PRESENCE OF VARIOUS FUNGI IN THE ORAL SMEARS OF HIV / AIDS PATIENTS

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ABSTRACT

This study was designed to observe the various fungi present in the oral buccal smears of HIV/ AIDS patients and their relation to CD4+lymphocyte count. Oral smears from n=35 patients taking antiretroviral therapy (ART) and twenty five (n=25) patients not taking ART were taken. These smears were stained with routine (H&E, Pap) and special stains recommended for fungi i.e. Grocott-Gomori's Methenamine Silver (GMS) and Periodic Acid Schiff (PAS). The CD4+ lymphocyte count was determined using flow cytometry. Candida albicans was the most common being present in 26% patients on ART and 44% in Non-ART patients. Cryptococcus neoformans was seen in 11.4% ART patients and 4% in Non-ART patients. Coccidioides immitis was observed in 2.9% ART patients and 4% of Non-ART patients. Aspergillus was seen in 1.6% Non-ART patients only. Mean CD4+ count at which various fungi were seen was <300 cells/mm3 in all types. The CD4+ lymphocyte count was quantitatively grouped as Group 1 with CD4 + lymphocyte count <350 cells/mm3 and Group 2 having CD4+ lymphocyte count >350 cells/mm3. Fungi was found to have significant (p=0.001) association with Group1 having CD4+ count less than 350 cells/mm3 which shows their association with immunosuppression. Determination of increased colonization of Candidal and Non-Candidal fungi in oral cavity by means of simple non-invasive methods like oral smears may be helpful in indirectly predicting the immune suffered status and such patients should be given early medical treatment to prevent from disseminated fungal infections.

Key Words: *HIV/AIDS, Cytology, Candida, Non-Candida, Fungi, CD4+ lymphocyte count.*

INTRODUCTION

In HIV/AIDS patients oral fungal infections can be caused by Candidal as well as non-Candidal fungi. Oral candidiasis caused by Candida albicans are essentially presented in HIV/AIDS patients and considered as the marker of immunosuppression and disease progression.[1] It can be used for indirectly predicting low CD4+ count in HIV/AIDS patients in resource limited countries where facility of measuring CD4+ lymphocyte count is limited or not available. Most commonly non-Candidal fungi in HIV/AIDS patients include Cryptococcus neo-formans, Coccidioides immitis and Aspergillus. The oral lesions caused by these fungi are usually subclinical but may manifest as non-healing oral ulcers.[2] Previously it was considered that these non-Candidal fungi are present only in endemic areas but now these are seen worldwide.[3] Special staining i.e. Grocott-Gomori's Methenamine Silver (GMS) and Periodic Acid Schiff (PAS) staining techniques can be used for diagnosis of fungi on cytology and histopathology.[4]

METHODOLODY

Oral smears from n=35 patients taking antiretroviral therapy (ART) and twenty five (n=25) patients not taking ART were taken. These were fixed in 95% ethanol and stained using routine (H&E, Pap) and special stains recommended for fungi i.e. Grocott-Gomori's Methenamine Silver (GMS) and Periodic Acid Schiff (PAS). The CD4+lymphocyte count was determined using flow cytometry.

Statistical Analysis

The data were analyzed using SPSS 18. Chi-square tests were used to find the associations between the CD4+ lymphocyte count and fungi.

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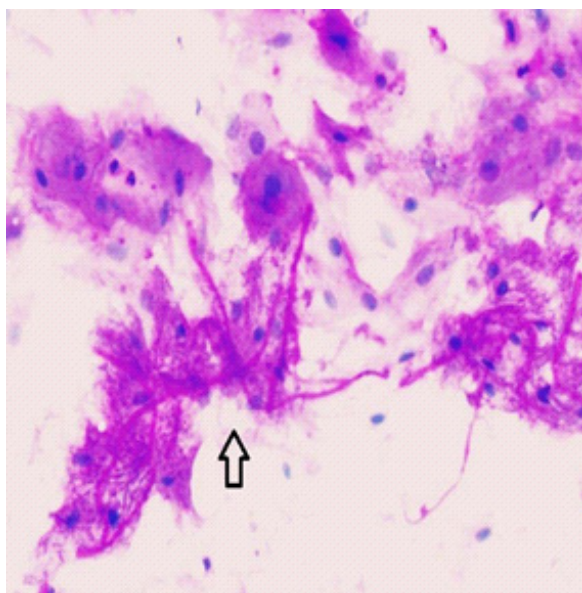Email: drahnagi@gmail.com

