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MOLECULAR DETECTION OF TUBERCULOSIS AND ITS COINFECTION IN PLHIV WITH CORRELATION TO CD4 COUNT AT A TERTIARY CARE HOSPITAL IN TRIBAL REGION OF EASTERN MAHARASHTRA

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ABSTRACT

BACKGROUND: HIV and tuberculosis (TB) are leading infectious diseases, with a high risk of co-infection. The risk of TB in people living with HIV (PLHIV) is high soon after sero-conversion and increases as the CD4 counts are depleted. This co-infection results in diagnostic difficulties and hampers treatment of TB resulting in increased mortality, morbidity, treatment failure and relapse. The present study was conducted at Government Medical College Gondia over period of one year commencing from January 2021 to December 2021 with.

AIM AND OBJECTIVES to determine the Occurrence of coinfection of TB in PLHIV and its co-relation with the CD4 counts of the affected individuals.

MATERIAL AND METHODS: Sputum and blood samples were collected from 288 HIV positive individuals who were clinically suspected to have TB as co-infection and samples were sent to Department of Microbiology, GMC Gondia. Sputum analysis was done by Ziehl Nielsen staining method and Catridge Based Nucleic Acid Amplification Technique (CBNAAT)/GeneXpert. CD4 counting of blood sample was done by Flow Cytometer.

RESULT: Among 288 individuals, 147 individuals were associated with HIV TB co-infection.115 patients (39.93%) were positive by CBNAAT and 32 (11.11%) were positive by sputum microscopy for acid fast bacilli. Out of these 115 CBNAAT positive patients, 23 (20%) showed rifampicin resistance. About 96 (65.30%) HIV and TB co infected individuals had a CD4 counts <200 cells/µl followed by 35 (23.80%) individuals with CD4 counts ranging between 200-300 cells/µl and in only 2 (1.36%) cases CD4 counts were more than 500 cells/µl.

CONCLUSION: The present study emphasizes that co-infection with TB has been a major concern in HIV/AIDS patients. TB is the the most common opportunistic infection in HIV positive patients even at higher CD4 counts and most of the time remains smear negative. Hence early diagnosis and optimum management is necessary to reduce mortality and morbidity.

KEYWORDS : Tuberculosis, PLHIV, CD4 Counts, CBNAAT

INTRODUCTION:

Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis, which can be latent in humans for long time without clinical symptoms. Active TB can present as Pulmonary Tuberculosis (PTB) or Extra-Pulmonary Tuberculosis (EPTB), with cardinal features of fever, productive cough, hemoptysis, weight loss, though the presentation among HIV infected individuals is often atypical. Several factors are associated with an increased risk of TB incidence like poverty, malnutrition and overcrowding but the risk of active TB is 16–27 times higher in people living with HIV (PLHIV) compared to those who are HIV negative [1].This is due to the impaired and lowered innate and passive immunity against TB among PLHIV[2] and increasing the risk of getting a new TB infection[3]

India has world's highest burden of TB.It is estimated that 60-70% of HIV positive person will develop tuberculosis in their lifetime[4]. Globally about 476774 cases of TB were detected in people living with human immunodeficiency virus infection (PLHIV) by the end of 2020[5]. The incidence of HIV TB co-infection is 1,63000 and mortality due to co-infection was about 35,000 in South East Asia as per WHO estimation in 2020[5]. About one third of HIV-TB cases goes underdiagnosed as there is scanty sputum production, lack of caseous necrosis leading to decreased number of bacilli in sputum and thus reducing sensitivity of smear microscopy[6]. Culture result for TB is available only after 2-8 weeks. GenXpert MTB/RIF holds a good promise by detecting 99% of smear-positive patients and >80% of patients with smear negative disease[7].

The pattern of clinical presentation of TB depends on the host immune status. The CD4 T cell count is one of the best indicators of immunological competence of the patient with HIV infection. Thus determination of CD4 cell counts provides a powerful tool for determining the prognosis and identification, assessment and induction of prophylactic therapies for various opportunistic infections in high risk individual. The present study was conducted to determine the occurrence of TB in PLHIV and its co-relation with CD4 counts of the affected individuals.

MATERIAL AND METHODS:

The Cross Sectional Descriptive study was conducted in the department of Microbiology, Government Medical College Gondia, Maharashtra, India from January 2020 to December 2021 in association with Antiretroviral therapy (ART) centre, Integrated Counselling & Testing Centre (ICTC) of our hospital. The Study was conducted after getting Permission from institution ethics committee and review board. HIV positive patients above the age of 18 years having manifestations of TB (cough for less than or up to 2 weeks) were included in the study. A total of 750 HIV positive individuals attended the ART centre for regular follow up out of which 288 individuals were clinically suspected to have TB as co-infection.

Immune Status Assessment

About 3 ml of blood was collected from each patient using aseptic precaution in ART centre and sent to CD4 lab. CD4 counting of blood sample was done by Flow Cytometer (PartecCyFlow Counter) as per manufacturer's instructions.

Sputum Analysis

Two samples of at least 1 ml sputum were obtained from each patient, in 2 sterile containers and sent to Microbiology department. One was used for Ziehl Nielsen stainng method. Another one was diluted with three times the reagent, incubated at room temperature and loaded into Xpert MTB/RIF cartridge for automated analysis. Results were obtained in 2 hours. Detection of Mycobacteria and rifampicin resistance was carried out in the same setting.

RESULTS:

21 – 40 years was the most commonly affected age group.

Males 88 (66%) were most commonly affected. The age and gender wise distribution were depicted in the table 1. Among 288 individuals participated in the study, 147 individuals were associated with HIV TB co-infection (Figure 1).

