Nepal J Biotechnol. 2021 Dec.9 (2): 29-32 Research article DOI: https://www.doi.org/10.54796/njb.v9i2.41912

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal
Journal Homepage: www.nepjol.info/index.php/njb
ISSN (Online): 2467-9313
ISSN (Print): 2091-1130

Sero-prevalance of Cryptococcal Antigenemia in HIV Positive Individual having CD4 Counts <100 Cells/mm^3

Sundar Khadka, Samikshya Kandel, Roshan Pandit, Rosham Manjhi, Subhash Dhital, Jagat Bahadur Baniya, Shravan Kumar Mishra, Raj Kumar Mahato

National Public Health Laboratory (NPHL), Department of Health Services, Ministry of Health and Population, Teku, Kathmandu 44-600, Nepal

Received: 5th Nov 2020; Revised: 21st Sep 2021; Accepted: 17th Nov 2021; Published online: 31st Dec 2021

Abstract

Cryptococcus neoformans is one of the foremost common opportunistic infectious agents in people living with Acquired Immuno Deficiency Syndrome (AIDS). It has been reported to cause about 1 million cases of cryptococcal meningitis per year among HIV/AIDS and 600,000 deaths annually. This study was done to find the prevalence of Cryptococcal antigenemia among HIV positive individuals having CD4counts <100 cells/mm^3.

A cross-sectional study was conducted in the HIV Reference unit, National public health laboratory from July to December 2015. The study comprised of 99 HIV positive individuals having CD4counts <100 cells/mm^3. CD4 T cell count was performed by flow cytometry (BD Biosciences, San Jose, CA, USA) and Cryptococcal antigen test by Latex agglutination assay. The overall prevalence of cryptococcal antigenemia was found to be 18.2%. Of the total ninety-nine subjects enrolled in the study, 72 (72.8%) were males and 27 (27.2%) were females. The mean age of the patients was 38 years ranging from 13 to 69 years. Higher percentage of female (22.2%) showed Cryptococcal infection in our study as compared to male (16.7%).

The study concludes higher prevalence of Cryptococcal antigenemia among HIV infected individuals and recommends Cryptococcal antigen screening to be made mandatory in HIV positive patients having CD4 T cells count below 100/µl.

Keywords: CD4, CrAg, Cryptococcosis, HIV, AIDS, Lateral Flow assay.

Corresponding author, email: cls.sundar@gmail.com

Introduction

Cryptococcus neoformans is one amongst the most common opportunistic pathogens infecting people with advanced Human Immune Deficiency Virus (HIV). Cryptococcal meningitis and Cryptococcal pneumonia are the common form of Cryptococcal infection in people living with Human Immune Deficiency Virus (PLHIV) [1]. Global data estimates in 2013 shows that 31.8 million people live with HIV among which 1.4 million were reported for Acquired Immuno Deficiency Syndrome (AIDS) related death [2]. Cryptococcal meningitis is supposed to cause 15% of AIDS-related mortality [2]. A study carried out in 2009 had estimated approximately 1 million cases of Cryptococcal meningitis [CM) with 600,000 deaths worldwide annually [3].

Cryptococcal meningitis represents a contagious form of the Cryptococcal disease that necessitates hospitalization and administration of drugs like amphotericin B. The infection is common mainly in immunocompromised HIV infected individuals having CD4 T lymphocytes count less than 100/µl. The infection can be treated easily if diagnosed earlier. However, in several cases the infection is asymptomatic and thus go unnoticed leading to complications that may further lead to death of patients [4].

The World Health Organization (WHO) guideline recommends two strategies for the prevention of Cryptococcal meningitis; 1) screening of patients with CD4+ T cell counts below 100 cells/mm^3 for Cryptococcal antigen (CrAg) and 2) primary prophylaxis with fluconazole for patients with CD4+ T cell counts below 100 [5]. Cryptococcal antigen (CrAg) is a biological marker of Cryptococcal infection that can be detected in serum of patients in an average of 21 days (range 5–234 days) before onset of symptoms of meningitis [6]. Cryptococcal infection can be identified using several laboratory tests such as Latex Agglutination Tests (LAT), Enzyme Immuno Assay (EIA), and Polymerase Chain Reaction (PCR); each having different sensitivity and specificity [6]. However, Cryptococcal antigen detection using Latex agglutination test is performed widely for screening of Cryptococococcal infection as it is easy to perform and do not require sophisticated laboratory set up. Timely identification of Cryptococcosis in PLHIV in the AIDS

stage prevents them from further risk of morbidity and mortality. There are different prevalence rates of Cryptococcal antigenemia from different parts of the world [2, 7-9]. However, there is no such study from Nepal till date to our knowledge. In this regard we carried out this study with the objective to find out the prevalence of co-infection by *Cryptococcus neoformans* in immunocompromised patients living with HIV in Nepal.

