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# Prevalence of HIV Type-1 along with fungal in Respiratory Tract Infection in Kadapa.

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**ABSTRACT**

The Human Immunodeficiency Virus (HIV) infection leading to AIDS has now emerged as a major public health problem. The focus is shifting fast to South-East Asia with over 7 million people infected . According to the numbers, The latest such numbers, however, show that HIV continue to spread at uncomfortable rate among Indians. The respiratory system is a system of organs functioning in respiration. It is made up of two parts namely the upper respiratory tract and lower tract. The upper respiratory tract consists of the nose, nasal cavity, pharynx and larynx and lower respiratory tract consists of the trachea, bronchi and lungs. The quality of the sputum was assessed mascroscopically. A good sputum sample must be viscous, mucoid or purulent. KOH wet mount, In a clean glass slide specimen/sputum was taken, 10% KOH added and cover slip was applied over it. The slide was heated gently over flame and examined under miscroscope under 10 X and 40 X objectives. The present study has been designed to assess prevalence of fungal respiratory tract pathogen in the HIV patients and the HIV sero-negative patients. Nine hundred sixty one (961) HIV sero-positive patients, sputa were screened for routine bacterial and fungal pathogens consisted test group(T)with 961 HIV sero-positive patients. All of them have at least one of the symptom of respiratory tract infection. While other 300 HIV sero-negative patients were also screened for routine bacterial and fungal pathogens consisted control group (C1)of 300 sero-negative patients who have also at least one of the clinical symptom of respiratory tract infection

**Keywords:** RTI, HIV, AIDS

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## INTRODUCTION

The Human Immunodeficiency Virus (HIV) infection leading to AIDS has now emerged as a major public health problem. The focus is shifting fast to South-East Asia with over 7 million people infected . According to the numbers, The latest such numbers, however, show that HIV continue to spread at uncomfortable rate among Indians.

The respiratory system is a system of organs functioning in respiration. It is made up of two parts namely the upper respiratory tract and lower tract. The upper respiratory tract consists of the nose, nasal cavity, pharynx and larynx and lower respiratory tract consists of the trachea, bronchi and lungs.The upper respiratory tract contains many normal flora which include Streptococcus species, Haemophilus species, Neisseria species, Corynebacterium species, Staphylococcus species and many anaerobes such as Bacteroides. Although the normal flora is generally harmless and beneficial to the host, they can cause diseases when the host defenses are impaired (Macowicak, 1982). Bacteria from the upper tract are washed downwards towards the lower respiratory tract, but the action of the ciliated epithelium and sticky mucus that cover the lining of the bronchial tubes keeps the lower respiratory tract free of these microorganism. Viruses may however, interfere with the ciliary function, allowing themselves or other microorganisms to invade such bacteria, to gain access to the lower respiratory tract. One among such viruses is HIV, the etiological virus of AIDS. HIV decreases the CD4 cells, creating the favorable conditions or micro-environment for other opportunistic organisms to initiate many infections in the host (Mohanty et al.,1993).

Various defects in immunity occur in the patients with HIV infections, which includes defects like humoral immune dysfunction, depressed IgA and IgG levels, and decreased T-lymphocyte cell mediated antibody-dependent cellular cytotoxicity.

## MATERIALS AND METHODS

A wide mouthed sterile leak proof specimen(Sputum) container was given to the patients. They were asked to go to quite isolated place, take deep breath and to cough up sputum directly into the sterile container provided to them. The patients were asked to provide sputum devoid of saliva and requested to return the samples as early as possible. The sputum specimen was then transported to the Clinical MicrobiologyLaboratory at FIMS for analysis. Patients' demographic data(required in proforma) such as age, gender, education, socio-economic condition, etc. were also taken.

