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Research Article

**STUDY TO DETERMINE VARIOUS FUNGI PRESENT IN
THE ORAL BUCCAL SMEARS OF HIV POSITIVE PATIENTS**Dr Sara Zubair¹, Dr Benish Pervaiz², Dr Sana Fatima³¹ De'Montmorency College of Dentistry, Lahore^{2,3} Nishtar Institute of Dentistry Multan**Abstract:**

Objective: The aim of this study was to observe various fungi in oral smears of HIV / AIDS patients and their relationship to CD4 + lymphocyte count.

Study Design: An Observational Study.

Place and Duration: In the Department of Oral Pathology, Mayo hospital Lahore for one year duration from March 2018 to March 2019.

Methods: Total 35 patients smear who were on antiretroviral therapy (ART) and twenty-five (n = 25) patients without antiretroviral therapy (ART) were included. These smears were routinely stained with H&E, Pap and the specific stains recommended for fungi, namely (PAS) Periodic Acid Schiff and (GMS) Grocott-Gomori Methenamine Silver. By flow cytometry; CD4 + lymphocyte count was determined.

Results: In 26% of patients; *Candida albicans* was found who were taking ART and in patients not taking ART therapy found in 44% of subjects. In 11.4% of subjects with ART, *Cryptococcus neoformans* was observed and in 4% of cases without ART. In 2.9% patients who were on ART, *Coccidioid immitis* was noted and in 4% of subjects without ART therapy. In 1.6% of cases who were not taking ART therapy; *Aspergillus* was observed. The average CD4 + number in which several fungi were observed was less than 300 cells / mm³ in all categories. The CD4 + lymphocyte count was quantitatively grouped as Group 1 with less than 350 cells / mm³ CD4 + lymphocyte count and Group 2 with above 350 cells / mm³ CD4 + lymphocyte count. The fungi were observed to have a strong association (p = 0.001) in Group 1 with a CD4 + count of less than 350 cells / mm³, indicating an association with immunosuppression.

Conclusion: It may be beneficial to predict the increase in the colonization of *Candidal* and non-*Candidal* fungi in the mouth by modest non-invasive approaches such as oral smears, and those subjects should receive prompt medical management to prevent their spread of fungal infections.

Keywords: *Candida*, cytology, non-*Candida*, HIV / AIDS, CD4 + lymphocyte count, fungi.

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INTRODUCTION:

Oral fungal infections in AIDS/ HIV patients may result from Candidal and non-Candidal fungi. The *Candida albicans* causes oral candidiasis occurs mainly in AIDS/ HIV individuals and is deliberated a sign of disease progression and immunosuppression¹⁻³. CD4 + can be used to circuitously estimate a low CD4 + count in AIDS/ HIV subjects in countries with inadequate resources where the ease of determining lymphocyte count is unavailable or limited⁴⁻⁵. The utmost common *Candida* fungi in AIDS/ HIV individuals are *Aspergillus*, *Coccidioides immitis* and *Cryptococcus neoformans*. These fungi causes' oral lesions which are usually subclinical, but apparent as non-healing oral ulcers⁶. Previously, fungi that were not present in this candida were thought to exist only in endemic areas, but are now seen all over the world⁷⁻⁸. Special staining, ie, Methenamine Silver (GMS) from Grocott-Gomori and Schiff from

periodic acid (PAS), can be used in the identification of fungi in cytology and histopathology⁹.

MATERIALS AND METHODS:

This study was held in the Department of Oral Pathology, Mayo hospital Lahore for one year duration from March 2018 to March 2019. Total 35 patients smear who were on antiretroviral therapy (ART) and twenty-five (n = 25) patients without antiretroviral therapy (ART) were included These smears were routinely stained with H&E, Pap and the specific stains recommended for fungi, namely (PAS) Periodic Acid Schiff and (GMS) Grocott-Gomori Methenamine Silver. By flow cytometry; CD4 + lymphocyte count was determined.

Using SPSS 18; the data was analyzed. To find the relationship between fungi and CD4 + lymphocyte count; Chi-square tests were used.

RESULTS:

In Table 1; the results are summarized.

