Efficacy of Fluorescence microscopy in diagnosis of Pulmonary Tuberculosis

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ABSTRACT

Introduction: Tuberculosis (TB), one of the oldest diseases known to affect humans, isa major cause of preventable death worldwide. This disease, which is caused by bacteria of the mycobacterium tuberculosis complex, usually affects the lungs, although other organs are involved in up to one-third of cases, If property treated, tuberculosis caused by drug-susceptible strains is curable in virtually all cases. **Materials and Methods:** This cross-sectional studywas conducted inthe Department of Microbiology. The sputum specimens of 200 patients were stained by both conventional Ziehl-Neelsen (ZN) and fluorochrome staining methods. **Results:** Out of the total 200 patients 26 were found positive for acid fast bacilli by ZN staining and 45 by Fluorescence staining. It is clear that scores are definitely higher by Fluorescence microscopy.

Key words: Tuberculosis, Ziehl-Neelsen, fluorochrome staining, efficacy.

Introduction

According to WHO (World Health Organization) 2 billion people that is one third of the world's population is suffering from tuberculosis (TB) [1,2].Annually 9.4 million people get infected by TB[3,4] with an estimate 2-3 million deaths every year, about 8000 people a day i.e. 1 person every 20 second[1]. TB was declared as a global emergency by WHO in the year 1993[5].ln India, an estimated 14 million people are infected with tuberculosis each year , 3.5 million of these are categorized as highly infectious[6].

Aim

To study the efficacy of Fluorescence microscopy (FM) in diagnosis of Pulmonary Tuberculosis in comparison to Ziehl- Neelsen (Z-N) staining of sputum samples from patients suspected of Pulmonary Tuberculosis.

Materials and Methods

This cross-sectional study was conducted inthe Department of Microbiology, Medical College and S.S.G. Hospital, Vadodara from October 2009 to February 2010, on the sputum specimens of 200 patients clinically suspected of pulmonary tuberculosis attending TB and Chest OPD. These smears were stained by both conventional Z-N and fluorochrome staining methods. Patients attending the TB & chest OPD and having cough for 2 weeks, or more, with or without other symptoms (having fever, loss of appetite. Loss of weight, chest pain, hemoptysis) suggestive of TB were included. Those unable to produce at least 5 ml of mucopurulent sputum and pediatric cases were excluded. Two sputum specimens, one is collected on the spot and other is an early morning specimen collected at home by the patient. Samples were collected in clean, sterile, leak –proof, wide - mouth containers. Ziehl-Neelsen smear reporting:Smears are examined using a light microscope scanning at least 300 oil immersion fields before reporting a smear as negative .AFB stain bright pink to red, beaded or barred forms are seen in Mycobacterium tuberculosis while the tissue cells and other organisms are stained blue.

Auramine O smear reporting: Switch on the mercury vapor lamp. The bulb takes approximately 10 minutes to reach full intensity. Using the low power objective (magnification 100-150x) first examine a known positive slide to ensure that the microscope is correctly set up. The films are examined with a 40x objective and a l0x eye piece. The tubercle bacilli arc seen as yellow luminous organisms in a dark field .For the present study 2+, 3+ and 4+ were classified as

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multibacillary an 1+ as paucibacillary. Doubtful was considered as negative.

Results

A total of 400 sputum specimens from 200 clinically suspected tuberculosis cases were examined in this study. Each sputum sample was stained by ZN and Fluorochrome staining methods. Out of the total 200 patients 26 were found positive for AFB by ZN staining and 45 by Fluorescence staining. The ZN smear positivity rate in the study was 13% (26/200) and the Fluorochrome smear positivity rate was 22.5% (45/200) respectively. The combined smear positivity using both the staining techniques was 22.5% (5/200) which is basically positivity rate of the Fluorochrome stained smear, as we did not have any smear which was positive by ZN and negative by FM staining. (Table 1)

Table 1: Comparative evaluation of Fl stain in all 200 tuberculosis cases

Kind of specimen	Z-N staining Positive	Fluorescent staining Positive	Z-N –ve Fl +ve	Total AFB +ve	Total number
Sputum	26	45	19	45	200

The ZN method missed 19 (42.22%) of the 45 cases found positive by auramine phenol method, these cases were all paucibacillary (Grade 1+). (Table. 2) Thus, it is clear that scores are definitely higher by Fluorescence microscopy: 45 (24+6+5+10) positive as against 26 (10+3+7+6) positive by ZN method (Table 2)

