Spectrum of Systemic Yeast Infections in Leukemia Patients in Cameroon and Sensitivity of Isolates to Griseofulvin, Ketoconazole and Organic Extracts of Four Medicinal Plants

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Abstract

Clinical management of leukemia patients with hydroxyurea - based chemotherapy drugs and radiotherapy often does not yield very quick therapeutic benefits in patients, especially in tropical resource limited countries. Evidence based supportive and palliative care plan is critical in the clinical management of leukemia patients, which clinicians and oncologists in Africa often do not adequately implement during in-patient management in hospitals for a number of reasons including cost and knowledge. The spectrum of opportunistic infections in leukemia conditions is not well known and considered during management plans. In this study, we report the spectrum of systemic yeast infections amongst leukemia patients attending Phytobiotechnology Research Foundation clinic in Cameroon from June 2012 to June 2015 for the presence of various opportunistic systemic mycoses. All the patients in this study were already receiving hydroxyurea therapy for more than one year at the hospitals in Cameroon. The screening and testing were done through visual observation, KOH microscopy, in addition to culture tests on potato and malt extract agars. Twenty patients screened, were found with systemic yeast infections. Culture of urine, mouth swabs, vaginal fluids and blood yielded a 80% isolates of Candida albicans, and 20% Non Albicans Candida (NAC) including Cryptococcus species isolated from oral swab only. The results generally show that systemic yeasts are prevalent in leukemia patients and its co morbidity may possibly complicate effective chemotherapy treatment. The anti-yeast activity of Persea americana, magnifera indica, Moringa oleifera, and Allium sativum was significant with zones of inhibitions exhibited by Moringa oleifera and Allium sativum showing a better ant yeast activities than with ketoconazole and griseofulvin. The results also suggested the need to exploit alternative botanicals in the co-management of leukemia as well as its attending opportunistic infections is a critical step toward generating a better treatment for leukemia.

Keywords: Leukemia; Phytobiotechnology; Human immunodeficiency virus; Acute lymphoblastic anemia; Acute myeloid leukemia, Chronic lymphocytic leukemia; Chronic myeloid leukemia

Introduction

Systemic fungal infections are increasing globally in patients with immunosuppression syndromes such as cancer and human immunodeficiency virus (HIV/AIDS) and even in those receiving various viral therapies and chemotherapies [1,2]. Although the disease burdens of fungal infections are potentially high, they are hardly considered as major public health problems both clinically and in literature and at the global health stage compared to malaria, tuberculosis and some neglected tropical diseases [3,4]. In Cameroon and most Sub Saharan Africa countries, fungal infections are increasingly competing on the scale of opportunistic infections associated with poor immunity such as with HIV/AIDS [2,5,6]. Limited or no studies in Africa that has studied co infection of systemic fungi and cancer exist, especially in patients with leukemia. The potential of confection of opportunistic systemic infection in leukemia patients may hinder effective treatment of leukemia with chemotherapy.

Leukemia is a group of cancer that usually begins in the bone marrow and results in high numbers of abnormal white blood cells. These white blood cells are not fully developed and are called blast cells or leukemia cells. Symptoms may include bleeding and bruising problems, feeling tired, fever, and an increased risk of infections. These symptoms occur due to a lack of normal blood cells, with diagnosis, typically, made by blood tests or bone marrow biopsy. The exact cause of leukemia is unknown. Different kinds of leukemia are believed to have different causes. Both inherited and environmental (non-inherited) factors are believed to be involved.

There are four main types of leukemia - acute lymphoblastic anemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML) - as well as a number of less common types. Leukemia and lymphomas both belong to a broader group of tumors that affect the blood, bone marrow, and lymphoid system, known as tumors of the hematopoietic and lymphoid tissues. Treatment may involve some combination of chemotherapy, radiation therapy, targeted therapy, and bone marrow transplant, in addition to supportive care and palliative care as needed.

In a therapeutic plan, the administration of hem tonics to treat anemia as well as antiinflammatorytory and pain management are usually considered; thrombocytopenia, leucopenia and low blood cell indices...
values are conditions in leukemic patients [7]. These treatment and management plan have been so uneventful in that it is most often based on performance management rather than an evidence based management plan. The latter is important in that a comprehensive laboratory tests that does not only quantify the virulence level of the cancer but the baseline documentation of associated infections is important. The working hypothesis in this study is that a management plan for leukemia patients should a critical treatment plan for opportunistic infections such as systemic yeast. Candidemia (yeast infection in the blood) can actually slow down the efficacy of chemotherapy in slowing down the rate of white blood cell proliferation. To this effect, it is advisable that a clinical management plan that incorporates the appropriate chemotherapy, antibiotics and antifungal agents as well as alternative and complementary management schemes could be the next generational clinical approach to treating leukemia or any of the cancers.

The report on the spectrum of yeast infections in leukemia patients in Cameroon with the effect of the isolates to some commonly used medicinal plants is presented.

