EFFICACY OF FLUOROCHROME STAIN IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS CO-INFECTED WITH HIV

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Abstract

The resurgence of Mycobacterium tuberculosis in association with HIV infection has focussed much attention in the rapid diagnosis of high risk cases. Infection with HIV is known to alter the presentation of pulmonary tuberculosis. The present study was undertaken to compare the efficacy of fluorochrome stain (Fl) with conventional Ziehl Neelsen (ZN) stain in the diagnosis of pulmonary tuberculosis. Two hundred cases of pulmonary tuberculosis were included in the study. Sputum smears were screened for acid fast bacilli (AFB) by ZN and Fl methods and blood samples were screened for HIV. Sputum positive cases detected by Fl stain were higher in number (69%) when compared to ZN stain (50%). Of the total cases studied 15.5% were HIV seropositive. Conclusions: Fluorochrome staining was found to be more efficient (45%) when compared to ZN staining (29%) in detecting cases associated with HIV seropositivity, especially paucibacillary cases.

Key words: Pulmonary tuberculosis, HIV, Fluorochrome stain, ZN stain

Tuberculosis (TB) was declared as a global emergency by WHO in the year 1993. In the world it kills 8000 people a day, that is 2.3 million people each year. In India, an estimated 14 million people are infected with tuberculosis each year, 3.5 million of these are categorized as highly infectious.1 HIV pandemic has a major impact on tuberculosis. It directly attacks the critical immune mechanisms involved in protection against tuberculosis. In the early stages of HIV infection, when CMI is only partially compromised, pulmonary tuberculosis presents typically as upper lobe infiltrates and cavitation with high bacillary load in the sputum, whereas in the late stages, primary tuberculosis like pattern with diffuse interstitial and miliary infiltrates, little or no cavitation is seen resulting in paucibacillary picture of sputum.2 Detection of smear positive cases is the highest priority in a TB control programme, as these cases are infectious and contribute substantially to the transmission of disease.3 Though smear positivity correlates well with infectivity, much of the transmission occurs before the level of bacilli reach 10⁵/mL in the sputum. Infection is a stochastic process and certainly occurs from paucibacillary and smear negative cases which are more common in HIV seropositives.4

The history of sputum examination dates back to 1882 when Robert Koch discovered the tubercle bacillus and confirmed the bacterial etiology of tuberculosis.5 The bacilli in the sputum can be detected by ZN stain and Fl stain. ZN stain is commonly used throughout the world and still remains the standard method against which new tests must be measured.6 The smears stained by ZN method can detect bacilli when they are at the order of 10⁵/mL of sputum, whereas a more sensitive staining technique like Fl stain detects the bacilli when they are at the order of 10⁴/mL of sputum.7 Fl staining is regarded as a more reliable method due to more intensive binding of mycolic acids of the bacilli to phenol auramine, and so that the bacilli stand out sharply against black background to allow rapid and accurate screening under low power objective.8

The present study was undertaken, 1) to determine the prevalence of HIV in pulmonary tuberculosis cases, 2) to compare the bacillary load in the sputum of pulmonary tuberculosis cases co-infected with HIV, and 3) to evaluate the efficacy of fluorochrome stain in detecting paucibacillary pulmonary tuberculosis cases in comparison to Ziehl Neelsen stain.

Materials and Methods

The present study was conducted at the department of microbiology, Gandhi Hospital, Secunderabad. The study comprised 200 clinically and radiologically diagnosed pulmonary tuberculosis cases, in the age group of 15-45 years attending the TB clinic during the period of June to December 2001. Three sputum samples were collected on three consecutive days as per the current guidelines of the National Tuberculosis Control Program. From each sputum sample two smears were made on clean glass slides, air dried and heat fixed.

One was stained by ZN stain and the other by phenol auramine stain (Fl stain). ZN stained smears were examined using oil immersion objective (x1000) and Fl stained smears using 40x objective (x400) under fluorescence microscope. The results were graded in a defined manner as per the guidelines of International Union Against Tuberculosis. For
the present study 2+ and 3+ were classified as multibacillary and 1+ and scanty as paucibacillary.

Using aseptic precautions five millilitre of blood was collected from each case and serum was obtained. All serum samples were screened for HIV seropositivity using Immunocomb II (PBS-Organics, France) and Capillus method (The Trinity Biotech, Ireland). All reactive samples were screened by ELISA (Lab systems) based on different antigens and different principles as per the WHO recommended strategies.