Table 2: Grade wise reporting of 200 cases

Grade	ZN Stain	Fl Stain
1^+	10	24
2^+	3	24
3^+	7	5
4^+	6	10

Discussion

Direct microscopic examination of sputum for AFB remains the cornerstone for the diagnosis of pulmonary tuberculosis in both the industrialized and low income countries. ZN and Fluorescence staining are the two staining techniques commonly used in clinical laboratory for acid – fast bacilli smear examination [7].ZN stain can detect the bacilli when they in the order of 10^5 /ml of the sputum whereas a more sensitive AO stain can detect in the order of 10^4/ml of sputum [8]. The other advantage of using Fluorescence staining is that slide can be scanned under lower magnification while a ZN prepared slide must be examined under oil immersion (100x magnification). Fluorescence stained slides can be examined with 40x or 60x magnification; this detection of acid - fast organisms with the Fluorescence stain takes less time than with the ZN stain and cause less eye strain[9].According to Harries et al. the sputum positive cases are most infectious& contribute substantially to transmission of disease [10]. But as per observations of Behr et al, though tuberculosis patients with sputum smear negative are less infectious, both theoretical and empirical evidence suggests that they can still transmit tuberculosis [11].In the present study we compared sputum smears with that of microscopy of ZN stained sputum smear with that of Fluorescent microscopy of phenol auramine stained smears for the detection of acid - fast bacilli. Out of 200 cases, 45 (22.5%) were found positive by Fluorochrome staining and 26(13%) by Z-N staining method. Sputum from an additional 19 cases(9.5%) were positive by phenol method (13% by ZN,22.5% by FM).Our study is in agreement with various studies carried out in different parts of the world and in India [12,13,14-17,18,19,20,21,9,23,24] .

Review of various studies

Sr. No	Study group	Year	No. of cases/samples studied	Z-N +ve	FM +ve	Reference
1	Truant J p et al[13]	1962	585	47(8.03)	56(9.57)	Culture
2	Githui et al[12]	1993	1480	718(48.51)	760(51.35)	Culture
3	Kumar V A et al[14]	1993	746	62(8.31)	66 (8.84)	Culture
4	F Ba,HLRieder[15]	1999	1491	287(19.24)	319 (21.39)	None
5	Mustafa Ulukangil et al[16]	2000	295	46 (15.59)	58(19.66)	Culture
6	A Jain et al[17]	2001	493	164(33.26)	208(42.19)	Culture
7	LEA Kivihya et al[18]	2003	993	332(59.90)	430(77.6)	Culture
8	K Prasanthj et ai[19]	2005	200	100(50.00)	138(69)	None
9	Singh NP et al[20]	2008	2600	975(37.5)	1104(42.46)	None
10	Ben J Marais et al[21]	2008	221	24(10.85)	27(12.22)	Culture
11	Laifangbam S et al[9]	2009	920	45(44.1)	73(71.6)	Culture
12	Khagi A R[23]	2009	250	134(53.6)	150(60.00)	Culture
14	Present study	2010	200	26(13.00)	45(22.5)	None

The sensitivity and specificities for the ZN staining and FM staining as reported by them ranges from (sensitivity: 47.67% for ZN; 73-97% for FM) and (specificity: 93-100% for ZN; 90-99.9% for GM)In a systematic review of 18 studies, Steingart et al [25] reported that Fluorescence microscopy of auramine stained smears provides similar specificity & sensitivity (mean improvement of 100%), compared with light microscopy of ZN - stained smears. Lempert described two reasons for the superiority of FM over the ZN method with respect to weekly positive sputum specimens: (i) an increased area of smear per field and, (ii) an increased contrast between the stained bacilli and the background. Furthermore, heating is not required during staining and immersion oil is also not required for smear examination.The Fluorochrome stain is more efficient over ZN stain detecting paucibacillary cases has been proved in the study done by Laifangbam et al they found that Fluorochrome stain could detect 20 paucibacillary cases whereas ZN detected only 3 of them [9]. We also observed that Fluorochrome stain could detect 24 paucibacillary .Similar results were also found by K. Prashanthi et al, Fluorochromestain (45%) was 16% times more sensitive in detecting paucibacillary tuberculosis than ZN stain (29%).

Conclusion

Two hundred (200) clinically suspected cases of pulmonary tuberculosis were examined for sputum smear microscopy TB & chest OPD. Fluorescence microscopy is easy because the Fluorescent contrast caused by stained AFB is seen much more quickly than the red of APB against a blue background in bright - field microscopy. and fluorescence microscopy(FM) are reportedly more sensitive than direct Ziehl-Neelsen (Z-N) sputum smears for tuberculosis detection, and might be particularly valuable for human immunodeficiency virus (HIV) positive patients excreting fewer bacilli. Introduction of this method is feasible and with a better yield could make a positive impact on the effectiveness of TB control programs

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