Materials and methods

A cross-sectional study was conducted in the HIV Reference unit of National Public Health Laboratory (NPHL), Kathmandu, Nepal from July to December 2015. Samples were collected from patients visiting NPHL for CD4 T cell count. All subjects enrolled in this study were HIV positive patients having CD4 T cells counts less than 100 cells/mm^3. A study questionnaire was used to collect demographic data from each patient. About 3 ml of EDTA whole blood was collected from each patient by trained professionals for the purpose of this study. The whole blood sample was used to determine CD4 T cell count by flow cytometry using the BD fluorescent-activated cell sorter system (BD Biosciences, San Jose, CA, USA) as per the manufacturer's instructions. Briefly, 20 µl of BD Tritest CD3/CD4/CD45 reagent was pipetted into a BD Trucount tube containing bead followed by 50 µl of well-mixed anticoagulated whole blood which was then vortexed and incubated in dark at room temperature for 15 minutes for staining of the cells. Following staining cells were lysed using BD FACS lysing solution and run in the flow cytometer to obtain absolute count.

For the purpose of Cryptococcal antigen testing plasma sample was separated from EDTA whole blood, which was then used for antigen detection using Cryptococcal Latex Agglutination test (Immuno Mycologics Inc, USA). The test kit employed is based on antigen-antibody agglutination reaction that detects capsular polysaccharide antigen of *C. neoformans*. The test kit is believed to have higher specificity and greater than 95% sensitivity. The assay procedure involves 5-easy steps with no specimen pretreatment. For test, at first a drop of lateral flow assay (LFA) specimen diluent was added to a disposable test tube. In the second step, 40 µL of the specimen was added to the tube and mixed. Afterward, a CrAg LFA test strip was inserted into the specimen and read at 10 min. The validity of the test was indicated by a single control line and the presence of line in test region indicated a valid positive test [10].

Informed consent was from each patient enrolled in this study after explaining them with the objective and outcome of this study. Ethical approval was taken from Nepal Health Research Council (ref no. 892) before carrying out this study.

Results

A total of Ninety-nine HIV positive patients were enrolled in the study during the period of six months. The study comprised 72 males and 27 females which were 72.8% and 27.2% of the total, respectively. The ratio of male to female patients was 8:3. The minimum and maximum age of study population was 13 years and 69 years, respectively with the mean age of 38 years. The mean CD4 T cell count was 60.4 that range from 2 to 98 cells/mm^3. Of the total patients enrolled in the study, 18 (18.2%) were found positive for Cryptococcal antigen. The positive to negative ratio for Cryptococcal antigen was observed to be 2:11. The prevalence rate of Cryptococcal antigen among male patients was found to be 16.7% while that for female patients was found to be 22.2%. In total, the prevalence rate was 18.2%. The prevalence rate was observed higher among female patients as compared to male patients **Table 1.**

Table 1. Prevalence of Cryptococcal antigen among HIV patients (N=99)

Sex	CrAg Positive	CrAg Negative	Total
Male	12 (16.7%)	60 (83.3%)	72 (100%)
Female	6 (22.2%)	21 (77.8%)	27 (100%)
Total	18 (18.2%)	81 (81.8%)	99 (100%)

Discussion

This study highlights the sero-prevalence of Cryptococcal antigen in HIV positive individuals having CD4 counts less than 100 cells/mm^3. HIV positive patients having CD4 count < 100 cells/mm^3 is referred to be in the AIDS stage. These patients are in immunocompromised state and are at higher risk of opportunistic infection. *Cryptococcus neoformans* is a fungus widely present in the environment and are able to infect immunocompromised host. Thus, HIV infected individuals in AIDS stage are highly susceptible for opportunistic infection by several other agents beside *C. neoformans*. In this study, we have estimated the prevalence rate of opportunistic infection by *C. neoformans* in immunocompromised HIV positive individuals.

The prevalence of Cryptococcosis was 18.2 % among 99 HIV positive patients having CD4 count < 100 cells/mm^3 in this study, which shows that a significant proportion of PLHIV has *Cryptococcus* infection in Nepal. This finding is comparable to a study by Micol et al. (2007) and Oyella et al. (2012) but, much higher than

the study by Lakoh et al. [9, 11, 12]. In one study done by Osazuwa et al. the prevalence rate was just 12.7% among 150 ART naïve populations, which is less as compared to our study [13]. ART naïve refers to a stage before receiving antiretroviral therapy (ART). It is believed that persons in ART have lower risk of opportunistic infection. However, the data in our study is much higher as compared to study by Osazuwa et al. The difference in data might be due to the difference in exposure to the causative agent of Cryptococcosis.

The ratio of female to male HIV patients in our study was 2:11. The number of males 72 (72.8%) were higher than the number of females 27 (27.2%). The data is in contrary to the study of Geda et al. that reported higher prevalence of HIV infection in females [14]. This may be due to differences of exposure as well as social stigma. Higher number of HIV-infected males as compared to females in Nepal might be another reason for the difference in enrollment of males and females in our study [15].

