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The Prevalence of Cryptococcal Antigenemia in Serum of Human Immunodeficiency Viruses-infected Patients of Iran

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Abstract

BACKGROUND: *Cryptococcus* species are the most prevalent opportunistic agents found in patients with HIV which may result in life-threatening cryptococcal meningitis (CM). A non-invasive way for diagnosis of CM is the detection of cryptococcal antigen (CrAg) in the blood to reduce either the mortality rate or the treatment complications associated with antiretroviral therapy. Not much information is available in CM among HIV patients in Iran.

AIM: Thus, in the current study, we aimed to evaluate the prevalence of cryptococcal disease by antigen testing, possible associated factors, and outcomes in HIV-infected patients being managed in a referral HIV/TB hospital in Tehran-Iran.

METHODS: In this cross-sectional study, blood samples were screened for CrAg using a rapid latex agglutination test between 2017 and 2018 at Masih Daneshvari Hospital (Tehran-Iran) as referral land center for HIV/TB patients. Based on CD4 counts, 106 HIV-positive infected patients including 101 (95.3%) males and 5 (4.7%) females with the mean \pm standard deviation age of 42.40 ± 10.687 and 36.50 ± 6.403 years enrolled in the study. The patients were categorized into 4 groups, (a) <50 , (b) $50-100$, (c) $101-200$, and (d) >200 CD4⁺ T cells/ μ L. Whole blood was obtained with EDTA (for flow cytometry of CD4 counts) or without for harvesting serum for determination of CrAg in serum.

RESULTS: The results showed only one positive case for CrAg, indicating that CrAg is rare in Iranian HIV patients (overall estimation is lower than 0.01%).

CONCLUSIONS: With the paucity of information about the prevalence of cryptococcosis in Iran, there is a need for better screening tests and strategies for detection of CrAg in addition to the prevention and treatment approaches of CM.

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Introduction

Cryptococcus species (mostly *neoformans* and *gatti*) are the most common opportunistic agents found in patients with HIV-infected patients leading to a fatal condition, referred to as cryptococcal meningitis (CM) [1,2]. It is associated with a high mortality rate among these patients with more than 600,000 deaths per year in 2009 and recent estimates from 2014 indicate that over 160,000 (95% confidence interval 113,600–193,900) cases of CM, including more than 130,000 deaths occurred in sub-Saharan Africa [3,4]. CM remarkably occurs in sub-Saharan Africa and Southeast Asia, which are known as the major prevalent sites for HIV [5]. Data from epidemiological studies suggest that increasing cryptococcosis is associated with increasing HIV infections. On the other hand, the prevalence of cryptococcosis was decreased with the implication of antiretroviral therapy (ART) regimens, which may be indicated a tight association between cryptococcosis prevalence and the immune system function [6]. Early screening for detecting the presence of CM in HIV

patients, especially those with CD4 counts <100 /ml, is highly recommended by the World Health Organization (WHO). This early tests are cost-effective and on the other hand, able to increase the survival rate of patients with HIV with treatment [7]. In addition, one of the complications associated with starting ART in HIV patients with a latent cryptococcosis is immune reconstitution inflammatory syndrome (IRIS), which arises after considerable improvement in the immune system of the HIV patients and leads to the inflammation of meninges. In fact, it is a very life-threatening extremely decreases the survival rate of the infected individuals [8]. Thus, antifungal therapy before initiating ART is very important in HIV patients with concurrent cryptococcosis [9]. Early cryptococcosis infection is asymptomatic and treatable which is characterized by the presence of cryptococcus antigens in the blood [10]. In contrast, symptomatic cryptococcosis, leading to meningitis or pneumonia, is hardly treated with high coast drugs such as amphotericin B [11]. Thus, early diagnosis and preemptive treatment of cryptococcosis is considered as cost-effective approaches for the cure of patients with HIV. Early detection of CM is achievable through cryptococcal antigen (CrAg) lateral flow assay

(LFA) for cryptococcosis as a rapid and inexpensive assay. CrAg is rapid latex agglutination test with lower sensitivity and specificity compared with LFA. However, there are other tests such as direct microscopy through staining lumbar puncture specimens with India ink, PCR, and enzyme-linked immunosorbent assay [12]. To date, there is not much information that is available about CM among Iranian HIV patients. Therefore, we aimed to investigate the prevalence of cryptococcosis antigenemia among HIV-infected patients from July 2017 to July 2018 in Masih Daneshvari hospital the referral center for HIV in land.

Materials and Methods

We conducted a cross-sectional study from July 2017 to July 2018. There were 106 HIV-positive subjects, including 101 male and 5 female, from Masih Daneshvari Hospital that is a national referral center for HIV Tehran-Iran.