115 patients out of 288 (39.93%) were positive by CBNAAT and 32 (11.11%) were positive by sputum microscopy for acid fast bacilli. Out of these 115 CBNAAT positive patients, 23 (20%) showed rifampicin resistance (Figure 2) About 96 (65.30%) HIV and TB co infected individuals had a CD4 counts <200 cells/μl followed by 35 (23.80%) individuals with CD4 counts ranging between 200-300 cells/μl and in only 2 (1.36%) cases CD4 counts were more than 500 cells/μl. The association of CD4 counts with the occurrence of Pulmonary Tuberculosis is shown in table 2.

In our study, male preponderance was seen which is in concordance with other studies like Rao et al.,[8], Sharma et al.,[9].This could be due to migration for employment and their high risk behaviour. The most commonly affected age group was 21-40 years which is similar to the findings of Nara et al.,[10] and Siddeshwari et al.,[11].

Table 1: Age Distribution Of Patients

AGE GROUP	GENDER		PERCENTAGE (%)
	MALES	**FEMALES**	
18 - 20	8	3	7.48%
21 – 40	45	33	53%
41 - 60	25	21	31.29%
61 and Above	10	2	8.16%
TOTAL	**88**	**59**	**100%**

Table 2: Correlation Of Cd4 Counts With Occurrence Of Pulmonary Tuberculosis

COUNTS: CELLS/μL	HIV WITH PULMONARY TUBERCULOSIS (n= 147)
<200	96 (65.30%)
200 – 300	35 (23.80%)
301 – 500	14(9.52%)
> 500	2(1.36%)
TOTAL	**147 (51.04%)**

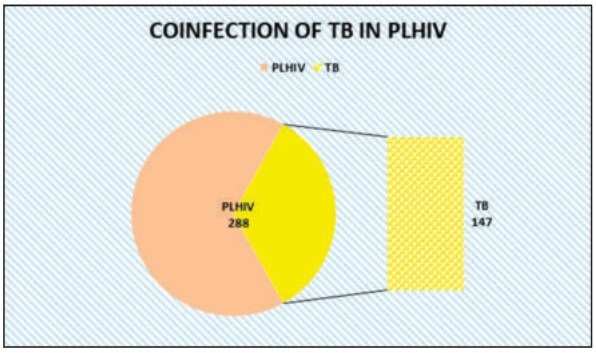

Figure 1: Coinfection Of Tb In Plhiv

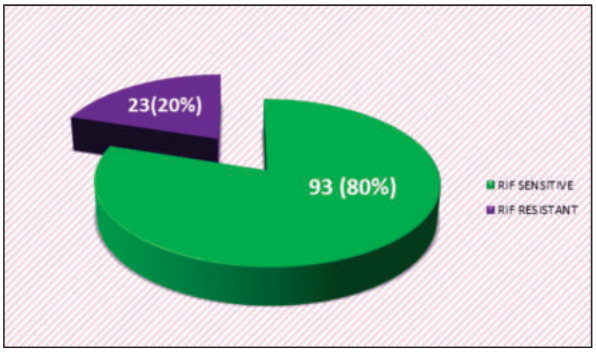

Figure 2: Rifampicin Resistance In Cbnaat Positive Cases

DISSCUSION

People living with HIV need early diagnosis and treatment of active TB disease. WHO recommends CBNAAT for diagnosis of pulmonary TB and detection of rifampicin resistance, especially in PLHIV and re-treatment cases who are at risk of MDR-TB. Major advantage of GenXpert is it is mycobacterium tuberculosis specific with no cross reaction to non-tubercular mycobacterium and simultaneous detection of rifampicin resistance in 2 hours helping in rapid initiation of MDR treatment. In our study prevalence of TB was 39.93% which is co-relating with findings of Sharma et al.,[9].Rao et al., [8] reported higher prevalence which could be attributed to factors like differences in screening of TB, gender, educational and occupational status, CD4 count.In our study, CBNAAT detected rifampicin resistance in 20% cases. Study by Siddeswari et al.[11] and Swaminathan et al., [12] showed rifampicin resistance in 13.55% and 27.3% cases respectively.

Though Sputum microscopy for AFB is economical, it possess some disadvantages. It is highly subjective (operator dependent), sensitivity ranges from 20% to 60% which is further reduced in PLHIV due to lower rates of caseous necrosis and sputum production [13]. In present study, out of 147 HIV-TB cases, 32 (11.11%) were positive by sputum microscopy for acid fast bacilli and 115 cases reported sputum smear negative. The consequences of this can be delayed or misdiagnosed cases, contributing to delayed treatment, increased morbidity and mortality rates and continued spread of TB to contacts. The appearance of many opportunistic infections correlates with CD4 cell count as it is an important indicator of CMI. HIV infection causes rapid decline in immune responses resulting in multiplication of mycobacterium within the granuloma. It is also theorized that there is an increased replication of HIV at sites of mycobacterium infection by multiplying within activated CD4+T cells. Hence HIV infected individuals with lower CD4 counts are more susceptible for TB than individuals with higher CD4 counts [14].

CONCLUSSION

The present study shows that patients with CD4 count less than 200 cells/μl are at higher risk of pulmonary TB. Thus the results of current study emphasizes that co-infection with TB has been major concern in HIV/AIDS patients. TB is the most common opportunistic infection in HIV positive patients even at higher CD4 counts and most of the time remains smear negative. Hence early diagnosis and optimum management is necessary to reduce mortality and morbidity. Utilization of culture sensitivity and GenXpert should be considered in individuals with potential risk factors.

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