Methodology/Research Results

Criteria for specimen collection

An exit pool system, where patients with leukemia being treated for with chemotherapy from nearby hospitals and opted to be treated using alternative and complimentary therapy at Phytobiotechnology Research Foundation Clinic (PRF) were considered.

The Phytobiotechnology Research Foundation Clinic is a registered research Non-Governmental Organization with the Cameroon Government and registered with the European Union.

Specimen collection and tests were selected based on the complains given by the patients as well as Para- clinical examinations. Only patients diagnosed with leukemia and placed on chemotherapy at the nearby hospitals were considered. Leukemia Patients’ without symptoms and signs of fungal infections were excluded from the analysis of fungi.

Specimen collection

The patients were 20 leukemia patients whose status was confirmed at the hospitals within Bamenda and beyond. This number represents patients received between 2012 and 2015 and was receiving Hydroxyurea and morphine for pain management and re-confirmed at the PRF Clinics through a total white blood cell count. The blood smears made and stained using Leishman stains to examine the blood picture for morphological anomalies with the blood cells. The anomalies confirmed were blast cells. Hemocytometer was used to re-establish the total white blood cells for each patient. Six specimens each was taken from each patient; two urine specimens, two oral swabs, two blood specimens. A total of 120 specimens were screened for opportunistic systemic fungal infections.

Microscopic examination and culture of oral swabs, urine, and blood for detection of fungi

The specimens were appropriately processed using the KOH method [8] and portions of each specimen were cultured aseptically by the streaking methods onto Potato and Sabouraud dextrose agars and incubated at room temperature at 27°C for 72 hours according to methods described by [9]. The blood specimens were initially suspended in selenite fluid and incubated at 25°C for 24 hours and sub cultured daily onto potato dextrose and sabaraud dextrose agars. The plates were incubated at 25°C for 7 days for any fungal growth. Each specimen was cultured in duplicate to minimize potential errors that can be due to contamination. Plates were examined for morphologically on culture plates and micro morphology of the yeast isolate under x10 and 40 objectives were described.

Sources, identification and processing of anti-yeast plant material

The plant materials (Avocado seeds (Persea americana) and mango seeds (magnifera indica and Garlic bulbs (Allium sativum) and Moringa oleifera) were collected from Boyo Division of North West Cameroon. The plants and parts were selected based on their ethnofolkloric history, in the use for traditional medical preparations in the treatment of multiple infections on cancer patients by traditional medical practitioners of the Kom kingdom. These plants have been used by the rural people in Northern Nigeria and many parts of rural Cameroon to manage and treat fungal related diseases. The plants were all collected and identified by botanists at the Catholic University of Cameroon, Bamenda with Voucher samples kept at PRF research laboratory. The seeds and bulbs were sun-dried for three weeks and then pulverized in a mortar using a pestle, sieved using sieves of 3 mm mesh and stored in brown khaki envelopes for extraction.

Extraction procedures

The sun-dried fifty grams (50 g) plant material was added to 250 ml of methanol (1:5w/v) in 250 beakers (pyrex) for each plant powder and allowed to extract for 72 hours [6]. The extracts were filtered using Whatman filter paper no 1 (Whatman, UK) and the filtrate solvent was evaporated under vacuum using rotary evaporator at 55°C and the resulting dried extracts were stored in sterile screw capped bottles and kept at room temperature.

Determination of anti-yeast activity of the extracts

Agar diffusion method according to [10] was employed. Zero point (0.2 g) of the plant extracts was reconstituted in 5 ml of distilled water and methanol. Each of the extracts was incorporated in 15 ml of each of the agars in molten state and allowed to solidify: A 6mm of steel boner was used to bore 2 week culture of each of the yeast isolates and carefully placed into each of the bored well at the center of the plate and placed at room temperature for the yeast to grow and spread out. A control set up by introducing the extracting solvent (methanol and water) into the different wells as well as 0.2 mls of ketoconazole 200 mg, and griseofulvin 500 mg the frequently prescribed antifungal drugs in hospitals was applied.

The plates were incubated at room temperature of 27°C for 72 hours. The development of inhibition of mycelia spread containing the extract indicated the anti-fungal activity of the plant extracts against the test organism. The differences between the inhibition rates of the mycelia spread observed for the test and that of the control was recorded as actual diameter of zones of inhibition caused by the plants extracts.

Results and Discussion

The results indicated in Figure 1 show that 60% of the patients with leukemia who consulted have urinary tract infected with yeast. A lot
budding yeast cells were observed during direct microscopic observation of centrifuged urine. A lot of epithelial cells were observed. These morphological structures in the urine of these patients with leukemia may suggest a high level of virulence. The results show that yeast was found in all the specimens from the patients with leukemia. The presence of budding yeast from the oral swabs and urine under the microscope possibly suggested invasiveness also by presence of keratinized epithelial cells and debris (Figure 1 and Table 1). All the patients with leukemia in this study had infectious oral candidiasis except 5% of the patients presenting with cryptococcosis.

