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A Comparative Study on CD4 Count and Sputum Smear Examination by Fluorescent Microscopy in Retroviral Positive Patients in a Tertiary Care Centre

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A B S T R A C T

TB is the most common opportunistic infection (OI) among HIV infected individuals, and co infected individuals are at high risk of death. TB is the largest single cause of death in the setting of AIDS, accounting for 26% of AIDS related deaths, 99% of which occur in developing countries. HIV Associated Tuberculosis remains a major global public health challenge. Hence routine TB screening among PLWHA (People Living with HIV-AIDS) offers the opportunity to identify those without TB, helps for early diagnosis and promptly treat TB. The aims of the study are 1. To know the prevalence of HIV/TB Co-infection in a Tertiary care centre in a rural area 2. To find the CD_4 count which gives Sputum smear positivity and negativity. Through Sputum smear examination by Fluorescent microscopy, routine HIV Testing by Rapid Test methods and CD_4 count by Flowcytometry method are planned for this prospective study. In this study, out of 65 HIV positive Patients 30 (46%) had TB HIV coinfection, were started on Antituberculous treatment and remaining 35 (54%) were retroviral positive only. Prevalence of HIV/TB co-infection is 46% among the sample size in this study. Prevalence is more in males and reproductive age group 16-45 years as 60% of patients fall into this group.

Introduction

Tuberculosis (TB) and Human Immunodeficiency Deficiency Virus/ Acquired Immunodeficiency Syndrome (HIV-AIDS) constitute the main burden of infectious disease in developing countries[1]. Around 14 million individuals worldwide are estimated to be dually infected[2,4]. Most TB cases are in South East Asia, African and Western Pacific regions and an estimated 11-13 per cent of incident cases were HIV Positive[2]. HIV-TB Co-infection most powerful risk factor for progression of *M. tuberculosis* infection. The two pathogens *M. tuberculosis* and HIV potentiate one another accelerating immunological deterioration[3]. TB may occur at any stage of HIV disease and is frequently the first recognized presentation of underlying HIV infection.[2,5] The two pathogens M. tuberculosis and HIV potentiate one another accelerating immunological deterioration. Various lines of evidence indicate that inborn errors of immunity, as

well as genetic polymorphisms, have an impact on susceptibility to TB and HIV[5].

The risk of TB in HIV continues to increase as CD4 cell counts progressively decline[6]. As a result of WHO's 3 by 5 campaign, >6 million HIV infected individuals in resource limited settings have had access to antiretroviral therapy (ART) since 2004, which is for short of actual need, although ART can reduce the incidence of TB both at the individual and population level. PLWHA on ART still have higher TB incidence rates and a higher risk of dying from TB[7], which may be due to delayed initiation of ART or the fact that patients present with advanced TB or both Routine TB screening among PLWHA offers[10].

-The opportunity to identify those without TB

-Prevent TB by chemoprophylaxis

-Diagnose and promptly treat TB

Materials and Methods

This is a Cross-sectional study conducted by the Department of Microbiology and ART, Govt. TVR Medical College and Hospital. After obtaining the Institutional Ethical committee approval, the study was conducted from January 2014 to August 2014.About 208 patients who attended the Integrated Counseling and Testing Centre were included in the study, of them only 65 were retroviral positive and they were subjected to the sputum smear examination by Fluorescent microscopy and CD_4 count.

HIV testing method

A total of 208 patients were screened for HIV by using WHO approved Elisa Rapid kits based on Immunoconcentration, Dot blot assay and Immunochromatography methods, 65 were found retroviral positive.

CD_4 counts

Blood samples were collected after obtaining written informed consent CD_4 counting done. Whole blood sample is collected from the 65 patients in EDTA liquid vacutainer tubes and the samples were processed on the same day using Fluorescence-activated cell sorting (FACS) COUNTER for determining the CD4 counts by the Flowcytometry method, (Fluorochrome labeled monoclonal antibodies to the CD_4T cells). Initially control run was done. Controls supplied with CD_4 kit were prepared by adding normal blood and fixative solution to the CD_4 reagent tube. Before running the reagent tubes on the FACS COUNTER control beads were added.

Patient samples were prepared by adding blood samples, fixative solution to the CD_4 tube.

A reagent tube is taken, labeled and vortexed. Then tubes were cored and 50 microlitre of patient's blood added, vortexed again and incubated, Fixative solution added and vortexed. Samples were run in instrument and CD_4 count results recorded

Sputum microscopy

Patients were asked to collect two sputum samples (1 early morning and 1 spot). Samples were labeled, smears were prepared from purulent part of the sputum and heat fixed.

- Staining was done using fluorescent stains
- 0.1% Auramine O was added and kept for 7 minutes
- Washed with water
- Decolourised with 0.5% acid alcohol for 2 minutes, washed with water
- Counterstained with 0.5% potassium permanganate for 30 seconds, washed and air dried
- Using LED fluorescence microscopy slides

were examined at low power magnification-250 X and 400X, which allows larger area per unit of time for examination and this is 6 % more sensitive than light microscopy.

