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Cryptococcal Meningitis in Senegal: Epidemiology, Laboratory Findings, Therapeutic and Outcome of Cases Diagnosed from 2004 to 2011

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Abstract
Background Cryptococcal meningitis is one of the most important opportunistic infection and a major contributor to early mortality. In sub-Saharan Africa, particularly in Senegal, prevalence of cryptococcal meningitis remains high. This study aimed to describe the epidemiology, laboratory profile, therapeutic and outcome of cases diagnosed in Dakar.
Methods We analyzed the cryptococcosis cases diagnosed at the department of parasitology–mycology in Fann Teaching Hospital in Dakar from 2004 to 2011. The diagnosis was confirmed by culture on Sabouraud's dextrose agar and/or by India ink preparation and/or by cryptococcal antigen detection. The diagnosis methods were assessed by using culture as reference.
Results A total of 106 cases of cryptococcal meningitis were diagnosed. The prevalence of cryptococcal meningitis was 7.8 %. The mean age of the patients was 40.17 ± 9.89 years. There were slightly more male (53.8 %) than female (46.2 %) patients; 89.6 % were found to be infected with HIV, and the median CD4+ count was 27/mm^3. Approximately 79.5 % of the patients had <100 CD4+ lymphocytes/mm^3. India ink staining presented sensitivity at 94.11 % and specificity at 100 %. Sensitivity and specificity of cryptococcal antigen detection in cerebrospinal fluid were, respectively, 96.96 and 15.78 %. The most frequently used antifungal drug was fluconazole (86.7 %), and the mortality rate was 62.2 % (66 deaths).
Conclusion Early diagnosis is essential to control cryptococcosis, and countries should prioritize widespread and reliable access to rapid diagnostic cryptococcus antigen assays. But it is important to make available conventional methods (India ink and culture) in the maximum of laboratory in regional health facilities.

Keywords Cryptococcal meningitis · Epidemiology · Laboratory profile · Therapeutic · Outcome

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Introduction

Cryptococcus neoformans is encapsulated yeast that is responsible for life-threatening infections, particularly in immunocompromised patients [1]; cryptococcal meningitis (CM) is one of the most important opportunistic infection and a major contributor to early mortality [2] accounting for between 13 and 44 % of deaths in HIV-infected cohorts in resource-limited countries [2]. Cryptococcal meningitis also occurs in

patients with other forms of immunosuppression and in apparently immunocompetent individuals [3, 4].

In sub-Saharan Africa alone, there are more than 500,000 deaths each year due to CM, which may exceed those attributed to tuberculosis [5].

Cryptococcal meningitis is also the leading cause of community-acquired meningitis in parts of sub-Saharan Africa where the HIV prevalence is high, ahead of *Streptococcus pneumoniae* and *Neisseria meningitidis* [6, 7]. Mortality from HIV-associated CM remains high (10–30 %), even in developed countries, because of the inadequacy of current antifungal drugs and combinations, and the complication of raised intracranial pressure [8, 9]. In the developing world, patients tend to present later [10–12].

In Senegal, prevalence of CM remains high in AIDS patients with a high mortality rate at 71.1 %. [13].

This study aimed to describe the epidemiology, laboratory profile, therapeutic and outcome of cases diagnosed in Dakar from 2004 to 2011.

Materials and Methods

Study Design

We analyzed the cryptococcosis cases that were diagnosed at the department of parasitology–mycology in Fann Teaching Hospital in Dakar retrospectively from January 2004 to December 31, 2010 and prospectively from January 2011 to December 2011.

Patients were included in this study if a clinical diagnosis of cryptococcal disease was made on or during the period of admission at the Infectious Diseases Clinic in Fann Teaching Hospital in Dakar. The diagnosis was confirmed by positive culture on Sabouraud's dextrose agar (SDA) and/or by microscopic detection of encapsulated yeast on India ink preparation and/or by cryptococcal antigen detection by latex agglutination (LA) in cerebrospinal fluid (CSF) and/or serum. Patients were excluded when all available specimens were negative for cryptococcal organisms by culture, India ink preparation and/or LA.

