

Prevalence of Cryptococcal Infection in Patients Clinically Diagnosed to Have Meningitis in Ethiopia

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To cite this article:

Adane Bitew, Mulu Hassen, Tigist Getachew, Surafel Fentaw. Prevalence of Cryptococcal Infection in Patients Clinically Diagnosed to Have Meningitis in Ethiopia. *Clinical Medicine Research*. Vol. 5, No. 4, 2016, pp. 73-76. doi: 10.11648/j.cmr.20160504.12

Received: May 21, 2016; **Accepted:** May 31, 2016; **Published:** June 14, 2016

Abstract: Cryptococcal meningitis is a fungal infection that causes significant morbidity and mortality worldwide. However, its incidence in Ethiopia is poorly known. The present study was undertaken to determine cryptococcal meningitis in Ethiopian patients diagnosed to have meningitis. Cerebrospinal fluid was collected from 201 patients with meningitis attending two tertiary hospitals for the isolation and characterization of major bacterial pathogens implicated in causing meningitis. Left over sample of each patient was screened for cryptococcal antigen using lateral flow Assay. Of the total number of 201 patients 17 (8.5%) were positive for cryptococcal antigenemia of which 8 (47.1%) were male patients while 9 (52.9%) were female patients. In conclusion a high prevalence of cryptococcal meningitis in the present study urges detection and /or isolation and characterization of the pathogen in patients diagnosed to have meningitis regardless of the immune status. Furthermore, in view of the increasing number of cryptococcal infections, rapid, sensitive, and specific diagnosis of cryptococcal disease has become more important than ever in Ethiopian health institutions.

Keywords: Cryptococcus, Meningitis, Ethiopia

1. Introduction

Cryptococcal meningitis is an infectious disease that affects predominantly immune compromised hosts causing significant morbidity and mortality. Although cryptococcosis has been recognized for more than 100 years, worldwide spread of HIV infection and other forms of immunosuppression are incriminated as the most important factors for its increase since the 1980's [1]. Currently, it has been estimated that there are about one million new cases of cryptococcosis and at least 600,000 deaths annually worldwide due to HIV-associated cryptococcosis [2]. In sub-Saharan Africa with the highest HIV prevalence, *Cryptococcus* species has become the leading cause of meningitis, preceding pneumococcal and meningococcal meningitis [3-9]. The infection accounts 13-44% of all deaths in HIV infected individuals in this geographical region [10-12].

Although cryptococcal meningitis is generally thought to be associated with immune-compromised patients (i.e., HIV-positive patients and non-HIV-infected immunocompromised patients), recent studies [13-15] showed that its incidence has increased among immunocompetent individuals. Ethiopia being located in sub-Saharan Africa, the morbidity and mortality of cryptococcal meningitis is poorly known. To the best of our knowledge, two case reports [16, 17] and three full flagged research articles in HIV infected patients [18-20] have been published with regards to cryptococcal infection.

An accurate diagnosis of cryptococcosis is important for its successful treatment. The cost and long duration of the therapy, the risk of developing adverse drug reactions, and possible interactions with concomitant medications all underline the importance of accurate diagnosis of the condition before commencing therapy. Therefore, early identification of cryptococcal infection regardless of the immune status of a host and clinical manifestations is

indispensable for appropriate management of the complication.

Demonstrating capsulated yeast by India ink preparation, culture and detection of capsular polysaccharide antigen serologically from various clinical samples (CSF, serum, urine) has been the mainstay for the diagnosis of cryptococcal infections. Although culture is considered as a gold standard for the isolation and characterization of the yeast, fungal culture is not performed in routine clinical laboratory practice in Ethiopia. Currently, the diagnostic tests most commonly used in the developed world for identifying cryptococcal antigen are the latex agglutination test (LA) and the enzyme immunoassay (EIA). These tests are not practiced in clinical laboratories in Ethiopia since they are resource intensive. Therefore, detection of the yeast by less sensitive and non-specific India ink preparation is the commonest test practice in clinical laboratories in Ethiopia. Lack of sensitive and specific test procedures in Ethiopian health institution have been identified as major problem in the under reporting of the infection. Difficulty in differentiating signs and symptoms of cryptococcal meningitis clinically from those of many other forms of meningitis has also hampered the study of cryptococcal meningitis. In line with, the aim of this study was to determine cryptococcal infection in patients diagnosed to have meningitis regardless of their clinical manifestations and immune status.

2. Materials and Methods

2.1. Study Patients

Patients diagnosed to have meningitis were enrolled between September 2006 and August 2007 from the outpatient department of Tikur Anbessa (Black Lion) and Yekatit twelve teaching hospitals for the isolation and characterization of major bacterial pathogens implicated in causing meningitis. CSF was collected from 201 patients and left over sample was used for the present study.

