Short Research Article

Cryptococcosis in HIV – infected hospitalized patients in Latvia.

ABSTRACT

Aims: to determine the prevalence of cryptococcal infection among HIV hospitalized patients, to evaluate clinical characteristics and outcomes in Latvia.
Study design: Prospective study.
Place and Duration of Study: Riga Eastern Clinical University Hospital, Latvian Center of Infectology, between January 2014 and February 2017.
Methodology: We conducted the study reporting demographics, epidemiological (age, sex, clinical aspects, paraclinical results (cryptococcal antigen in cerebrospinal fluid, serum, urine, cryptococcal DNA, HIV RNA and lymphocyte T CD4+ count), treatment and outcome aspects. We included 69 patients (71% men, 29% women) with HIV infection and cryptococcosis.
Results: 69 cases of cryptococcosis were confirmed for 699 HIV infected hospitalized patients tested, giving a prevalence of 9, 9 %. 38 % (n=26/69) of patients were with clinical signs of infection with the central nervous system involvement, 19% (13/69) patients had pulmonary involvement. Other 43 % (n=30/69) of patients had disseminated non-CNS disease (elevated serum cryptococcal Ag or DNA). Most patients had advanced HIV disease (Median lymphocyte T CD4+ count=48, 5 cells/uL, (1-1041), the average was 112, 9 cells/ uL (SD 184.98). 87 % (n=59/68) of patients had lymphocyte T CD4+ cell count < 200cells/µL Only 25 % (n=14) of the patients known to have HIV infection (n=56/69) were receiving antiretroviral therapy at the time of presentation. Overall mortality rate was 25% (n=41/69).
Conclusion: prevalence of cryptococcal antigenemia was 9, 9 %, indicating that the prevalence of cryptococcal infection among HIV patients in Latvia may be high enough to consider targeted screening.HIV positive patients have high mortality (35%) following cryptococcal infection which persists beyond their initial hospitalization. Follow-up studies of late mortality would be beneficial.

Keywords: Cryptoccocal infection; cryptoccocal antigenemia; invasive fungal infection; HIV; AIDS.

1. INTRODUCTION

Cryptococcus neoformans infection is a systemic invasive fungal infection (IFI) and is seldom among people who have healthy immune system. However, C. neoformans is a major cause of illness in people living with Human Immunodeficiency Virus (HIV), with an estimated 220,000 cases of cryptococcal meningitis occurring among people with HIV worldwide each year, resulting in nearly 181,000 deaths [1, 2]. Before antiretroviral therapy (ART) was discovered, fungal and other opportunistic infections were an ~~essential~~ common problem for people with advanced HIV/AIDS. Since then, the numbers of fungal infections and deaths due to fungal infections in people with advanced HIV/AIDS have decreased significantly in the developed countries [3, 4]. Although the widespread availability of ART in developed countries has helped improve the immune system of many HIV patients so that they don't become vulnerable to infection with Cryptococcus. Cryptococcal meningitis is still a great problem in resource-limited countries where HIV prevalence is high and access to healthcare is limited. Most cryptococcal meningitis cases occur in sub-Saharan Africa (estimated in 2014 in sub-Saharan Africa 162,500 cases, 43,200 cases in Asia and Pacific, 9,700 cases in North/South America and Carribbean, 3,300 cases in North Africa and Middle East) [5]. However, fungal diseases, especially cryptococcosis, are still a concern for people living with HIV in Europe, for instance, in 2014 4,400 cases were estimated [5] despite the widespread availability in Europe of ART.

Mainstay therapy includes an induction phase with amphotericin B (Amb), either the lipid or deoxycholate formulation, combined with flucytosine (5-FC), followed by the consolidation and subsequent maintenance phases, where higher and lower doses of fluconazole are used. Lipid soluble formulations of Amb are preferred over deoxycholate Amb due to their better tolerability and lower nephrotoxicity. However, cost and availability of lipid formulations of Amb and 5-FC are major limitations in resource-limited settings [6].