Collection of Data

Data were recorded by using a questionnaire which included administrative data (first three letters of the patient's last name and first initial; patient's sex and age; hospital ward; and city of diagnosis), epidemiologic data (exposure category for HIV infection, stage of the HIV disease), clinical data and laboratory data (CD4+ lymphocyte count at the time of the diagnosis of cryptococcosis, culture-positive specimens, results of India ink preparation and LA).

Laboratory Examination

Cerebrospinal fluid specimens for culture of *C. neoformans* were centrifuged and the sediment inoculated onto Sabouraud's dextrose agar and incubated at 37 °C for 14 days. The suspected colonies were identified by microscopic examination and its ability to produce urease on Christensen's urea medium.

Cerebrospinal fluid or serum cryptococcal antigen was detected using a LA test (PASTOREX^{TM} CRYPTO PLUS) following the manufacturer's instructions. The specimens were heat-inactived (30 min at 56 °C) to eliminate the risk of HIV contamination. Prior enzymatic treatment of all samples was performed in order to eliminate interferences and enhance detection sensitivity. After preparation, agglutination card was placed on the shaker for 5 min (160 rpm), at room temperature (+18–30 °C). A positive reaction was indicated by an agglutination of the latex particles with the test sample.

Microscopic examination was made by adding one drop of India ink on the CSF specimen.

Statistical Analysis

All analyses were performed with the statistical software R2.15.0 (R Foundation for statistical computing, Vienna, Austria). Medians and frequencies (%) were used to describe patients' characteristics. A comparative evaluation of methods was done using culture as reference. Sensitivity and specificity were calculated.

Results

Patients' Characteristics

From 2004 to 2011, 1,342 patients hospitalized at the Infectious Diseases Clinic in Fann Teaching Hospital in Dakar were screened. Only 106 patients screened met the eligibility criteria and the prevalence of CM was

Table 1 Characteristics of patients

	Number	Percentage	IC 95 %
Age range (years)			
0-25	5	4.7	[1.5–10.7]
26-35	25	23.6	[15.9–32.8]
36-45	54	50.9	[41.0–60.8]
> 46	22	20.8	[13.5–29.7]
Sex			
Male	57	53.8	[44.3–63.2]
Female	49	46.2	[36.7–55.6]
Origin			
Urbain	38	35.9	[26.7–45.0]
Suburbs	37	34.9	[25.8–43.9]
Rural	9	8.5	[3.1–13.8]
Status			
Married	58	54.7	[45.2–64.1]
Single	14	13.2	[6.7–19.6]
Veuf (ve)	4	3.8	[0.16–7.4]
Divorcé	9	8.5	[3.1–13.8]

Table 2 HIV status of patients

	Number	Percentage	IC 95 %
HIV			
Positive	95	89.6	[82.2–94.7]
Negative	11	10.4	[5.3–17.8]
Type HIV			
HIV 1	80	75.4	[62.2–83.5]
HIV1 + HIV2	5	4.8	[0.7–8.8]

7.8 %. The mean age of the patients was 40.17 ± 9.89 years (range 4–78). The number of cases was more important in patients aged between 36 and 45 years (50.9 %) than in other groups. There were slightly more male (53.8 %) than female (46.2 %) patients. The majority of patients were originated from urban (35.9 %) and suburbs (34.9 %) areas of the country, while few participants were originated from rural areas (8.5 %). Most of patients were married (54.7 %) (Table 1). The majority of patients were farmers (16 %) and housewives (16 %).

Of the 106 cases of CM, 89.6 % were found to be infected with HIV and 75.4 % were type 1 (Table 2). The median CD4+ count was 27/mm^3 (range 1–375/mm3). Approximately 79.5 % of the patients had <100 CD4+ lymphocytes/mm^3.