Fig 1: Pseudohyphae of Candida albicans (arrow) (PAS Stain) (400X)
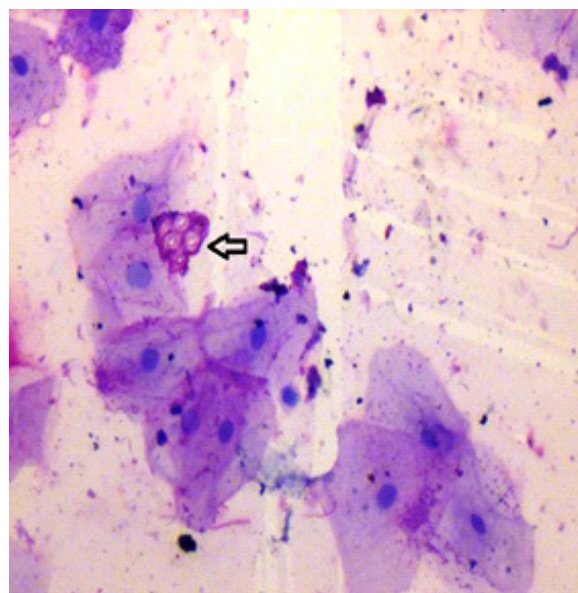
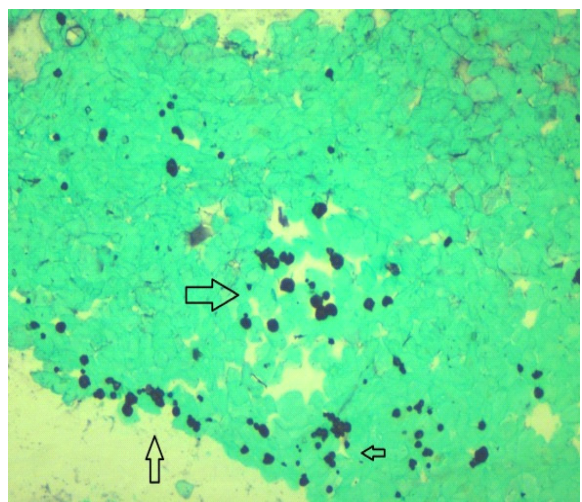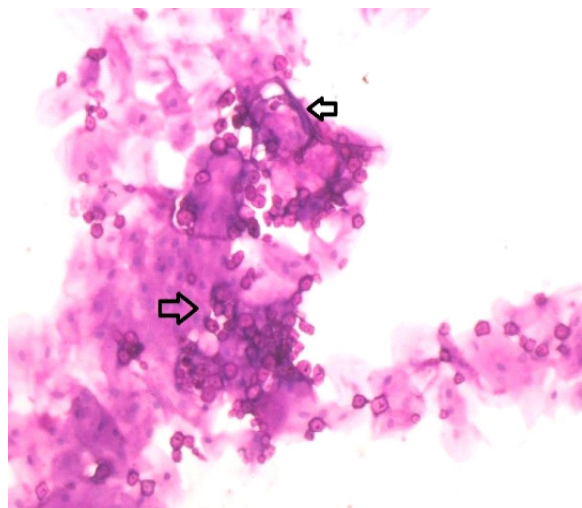Fig 3: Endospores of Coccidioides immitis on oral squamous cells (arrow) (PAS Stain) (400X)

Fig 4: Dichotomous septate hyphae of Aspergillus on oral squamous cells (arrow) (GMS Stain) (400X)