The study was reviewed and approved by the University Ethics Committee. All procedures performed were in accordance with the ethical standards of the institutional and national research. After obtaining written consent to participate from the participants, demographic data were collected. Remel Cryptococcus Antigen Test Kit (Thermo Scientific™, USA), a rapid latex agglutination test was employed for qualitative or semi-quantitative detection of polysaccharide antigens associated with *Cryptococcus neoformans* infection in serum or CSF of patients. Frequency and percentage were reported for categorized variables. All data were analyzed with SPSS version 22 (SPSS Inc., Chicago, Illinois, USA). The normality of the data was evaluated by the Kolmogorov–Smirnov test. The Kruskal–Wallis H test was used to assess significant intergroup variations.

Results

In this study, 101 (95.3%) males and 5 (4.7%) females were screened for CrAg antigen. The mean age \pm standard deviation of them was 42.40 ± 10.687 and 36.50 ± 6.403 , respectively. About 50% of individuals were 30–40 years old and other age groups have a relatively lower frequency. The demographic data were presented in Table 1. Based on the CD4 counts, the patients were categorized into the following groups: 33 (31.1%) of the subjects had CD4 counts of <50 cells/ μ L, 9 (8.5%) had CD4 counts of 50–100 cells/ μ L, 19 (17.9%) had CD4 counts of 101–200 cells/ μ L and 42 (39.7%) with CD4 counts of >200 cells/ μ L, and 3 (2.8%) had missing CD4 cell counts. Pulmonary symptoms were negative for

Table 1: Demographic characteristics of HIV-infected patients

Variable	Group	Frequency	Percent
Sex	Male	101	95.3
	Female	5	4.7
Age	0 (<20)	1	0.9
	1 (20–29)	4	3.8
	2 (30–39)	46	43.4
	3 (40–49)	39	36.8
	4 (50–59)	12	11.3
	5 (≥ 60)	4	3.8

73 (70.9%) of the subjects and positive for 30 (29.1%) individuals. Furthermore, CNS symptoms were negative for 25 (24.3%) of the individuals and positive for 78 (75.7%) subjects. CrAg evaluation was positive in one patient 41 years man with CD4 counts <50 . He was from Bandar Abbas city, in the South of Iran with tropical climate while other subjects with negative CrAg results are not from cities with such climate. In fact, the prevalence of CrAg was $<1\%$ among whole patients with HIV (Table 2).

Table 2: Indicating for CD4+ T cell counts, clinical symptoms, and serum CrAg

Variable	Group	Frequency	Percent
CD4 counts	<50 cells/ μ L	33	31.1
	50–100 cells/ μ L	9	8.5
	101–200 cells/ μ L	19	17.9
	>200 cells/ μ L	42	39.7
	Missing CD4 cell count	3	2.8
Pulmonary symptoms	Yes	32	30.2
	No	74	69.8
CNS symptoms	Yes	81	76.4
	No	25	23.6
CrAg results	Negative	105	99.1
	Positive	1	0.9

CrAg: Cryptococcal antigen, CNS: Central nervous system.

The association of CNS symptoms and CrAg results is shown in Table 3. According to the results, 91 (85.9%) subjects were presented negative CNS symptoms and CrAg, 1 (0.9%) subjects were presented negative CNS symptom meanwhile positive CrAg results, and 14 (13.3%) subjects presented positive CNS symptoms meanwhile CrAg-negative results.

Table 3: Indicating for CNS symptoms in HIV patients with serum CrAg

CNS symptoms	CrAg results	Frequency	Percent
Negative	Negative	91	85.9
	Positive	1	0.9
Positive	Negative	14	13.2
	Positive	-	-

CrAg: Cryptococcal antigen, CNS: Central nervous system.

Discussion

Cryptococcosis is an opportunistic infection which HIV patients due to the lower immune response have the highest prevalence with an average of 6%.

Cryptococci infection in HIV patients induces the life-threatening condition and usually leads to developing CM and considered approximately 15% of HIV-related deaths.

In addition, it is a contraindication of the early beginning of ART due to the IRIS phenomenon which arises after considerable improvement in the immune system of the HIV patients and leads to the

inflammation of meninges [4,8]. Therefore, early detection and preemptive treatment of this infection is highly recommended due to the patient's survival and treatment costs [10]. In this study, it was found that despite having neurologic and respiratory symptoms, the prevalence of cryptococcosis among the study of HIV patients was lower (1.0%). Among the 106 participants of this study, only one person was positive for CrAg from a tropical climate area with poor hygiene and low resources setting which are favorable for the transmission of *Cryptococcus* species.