Fungal culture is the gold standard for the detection of *Cryptococcus neoformans*. However, some literature suggest that the latex agglutination test for Cryptococcal antigen has 100% sensitivity and 96% specificity compared to culture [10]. Our study shows that there is high prevalence of Cryptococcal antigenemia among immunocompromised HIV positive individuals in Nepal. Though our national guidelines do not recommend Cryptococcal screening and primary prophylaxis mandatory for every HIV infected individuals having CD4 T cell count less than 100 cells/mm^3, this study recommends testing for Cryptococcal infection at least by latex agglutination assay to be made as a routine test for such cases. This may be helpful in preventing morbidity and mortality caused due to meningitis among HIV infected individuals.

Conclusion

The study revealed that 18.2 % of people living with HIV in AIDS stage in Nepal are seropositive for the Cryptococcal antigen. Furthermore, in absence of gold standard test available for detection of Cryptococcal infection, serological antigen screening by latex agglutination assay is helpful to detect the cases of Cryptococcosis among immunocompromised HIV infected individuals.

Abbreviations

HIV: Human immunodeficiency virus

AIDS: Acquired Immuno Deficiency Syndrome

ART: Antiretroviral therapy

CD4: Cluster of differentiation 4

CrAg: Cryptococcal antigen

LAT: Latex Agglutination Tests

Consent for publication

Not applicable

Competing Interests

All the authors declare that they have no any competing interests.

Author's contributions

SK, SD, SKM, and RKM were responsible for study design, supervision of work, and guidance. SK, SK, RP, RM, SD and JBB performed laboratory work and data analysis. SK, SK, RP, RM and SD prepared the draft and wrote the manuscript. Finally, all the authors read the manuscript and approved for publication.

Acknowledgment

We would like to acknowledge the all staffs of HIV Reference Unit, National Public Health Laboratory, Nepal for their valuable support in completing this work

Ethical Approval and Consent

Ethical approval was taken from Nepal Health Research Council (ref no. 892) before carrying out this study

References

1. Osazuwa F, Dirisu JO, Okuonghae PE, Ugbebor O. Screening for cryptococcal antigenemia in anti-retroviral naïve AIDS patients in Benin City, Nigeria. Oman Medical Journal. 2012;27(3):228.
2. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. The Lancet infectious diseases. 2017;17(8):873-81.
3. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. Aids. 2009;23(4):525-30.
4. Smith RM, Nguyen TA, Ha HTT, Thang PH, Thuy C, Xuan Lien T, et al. Prevalence of cryptococcal antigenemia and cost-effectiveness of a cryptococcal antigen screening program–Vietnam. PloS one. 2013;8(4):e62213.
5. WHO. Guideline for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. World Health Organization (WHO); 2018.
6. Saha DC, Xess I, Biswas A, Bhowmik DM, Padma M. Detection of Cryptococcus by conventional, serological and molecular methods. Journal of medical microbiology. 2009;58(8):1098-105.
7. Letang E, Müller MC, Ntamatungiro AJ, Kimera N, Faini D, Furrer H, Battegay M, Tanner M, Hatz C, Boulware DR, Glass TR. Cryptococcal antigenemia in immunocompromised human immunodeficiency virus patients in rural Tanzania: a preventable cause of early mortality. InOpen forum infectious diseases 2015 Apr 1 (Vol. 2, No. 2). Oxford University Press.
8. Ezenabike C, Ashaka OS, Omoare AA, Fadeyi A, Salami AK, Agbede OO. Cryptococcal antigen among HIV1-infected individuals in north-central Nigeria. Current Medical Mycology. 2020; 6(2):43.

9. Lakoh S, Rickman H, Sesay M, Kenneh S, Burke R, Baldeh M, et al. Prevalence and mortality of cryptococcal disease in adults with advanced HIV in an urban tertiary hospital in Sierra Leone: a prospective study. BMC infectious diseases. 2020;20(1):1-7.
10. CrAg® LFA Cryptococcal Antigen Lateral Flow Assay For the Detection of Cryptococcal Antigen. IMMY.
11. Micol R, Lortholary O, Sar B, Laureillard D, Ngeth C, Dousset J-P, et al. Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2007;45(5):555-9.
12. Oyella J, Meya D, Bajunirwe F, Kamya MR. Prevalence and factors associated with cryptococcal antigenemia among severely immunosuppressed HIV-infected adults in Uganda: a cross-sectional study. Journal of the International AIDS Society. 2012;15(1):1-7.
13. Osazuwa OF, Dirisu O, Okuonghae E. Cryptococcal antigenemia in anti-retroviral naive AIDS patients: prevalence and its association with CD4 cell count. Acta Medica Iranica. 2012:344-7.
14. Geda N, Beyene T, Dabsu R, Mengist HM. Prevalence of Cryptococcal Antigenemia and associated factors among HIV/AIDS patients on second-line antiretroviral therapy at two hospitals in Western Oromia, Ethiopia. PloS one. 2019;14(12):e0225691.
15. Government of Nepal MoHaP. National HIV Strategic Plan 2016-2021. In: control NCfAaS, editor. Nepal2017.