Results

In the study group, 70% were males and 30% were females. Maximum number of TB cases were observed in the economically most productive age group of 15-35 years (Table 1). It was observed that a total no. of 38 sputum smears which were negative by ZN method were positive by fluorochrome stain (Table 2). The overall prevalence of HIV in the study population was 15.5%. It was also observed that sputum positivity was less in HIV positive patients than in patients negative for HIV; and fluorochrome stain was more sensitive in detecting sputum positivity in HIV cases than ZN stain (26% more in HIV seropositives and 18% more in HIV seronegative cases) (Table 3).

Bacillary load was less in HIV seropositives and fluorochrome staining could pick up more number of paucibacillary cases (14 cases), whereas ZN stain was positive only in nine paucibacillary cases (Table 3). The higher sensitivity of FI stain (45%) than that of ZN stain (29%) in detecting the paucibacillary cases associated with HIV co-infection was seen in this study.

Discussion

In this study, the prevalence of pulmonary tuberculosis was high in the age group of 26-35 years; and in males (70%), as compared to females (30%). A nationwide study conducted by the ICMR in 1969 has reported increased incidence of Tuberculosis in 25-34 years and in 63% of males. Similar results were reported by Banvalikar et al in 1997 (79% males and 21% females) and by Rajasekaran et al in 2000 (76.3% males and 23.7% females). This does not however reflect an increase in the occurrence of disease in males, since the attendance of females to OPD is lower than males.

According to Harries et al, the sputum positive cases are most infectious and contribute substantially to transmission of disease. But as per observations of Behr et al, though tuberculosis patients with sputum smears negative are less infectious, both theoretical and empirical evidence suggests that they can still transmit tuberculosis. Laboratory diagnosis of pulmonary tuberculosis rests on the bacteriological examination of sputum smears stained by the ZN method for AFB. In the present study we compared light microscopy of ZN stained smears with that of fluorescent microscopy of phenol auramine stained smears for the detection of acid-fast bacilli. Sputa from additional 38 cases (19%) were positive by phenol auramine method (50% by ZN, 69% by FL). This shows that fluorochrome staining of sputum smears in comparison to that of ZN staining is a better method of microscopy (χ² = 4.16, p < 0.05) for demonstration of acid-fast bacilli. FI method has an added advantage of allowing a large number of sputum specimens to be examined in a given time as low power is used for examination. Our study correlates with the results of Githui et al (65% by ZN, 80% by FL), Singh et al (abstract) (37.5% by ZN, 42.5% by FM) and Ulukanligil et al (67.6% by ZN, 85.2% by FM). The use of fluorochrome stain greatly improves the diagnostic value of the sputum smears especially in patients with a low density of bacilli who are likely to be missed on ZN stained smears (χ² = 6.54, p < 0.05).

HIV sentinel surveillance among tuberculosis patients is being carried out in many countries in order to ascertain the level of HIV prevalence and trends over time in them, since these data reflect the association between TB and HIV. HIV prevalence of 20.1% has been reported by Paranjape et al from Pune in 1996 and 14% by Jain et al from Delhi in 2000 in TB patients. Our study is also showing 15.5% of HIV prevalence in Hyderabad area, which stresses the need for screening every tuberculosis patient for HIV status. Difficulties in the diagnosis of tuberculosis in HIV positive patients could arise chiefly because of the reduced sensitivity of the direct sputum examination and the higher frequency of atypical lesions in chest X-ray. Sputum smear examination remains an essential component of diagnosis, even in countries where HIV infection is common, because of the need to identify infectious cases. In our study (Table 3), sputum positivity was less (32%) in HIV seropositives than in HIV seronegatives (53%). Similar results have been shown by other investigations. Tuberculosis patients with sputum
smears negative for AFB are less infectious than those with positive smears. But patients with smear negative pulmonary tuberculosis appear responsible for about 77% of TB transmission. Table 3 demonstrates that bacillary load in sputum is less in HIV seropositives. In ZN stained smears, one multibacillary and nine paucibacillary pulmonary tuberculosis cases were detected whereas in fluorochrome stained smears four multibacillary and fourteen paucibacillary cases were detected. By both techniques, it was found that paucibacillary cases are more than multibacillary cases in HIV seropositives. This is because of atypical manifestations such as non-cavitary lesions with dissemination of disease that are encountered in HIV-TB co-infected patients. In such cases sputum positivity decreases, paucibacillary status and smear negativity increases. Point to be noted is that in HIV seropositives Fl stain (45%) is 16% more sensitive in detecting paucibacillary pulmonary tuberculosis cases than ZN stain (29%).

In conclusion, fluorochrome stain is more efficient over ZN stain in detecting paucibacillary pulmonary tuberculosis cases co-infected with HIV, for early diagnosis. Since screening is done under low power, much time is likely to be saved. Fluorescent microscopy though expensive is appropriate where the work load is high and screening needs to be done on a routine basis.

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