Results and Discussion

Among the 208 patients screened for HIV, 65 were retroviral positive and were screened for pulmonary tuberculosis by sputum smear for Acid Fast Bacilli (AFB) using fluorescent Microscopy and by chest X ray. Sputum smear positive for AFB received Directly Observed Therapy Short course (DOTS). The results were analysed using SPSS (version 13) with the level of significance p= 0.05

Table.1 Statistical analysis

Gender	HIV reactive Sputum negative No %	HIV Reactive Sputum Positive No %	Total No %
Male	23 66 %	20 67 %	43 66 %
Female	12 34 %	10 33 %	22 34 %
Total	35 100 %	30 100 %	65 100 %

HIV/TB Co-infection

Of the 65 retroviral positive cases, 30 had HIV/ TB co-infection, thus 46% of patients had dual infection. Co-relating with gender 20 (67%) were males and 10 (33%) were females.

Table.2 HIV/TBCO-Infection and age

Age	Male	Female	Total
1-15	1	0	1 (3.3%)
16-30	4	3	7 (23.3%)
31-45	7	4	11 (36.6%)
46-60	5	3	8 (26.6 %)
61-75	3	0	3 (10%)
TOTAL	20	10	30 (100%)

Table.3 HIV/TB and CD_4 count

CD_4 Count	HIV/TB Co-Infection	HIV Alone	Total
<50	13 (43.3%)	2 (5.7%)	15
50-150	11 (36.6%)	9 (25.7%)	20
151-250	2 (6.6%)	8 (22.8%)	10
251-350	2 (6.6%)	2 (5.7%)	4
>350	2 (6.6%)	14 (40%)	16
TOTAL	30 (100 %)	35 (100%)	65

Table.4 Sputum Negativity and CD_4 Count

CD_4 Count	**<50**	**50-150**	**151-250**	**>250**
No. of Patients	4	11	4	15
P Value of < 0.001				

Prevalence of HIV/TB co-infection, a global estimate shows around 5.1 million people infected with HIV and about half of them are co-infected with TB[2]. In our study out of 65 Retroviral positive patients, 30 (46%) had HIV/TB co-infection and were started on DOTS and the remaining 35 (54%) Retroviral positive alone. Our study correlates with a North Indian study done by Naren et al in New Delhi. As per his studies, in developing countries TB is the most common life threatening, opportunistic infection in patients with dual infection[6]. He narrates 35-65% patients of PLHA having TB of any organ. The incidence of dual infection was reported to be very high (50%) in Sub Saharal Africa compared to that of Asia. The rate of dual infection varies in different regions of India, found to be between 0.4 and 20.1% in North India, 3.2% in South India two decades back which increase to 20.1% now. And this increase may be due to improvement in diagnostic methods to detect TB[10].

Dual infection and age group

In our study, the dual infection is higher in the reproductive age group of 16-45 years, 60 % of the co-infected belong to this age group.Similarly Sameer Singhal et al study in co-infection from Wardha showed prevalence of dual infection was higher 55(84%) in the age group og 16-45years[12,13].

Dual Infection and CD_4 count

Among other OI's like Cryptococcal meningitis or toxoplasmosis which occur in very low CD_4 count, TB is unique it occurs over a wide range of CD_4 count < 300 cells per microlitre CD_4 count[14]. In our study of 30 dual infection patients 93.4 had CD_4 counts below 350 cells per microlitre. In sputum negative and retroviral positive cases 16 patients had a high CD_4 counts, indicating sputum negativity has positive co-relation with high CD_4 counts[15]. This is similar to the study done by Purushottam et al in Prevalence of Pulmonary TB among HIV positive patients attending Antiretroviral Therapy Clinic[11].

Summary and conclusion of the study are as follows

Prevalence of HIV/TB co-infection is 46% among the sample size in this study.

Prevalence is more in reproductive age group 16-45 years as 60% of patients fall into this group

Sputum positive PTB had positive correlation with low CD_4 counts as 93.4% had CD_4 counts < 350 cells per microlitre

Sputum negative PTB had positive correlation with high CD_4 counts

Recommendations

In a study from South India, the medium survival in HIV infected presenting with PTB and EPTB(Extra Pulmonary TB) were found 45 and 40 months respectively

Most of the EPTB is missed in resource limited settings. About 30% of TB in HIV

extra pulmonary. A Battery of tests are available including molecular techniques like NAAT- CBNAAT, PCR. So in resource limited settings at least we can do Sputum smear microscopy, chest Xray, which are cost effective and CB NAAT which is rapid and advanced molecular method which helps in early diagnosis and treatment, reduce the community spread of TB morbidity and mortality.

In 2011, app. 5% of all diagnosed TB cases in India came from ICTC's which proves to be excellent sites for active TB case finding. Though close synergy between TB and HIV / Aids control programs were launched for active place finding with more advanced and rapid diagnostic mythology like CBNAAT to evaluate the resistance patterns also have to be provided for early diagnosis and treatment in rural areas. So these points should also be considered to improve the active TB case finding in HIV patients and hence will improve the early diagnosis and treatment, thus will reduce the morbidity and mortality

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