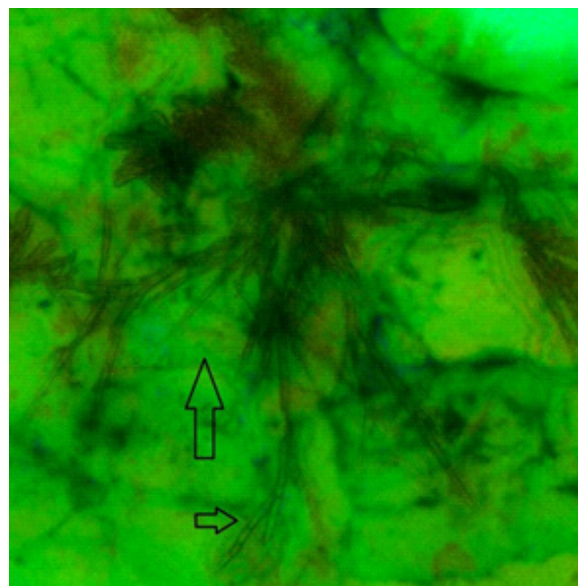Fig 2: Thick round encapsulated spores of Cryptococcus neoformans (arrow) (PAS Stain+ GMS Stain) (400X+200X)

TABLE 1: VARIOUS FUNGI IN ART AND NON-ART GROUPS

Fungi	Art	Non-Art	Total	Mean CD4+ count
	48.5% (n=17)	56%, (n=14)	52%, (n=31)	
Candida albicans	25.7%, (n=9)	44%, (n=11)	33.3%, (n=20)	249±106
Cryptococcus neoformans	11.4%, (n=4)	4%, (n=1)	8.33%, (n=5)	200±61
Coccidio immitis	2.9%, (n=1)	4%, (n=1)	3.33%, (n=2)	281±53
Aspergillus	—	4%, (n=1)	1.66%, (n=1)	162±0
Mixed	8.6%, (n=3)	—	5%, (n=3)	224±120

RESULTS

The results are summarized in Table 1. Candida albicans (Fig 1) was seen as budding yeast cells, pseudo-hyphae and true hyphae in the oral sears. Cryptococcus neoformans (Fig 2) was observed as pleomorphic yeast-like cells with thick capsule and narrow-based budding. Coccidioides immitis (Fig 3) was seen as spherule containing multiple endospores. Aspergillus (Fig 4) was observed as dichotomous septate hyphae branching at acute angles.

CD4+ lymphocyte count was quantitatively grouped as Group 1 with CD4 + lymphocyte count <350 cells/mm3 and Group 2 having CD4+ lymphocyte count >350 cells/mm3. This cut point of 350 cells/mm3 was used because it is considered as the level of advanced immunosuppression according to WHO immunological classification.5 Group 1 had n=37 while Group had n=23. Chi-square test was used to find the association between fungi and CD4+ lymphocyte count groups. Fungi was found to have significant (p=0.001) association with Group1 having CD4+ count less than 350 cells/mm3 which shows their association with immunosuppression.

DISCUSSION

No study has been reported that describes the type of fungi present in the oral squamous cells of HIV/AIDS patients although much literature is available on the clinical oral candidiasis in these patients. Hence the results of the present study cannot be compared to the other studies. In the present study most of fungi were seen at the mean CD4+ count of <300 cells/mm3 and fungi were significantly associated (p=0.001) with CD4+ group 1. These results show that Candida and non-Candida fungi are associated with low CD4+ lymphocyte count and hence immunosuppression. Increased colonization of Candida albicans as well as other non-candidal fungi (Cryptococcus neoformans, Aspergillus, Coccidiod immitis) in the oral cavity of HIV/AIDS patients is alarming as these patients cannot actively respond to these microorganisms because of immune failure. Cryptococcus neoformans have been reported to be associated with increased immunosuppression.[6] These fungi can reach to the deeper oral mucosal layers and may disseminate to distant body organs via blood and multiply their causing more serious damages to the body and overall health. Hence oral cavity can become a source of serious fatal disseminated fungal infection in such cases. Regular oral screening via oral smears to detect the increased colonization of Candida and Non-Candida fungi in HIV/AIDS patients can be helpful to these patients and proper medical measures taken at earlier stages may prevent the patients from serious consequences.

CONCLUSION

Different types of fungi including Candida albicans, Cryptococcus neoformans, Coccidiod immitis and Aspergillus were observed in the oral squamous cells of HIV/AIDS patients having mean CD4+count less than <300 cells/mm3 which shows their relation to the immunosuppression state. Determination of increased colonization of Candidal and Non-Candidal fungi in oral cavity by means of simple non-invasive methods like oral smears may be helpful in indirectly predicting the immune suffered status and such patients should be given early medical treatment to prevent from disseminated fungal infections.

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