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)	
<i>Candida albicans</i>	25.7%, (n=9)	44%, (n=11)	33.3%, (n=20)	249±106
<i>Cryptococcus neoformans</i>	11.4%, (n=4)	4%, (n=1)	8.33%, (n=5)	200±61
<i>Coccidio immitis</i>	2.9%, (n=1)	4%, (n=1)	3.33%, (n=2)	281±53
<i>Aspergillus</i>	—	4%, (n=1)	1.66%, (n=1)	162±0
Mixed	8.6%, (n=3)	—	5%, (n=3)	224±120

Candida albicans (Fig 1) was perceived as true hyphae in the oral sears, pseudohyphae and budding yeast cells.

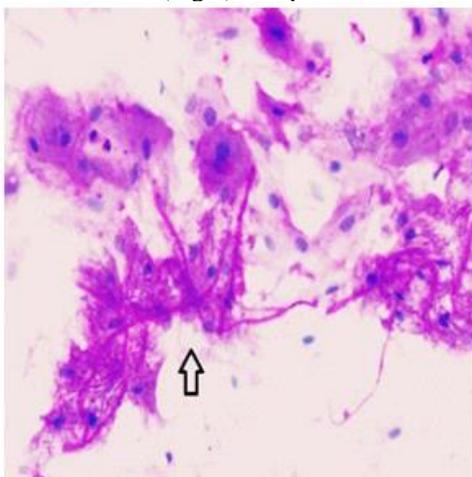
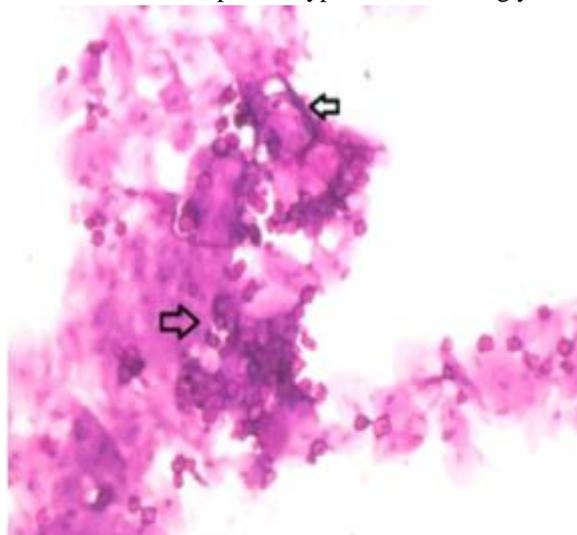


Fig 1: Pseudohyphae of *Candida albicans* (arrow) (PAS Stain) (400X)



Cryptococcus neoformans (Figure 2) were seen as pleomorphic yeast-like cells with narrow-based budding and thick capsule.

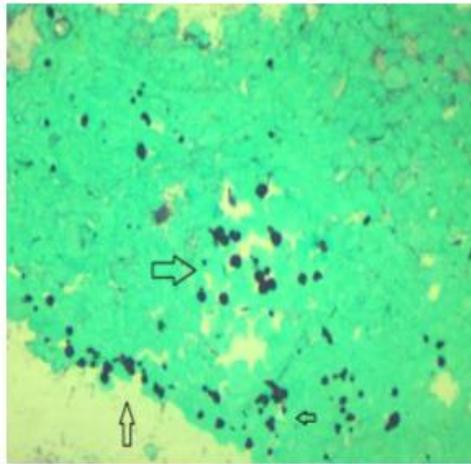


Fig 2: Thick round encapsulated spores of *Cryptococcus neoformans* (arrow) (PAS Stain+ GMS Stain) (400X+200X)

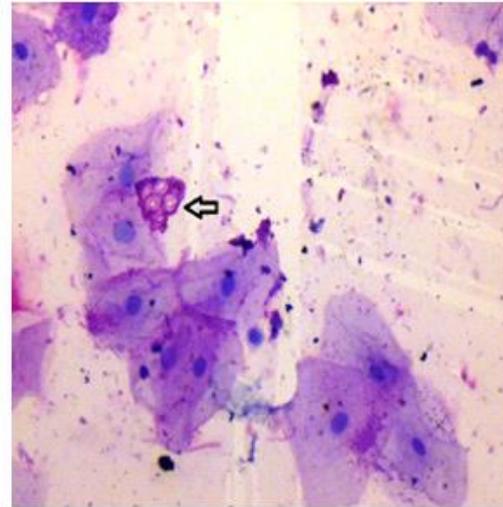


Fig 3: Endospores of *Coccidioides immitis* on oral squamous cells (arrow) (PAS Stain) (400X)

Coccidioides immitis (Figure 3) was observed as a spherule having numerous endospores. The dichotomous septate hyphae is the feature of *Aspergillus* (Fig 4) seen as branching at acute angles.

