Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/17128
DOI URL: http://dx.doi.org/10.21474/IJAR01/17128

RESEARCH ARTICLE

CRYPTOCOCCAL INFECTION IN PLHIV : SERIES OF 12 CASES AT THE LABORATORY OF MOHAMMED VI UNIVERSITY HOSPITAL OF MARRAKECH

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Manuscript Info

Manuscript History
Received: 20 April 2023
Final Accepted: 24 May 2023
Published: June 2023

Key words:-
Cryptococcosis, HIV/AIDS, Opportunistic Infection, Cryptococcal Meningitis

Abstract

Background: Cryptococcal infection is opportunistic and causes high morbidity and mortality among severely immunocompromised patients, specially those living with HIV/AIDS (PLHIV). Cryptococcus neoformans is the most frequently identified species, incriminated in 90% of cryptococcal meningitis (CM).

Methods: This is a descriptive retrospective study over a 2-years period (2021 - 2022) involving 12 PLHIV in whom we have isolated Cryptococcus neoformans from cerebrospinal fluid (CSF), blood culture, respiratory or skin specimen. All patients received antifungal therapy, associated to therapeutic lumbar punctures for those who presented with increased intracranial pressure. The clinical examination findings, laboratory data and evolution under treatment of these patients were reviewed.

Results: The twelve patients were all HIV-infected adults, with median age of 41.25 ± 12.22 years and male predominance with a sex ratio of 1:2. All patients had low CD4 T-cell counts at diagnosis (<100 cells/µl). Neurological involvement existed in all patients of this cohort : Isolated CM was diagnosed in three cases (25%). Nine patients (75%) had disseminated cryptococcosis: Central nervous system (CNS) with positive hemoculture in six cases, CNS and pulmonary involvement with positive hemoculture in two cases, CNS and cutaneous involvement with positive hemoculture in one case. Cryptococcus neoformans was identified in all cases. Of the twelve patients, seven (58%) survived with good response to the treatment. Lethality rate was 42%.

Conclusion: This study demonstrates cryptococcal disease is a high mortality infection in PLHIV.Priority should be given to access to rapid diagnostic CrAgtests to accessibility to liposomal amphotericin B. This could improve the clinical outcome of the patients in our moroccan context.

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Introduction:-

Cryptococcosis is a fungal infection with worldwide distribution caused by pathogenic encapsulated yeasts in the genus Cryptococcus. Two species of Cryptococcusare frequently responsible for human illness: Cryptococcus neoformans and Cryptococcus gattii. Over the last decades, as population of immunocompromised patients have expanded, cryptococcal meningitis (CM) became an infection of global significance [1]. Cryptococcal infection (CI) is one of the prevalent opportunistic infections that has a high morbidity and fatality rate among patients living with HIV (PLHIV). CI still accounts for 19% of AIDS-related deaths in 2020 [2, 3]. CM is by far the most frequent manifestation, accounting for 70–90% of HIV-related CI. Cryptococcal disease can also present with pulmonary, skin, lymph node or bone involvement.Despite efforts to improve immunological response in PLHIV by early antiretroviral treatment (ART), certain patients still exhibit a high prevalence of HIV-associated cryptococcosis[4]. In Morocco, HIV prevalence remains low, estimated at around 0.08%, with a dynamic stability in the general population for several years. It is higher in men (0.09%) than women (0.07%). The 2017-2023 National Strategic Plan to Combat AIDS has been extended and aims, among other objectives, to reduce AIDS-related mortality by 50% by 2023. It is based on UNAIDS guidelines [5]. Moroccan studies focusing on cryptococcosis in PLHIV are rare. To date, only four retrospective studies have been published and national epidemiological data are lacking on cryptococcosis in PLHIV [6].

Materials And Methods:-

This case series was conducted in the Parasitology-Mycology Laboratory at Mohammed VI University Hospital of Marrakech, Morocco. This is a descriptive retrospective study over a 2-years period (2021 - 2022), involving 12 PLHIV that were hospitalized at Mohammed VI University Hospital of Marrakech and from whom we isolated Cryptococcus neoformans from cerebrospinal fluid (CSF), blood cultures, bronchoalveolar Fluid (BALF) or skin biopsy samples.

