

Original Research Article

Prevalence of Pulmonary Mycoses among HIV infected clients attending anti-retroviral therapy clinic at Kisoro district Hospital, Western Uganda.

ABSTRACT

Aim: To determine the prevalence of pulmonary mycosis among HIV infected clients attending anti-retroviral therapy clinic at Kisoro district Hospital in Western Uganda.

Study design: This was a cross- sectional study.

Place and Duration of Study: This was conducted in the anti-retroviral therapy (ART) clinic at Kisoro District Hospital (KDH) during the months of March to July, 2016.

Methodology: We collected sputum samples from HIV sero positive clients that were microscopically examined using Ziehl Neelsen (ZN) staining technique. Also, sputum was cultured on sabroid dextrose agar (SDA), and identified to isolate fungal pathogens. Further, we obtained about 4.0 milliliters of ethylene-di-amine tetra acetic acid (EDTA) blood that was used to assay CD₄⁺ cell counts. Data was analyzed, and presented as proportions.

Results:

Conclusion: Of the 252 participants, 136 (53.97%) were females. The mean and median age was 38.94 (SD±12.64) and 38 years, respectively. The prevalence of pulmonary mycosis was 34/252 (13.49%; 95% CI=9.53-18.34), of which 12(35%; 95% CI=19.75-53.51) were molds and 22 (64.71%; 95% CI=46.49-80.25) were yeasts. The species isolated and their proportions were: *Candida albicans* (10/252, 3.97%), other *Candida* species (7/25, 2.78%), *Aspergillus* species (6/252, 2.38%), *Penicillium* species (5/252, 1.98%), *Cryptococcus* species (4/252, 1.59%), *Basidiomycetes* (1/252, 0.40%) and *Acremonium* species (1/252,

0.40%). From this study, we report a high prevalence of pulmonary mycosis that is associated with deteriorating immunological state. In addition, it presents with signs and symptoms aping *Mycobacterium tuberculosis*.

Keywords: opportunistic infections, pulmonary mycosis, HIV, Uganda.

1. INTRODUCTION

The burden of human immune deficiency virus (HIV) infection in Uganda remains high [1]. This risks an estimated 60% of people living with HIV (PLWHIV) to acquire opportunistic infections (OIs) [2]. The OIs further weakens the immune system indicated by very low clusters-of differentiation-4 (CD₄+) cell counts, leading to acquired immune deficiency syndrome (AIDS) that may aggravate deaths [3, 4]. Pulmonary mycosis is associated with 58% - 81% of morbidity and mortality among PLWHIV [5]. Infected individuals present with features similar to those of pulmonary tuberculosis (PTB), and this makes both diagnosis and management difficult [6]. Whereas prevention of pulmonary mycoses would offer a seemingly better approach among the at risk population, this is complicated by the virtue that fungi are ubiquitous, which propagates their rapid spread. Though it's commended to screen suspected cases of PTB for mycosis, Kisoro district Hospital (KDH) has no strategy for this. As such, there is missed diagnosis, delayed therapeutic intervention which augments poor outcome. Besides, cases which are treated with anti-TB therapy have a poor prognosis as these drugs have no effect on pulmonary fungal pathogens, also, such cases ought not to be initiated on anti-fungal empirically due to their hepatotoxicity [7]. To this, we sought to determine the prevalence of pulmonary mycosis, as a guide on infection burden and implication on diagnosis in a low resource set up.

37 2. MATERIAL AND METHODS

38 2.1 Study site

39 The study was conducted in the anti-retroviral therapy (ART) clinic at Kisoro District Hospital
40 (KDH) during the months of March to July, 2016. KDH is government hospital located in
41 Kisoro District, Kisoro Town Council in Western Uganda along Kabale-Bunagana road. The
42 clinic has 3,012 clients from within the district, neighboring districts, parts of Democratic
43 Republic of Congo (DRC) and Rwanda. It attends to approximately 250 clients daily.

44 2.2 Sample collection

45 2.2.1 Sputum: Samples were obtained after one had inhaled deeply 3 times before coughing
46 from their chest and carefully spat into the container and screwed tightly.

47 2.2.2 Blood: We collected about 4.0 millilitres (mL) of blood into a well labeled ethylene di-
48 amine tetra acetic acid (EDTA) vacutainer. This was collected by applying a tourniquet to the
49 upper arm, then disinfecting the site with a moist 70% alcohol swab. Venipuncture was then
50 done to the prominent vein, with care to avoid hemolysis.