Screening of cryptococcal antigenemia in HIV-patients at risk allows early identification of asymptomatic cases. Cryptococcal antigenemia in the absence of meningitis can represent early-stage cryptococcosis during which antifungal treatment might improve outcomes. However, patients without meningitis are seldom tested for cryptococcal infection [7].

(You need to write a longer Introduction/Background around and in relation to the Objective/Aim of your study providing and discussing additional References in relation to the Objective/Aim of your study)

1.1 Objective

A prospective analysis of patients with HIV and cryptococcal infection was conducted to evaluate clinical characteristics and outcomes in Latvia. Until today, there was no research in Latvia about prevalence of cryptococcal infection and associated factors, characteristics of infection among HIV adults hospitalized patients.

2. MATERIAL AND METHODS

2.1 Study population

We conducted a prospective study of hospitalized patients older than 18 years with HIV infection and cryptococcosis of a 3-year period (from January 1, 2014 to February 1, 2017) at Riga Eastern Clinical University Hospital, Latvian Center of Infectology (LCI) located in the capital city of Latvia - Riga. (Prospective studies study outcomes at a future point in time in relation to factors at the start of the study. Thus, this study is a cross-sectional study even though it is done over 3 years, and not a prospective study)

An anonymity number was given to each patient to preserve the confidentiality. Personal data from participant and all diagnostic results was kept strictly confidential.

IFIs were classified according the European Organisation for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria [8]. The diagnosis of cryptococcosis was made according to EORTC/MSG criteria; additionally we used EQUAL Cryptococcus Score 2018: A European Confederation of Medical Mycology Score Derived from Current Guidelines to Measure QUALity of Clinical Cryptococcosis Management [6].

2.2 Data Collection

Data included demographics, epidemiological (age, sex, clinical aspects, paraclinical results (cryptococcal antigen (CrAG) in cerebrospinal fluid (CSF), serum, urine, cryptococcal DNA in serum and CSF, HIV RNA and Lymphocytes T CD4+ (LT CD4+) count), treatment aspects were collected from the patient folders and LCI hospital HIV database.

Death date was obtained from the LCI hospital HIV database and country system's Medical Informatics database and Population Register.

The mycological diagnosis of cryptococcosis was done in the Eastern Clinical University Hospital, LCI reference laboratory. CrAG was detected using a latex agglutination test (PASTOREXTM CRYPTO PLUS) following the manufacturer's instructions. CSF and urine was examined for research of antigen with the same technique. Cryptococcus DNA was detected with polymerase chain reaction.

(Was a Questionnaire used? If yes, briefly outline the contents of the Questionnaire, the Language of the Questionnaire and describe whether the Questionnaire was validated? Briefly outline the pilot-study which validated the Questionnaire. Do you think the subjects understood the questions in the same manner as the researchers? Who administered the Questionnaire, and where? What is they qualification? In case you have answers to these questions, include under Methodology).

2.3. Statistical Analysis

Statistical analysis was performed using SPSS.16 (California, USA, 2007) [9]. Means, medians and frequencies (%) were used to describe patients' characteristics. Fisher's exact test was used to compare categorical variables where appropriate. A p-value ≤ 0.05 was chosen as a level of statistical significance. (You have not indicated at all in your Results and Discussions below, where you have done analytical statistics using the Fischer's Exact Test, or any other, in what manner and what is the p-value of significance ascertained. What you present and discuss below is entirely descriptive statistics).