### Processing of Sputum

The quality of the sputum was assessed mascroscopically. A good sputum sample must be viscous, mucoid or purulent. If the sputum sample turned out to be thin, watery and with no purulent matter it was considered unsuitable for processing. Bartlett's scoring method was used for microscopic evaluation of the expectorated sputum. A sputum was considered unsuitable if it had a final score of 0 or less. All unsuitable specimenswere discarded and a repeat specimen was collected.

**COLLECTION OF SAMPLE:** Early morning sputum sample was collected by deep cough after rinsing mouth with water. These sputum samples tested in the laboratory as follows.

### Mycological Procedure

### Direct Microscopy

### KOH wet mount

### Principle

The aqueous potassium hydroxide digest protein debries and dissolves connecting substances of keratinized cells so that yeast cells and pseudohyphae are seen clearly. Concentration of KOH can be increased depending upon the nature of specimens. As high as 40% KOH can be ued for solid specimen.

**Procedure**

**Slide KOH:** In a clean glass slide specimen/sputum was taken, 10% KOH added and cover slip was applied over it. The slide was heated gently over flame and examined under miscroscope under 10 X and 40 X objectives.

**Gram staining:** Yeast cells usually show well stained morphology but not the filamentous fungi. Method is same as that used for bacteriological staining. Candida was seen as gram positive budding yeast cells with or without pseudohyphae.

**Nigrosin staining**

**Principle**

It is a type of negative staining used for encapsulated organism like Cryptococcous. It contain Nifrosin granules in 10 % formalin solution. It is better than ink preparation as there are no carbon particles.

**Procedure**

One drop of sputum was examined with one drop of Nigrosin stain on a clean sterile glass slide and a wet mount was prepared. It was examined under low objective.

The sputum was also inoculated on to Sabouraud Dextrose Agar(SDA) with antibiotics, SDA without antibiotics in duplicate (incubated at $37^0$C and $25^0$C) and BHI agar (incubated at $37^0$C). Any significant growth of fungal species was further identified as per standard protocol(Procop et al,1998).

**Giemsa staining:-** For the detection of Pnumocyctis carinii pneumoniatiis staining technique was carred out.

**Detection of HIV Status**

The HIV status was confirmed by three tests with different principles/antigens (Enzaids ELISA, i.e. enzyme-linked immunosorbent assay, Combaids-RS, HIV Tridot) in the Integrated Counselling and Testing Centre attached to the department as per NACO guidelines(HIV testing Manual on Laboratory Diagnosis, Bio safety and Quality Control by NICD and NACO. March 2007). Antibodies against immunodominant regions of HIV 1 (gp 120, gp 41) and HIV 2 (gp 36) and p24 antigens were detected. Necessary pre and post-test counseling of the patients was carried out and detailed history was taken (HIV testing Manual on Laboratory Diagnosis, Bio safety and Quality Control by NICD and NACO. March 2007). Sputum samples, were collected taking all aseptic precautions.

**Principles of flow cytometry for CD4 count estimation**

The advent of monoclonal antibody technology and the development of flow cytometry have facilitated the phenotypic characterization of functionally different types of lymphocytes. In addition, the availability of multi-parametric flow cytometry using a variety of fluorochrome has made it possible for simultaneous measurements of large numbers of subsets of cells. Flow cytometry has added a new dimension to medical and biological research, including the immunology of HIV.

**Collection and transport of blood samples for CD4 Count**

Quality laboratory results begin with proper collection and handling of the specimen submitted for analysis. Correct patient preparation, specimen collection, specimen packaging and nsportation are of vital importance. All necessary biosafety precautions should be followed during collection and transport of the blood samples. The Standard Operating Procedures (SOPs) should be available and should be followed strictly. Errors during these procedures can lead to serious consequences and affect patient a subsequent patient management. The collection procedures may vary depending upon the methodology used for the enumeration of absolute CD4 T lymphocytes, although Ethylenediamine tetra-acetate (EDTA) is generally recommended by

most of the manufacturers. For dual platform approach the whole blood collected in EDTA should reach and be processed in the laboratory for lymphocyte counts within six hours.