51 2.3 Laboratory sample analyses

52 Microscopic wet preparations using 10% Potassium Hydroxide (KOH) was done and
53 examined. In addition, India ink preparation and Lacto phenol cotton blue (LPCB) for
54 morphological features were done. These were stained using Gram staining technique for
55 fungi shape and size of fungal arrangement. Culture was done on SDA with
56 Chloramphenicol (CONDA pronadisa, Spain) for general fungal characteristics, and *Candida*
57 *albicans* were identified by a positive Germ tube. Urease test was performed to identify
58 other fungal isolates. CD₄⁺ cell counts were determined using a Becton, Dickson and
59 company (BD) FACSCount (BD 2008, USA). The following approved standard operating
60 procedures were used;

61

62 **2.3.1 Identification of fungal pathogens**

63 2.3.1.1 10% Potassium Hydroxide (KOH): using a clean glass slide, a drop of homogenized
64 sputum sample was mixed with a drop of 10% KOH solution. The mixture was then
65 incubated in a moist petri dish at room temperatures for 30 minutes. This was then
66 microscopically examined for identification features.

67 2.3.1.2 India ink preparation: This was done by mixing a drop of sputum and a drop of India
68 ink. The mixture was covered with a coverslip and microscopically examined using x10 and
69 followed by x40 objectives looking for capsule of *Cryptococcus* species which appeared as
70 round cells, against the dark back ground.

71 **2.3.2 Culture on SDA with Chloramphenicol**

72 We dried SDA plates at 37°C for 15 minutes. This was followed by streaking the sputum
73 sample onto the surface of SDA plate containing chloramphenicol. We then incubated the
74 plates at 37°C for 2 weeks and were examined for growth after every two days.

75 **2.3.3 Gram staining technique**

76 With a sterile wire loop, we picked a pure single colony, then spread it evenly over an area of
77 2x1cm on a clean slide and left it to air dry. This was heat fixed by slowly passing the slide
78 smear uppermost, three times through a flame and allowed to cool. We then flooded the
79 slide with Gentian violet stain for 60 seconds, washed the stain with clean water, and tipped
80 off excess water. We added Gram's iodine for 60 seconds, washed the stain with clean
81 water and tipped off excess water. We decolorized using 50% Acetone Alcohol for 5
82 seconds and washed off immediately with water. Flooded the smear with dilute carbol
83 fuschin (1 in 20) stain for 3 minutes then washed off the stain with clean water and wiped the
84 back of the slide clean and air dried. We examined the smear microscopically under the
85 X100 objective for fungi shape and size of fungal arrangement.

86

87 **2.3.4 Urease test on yeasts**

88 We dried urease agar in the hot air oven at 37°C for 30 minutes and then streaked the
89 colonies from the growth on SDA media. We incubated at 37°C with the lid of the universal
90 bottle loosely tightened for 24 hours and examined for color change to pink for *Cryptococcus*
91 species and no color change for *Candida* species. This was done to differentiate between
92 the *Cryptococcus* species from *Candida* species among yeast growth on SDA media.

93 **2.3.5 Germ tube technique**

94 To 1 ml of human serum in a labeled small tube, we emulsified yeast colonies and the
95 mixture was incubated at 37°C for one hour then transferred a drop of serum onto the slide
96 for microscopic examination under X40 objective for the presence of Germ tube. This was
97 used to differentiate *Candida albicans* from other *Candida* species whereby *Candida*
98 *albicans* produced a short hyphal extension that arose laterally from a yeast cell and were
99 reported as Germ tube positive and other non-*Candida albicans* were negative.

100 **2.3.6 Determination of CD₄⁺ counts**

101 We labeled each reagent tube according to the patients' identification number and vortexed
102 upside down and upwards for 6 seconds each. We opened the reagent with the coring
103 station by sliding the reagent tubes upright into the coring station. We then transferred the
104 tubes from the coring station to the workstation, keeping the tubes upright. We then added
105 50 µl of the patient's blood to 50 µl of a fixative solution into the tube, then vortexed upright
106 for 6 seconds. We then switched the on the BD FACSCount machine then entered the
107 patient identification number and ran samples.

