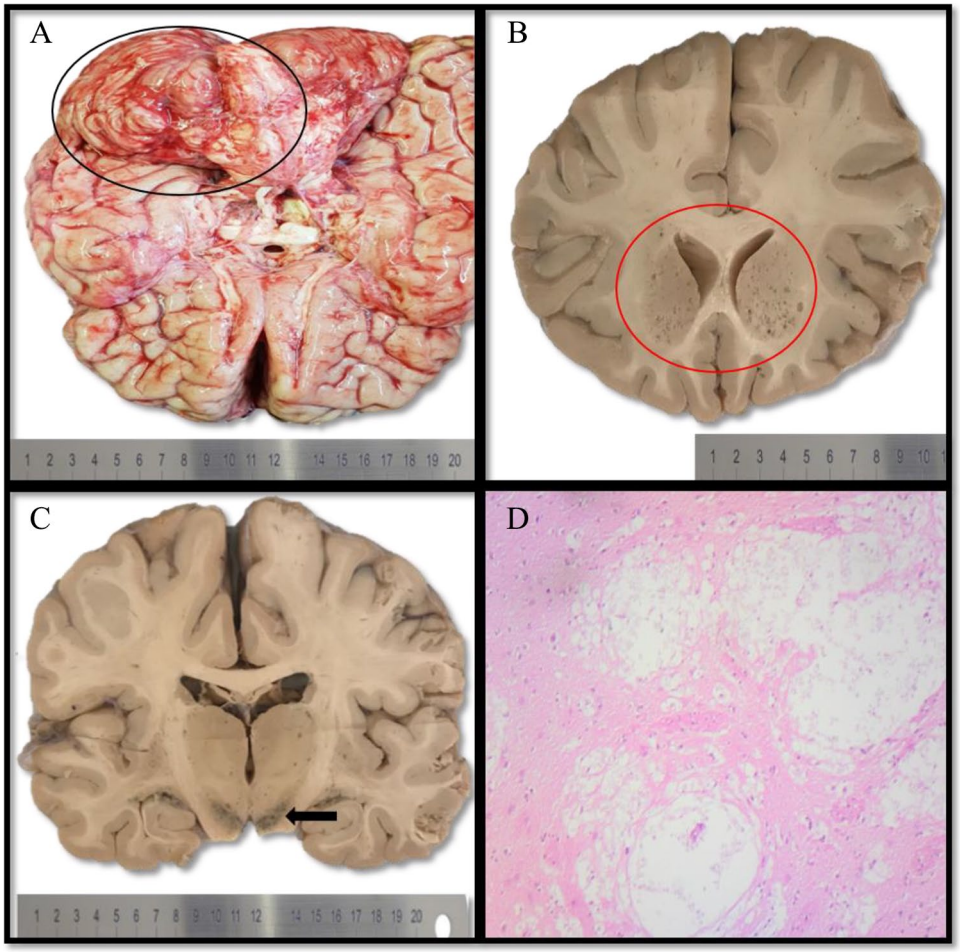

Figure 2. Brain: (A) gross features demonstrating widened sulci and flattened gyri with associated tonsilar herniation (black circle), (B) microcysts at the thalamus (red circle), (C) necrosis of the anterior commissure (black arrow) and (D) microscopic image showing soap bubble appearance.

Discussion

Central Nervous system (CNS) and lung are commonly affected in cryptococcosis.[2,5] Whilst CNS symptoms are often easier to identify, lung infection is probably underdiagnosed clinically.[13] Pulmonary CI still poses a clinical diagnostic conundrum with other infections, especially tuberculosis and *Pneumocystis jirovecii* pneumonia in HIV endemic areas.[3,14] This was evident in 2 of the cases who presented with pulmonary symptoms in which an antemortem diagnosis was challenging.

Our study shows lung abnormalities of varying degree on all 4 cases. Two cases showed parenchymal nodules with associated friability. The other 2 cases showed oedema, congestion and friability without nodules.

Cryptococcal neoformans is an opportunistic pathogen, therefore, it is expected that HIV situation would provide enabling environment for the fungus to thrive. Articles abound on pulmonary cryptococcosis and they do not present special pulmonary lesions in HIV patients different from the lesions in other immunocompromised patients. What may vary may be in the severity and distribution of the lesions, which may likely be on a case by case basis.