In the Mycology Laboratory, we performed direct microscopic examination by India ink preparation of CSF and bronchoalveolar lavage fluid (BALF) samples. Skin biopsy smearwas stained with May Grunwald Giemsa (MGG). Sabouraud Dextrose Agar (SDA) and SDA with Chloramphenicol were used for the isolation and cultivation of the yeast. Bactec Mycosis IC/F vials were used for blood cultures. Identification was performed using VITEK 2 AST cards, BioMérieux®. Detection of Cryptococcus neoformansglycuronoxylomannan antigen (CrAg) by latex agglutination technique (Pastorex Crypto Plus BioRad®) was systematic for negative India ink CSFs, and for serum and BALF samples.

All patients received antifungal therapy. As flucytosine and liposomal amphotericin B were not available, patients were treated with the combination of amphotericin B deoxycholate (3 mg/kg) and fluconazole (1200 mg) in the induction phase followed by treatment with fluconazole (400-800 mg/day) in the consolidation phase. Patients with CM complicated by increased intracranial pressure underwent serial therapeutic lumbar punctures combined with antifungal therapy.

The clinical examination findings (age, sex, symptoms at diagnosis, treatment regimen, evolution under treatment) and laboratory data (cellular and biochemical CSF findings, direct examination results either by India ink or MGG staining, CrAg detection result, growth times of colonies for each sample, species identification, T-CD4 cell count, viral load) of all patients were collected and reviewed.

Results:-

The twelve patients were all HIV-infected adults, with a median age of 41.25 ± 12.22 years (24 - 70) and male predominance (eight are men against four women), giving a sex ratio of 1:2 (F/M). Neurological involvement existed in all patients of this cohort. Isolated CM was diagnosed in 3 cases (25%). Nine patients (75%) had disseminated cryptococcosis: Central nervous system (CNS) with positive hemoculture in six cases (50%), CNS and pulmonary involvement with positive hemoculture in two cases (16.7%), CNS and cutaneous involvement with positive hemoculture in one case (8.3%). Clinically, CM was revealed by impaired consciousness in 4 patients (33%), febrile meningeal syndrome in 3 patients (25%), intracranial hypertension syndrome in 3 patients (25%) and febrile seizures in 2 patients (17%). Direct detection of the fungus by India ink staining was positive in 75% of CSF samples (9/12) showing round yeasts sometimes budding surrounded by a capsule highlighted in negative and forming a peripheral halo(Figure 1). The skin biopsy smear was stained with MGG and showed round capsulated yeasts (Figure 2). All CSF samples were clear in appearance and biochemically normal, the cellularity was variable

from normal to lymphocytic. CrAg detection by latex agglutination technique was positive in 92% of cases (11/12) and culture positive in 100% of cases. The growth times of Cryptococcus colonies were variable, ranging from 2 days to 11 days.Colonies were mucous in appearance and white to beige in color.

Cryptococcus neoformans was identified in all cases. All patients had low CD4 T-cell counts (<100 cells/µl), the mean CD4 T-cell count was 41 cells/µl. The mean viral load was 942851.75 copies/mL.In two patients (16%), HIV infection was diagnosed during the investigation of cryptococcosis. Of the twelve patients, seven (58%), including those who presented increased intracranial pressure at diagnosis, survived with good response to the treatment. Lethality rate was 42%.

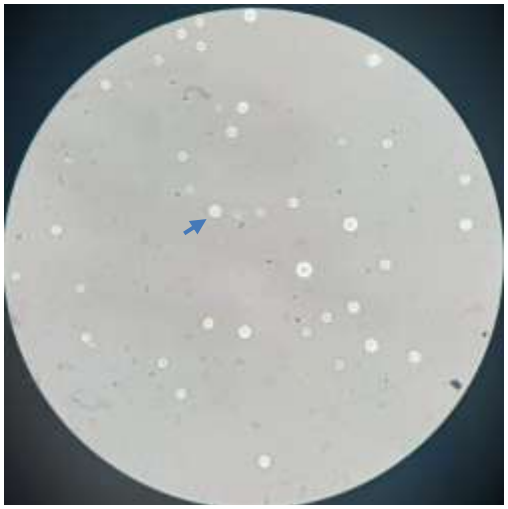

Figure 1: India ink staining of CSF.

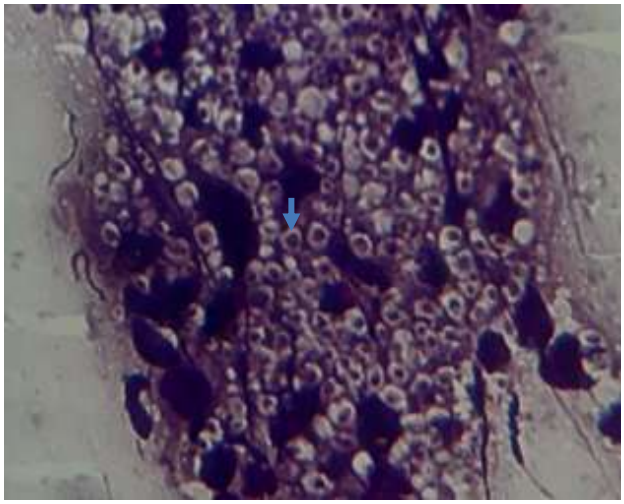

Figure 2: Skin biopsy smear, MGG staining.