2.2. Laboratory Diagnosis

Left over CSF sample was transferred to the Department of Medical Laboratory sciences and kept in a deep freezer at minus 80°C until use. Each sample was then screened for CrAg using CrAg Lateral flow assay (Immuno-Mycologics Inc., Oklahoma, USA) as per the instruction of the manufacturer. The Lateral flow assay uses immunochromographic test strips that have been impregnated with monoclonal antibodies against capsular polysaccharide antigens common to fungi in the Cryptococcal species complex (*C. neoformans* and *C. gattii*). To perform the assay, 40 microliters of CSF was mixed with one drop of LF specimen Diluent (GLF025). Then the white end of a Cryptococcal Antigen Lateral Flow Test Strip (LFCR50) was submerged into the preparation and the result was read after 10 minutes at room temperature. The presence of two lines (test and

control) on the test strip regardless of the intensity of the test line were interpreted as positive result, while the presence of a single control line was read as a negative result.

2.3. Ethical Clearance

All ethical considerations and obligations were duly addressed and the study was conducted after the approval of the Department Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University and the Addis Ababa City Health Bureau. Informed written consent was obtained from participants before data collection. The respondent was given the right to refuse to take part in the study and to withdraw at any time during the study period. Assent form was completed and signed by family member and/or adult guardian for participants under the age of 18 years.

3. Result and Discussion

With a declining incidence of AIDS-related cryptococcosis in the Highly Active Antiretroviral Therapy (HAART) era, and increasing use of immune-suppressive agents worldwide [21, 22], non-HIV infected individuals (with or without predisposing factors) have become more frequently infected by the yeast. Ethiopia being a developing nation located in sub-Saharan Africa where anti-retroviral therapy is less available appears to fall into the category of regions with high prevalence of cryptococcosis. Furthermore, in contrast to immune-compromised patients, few reports have described the variety of *C. neoformans*, clinical presentation, cerebrospinal fluid (CSF) parameters and prognosis associated with cryptococcal infections among immunocompetent individuals [13]. To this effect, screening of cryptococcal infection in Ethiopian patients clinically diagnosed to have meningitis regardless of the immune status appears to be of a highest priority. Consequently, in the present study a total of 201 samples were collected from patients clinically diagnosed to have meningitis. Among study subjects, 106 (52.7%) were males and 96 (47.8%) were females. The ages of study subjects ranged from 1 day to 75 years with a mean age of 26 years. Of the total number of 201 patients 17 (8.5%) were positive for cryptococcal antigenemia of which 8 (4%) were male patients while 9 (4.5%) were female patients. As shown in table 1, the finding of the present study was comparable with the findings of previous studies conducted in Ethiopia with HIV infected patients [18-20] but much lower than similar studies conducted in AIDS patients in other sub-Saharan African countries [3, 9, 23-28]. Index of suspicion, patient selection and intensity of evaluation, variation in test procedures and type of clinical sample used for detection and/or isolation and characterization the etiologic agent may have attributed to such a variation.

Table 1. Prevalence of cryptococcal meningitis in HIV patients: review of available literature in sub Saharan African countries.

| Country | References | Total cases of meningitis | Cryptococcal meningitis, n(%) | Study population | Sample | method of identification |
|--------------------------|----------------------------|---------------------------|-------------------------------|------------------|--------|-------------------------------|
| Ethiopia | Seboxa et al [18] | 375 | 31 (8.3%) | HIV patients | CSF | India ink & culture |
| Ethiopia | Alemu et al [19] | 369 | 31 (8.8%) | HIV patients | Blood | Serology |
| Ethiopia | Tafese et al [20] | 254 | 26 (10.2%) | HIV patents | Blood | Serology |
| Central African Republic | Bekondi, et al [3] | 215 | 84 (39.1) | HIV patients | CSF, | culture & India ink |
| South Africa | Schutte [9] | 44 | 22 (50.0) | HIV patients | CSF | India ink, culture & serology |
| South Africa | Bergemann & Karstaedt [23] | 106 | 37 (34.9) | HIV patients | CSF | India ink & serology |
| Zimbabwe | Hakim et al, [24] | 170 | 80 (47.1) | HIV patients | CSF | serology & India ink |
| South Africa | Silber et al [25] | 41 | 8 (19.5) | | CSF | Culture |
| South Africa | Jarvis et al [26] | 492 | 337 (68.5) | HIV patients | CSF | culture, India ink & serology |
| Malawi | Cohen et al [27] | 235 | 110 (46.8) | HIV patients | CSF | culture India ink & serology |
| Zambia | Mwaba et al [28] | 230 | 210 (91%) | HIV patients | CSF | India ink& culture |