Table 3 Laboratory parameters

	Number	Percentage	IC 95 %
India ink staining			
Positive	35	55.6	[42.5–68.1]
Negative	28	44.4	[31.9–57.5]
Culture			
Positive	35	56.5	[43.3–69.0]
Negative	27	43.5	[31.0–56.7]
CSF crypt antigen			
Positive	48	92.3	[81.5–97.9]
Negative	4	7.7	[2.1–18.5]
Serum crypt antigen			
Positive	86	95.6	[89.0–98.8]
Negative	4	4.4	[1.2–11.0]

Table 4 Assessment of the performance of India ink staining compared to culture

		Culture		
		Positive	Negative	Total
India ink	Positive	32	0	32
	Negative	2	26	28
Total		34	26	60

Laboratory Findings

It was observed that 35 patients of the 63 tested (55.6 %) were positive by India ink preparation. Culture on Sabouraud dextrose agar was positive in 35 cases of the 62 tested (56.5 %) within 72 h of inoculation. Cryptococcal antigen was detected in cerebrospinal fluid in 48 patients of 52 tested (92.3 %). Cryptococcal diagnosis was made by positive serum cryptococcal antigenemia in 86 cases (95.6 %). (Table 3).

Assessment of the Performance of Diagnosis Methods

A comparison of the different methods by using culture as reference revealed that India ink staining presented sensitivity at 94.11 % and specificity at 100 % (Table 4). Cryptococcal antigen detection in CSF was also compared to culture and shown sensitivity and specificity, respectively, at 96.96 and 15.78 % with 16 cases of false positive (Table 5).

Antifungal Treatment and Outcome

Of the 106 cases recorded, ninety-two patients were treated with fluconazole in monotherapy (86.7 %) while five (4.7 %) received Amphotericin B. The combination of these two drugs was administered to five patients (4.7 %). For the remaining four cases, the antifungal treatment has not been clarified. (Table 6). Among these 106 cases, 38 patients (35.8 %) received ARV treatment.

Despite treatment, the mortality was high with 62.2 % deaths. However, 39 patients survived the infection and left the hospital with fluconazole treatment (Table 6). In one case, the final clinical outcome was not available as the patient was discharged before a fungal etiology could be confirmed or he left the hospital against medical advice.

Discussion

The incidence of infections caused by the encapsulated yeast *C. neoformans* has risen markedly over the past 20 years as a result of the HIV epidemic and increasing use of immunosuppressive therapies [14]. This study describes the epidemiology of the cases diagnosed in our laboratory from 2004 to 2011.

Table 5 Assessment of the performance of CSF cryptococcal antigen detection compared to culture

		Culture		
		Positive	Negative	Total
CSF Crypt Antigen	Positive	32	16	48
	Negative	1	3	4
Total		33	19	52

The prevalence of cryptococcosis meningitis noted in this study is more important than those described in the region by some authors in Abidjan and Libreville with prevalence, respectively, of 5.4 % [15] and 1.7 % [16]. But this prevalence is similar to findings in Cameroon [17]. Compared to the results from other parts of the world, the prevalence of cryptococcosis varies from place to place [18, 19]. The mean age of the patients in this study is comparable to the results reported in many studies carried out in Europe [19, 20], India [21] and Africa [22] varying between 34 and 39 years. The age ranges in *C. neoformans*-positive patients (36–45 years) obtained in this study fit in with findings from previous studies [17]. A high incidence of cryptococcosis among patients ranging from 20 to 49 years has been documented by investigators from many parts of the world [23, 24]. It should be noted that this age group is probably the most infected by HIV. It was also observed in this study that there were more male than female as described in other studies [16, 22]. This may reflect the difference of exposure rather than difference in host susceptibility [17].

We noted during this study that the majority of patients were farmers (16 %) and housewives (16 %). It can be explain by the proximity of this category of persons with soil contaminated by excreta of pigeon.

HIV infection remains the main factor predisposing to cryptococcosis infection in Senegal. It was noted that 89.6 % of the cases in this study were found to be infected with HIV with the predominance of virus type 1 (94.1 %). This study confirms also that cryptococcosis affect severe immunocompromised patients because 79.5 % of our patients had <100 CD4+ lymphocytes/mm^3. These findings are in accordance with results reported in the literature describing the high incidence of cryptococcosis in patients with CD4 counts <200 cells/mm^3 [14]. However, it should be

Table 6 Antifungals treatments and outcome of cryptococcosis cases

	Number	Percentage	IC 95 %
Antifungal treatment			
Fluconazole	92	86.7	[80.2–93.1]
Amphotericin B	5	4.7	[0.6–8.7]
Fluconazole + Amphotericin B	5	4.7	[0.6–8.7]
Outcome			
Survived	39	36.7	[27.5–45.8]
Died	66	62.2	[52.9–71.4]
Not available	1	0.9	[0.03–6.08]

noted that 11 patients in this study were not infected by HIV. Unfortunately the predisposed factor was not known because CD4 lymphocytes were not counted in order to know whether these patients were immunocompromised or not.