Discussion:-

All the patients in this series were adults with age at diagnosis ranging from 24 - 70 and male predominance (sex ratio of 1:2). One of the risk factors for cryptococcosis in HIV-infected people is a CD4 T-cell count of less than 100 cells/µl [4]. However, certain people still exhibit a high frequency of HIV-associated cryptococcosis despite efforts to restore and maintain immunological response with early antiretroviral therapy (ART) among HIV-infected patients with greater CD4 T cells [7]. In all our HIV co-infected patients, we identified Cryptococcus neoformans, and all patients had severe immunosuppression, as evidenced by their low T-CD4+ counts. Although it has been reported that cryptococcosis can affect children, the condition is rare, and little is understood about why immunocompromised children are less likely to contract it than immunocompromised adults [3]. In observational studies conducted in South Africa, North and South America, cryptococcosis in children was reported in only 2-2.6% of all cryptococcosis cases [8].

Globally prevalent and isolated from the excrement of a wide range of birds and from various mammals, Cryptococcus neoformans also makes up the bulk of clinical isolates, and is typically recovered from PLHIV or otherwise immunosuppressed [9]. Approximately 95% of CI are caused by C. neoformans (serotype A) strains with the remaining 4% to 5% of infections caused by C. neoformans (serotype D) or C.gattii (serotypes B/C strains) [1]. As soon as the T-CD4+ cell count drops below 100 cells/µL, CM becomes a serious opportunistic infection in AIDS patients. It mainly affects patients with compromised cell-mediated immunity[1].

Most developed countries have seen a significant decrease in the frequency of HIV-associated cryptococcosis thanks to the widespread use of effective antiretroviral therapy (ART) [10], yet, the prevalence of CM is still significant in countries with poor access to medical resources [11].

CNS and lung are commonly affected in cryptococcosis. Other less common locations for infection include the skin, prostate, eyes, and bones or joints, however in individuals with severe immunodeficiency this yeast may spread broadly and infect any organ [12].

Combined lung and CNS infection is common [13]. CNS symptoms are easier to identify while lung infection is probably underdiagnosed clinically [14]. Manifestations of pulmonary cryptococcosis (PC) can range from an isolated lung mass to multiple lung nodules or disseminated interstitial infection. To rule out CNS infection, all patients with PC should undergo lumbar puncture and cerebral imaging. Radiology is crucial, both as a supplement to laboratory testing and as the main way of detection in patients who are asymptomatic or have non-specific symptoms. Single or numerous pulmonary cryptococcomas may be accompanied by widespread cryptococcosis and/or CM, necessitating lengthy treatment regimens [12]. PC is typically symptomatic in immunocompromised people and can advance quickly to acute respiratory distress syndrome [15]. High mortality rates are associated with acute respiratory failure; a small series of cases reported a mortality rate of 100% [16]. Despite the fact that CNS symptoms are typically more prominent, PC affects 10% to 55% of patients with AIDS-associated cryptococcal meningoencephalitis [15].

The clinical appearance of PC may not be differentiated from other opportunistic pulmonary infections as tuberculosis (TB) in regions with a high TB burden, as in our context, and associated infections are described [17].

In AIDS patients, PC can also be confused with Pneumocystis jirovecii pneumonia which necissitates accurate diagnostic tests [13]. To optimise the diagnostic yield in PC, the optimal specimen types are serum for CrAg detection and lung tissue biopsies. BALF samples, especially in immunocompromised individuals, provide high-quality lower airway specimens in the absence of tissue samples [18].