McDonnell and Hutchins classifies pulmonary cryptococcosis into peripheral pulmonary granuloma, granulomatous pneumonia, intracapillary-interstitial involvement, and massive pulmonary involvement.[12] In this study, all 4 cases had intracapillary-interstitial involvement. Case 3 showed multinucleated giant cells while case 4 showed acute suppurative inflammation with micro-abscess and necrosis (Figure 1E and F).

Gross CNS features of CI include intracerebral multiple small and larger cysts within the cortical and subcortical areas.[12,15] The most involved areas include the basal ganglia, midbrain and the cerebellum.[15] All 3 cases presented with small cysts in the thalamus with necrosis of the anterior commissure (Figure 2B and C). The significance of the latter is unknown as it has not been a frequent feature in previous studies.

Microscopically, the CNS involvement is classified as meningitis, meningoencephalitis and encephalitis. This is based on the pattern of distribution and amount of fungal yeasts, and inflammatory response. Furthermore, granulomas, pseudocysts, oedema, fibrosis and necrosis are seen.[15] None of the cases showed inflammatory reaction nor granulomas. Visible gross

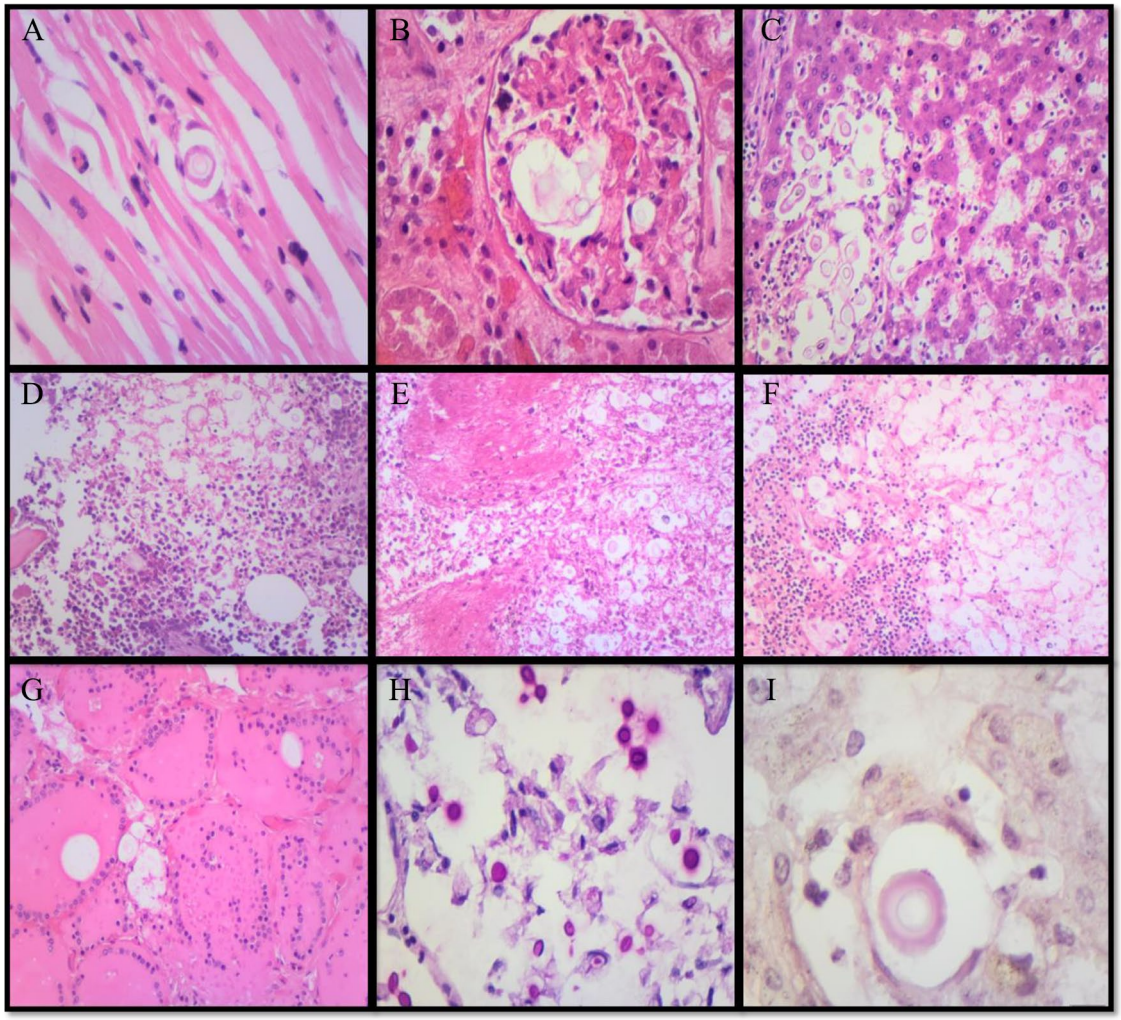

Figure 3. (A-G) Microscopy of affected organs. (A) Heart, (B) kidney, (C) liver, (D) bone marrow, (E) spleen, (F) lymph node, (G) thyroid; histochemical stains, (H) Periodic Acid Schiff (PAS) highlights the fungal yeast and (I) mucicarmine highlights thick mucinous capsule.