To make an appropriate decision for preparing national guidelines regarding the policies related to cryptococcus screening, it is highly recommended to assess the prevalence of cryptococcosis in different areas and different referral centers for HIV in the country for the prevalence. One of the best ways for estimating cryptococcosis prevalence is testing for the presence of CrAg using different screening tests. These screening tests not only provide a measure for assessing the outcome of successful strategies for the management of AID-associated complications but also could help the physicians to make the best decisions regarding the treatment options and also the timing of ART. Most of the screening studies have been done so far in sub-Saharan Africa and also in Southeast Asia. These two locations are known for having the most HIV prevalence and also their climate and poor settings support the survival and transmission of cryptococcosis.

Most of the retrospective and cross-sectional studies were done in these regions to support the WHO guidelines regarding the early screening tests for HIV patients with blood CD4 counts $<100/\mu\text{l}$ and warrant for pre-emptive treatments which by oral fluconazole [13].

Thus, the WHO introduces CrAg antigenemia as an effective predictor of CM. In correspondence, Smith *et al.* estimated that the average prevalence of CrAg in Vietnam as a Southeast Asian countries around 4% with a higher prevalence (6%) in South Vietnam compared to North (2%) Vietnam, which could indicate the necessity of investigating other parts of a country to determination and making a decision for the prevalence of cryptococcosis for employment of the right strategies for screening and treatment [14]. Moreover, a study done in Namibia for estimation of CrAg prevalence in immunocompromised adults reported an average prevalence of 3.3% among the study groups and suggested the implementation of screening tests for HIV patients with a CD4 count lower than $100/\mu\text{l}$ and employing preemptive treatment for the positive tested individuals [15]. Another study conducted in the Guinea-Bissau showed that 20% of ART-naive patients which were selected randomly, showed positive results for CrAg and thus emphasized on early screening and preemptive treatment for cryptococcosis [16]. In support of early screening and preemptive treatment for cryptococcosis, Longley *et al.* conducted a prospective

study in South Africa and confirmed that preemptive treatment of cryptococcosis with fluconazole along with ART significantly reduced the risk of developing CM and CM associated death in HIV patients [17].

There is a paucity of data regarding the cryptococcosis prevalence in Iran and only one study has considered the concurrent infection of cryptococcosis with AIDS, which has found that among the 86 patients [18]. This patient had CD4 count $<100/\mu\text{l}$; no one was identified as a serum positive for CrAg through lateral flow immunochromatographic assay [18]. The lower ratio of prevalence in this study and the current one is not well understood and needs to be further explored.

In the current study, latex agglutination test was used which possesses lower sensitivity and specificity in comparison to LFA. However, there are some studies on the environmental distribution of *Cryptococcus* species in Iran; for example, Kamari *et al.* have shown that *Cryptococcus* species could be isolated from nearly 18% of pigeon nests and around 12% of eucalyptus trees in Ilam, Iran [19]. Furthermore, Hedayati *et al.* in North of Iran found that 5.2% of collected samples from swallow (*Hirundo rustica*) excretions contained *C. neoformans* [20]. Soltani *et al.* showed that 2.5% of pigeon dropping samples from Isfahan, Iran, showed contamination with *C. neoformans*. Furthermore, a similar study by agha Kuchak Afshari *et al.* in North of Iran (Mazandaran) has revealed that 5% of the pigeon droppings showed contamination with *C. neoformans* [21]. These data collectively indicate the possibility that immunocompromised patients, especially those affected with HIV, could be infected with *Cryptococcus* species in different regions of Iran, and therefore it seems necessary to accurately estimate the prevalence of cryptococcal diseases in other parts of land, which requires integrated multicenter studies in land. In referral centers for HIV, which accept patients from different populations and areas, investigating the prevalence of opportunistic infections such as cryptococcosis could be very beneficial. Moreover, identification of the infected individuals can help decrease the chance of direct contact transmission by early treatment of the affected individuals in such centers containing immunodeficient patients.

Conclusions

We found a prevalence of CrAg of approximately 1% among HIV-infected patients with CD4 counts <50 cells/ μL . With the paucity of information about the prevalence of cryptococcosis in Iran, there is a need for further investigation of the prevalence of this disease using rapid tests, such as latex agglutination test and LFA in different regions of Iran; so as to come to a national agreement about deciding whether to implement cryptococcus screening

tests and preemptive treatments in HIV care systems according to the WHO guidelines. Indeed, it requires the exact estimation of screening methods and cost burden of strategies compared with the cost burden of the treatment of cryptococcus-related meningitis and other complications.

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