108 **2.4 Quality Control**

109 Sample analysis was done in strict adherence to SOP. In addition, standard strains of
110 *Candida albicans* (ATCC 60193) as a positive control, *Staphylococcus aureus* (ATCC-
111 25923) and *Escherichia coli* (ATCC-25922) were included as a negative control for

112 performance of culture media. We tested for sterility of the culture media, and daily controls
113 for the BD FACS Count machine were done before running patient samples.

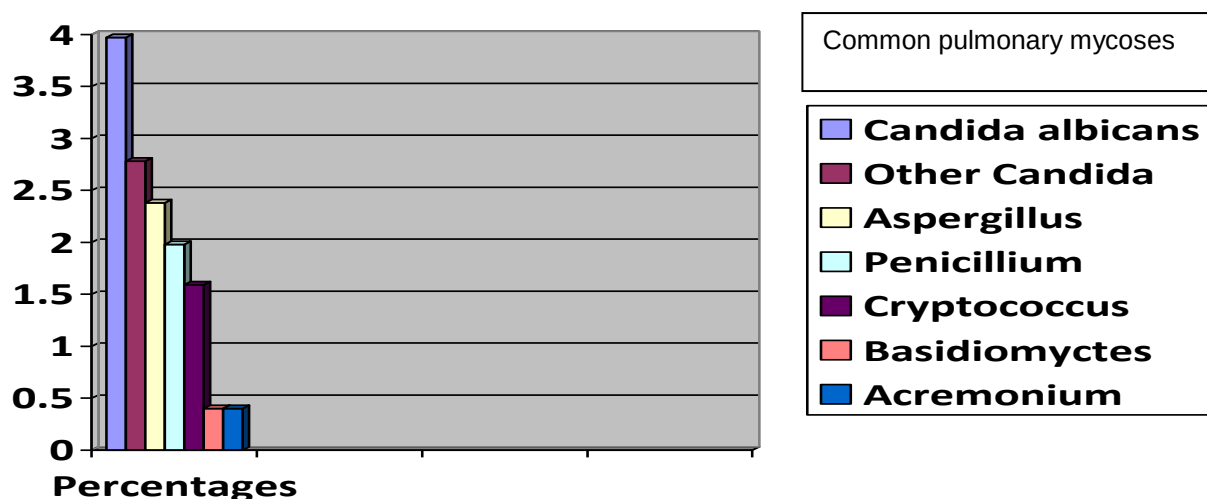
114 2.5 Data Analysis

115 Data was entered in Microsoft Excel, and imported to STATA software for analysis. Data
116 was presented using frequencies and percentages, the differences in proportions were
117 assessed using the Chi-square test at 95% CI.

118 3. RESULTS 119

120 We enrolled 252 adult HIV sero-positive participants. Of these, 136 (53.97%) were females.
121 Their mean and median age were 38.94 (SD±12.64) and 38 years, respectively. Out of the
122 252 sputum samples cultured, 34/252 (13.49%; 95% CI=9.53-18.34) had growth that tested
123 positive for fungal infections. Twelve (35%; 95% CI=19.75-53.51) were molds and 22
124 (64.71%; 95% CI=46.49-80.25) were yeasts. The species isolated were *Candida albicans*
125 (10/252, 3.97%), other *Candida* species (7/25, 2.78%), *Aspergillus* species (6/252, 2.38%),
126 *Penicillium* species (5/252, 1.98%), *Cryptococcus* species (4/252, 1.59%), *Basidiomycetes*
127 (1/252, 0.40%) and *Acremonium* species (1/252, 0.40%) as shown in figure 1.

128 Figure 1: A bar graph illustrating common pulmonary mycoses
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131

132 When a comparison with CD₄⁺ cell count was made, majority (33/34, 97.1%) had CD₄⁺ less
133 than 350 cells/μl ($\chi^2 = 80.37$, $p\text{-value} = <0.001$), as indicated in table 1.

134 **Table 1: Frequency of the relationship between CD4 counts and pulmonary mycoses**

Variables	Culture results		Total	χ^2	P. Value
	Growth (%)	No growth (%)			
CD4 counts					
<350	33 (97.06)	45 (20.64)	78 (30.95)	80.365	<0.001
≥350 cells/μl	1 (2.94)	173 (79.36)	174 (69.05)		
Total	34 (100.00)	218 (100.00)	252 (100.00)		

135

136 4. DISCUSSION

137 The prevalence of pulmonary mycosis was 13.49%. This is lower than 42.96% that was
138 earlier reported in India [8], 68.5% in Nigeria [9], 48% in Cameroon [10] and 44.18% in
139 Kenya [11]. The lower prevalence in this study is attributed to two factors: Firstly, our
140 participants had CD₄⁺ cell count above 350 [5]. Secondly, unlike the previous studies, the
141 'test and treat' HIV care approach in our set up implies that our participants were enrolled on
142 ART, which lessens the risk of OIs [9].