All the cryptococcosis meningitis cases in this study were diagnosed by using conventional approach such as culture on SDA and/or microscopic detection of encapsulated yeast on India ink preparation and/or cryptococcal antigen detection by LA in CSF and/or serum. In this study, the number of sample positive in India ink preparation (55.6 %) and in culture (56.5 %) is less than that obtained in a similar study (70 %) in the same locality about fifteen years back by Soumare et al. [13]. It is also less than results described in many other studies in the rest of the world suggesting that India ink preparation and culture can make the diagnosis in 70–90 % of cases [25–27]. One of the reasons in this study can be early treatment before lumbar puncture. However, we noted that India ink preparation presented excellent sensitivity (94.11 %) and specificity (100 %) compared to the culture considered as reference. This finding demonstrated the importance of this method in resource-limited settings like our context because it is characterized by its low cost and can be performed with minimal laboratory infrastructure. But in order to improve an effective diagnosis of cryptococcosis, it is necessary to complete this method by other tests such as *Cryptococcus* antigen detection in the samples.

Indeed, cryptococcal antigen was detected in our study in cerebrospinal fluid in 48 patients of 52 tested (92.3 %) and in serum in 86 cases (95.6 %). And when it was compared to culture it showed a high sensitivity (96.96 %) but a poor specificity (15.78 %) with 16 cases of false positive. The results of the sensitivity observed in this study are comparable to those reported in the literature [14, 27], but the specificity is less than data from the same studies. Our findings emphasize the problem of the specificity of these tests which can provide positive results in many other fungal infections (*Trichosporon asahii* or other yeast of *Cryptococcus)* or in cases with positive rheumatoid factors [27]. Despite the possibility of false positive, C*ryptococcus* antigen detection remains an excellent tool essential for early diagnosis of cryptococcal disease.

According to WHO, in HIV-infected adults, adolescents and children with suspected first episode of (CM), prompt lumbar puncture (LP) with measurement of CSF opening pressure and rapid CSF cryptococcus antigen assay (either LA or lateral flow assay) or rapid serum or plasma CrAg (either LA or LFA) are recommended as the preferred diagnostic approach. The Guideline Development Group recognized the need for cost reduction in CrAg assays to make them more widely available in resource-limited settings. Countries should develop plans to improve access to rapid CrAg assays, although the speed and completeness of access will be determined by each country's health system capacity, cryptococcal burden, ART coverage and available funding [2].

Regarding the management cases, the majority of our patients received intravenous or oral fluconazole treatment due to its availability in hospital. Only four patients were treated with amphotericin B. Despite these treatments, 62.2 % died during their hospitalization. This high mortality rate can be explained by the unavailability in most cases of amphotericin B and flucytosine which are recommended by the WHO as the first-line treatment in combination [2]. These findings are similar to results described by Soumaré et al. in a study carried out in Senegal 10 years ago with the same drugs used and a high mortality rate at 71.1 % [13]. However, the mortality rate noted in this study is more important than that observed in burkina where 15/36 (41.6 %) patients died during their hospitalization [28]. Other studies carried out in Gabon [29], Mali [30] and other parts of the world [5] revealed that Mortality from CM remains high in developing countries in particular in sub-Saharan Africa despite treatment with fluconazole and amphotericin B. Regarding these results, countries should prioritize the best options for diagnosis, prevention and treatment of cryptococcal disease, and propose alternatives if the best option is not available.

Conclusion

Early diagnosis and treatment are key to improving mortality from cryptococcal disease. Health care professionals need to have a low threshold for suspecting CM. Countries should prioritize widespread and reliable access to rapid diagnostic cryptococcus antigen assays, but it is important to make available conventional methods (India ink and culture) in the maximum of laboratory in regional health facilities.

Conflict of interest None.

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