2.4 Country's Profile

According to the Latvian Central Statistical Administration data in 2018, the Latvia total population was estimated to be 1 million 934 thousands, with 54 % females. Of the general population, 15.8 % were younger than 15 years and 20.1 % were above 65 years of age [10]. According to the Centre for Disease Prevention and Control of Latvia report, in 2018, Latvia had 326 new HIV infections including 89 AIDS-related deaths. There were 7669 people living with HIV on January 1, 2019, including 2036 in AIDS stage and 1121 AIDS-related death. An estimated number of children infected with HIV was 80 due to mother-to-child transmission in January 2019, and 5 in 2018 [11]. Also in 2017 Latvia reported the highest number of new HIV cases in the EU and European Economic Area (EEA). The HIV/AIDS Surveillance Report of the World Health Organization and the European Center for Disease Prevention and Control (ECDC) about the situation in Europe 2018 (2017 data) suggests that last year there were 18.8 new HIV cases per 100,000 residents in Latvia, which is higher than average of 6.2 cases in other countries [12,13].

3. RESULTS AND DISCUSSION

3.1 Patient characteristics

During the study period, a total of 699 patients infected by HIV were admitted in the hospital. A CrAG test in serum and urine and DNA assay in serum was performed for all patients. Of these, 69 hospitalized HIV positive adult patients ~~all~~ were diagnosed positive Cryptococcus Ag or DNA, meaning a prevalence of 9, 9 %. (The standard accepted method in journals of denoting decimal is with a period (.) and not a comma (,)) They were included in this study. The mean follow-up was 24.6 months (SD 24, 63; Median 16, 5 (0-62). (This paragraph is best included under Methodology. How did you arrive at the sample-size? Was any formula used – what formula? Was any previous study based upon in arriving at sample-size? Reference the Study. Was random-sampling done? What method of random-sampling? In case you have answers to these questions, include under your Methodology).

The different characteristics of our study population and groups of patients depending on the result of antigenemia are described **(Table 1).**

Table 1. Characteristics of hospitalized HIV patients screened for cryptoccocal antigen and DNA in Riga, Latvia.

Characteristics	Cryptococcal Ag Positive Total n (%)
Gender: n (%):	
Male	49 (71)
Female	20 (29)
Job: n (%)	
Work	16 (23,19)
Disability	2 (2,90)
Not working	50 (72,46)
Prisoner	1 (1,45)
Mean age (SD)	38,2 (8,18)
Min	23
Max	57
Age groups: n (%)	
15-30	11 (16)
31-45	44 (64)
>45	14 (20)

HIV serotype: n (%) HIV -1 HIV 2	 69 (100) 00
LT CD4+ (cells/mm^3): n (%) 0-199 200-499 >500 Unspecified	 59 (87) 6 (9) 3 (4) 1
Mean HIV RNS (cop/ml) (SD) Median Min Max	671520,7 (1682950) 55950 40,8 9800000
Antiretroviral therapy n (%): Yes No Discontinued due to the lack of compliance	 14 (20) 43 (62) 12 (18)

3.1.1 Epidemiological aspects

Of the 69 HIV-seropositive patients who were included in this study, the majority were male (n= 49/69, 71%). The predominance of adult patients was noted in our study population. The average age was 38, 2 years and patients ranged from 23 to 57 years. There were 57/69 patients with information on a possible HIV transmission route. 72% (n=41/57) were intravenous drug users.

3.1.2 Clinical aspects

More patients (81%) were known to have HIV infection at the time of their presentation, average 6, 2 years (SD 4, 98; min – less than one year; max – 16 years). The remaining 19% (n=13/69) of patients had HIV infection diagnosed during this hospitalization. 59 % (n=41/69) of patients had viral ~~virus~~ hepatitis ~~were the~~ as a co-existent disease ~~associated~~ underlying ~~diseases~~. 40/41 of patients were hepatitis C virus co-infected, 1/41 - was hepatitis B virus co-infected. 38 % (n=26/69) of patients were with clinical signs of infection with the central nervous system (CNS) involvement, and 4 of them were CrAG in serum negative. 19% (n=13/69) patients have pulmonary involvement. Only two of them were CrAG in serum positive, others were CrAG in serum negative (respectively had localized pulmonary disease). Other 43 % (n=30/69) of patients had disseminated non-CNS disease (elevated serum CrAG or DNA). The prevalence of neuromeningeal signs was 22 % (n=15/69). They predominated in patients with positive cryptococcal antigenemia. 13% (n=9/69) of patients had concurrent final diagnoses. Final diagnoses included Pneumocystis jirovecii pneumonia (n=7/9) and Pneumocystis jirovecii colonization (n=2/9). 54 % (n=37/68) patients with medical charts available did not have evidence of any OI. Other 46% of patients (n=31/68) had past OI, half of them (n=15/31) had tuberculosis (TBC). 20% (n=14/69) of patients had a prior hospitalization coded for cryptococcal disease.