CM represents 70-90% of HIV-related cryptococcal disease [4]. Neurological involvement exists in all patients of this cohort. Clinical manifestations of CNS involvement include a myriad of signs and symptoms, such as headache, fever, cranial neuropathies, altered mentation, lethargy, memory loss, and signs of meningeal irritation [19]. In areas where other endemic diseases also present with symptoms similar to those of CM, early signs such as fever and headache may confound accurate identification and postpone antifungal treatment. In severely immunocompromised HIV-infected patients with CM, yeasts can reach levels of more than 10^6 /ml of CSF. As a result, these patients might experience symptoms more quickly, have higher CSF CrAg titers, and have higher intracranial pressures [1]. Increased intracranial pressure is associated with increased morbidity and mortality [20]. Among this cohort, 25% presented an increased intracranial pressure. They underwent serial therapeutic lumbar punctures associated to antifungal therapy with good evolution under treatment. These patients are known to have poor antifungal medication response and require early neurosurgery intervention to manage intracranial pressure, in association with longer antifungal therapy for a successful outcome [21]. Actually, no clinical trials have examined the ideal timing and volume of CSF draining necessary to enhance clinical outcomes in patients with high intracranial pressure, nor have they examined whether this can be determined just by clinical symptoms or whether it necessitates monitoring and measuring CSF opening pressure. The maximum amount of CSF that can be securely drained with a single lumbar puncture is unknown. After every 10 ml is removed, the CSF opening pressure can be evaluated once again. Typically, 20 to 30 ml are adequate to significantly lower the opening pressure [3]. In this series, only one patient presented with cutaneous cryptococcosis, associated with CNS involvemennt and positive hemoculture. Cutaneous cryptococcosis in PLHIV is often a marker of disseminated infection and can present with a wide range of skin manifestations. Disseminated cryptococcosis commonly presents as meningitis and cutaneous papules, nodules, or ulcers, and because lesions are sometimes difficult to identify, a skin biopsy with culture and histopathology is crucial for making a conclusive diagnosis [1,22]. As diagnosis is key to improving mortality from cryptococcal disease and in order to rule out CM, all PLHIV with a positive CrAg result on screening should be thoroughly examined for meningitis symptoms and, if possible, have a lumbar puncture with CSF analysis and CrAg test (or India ink if CrAgtest is not available). Rapid diagnostic tests, ideally lateral flow assays for CrAg detection in CSF, serum, plasma or whole blood, should be available [3].

In our series, lethality rate was 42%. Low-income countries continue to have the greatest rates of CM mortality, the projected one-year death rate for PLHIV who get care for CM is 70%, compared to 20-30% in high-income nations [3]. Radha Rajasingham and al. reported an annual estimate of 181 100 deaths due to CM in 2014. However, the proportion of AIDS-related deaths due to CI did not improve between 2014 (15%) and 2020 (19%), which raises serious concerns regarding the prevention and control of the HIV epidemic [2]. Delay in diagnosis is a crucial factor in this high fatality rate. First-line antifungal medication's limited accessibility and high price, in addition to the incapacity in low-income countries to detect and control treatment-limiting side effects and the problems associated with increased intracranial pressure are further contributing factors to mortality [23, 24]. Our patients were treated according to the 2018 WHO guidelines by a 14 days regimen of amphotericin B deoxycholate + fluconazole in the induction phase because liposomal amphotericin B and flucytosine were not available. Given its superior safety and

comparable effectiveness, liposomal amphotericin B is recommended over amphotericin B deoxycholate. In low- and middle-income countries, however, access to liposomal amphotericin B is still very restricted, mostly due to the drug's high cost and lack of registration. In 2022, WHO recommands that a single high dose (10 mg/kg) of liposomal amphotericin B with 14 days of flucytosine (100 mg/kg per day divided into four doses per day) and fluconazole (1200 mg/daily for adults; 12 mg/kg per day for children and adolescents up to a maximum of 800 mg daily) ought to be employed as the optimum induction regimen for CM treatment. Since intravenous therapy is not necessary after the first dose and the requirement for toxicity monitoring is decreased, this may allow patients to leave the hospital earlier. If liposomal amphotericin B and flucytosine are not available: 14 days of amphotericin B deoxycholate (1 mg/kg per day) + fluconazole is strong recommendation. Because flucytosine-containing regimens are more effective, measures should be taken to guarantee that both liposomal amphotericin B and flucytosine are available [3].

Conclusions:-

Cryptococcosis is a frequent opportunistic infection among PLHIV and an alarming cause of morbidity and death. Cryptococcal meningitis is by far the most frequent manifestation. In low- and middle-income nations, a reduction in the high death rate of cryptococcal meningitis in PLHIV can be achieved via early detection and treatment of the disease and its consequences. Access to rapid diagnostic CrAg tests, ideally lateral flow assays should be given priority. The course of therapy has to be improved, primarily by lowering drug costs and encouraging the manufacturing of generic medications, particularly for liposomal amphotericin B and oral flucytosine.

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