<table>
<thead>
<tr>
<th>Total Number of Specimen analyzed from leukemia patients</th>
<th>Total number of specimen with yeast (direct microscopy)</th>
<th>Positive culture cases</th>
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<tr>
<td>20 patients, 40 specimens</td>
<td>40 (100%)</td>
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**Table 1:** Prevalence of yeast isolated from oral swabs (Buccal cavity) from leukemia patients at PRF clinic.

Clinical examination of the tongue showed that all the patients had it coated with white flakes, diagnostic of oral thrush. The infectiology and immunology of systemic yeast infections amongst leukemia patients has not been previously reported. Yet, the infectiology may possibly generate poor treatment response in leukemia management plan with hydroxyurea. In this study, the average white blood cell counts of the patients were 65000 cells/mm³, and all patients were on the chemotherapy with 6 patients with chronic pain being managed with periodic high doses of morphine. In Table 2, the results indicated that 40% of the patients with leukemia had Candida isolated from their blood. Although, blood cultures had lower Candida isolates compared to a standard drug—ketoconazole and griseofulvin as well as both water and methanol controls. Persea americana seeds extracts also demonstrated an inhibition of yeast but lesser than with Magnfera indica (mango) seeds (Figure 3). In a related study reported the activity of a number of medicinal plant extracts on yeast isolates from clinical specimens from HIV patients in Cameroon. Extracts from Allium sativum (garlic) and Moringa oleifera exhibited very high anticaandidal activity more than with griseofulvin and ketoconazole. This is an important observation in that not only can griseofulvin and ketoconazole be used in the treatment of systemic mycosis, but that these plant extracts can be further purified as potential anti yeast candidate drugs to treat infections caused by Candida species from leukemia patients.

It is difficult to conclude at this stage, that the level of the white blood cells counts of the patients did not significantly drop despite treatment with chemotherapy-hydroxyurea, with reason associated with co morbidity due to candidemia. Although, too premature to make this conclusion, the possibly of co morbidity of candidemia complicating chemotherapy and leading to poor or slow response cannot be easily dismissed.

One Candida species—Candida albicans was frequently isolated from all the specimens (Figure 2). Other Non Albican Candida (NAC) were observed (20%) but due to limitation of molecular tools to carry out a full characterization of the isolates. Cryptococcus species was isolated from oral swabs taken from the patients (Table 1). Cryptococcus has frequently been isolated from HIV/AIDS patients with upper respiratory tract infections, but there is dearth of information on the incidence of Cryptococcus on cancer patients. This report potentially fills this gap.

The data in Figure 3 shows that the methanol extracts of the plants used in this study demonstrated a greater yeast inhibition on all the yeast isolates compared to a standard drug—ketoconazole and griseofulvin as well as both water and methanol controls. Persea americana seeds extracts also demonstrated an inhibition of yeast but lesser than with Magnfera indica (mango) seeds (Figure 3). In a related study reported the activity of a number of medicinal plant extracts on yeast isolates from clinical specimens from HIV patients in Cameroon. Extracts from Allium sativum (garlic) and Moringa oleifera exhibited very high anticaandidal activity more than with griseofulvin and ketoconazole. This is an important observation in that not only can griseofulvin and ketoconazole be used in the treatment of systemic mycosis, but that these plant extracts can be further purified as potential anti yeast candidate drugs to treat infections caused by Candida species from leukemia patients. The potential of a treatment of systemic opportunistic fungi, especially due in leukemia patients, at low cost in the future is perceivable.

Long term clinical use of imidazole- antifungal derivatives such as ketoconazole, flucanazole and econazole are becoming ineffective to treat many of the systemic fungal infections seen in these countries and these have been blamed largely on the unavailability of established antifungal gram guided prescription in health centers in these countries [2].

Similarly, other antifungal drugs such as griseofulvin, nystatin, diflucan, benzoic acids, and salicylic acid have also been over prescribed in sub Saharan Africa and over the years; leading to the...
development of resistant strains of many fungal species especially yeast [7,11]. The thrust of this study were to (I) report the prevalent systemic yeast amongst leukemia patients in Cameroon receiving chemotherapy drugs but with a clinical presentation of a systemic mycosis.

Figure 3: Zone of inhibition.

The conclusion can be drawn that systemic yeast infections is prevalent amongst patients with leukemia in Cameroon, and that the possibility of co morbidity of these opportunistic infections may be slowing down the efficacy of chemotherapy. The potentials of an alternative and complementary management plan for patients with leukemia are critical and should be considered by oncologists and cancer specialists. The broader lessons from this study are that a linear clinical management plan for leukemia is outdated, and unlikely to produce better health outcomes for leukemia patients. This probably accounts for the fact that most leukemia patients die too early, whereas an integrative evidence based management plan could potentially prolong their lives.

It recommended that a comprehensive characterization of the yeast strains as well as other opportunistic infections profiling, from leukemia patients as well as an anti-fungi gram and antibiograms be considered in the management plans for such patients under hospital conditions in Africa.

Finally, the need to exploit alternative botanicals in the co-management of leukemia as well as its attending opportunistic infections is a critical step toward generating a better treatment for leukemia.

References