(Try to tabulate as much of the contents of this paragraph as is possible, for maximum impact and easy readership)

3.1.3. Paraclinical aspects

HIV-1, the predominant serotype, was found in all of cases. In none of samples Cryptococcus neoformans was detected by fungal culture. CrAG screening in serum was performed in all 69 patients. In 32% (n=22/69) of cases CrAG was negative, including 4 patients with CNS involvement and CrAG positive in CSF, 11 patients with pulmonary disease, and 7 patients with positive Cryptococcus DNA in serum. A lumbar puncture (LP) was performed in individuals who had CNS symptoms (headache, neck stiffness, confusion, ataxia, vomiting, photophobia), and if the patient had focal neurological symptoms. LP was performed in 46% (n=32/69) of patients. (Include this under Methodology) CrAG or DNA screening in the CSF was positive in 26 cases. 90% CSF characterised with lymphocytic pleocytosis with the average 60, 5 cells/µL (SD 99, 58; Median 19, 5 (2-373). Patients with pulmonary involvement (infiltrates or nodule on imaging, respiratory symptoms) underwent diagnostic bronchoscopy (n=18/69). 13 patients´ bronchoalveolar lavage fluid (BAL) samples were positive in 'Pastorex Crypto-Plus' test (Bio-Rad). (No need to repeat. Already stated under Methodology) CrAG screening in urine was performed in all 69 patients. 7% (n=5/69) of patients had positive CrAG in urine. LT CD4 count was available for 68/69 participants. Most patients had advanced HIV disease (Median LT CD4+ count=48, 5 cells/uL, (1-1041) .The

average LT CD4 + was 112, 9 cells/ uL (SD 184.98) 87 % (n=59/68) of patients had CD4+ cell count < 200cells/μL. Mean Log viral load was 5.

(Try to tabulate as much of the contents of this paragraph as is possible, for maximum impact and easy readership).

3.2 Therapeutic and outcome aspects

Despite that most patients had advanced HIV disease, only 25 % (n=14) of the patients known to have HIV infection (n=56/69) were receiving antiretroviral therapy at the time of presentation. Antifungal therapy of cryptococcosis with Fluconazole (800mg/day per os or intravenous) was administered to all 69 cases during hospitalization. And 22% (n=15/69) received combination therapy with Lipid soluble formulations of Amb due to severe illness. 20% (n=14/69) of patients in ~~additions~~ addition received corticosteroids.

Overall mortality rate was 59% (n=41/69). 37% died within 90 days of cryptococcal diagnosis (early mortality), and 63 % died after 90 days (late mortality). In hospital mortality rate was 25% (n=17/69), with mean days at hospital – 16, 1 days (SD 8, 72). 41 % (n=7/17) died in Intensive Care Unit. After hospitalization mortality rate was 35% (n=24/69).

In 88% (n=15/17) patients who died in hospital, the cause of death was IFI – cryptococcosis. In 12% (n=2/17) the cause of death was other reasons (1- renal insufficiency glomerulonephritis due, 1- hepatic insufficiency).The cause of death after hospitalization was IFI (including cryptococcosis) in 21% (n=5/24) of cases. In 46% (n=11/24) of cases the cause of death were other reasons, from them 4 cases – TBC,3- hepatic insufficiency,2 – bacterial meningoencefalitis , 2 – lymphoma. And in the remaining 33% (n=8/24) the cause of death was unknown.