## RESULTS

The present study has been designed to assess prevalence of fungal respiratory tract pathogen in the HIV patients and the HIV sero-negative patients. Nine hundred sixty one (961) HIV sero-positive patients, sputa were screened for routine bacterial and fungal pathogens consisted test group(T)with 961 HIV sero-positive patients. All of them have at least one of the symptom of respiratory tract infection. While other 300 HIV sero-negative patients were also screened for routine bacterial and fungal pathogens consisted control group (C1)of 300 sero-negative patients who have also at least one of the clinical symptom of respiratory tract infection. To study predisposing factors like age, smoking, chewing tobacco and cooking food with biomass fuel like wood, waste garbage and cow-dung, an another control group (C2) was analysed, which consisted of 300 HIV sero-positive patients without any clinical symptoms of respiratory tract infections. The data were noted and analysed in tabular forms as follows:

The respiratory tract infections in female and male in group T(HIV+VE/ RTI+ve) and C1(HIV-ve/RTI+ve) were found almost comparable i.e. 39.85 % and 38.00 % in female and 60.04 and 62.00 in male respectively(Table No. 1). One patient of RTI in group T was found trans-sexual or trans-gender. In group C2 (HIV +ve/RTI –ve) the number of female was found to be. 44.33 % and 55.67 % respectively when compared with group T and C1(Table No.1).

The cough was taken as the primary symptom of respiratory tract in our study in both the group, fatigue was seen in 92.50%(889/961) of patients in HIV-infected pateints of group T, while in HIV-uninfected RTI patients, main complain noted was nasal discharge, seen in 54.33%(1623/300) patients. out of 300 patients fungal element were detected in 14 patients

**Prevalence of pathogenic bacterial &Fungalisolates from both HIV seropositive(T) and HIV seronegative(C1) groups**.

| HIV +ve | % | RT +ve | % | HIV -Ve | % |
|---|---|---|---|---|---|
| 66 | 6.87 | 04 | 1.33 | 2.9 | 0.1 |

**Microbial profile of patients of both the groups studied(HIV reactive and HIV non-reactive patients)**

| | HIV +ve | % | RTI +ve(T) | HIV -Ve |
|---|---|---|---|---|
| **Candida albicans** | **47** | **4.89** | **4** | **1.33** |
| **Candida (Non albicans** | **63** | **6.56** | **12** | **4.00** |
| **Aspergellus** | **3** | **0.31** | **0** | **0.00** |

In the present study in the control group C2, there were 300 patients, out of which 64 were pre-ART. The highest number of patients were on 6ZLE(Zidovudine, Lamivudine, and Efavirenz), followed by 92 patients who were taking 3SLE. In this study group, onl patients was found on seond line treatment with 3DF/3TC/ATVr. While talking about death five patients expired in preART group, followed by one in each expired in drug group of SLE and SLN.

## DISCUSSION

The occurrence of opportunistic fungal infections has risen progressively in recent years. Invasive fungal infections had been reported in 26% of chronically and intensively immunosuppressed patients (Topley and Wilson's 2005).Infections with Candida albicans appear when CD4 count is between 500-200cells/µl and may be the first indication of immunodeficiency. In the present study the mean of CD4 count for C. albicans was found to be 257.12$\pm$ 82.86 cells/µl, while the mean CD4 count for non-albicans Candida was found to be 499.73$\pm$196.24 cells/µl in HIV-infected patients with RTI of group T.