Quite a good number of studies on the prevalence of cryptococcal meningitis on immunocompetent and apparently immunocompetent patients have been conducted. Prevalence of cryptococcal meningitis among immunocompetent and immunocompromised individuals has been conducted by Mahale, et al [29]. A total of 242 CSF samples collected from patients clinically suspected for cryptococcal meningitis were screened for *Cryptococcus neoformans* irrespective of their immune status. The result of the study depicted an overall prevalence of 8.3% cryptococcal meningitis and prevalence 16.6% cryptococcal meningitis among immunocompromised patients. The result of this study was in good agreement with our finding. Similarly, the prevalence of cryptococcus in apparently immunocompetent patients was conducted by Lui et al [13]. Out of 46 patients with Cryptococcosis, twenty patients (43.5%) were apparently immunocompetent. The finding of Lui et al [13] is supported by the report of Pappas et al [30] that showed cryptococcosis in non-HIV patients of which 10–40% were immunocompetent. To determine the prevalence of cryptococcal infection, a retrospective analysis of 326 clinically diagnosed cases with meningitis over a period of five-and-a-half years was carried out by Prasad et al [31] of which fifty-four (16.6%) patients were found to have cryptococcal meningitis. Our finding was not in accordance with the findings of Prasad et al [31], which has demonstrated a higher prevalence of cryptococcal meningitis.

As a result of differences in exposure to the pathogen, cryptococcal meningitis is more frequently reported in men than women and rarely from children [4]. The finding of the present study in this regard was not in line with the findings of this study where the number of male and female patients with cryptococcal meningitis was more or less the same (8: 9).

4. Conclusion

Since clinical manifestations of cryptococcal meningitis are indistinguishable from other causes of central nervous system infection, all patients clinical diagnosed as having meningitis must be investigated for cryptococcal

meningitis irrespective of their immune status. Furthermore, in view of the increasing number of infections, rapid, sensitive, and specific diagnosis of cryptococcal disease has become more important than ever in Ethiopian health institutions.

5. Limitation of the Study

Unable to perform other laboratory procedures as the result of inadequate clinical sample was the limitation of this work.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgement

The authors would like acknowledge all patients that participated in the present study

References

- [1] Dromer F, Mathoulin S, Dupont B, and Laporte A. "Epidemiology of cryptococcosis in France: a 9-year survey (1985– 1993). French Cryptococcosis Study Group." *Clin. Infect. Dis* 1996; 23: 82–90.
- [2] Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 2009; 23: 525–530.
- [3] Bekondi C, Bernede C, Passone N, Minssart P, Kamalo C, Mbolidi D, Germani Y. Primary and opportunistic pathogens associated with meningitis in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. *Int J Infect Dis* 2006; 10: 387–395.
- [4] Bogaerts J, Rouvroy D, Taelman H, Kagame A, Aziz MA, Swinne D, Verhaegen J. AIDS-associated cryptococcal meningitis in Rwanda (1983–1992): epidemiologic and diagnostic features. *J Infect* 1999; 39: 32–37.