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4. DISCUSSION

Advanced HIV disease remains an essential challenge. Despite major progress over the last decade in expanding access to ART and reducing HIV-related deaths, up to half of people living with HIV present to care with advanced disease, and many continue to die from HIV-related opportunistic infections. We need to find better ways to identify and manage advanced HIV disease, in order to reach the global goal to reduce HIV deaths by 50% by 2020. Cryptococcal meningitis is a serious opportunistic infection and a major cause of morbidity and mortality among HIV positive people with advanced disease. Most people dying of cryptococcal meningitis live in low-income countries. Often people are not diagnosed early enough because rapid diagnostic tests and lumbar puncture, in the diagnosis of cryptococcosis, are unavailable. The first-line antifungal drugs that are used for treatment are costly and often not available to save the lives of people infected with cryptococcal meningitis. In Europe important death factors are antifungal drug toxicity, intracranial pressure and immune raised reconstitution inflammatory syndrome [14].

In this prospective study we report a prevalence rate 9, 9 % of cryptococcosis among hospitalized patients with advanced HIV infection. Other published research data, shows a different situation to Latvia in other European countries. (Provide all the References here, and discuss in much detail as is possible) For instance, in Germany Cryptococcal antigenaemia was found in 1, 6% of patients with LT CD4 <100 cells/uL [15]. The data about the burden of cryptococcal disease in the Baltic States is scarce.

According to the Centre for Disease Prevention and Control of Latvia report, in 2018, Latvia had 326 new HIV infections, including 99 AIDS stage and 89 AIDS-related deaths [10]. According to these findings, one quarter of individuals were recently diagnosed at such advanced stage. Late HIV diagnosis remains a problem in Latvia. This could be one of the reasons why the disease is more common. In our study 19% of patients had HIV infection diagnosed during the hospitalization with an existing opportunistic AIDS disease – cryptococcosis. (This is not presented under your Results. Do you mean they were not diagnosed HIV prior to being diagnosed cryptococcosis? But you say, your Study-subjects comprise entirely of those already diagnosed HIV – totaling 699)

Although access to HIV diagnosis, and immediate start to ART (regardless of the stage or LT CD4 counts) constitutes a major medical advancement in the clinical management of HIV in Latvia, its success depends on strict adherence to prescribed regimen. 18 % of our patients with cryptococcosis were on ART, with poor adherence to medical treatment.

In our study in 43% of cases of ~~occurs~~ occult cryptococcal antigenemia is seen among hospitalized HIV-seropositive patients. Health care providers should evaluate HIV-infected patients for cryptococcal antigenemia, even in the absence of meningitis. Based on World Health Organization and European AIDS clinical society guidelines, it is recommended to screen all HIV-infected people with LT CD4 <100 cells/uL for CrAg, and based on World Health Organization guidelines it

may be considered at a higher LT CD4 cell count threshold of <200 cells/uL [16,17]. Routine screening might identify asymptomatic meningitis, too. Symptom-based diagnosis is not a reliable predictor of the central nervous system involvement [18].

5. CONCLUSION

Cryptococcal disease often has an insidious presentation and can be difficult to recognize. However, delayed diagnosis can lead to increased morbidity and mortality. In our study the prevalence of cryptococcal antigenemia was 9, 9 %, indicating that the prevalence of cryptococcal infection among HIV patients in Latvia may be high enough to consider targeted screening.

HIV+ patients have high mortality (35%) following cryptococcal infection which persists beyond their initial hospitalization. Identifying patients at higher risk for mortality is critical for successful treatment and outcomes. Follow-up studies of late mortality would be beneficial.

Our research data again highlights that there is a need for a broader body of society, educating the public to discover HIV infection more quickly and reduce the number of patients at high risk of opportunistic infection (OI) developing; already known HIV positive patients should improve their adherence. This will improve the health and survival of a particular individual and reduce the public's financial costs of OI treating.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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(Make certain of listing the References and the style conforms with that required under the Instructions for Authors of this Journal)

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