143 In the present study, the most dominant isolates were *Candida* species which was in line
144 with other findings reported in India [6], Cameroon [10] and Nigeria [5]. Among the *Candida*
145 species, *Candida albicans* was the most isolated which agrees with findings from other
146 studies in India [6], Kenya [11], and Nigeria [5, 9]. *Candida* is a normal flora that becomes
147 opportunistic following low immunity [12, 13], and this could be the basis as to why it was the
148 most common isolate. *Aspergillus* species were the second most prevalent with a
149 prevalence of 2.38% which was a similar observation in Kenya [11] and India [6]. This could
150 have been due to presence of spores of *Aspergillus* species in the atmosphere, their ability
151 to grow in abundance everywhere, production of small conidia that easily penetrate deep in
152 the alveoli region and grows at 37°C [14], also the possibility of ingesting contaminated
153 grains. The isolates of *Penicillium* species were in agreement with a study in Cameroon [10]

154 but slightly lower compared to 4.1% in Nigeria [5] and 6.25% in India [15]. *Cryptococcus*
 155 species was among the less common isolates in our study with a low rate, similar to studies
 156 in Cameroon [10] and India [5], but highly differing from other studies in India [8] and in
 157 Nigeria [9]. Soils and bird droppings predispose immune-suppressed patients to
 158 Cryptococcosis, and this could be the basis for the observed high prevalence [12]. Whereas
 159 a study in India isolated *Histoplasma capsulatum* and *Pneumocystis jirovecii* [6], we did not
 160 isolate them in this study. This is probably due to their isolation requirements (like scrapings
 161 from foci of infection, bronco-alveolar lavage and pulmonary biopsies) which we never
 162 performed. Further, their identification techniques like, complement fixation test, Wright's
 163 stain, Giemsa stain, direct immunofluorescence were not done; Albeit we cannot rule out
 164 their occurrence in our set up.

165 Pulmonary mycosis were seen more among patients with CD₄⁺ counts less than 350 cells/μl
 166 which was in agreement with a study in Nigeria [9]. Furthermore *Cryptococcus* species were
 167 isolated among patients whose CD₄⁺ counts were below 100 cell/ μl, and *Aspergillus*
 168 species at CD₄⁺ less than 350 cells/μl and this was similar to findings in Nigeria [3, 9].

169

170 5. CONCLUSION

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172 The findings in this study reveal a high prevalence of pulmonary mycosis. It is more among
 173 those with deteriorating immune status indicated by CD₄⁺ cell counts below 350 cell/μl. As
 174 this infection presents with signs and symptoms aping *Mycobacterium tuberculosis*, apt
 175 laboratory diagnosis is key to reduce its mismanagement, and its effects.

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177

178 COMPETING INTERESTS

179

180 The authors declare that they have no financial or personal relationship(s) that may have
 181 inappropriately influenced them in writing this article.

182 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**

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184 Ethical approval was obtained from research and ethics committee of International Health
185 Science University. We ensured strict confidentiality and high ethical standards. We obtained
186 written informed consent from each participant. Results were only availed to the attending
187 doctor for proper management. All authors hereby declare that all experiments have been
188 examined and approved by the appropriate ethics committee and have therefore been
189 performed in accordance with the ethical standards laid down in the 1964 Declaration of
190 Helsinki.

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236 **LIST OF DEFINITIONS**

237 -Opportunistic fungal infections: infections caused by fungal pathogens when the body
238 defense mechanism has been weakened.

239 -Pulmonary fungal infections: infections of the lungs caused by fungal microorganisms

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241 LIST OF ACRONYMS, ABBREVIATIONS: AIDS: Acquired Immune Deficiency Syndrome; ART:

242 Anti-retroviral therapy; CD₄⁺: Cluster of differentiation-4cells; HIV: Human Immune deficiency Virus;

243 SDA: Sabouraud Dextrose Agar; OIs: Opportunistic Infections; WHO: World Health Organization.

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