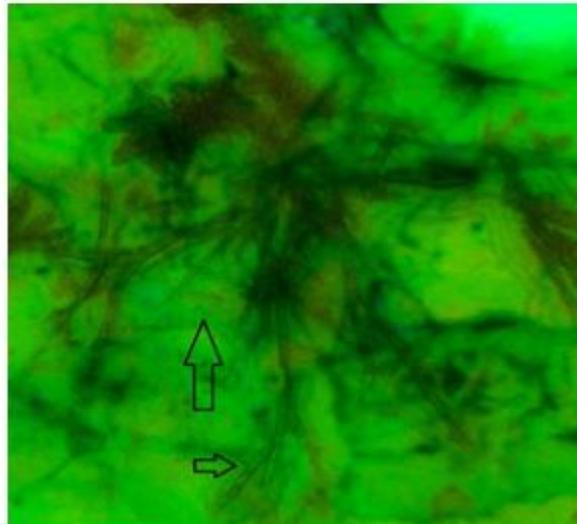


Fig 4: Dichotomous septate hyphae of *Aspergillus* on oral squamous cells (arrow) (GMS Stain) (400X)

The CD4 + lymphocyte count was quantitatively grouped as Group 1 with less than 350 cells / mm³ CD4 + lymphocyte count and Group 2 with above 350 cells / mm³ CD4 + lymphocyte count. According to the WHO immunological classification; this 350 cells / mm³ cut-off value was used because it was deliberated as better immunosuppression level. To determine the relation between CD4+ lymphocyte count and fungi among groups; Chi-square test was used. The fungi were observed to have a strong association ($p = 0.001$) in Group 1 with a CD4 + count of less than 350 cells / mm³, indicating an association with immunosuppression.

DISCUSSION:

Although there is much literature on clinical oral candidiasis in these patients, no studies have been reported describing fungal species found in oral squamous cells of HIV / AIDS patients. Therefore, this study results cannot be matched with other analysis¹⁰. In this study, most fungi were observed with the mean CD4 + number of <300 cells / mm³, and fungi were significantly associated with CD4 + group 1 ($p = 0.001$)¹¹. These results indicate that *Candida* and non-*Candida* fungi are associated with low CD4 + lymphocyte count and hence

immunosuppression¹². The increased colonization of *Candida albicans* and other non-candidate fungi (*Cryptococcus neoformans*, *Aspergillus*, *Coccidioides immitis*) in the oral cavity of HIV / AIDS patients is of concern that these patients cannot actively respond to these microorganisms due to Immunodeficiency¹³. *Cryptococcus neoformans* have been reported to be associated with increased immunosuppression. These fungi have been reported to reach the deepest layers of the oral mucosa and spread to the distal organs of the body through blood and cause more serious damage to the

body and the body¹⁴. Therefore, oral cavity in general health can be a source of serious spread fungal infection in such cases. Regular oral examination by oral smear to detect increased colonization of Candida and Candida fungi in HIV / AIDS patients may be beneficial for these patients and appropriate medical measures taken at an early stage may prevent patients from taking serious results¹⁵.

CONCLUSION:

Different fungal species including Cryptococcus neoformans, Candida albicans, aspergillus and Coccidioid immitis were noted in the AIDS/ HIV patients and their oral squamous cells having less than 300 cells/mm³ CD4+count mean which demonstrate association to the immunosuppression state. Determination of the increase in the Candidal and non-Candidal fungi colonization in the oral cavity through modest non-invasive techniques such as oral smears may be useful to ultimately predict immune grade, those subjects should receive prompt medical management to prevent their spread of fungal infections.

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