- [5] Gordon SB, Walsh AL, Chaponda M, Gordon MA, Soko D, Mbwvinji M, et al. Bacterial meningitis in Malawian adults: pneumococcal disease is common, severe, and seasonal. *Clin Infect Dis* 2000; 31: 53–57.
- [6] Helbok R, Pongpakdee S, Yenjun S, Dent W, Beer R, Lackner P, et al. Chronic meningitis in Thailand. Clinical characteristics, laboratory data and outcome in patients with specific reference to tuberculosis and cryptococcosis. *Neuroepidemiology* 2006; 26: 37–44.
- [7] Heyderman RS, Gangaidzo IT, Hakim JG, Mielke J, Taziwa A, Musvaire P, et al. Cryptococcal meningitis in human immunodeficiency virus-infected patients in Harare, Zimbabwe. *Clin Infect Dis* 1998; 26: 284–289.
- [8] Hovette P, Soko TO, Raphenon G, Camara P, Burgel PR, Garraud O. Cryptococcal meningitis in AIDS patients: an emerging opportunistic infection in Senegal. *Trans R Soc Trop Med Hyg* 1999; 93: 368.
- [9] Schutte CM, Van der Meyden CH, Magazi DS. The impact of HIV on meningitis as seen at a South African Academic Hospital (1994 to 1998). *Infection* 2000; 28: 3–7.
- [10] Okongo M, Morgan D, Mayanja B, Ross A, Whitworth J. Causes of death in a rural, population-based human immunodeficiencyvirus type 1 (HIV-1) natural history cohort in Uganda. *Int J Epidemiol* 1998; 27: 698–702.
- [11] French N, Gray K, Watera C, Nakiyingi J, Lugada E, Moore M, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS* 2002; 16: 1031–1038.
- [12] Corbett EL, Churchyard GJ, Charalambos S, Samb B, Moloi V, Clayton TC, et al. Morbidity and mortality in South African gold miners: impact of untreated disease due to human immunodeficiency virus. *Clin Infect Dis* 2002; 34: 1251–1258.
- [13] Shih CC, Chen YC, Chang SC, Luhi KT, Hsieh WC. Cryptococcal meningitis in non-HIV-infected patients. *Q J Med* 2000; 93: 245–251.
- [14] Lui G, Lee N, Ip M., Choi KW, Tso YK, Lam E, et al. Cryptococcosis in apparently immunocompetent patients *Q J Med* 2006; 99: 143–151.
- [15] Bello YB, Machado HG, Silveira JF, Schettini F, Junior GM, Junior SD et al., Cryptococcal meningitis in immunocompetent patient-case report. *American Medical Journal* 2013; 4: 100-104.
- [16] Lester FT. Cryptococcal meningitis complicating remoteskullfracture. *J. Neurology, Neurosurgery, and Psychiatry*, 1978; 41: 672-673.
- [17] Gebremedhin A. Cryptococcal meningitis in a young Ethiopian woman with AIDS. *Ethiop Med J.* 199; 1; 30: 169-73
- [18] Teshale S, Shitaye A., Abraham A, Atakilti A, Ermias D. Cryptococcal meningitis in patients with acquired immunodeficiency syndrome in pre-HAART era at Gondar College of Medical Sciences Hospital north-west Ethiopia. *Ethiop Med, J*, 2010; 48: 5-15.
- [19] Alemu AS, Russell R, Kempker, Admasu T, Smitson C., Nega B et al. High Prevalence of Cryptococcal Antigenemia among HIV-infected Patients Receiving Antiretroviral Therapy in Ethiopia. *PLoS ONE* 8 (3): e58377.
- [20] Tafese B, Yimtubezinash W, Daniel A, Gonfa A, David Boulware R. Comparison of Cryptococcal Antigenemia between Antiretroviral Naïve and Antiretroviral Experienced HIV Positive Patients at Two Hospitals in Ethiopia. *PLoS ONE* 8 (10): e75585.
- [21] Chen S, Sorrell T, Nimmo G, Speed B, Currie B, Ellis D et al. Epidemiology and host- and variety-dependent characteristics of infection due to *Cryptococcus neoformans* in Australia and New Zealand. Australasian Cryptococcal Study Group. *Clin Infect Dis* 2000; 31: 499–508.
- [22] Friedman GD, Jeffrey Fessel W, Udaltsova NV, Hurley LB. Cryptococcosis: the 1981–2000 epidemic. *Mycoses* 2005; 48: 122–125.
- [23] Bergemann A, Karstaedt AS. The spectrum of meningitis in a population with high prevalence of HIV disease. *QJM.* 1996; 499–504.
- [24] Hakim JG, Gangaidzo IT, Heyderman RS, Mielke J, Mushangi E, Taziwa A, et al. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. *AIDS.* 2000; 14: 1401–7.
- [25] Silber E, Sonnenberg P, Ho KC, Koornhof HJ, Eintracht S, Morris L, et al. Meningitis in a community with a high prevalence of tuberculosis and HIV infection. *J Neurol Sci.* 1999; 162: 20–6
- [26] Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect Dis.* 2010; 10: 67.
- [27] Cohen DB, Zijlstra EE, Mukaka M, Reiss M, Kamphambale S, Scholing M, et al. Diagnosis of cryptococcal and tuberculosis meningitis in a resource-limited African setting. *Trop Med Int Health.* 2010; 15: 910–917.
- [28] Mwaba P, Mwansa J, Chintu C, Pabee J, Scarborough M, Portsmouth S, Zumla A., Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions. *Postgrad Med J* 2001; 77: 769–77.
- [29] Mahale K, S Patil S, Ravikumar, Nagabhushan, Ramanath Mahale R, prevalence of cryptococcal meningitis among immunocompetent and immunocompromised individuals in Bellary, South India – a Prospective Study *Journal of Clinical and Diagnostic Research.* 2012 May (Suppl-1), 6: 388-392.
- [30] Pappas PG, Perfect JR, Cloud GA, Larsen RA, Pankey GA, Lancaster DJ et al Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. *Clin Infect Dis* 2001; 33: 690–699.
- [31] Prasad KN, Agarwal J, Nag VL, Verma AK, Dixit AK, Ayyagari A Cryptococcal infection in patients with clinically diagnosed meningitis in a tertiary care center. *Neurology India,* 2003; 51: 364-366.