cystic spaces in the brain corresponded to the 'soap bubble appearance' microscopically (Figure 2D).

Other affected organs identified at autopsy in patients with CI include lymph nodes, thyroid, bone marrow, liver, spleen and kidneys; however, during life, these localisations are rarely suspected and identified.[17] In our study, the brains, lungs, heart, kidneys, spleens and thyroids were increased in size and weight without overt gross features and microscopic inflammatory response.

Previous autopsy studies on CI in HIV patients focussed mainly on CI in CNS involvement and DCI without histomorphological descriptions.[15]

Whilst an autopsy study by Klock et al.[15] seems to be largest to date, it only concentrated on CNS features of CI rather than on all organs in contrast to studies by Hurtado et al.[2] and Subedi et al.[16] Hurtado et al.[2] compared autopsy cases from Mozambique and Brazil in which the Brazilian cohort consisted of a total of 17 cases from total of 284 cases which consisted of 163 of HIV positive cases; whilst the Mozambican cohort consisted of 11 cases from a total of 169 patients from which 109 were HIV-infected. The most recent case report has been described by Subedi et al.[16] in 2021 about an HIV positive patient with DCI. In all these studies, the cases were HIV positive except on only one case in a study by Hurtado et al. where the patient was HIV negative. Whilst the CNS and lung involvement were the commonest in all these studies, other organs such as spleen, liver, bone marrow, lymph nodes, etc were involved as evident in our cohort.[2,16] Furthermore, there was no co-infection such as candidiasis, tuberculosis and histoplasmosis in our cases as was evident in some of the cases from a study by Hurtado et al.[2]

A case by Subedi et al.[16] may be a lesson that minimally invasive autopsy could be an important alternative to complete autopsy in the diagnosis of CI or any other instances where complete autopsy is not feasible. All these studies further highlight the importance of performing autopsy in HIV infection to determine the cause of death, especially where an antemortem diagnosis is not obvious and/or challenging. Microscopic assessment of organs involved in DCI may aid in describing different reaction patterns identified on individual organs.

The recommended treatment for disseminated infection and cryptococcal meningitis includes Amphotericin B and Fluconazole.[6,17,18] However, treatment relapse, failure, or immune reconstitution syndrome (IRIS) may be encountered. Resolution of symptoms and signs should be used to monitor the response to treatment.[1,19] Persistent infection should immediately raise concern for the presence of fluconazole resistance, IRIS and treatment failure. With regards to case 2, it is possible that the patient might have had treatment failure, as there was no improvement after completing 2 weeks of induction phase. Moreover, microscopic features related to treatment were not identified on autopsy assessment. According to Li et al.[20] morphological changes of the fungi after treatment include yeasts changing from round cells with single budding, to enlarged multiple budding. This was not evident in case 2 which may further raise concern for treatment failure.

Strength of the Study

According to our knowledge, this is the first autopsy study from South Africa with disseminated cryptococcal infection. This study will add to the scarce knowledge on disseminated cryptococcal infection from autopsy studies.

Limitation to the Study

This was a retrospective study with some information missing, therefore, compromising comprehensive appraisal of all these cases. In addition, the sample size was very small and therefore, statistically insignificant.

Conclusion

There are still cases of individuals living with HIV who die before a definitive diagnosis is made and deemed natural cause. CrAg screening and prophylactic treatment are fundamental in HIV patients with advanced disease. Autopsy plays an important part in determining the cause of death in HIV infected patient and the extent of disease. Clinicians have a critical role to play in determining the final cause of death by requesting autopsies. Moreover, more autopsies including minimally invasive will aid in further elucidating gross morphological and inflammatory reaction patterns associated with disseminated cryptococcal infection.

Author Contributions

All authors wrote the manuscripts. Dr MC Khaba and Dr N Makhado organized and critically revised the manuscript.

ORCID iD

Moshawa Calvin Khaba https://orcid.org/0000-0001-6999-6836

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