Apergillus infection were only seen in tremendous immunosuppression with CD4 count when decreases below 50(Meyohas MC et al,1995). In the present study the mean CD4 for Aspergillus was found to be 29.00±2.16cells/µl, which matches well with the findings of other workers. The phagocytic cells and lymphocytes (T & B both) are believed to function together in protecting the host against fungal pathogens but the exact degree to which each is involved is not yet fully known. It has been shown that vegetative hyphal structures of Aspergillus and Candida are ingested and killed by neutrophils (Jagdish,2009). Skin and mucosal surface play an important role in primary defence against pathogens. The muco-ciliary action of mucus membrane is the prime clearance mechanism active against inhaled fungal spores. As the HIV sero-positive individuals are prone to get recurrent respiratory infections, the mucosal barrier may be damaged and they are more vulnerable to develop fungal respiratory tract infections(Topley and Wilson's, 2005).

The fungal infections depends on exposure to sufficient inoculum size of organism and general resistance of the host. With introduction of antifungal agents, the cause of candida infections shifted from C. albicans to C.glabrata and other non-albicans species, as C. glabrata and C. krusei develop resistance to fluconazole(Topley and Wilson's, 2005). Fungal infections may disseminate and cause fungaemia, which is a grave condition in immunosuppressed individuals. Thus prompt diagnosis by standard microbiological methods and treatment are crucial.

In the present study Candida albicans were isolated from 4.89% (47/961) of HIV-infected patients with RTI of group T. This matches well with the findings of V.V. Shailaja et al,(2004), who also found 5 % C.albicans in their study, while they found 3.33% C.albicans from HIV-uninfected patients of RTI, in the present study also C. albicans were isolated from 1.33%(4/300) of HIV-uninfected patients. Aspergillus was isolated from 0.3%(3/961) patients from HIV-infected patients of RTI, in the present study, while they had been isolated in 2% patients in the study of Bharathi M and Usha Rani,(2011). Non-albicans Candida were isolated from 29% of patients while C.albicans were isolated from just 26% of patients in study of Bharathi M and Usha Rani(2011),which showed the change in the trend of candida infections towards non albicans spp. In the present study also nonalbicans Candida were isolated in 6.56%(63/961) HIV-infected patients, while C.albicans were isolated from 4.89%(47/961) patients of the same group proved changed trend of candida infection towards nonalbicans Candida. This changed trend is due to resistance to fluconazole developed by non-alnicans spp. like C.krusei and C.glabrata. As fluconazole is commonly used antimycotic drug for prophylaxis. Shailaja et al(2004) in Hyderabad, India also isolated more number of non albicans spp. in 18 cases than C. albicans in 6 cases. Aruna Aggrwal et al(2005), Punjab isolated C.albicans in 20(62.5%) out of 32 isolates. Jha et al.(2006) from Khatmandu isolated 20 C.albicns and 10 non albicans from 462 samples of lower respiratory tract infections.Prasobh et al.(2009) Tamilnadu, performed resistotyping of 350 C. albicans isolated from sputum samples.All the above studies were geographically from the same region. C. albicans was isolated from sputum samples in all these studies. In the study of opportunistic fungal infection in AIDS patients by Rakhamanova et al(1998), the culture positivity for C. albicans and C.neoformans in pulmonary infections was 4% each. Yongabi et al (2009) isolated 12 strains of C.albicans from 98 sputum samples. Non albicans spp was found predominating in the present study, study by Shailaja et al(2004) and Bharathi M et al(2009) where as C. albicans was found predominate species in Jha et al(2006) study. In rest of the other studies only C. albicans was mentioned. But over all, the importance of C. albicans as causative agent in pulmonary infection was proved by all these studies.

Aspergillus spp were the next commonest fungi isolated in the present study in 3 samples(0.31%). Bharathi M et al(2009) had isolated 13.5% Aspergillus in their study where as Shailaja et al(2004) isolated isolated Aspergillus in one case. Nash et al (1997) identified 17 cases of AIDS related pulmonary aspergillosis at autopsy. Mylonakis et al (1998) after reviewing 342 AIDS cases concluded that invasive pulmonary aspergilosis usually occure among **patients** with less than 50 CD4 clls/µl, which matches well in the present study as in the present study mean CD4 count for Aspergillus was found to be 29±2.16 which is less than 50.

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