



Rapid Diagnosis of Tubercular Bacilli in Sputum Cytology

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ABSTRACT

Background: Tuberculosis is a most common infectious disease which is still remains a leading cause of adult mortality. There are various methods for the diagnosis of tuberculosis, such as sputum examination of tubercular bacilli by Ziehl-Neelsen(ZN) staining, demonstration of tubercular bacilli by Auramine-O, Papanicolaou(Pap) stain staining and culture in LJ medium.

Aim: The aim of present study was to evaluate the diagnostic utility of Pap stain, Auramine-O and ZN stain in rapid diagnosis of pulmonary tuberculosis in sputum cytology.

Material & Methods: Study was done on Sputum of clinically suspected case of pulmonary tuberculosis from June 2017 to August 2017. On all received sample Z N, Pap, Auramine-O staining done.

Result: Among clinically suspected 150 patients. Tuberculosis was diagnosed in 74 patients. Pap shows 49 (66.21%) , Auramine-O shows 42(56.75%) ; ZN shows 35 (47.29%) cases positive.

Conclusion: Evening rise of Temperature, Weight Loss, and Cough more than 2 weeks, CXR reports are the main evidence to suspect a case of Tuberculosis. Males are more affected than female and more in case of seropositive patient. Pap is slightly more sensitive than Auramine-O in diagnosis of Pulmonary tuberculosis in Fluorescent Microscopy.

Keywords: Pap, Auramine-O, Sputum, ZN stain, Fluorescent Microscopy.

Introduction

Mycobacterium Tuberculosis (MTB) is most common communicable disease in low socioeconomic population. It is most common in developing countries like India.

Causative organism was discovered more than 100 years ago and highly effective drugs and vaccine are available, still it is prevalent in our society.^[1]

Koch first described the tubercle bacilli in 1882.^[1] Mycobacteria are now known to comprise a large group of acid-fast, alcohol-fast, aerobic or micro-aerophilic, non-spore forming, non-motile bacilli.^[1]

World Health Organization (WHO) statistics as of 2011 estimates gives global incidence of 8.7 million cases, about 2.2 million from India. About 40% of Indian population is infected with tuberculosis.^[2]

In developing countries like India with deficit of resources compared to high TB burden, culture facility is not adequately available. Hence most of the TB cases are diagnosed based on Sputum Smear Microscopy (SSM).^[5] Hence, Rapid and effective methods are required for detection of Tubercle bacilli in sputum in limited resource setup.

Many laboratories prefer Rhodamine Auramine method of staining AFB over carbol fuchsin stain because the fluorescing bacilli are more readily detected. Auramine stained smears are scanned under low magnification than fuchsin stained smears, thus permitting a large area of the smear to be examined in shorter period of time. Diagnosis of pulmonary tuberculosis by demonstration of Acid Fast Bacilli (AFB) in sputum smears by ZN staining is known to be economical and less time consuming.^[6]

Papanicolaou stain is widely used in routine cytological evaluation of samples derived from the respiratory tract. Its value in the fluorescence microscopic diagnosis of various pulmonary infectious diseases is well established.^[7] In Pap stained smears the slightly curved, beaded tubercle bacillus may be readily identified.^[8] Limited data are available regarding efficacy of different types of stain especially Pap stain in detection of tubercular bacilli.

So, we studied the efficacy of different types of stain i.e ZN, PAP, Auramine-O in the diagnosis of tuberculosis in sputum sample.

Material & Methods

Study was done on Sputum of clinically suspected case of pulmonary tuberculosis from June 2017 to August 2017 in

Department of Pathology, S N Medical College & HSK Hospital, Bagalkot. Study was done on clinically suspected cases. Usual clinical presentation is fever, cough, sputum and weight loss.

Sputum collection: Sputum of 24 h duration was collected in dry, sterile bottles.

Three smears were prepared from each sample (Two dry and one wet smear). Three smears were stained by routine Ziehl-Neelsen (ZN) method and other by fluorescence stain Auramine–Rhodamine and Pap (Papanicolaou) stain.

Ziehl-Neelsen stain shows-AFB stain pink curve/straight beaded rods against blue background.

Auramine–O stain shows-AFB appears as bright reddish–yellow fluorescing rods against a dark background and

Papanicolaou stain fluorescent bacilli shows- slender, often beaded, yellow-green, straight or slightly curved rods of relatively uniform length against a dark background.

Grading of PAP & Auramine-O smears done as: >10 bacilli/field= 2+; 2-9 bacilli/field=1+; 10-99 bacilli/100 fields =+; 1-9 bacilli/100 fields= Scanty; No bacilli in 100 fields= Negative. ^[14]

RESULT (Total case =150, Positive case (n)=74)

Stain	Positive cases (n)	Percentage
Pap	28	37.83%
Auramine-O	24	32.43%
ZN	22	29.72%

*FMP=Fluorescent Microscopy

Result compared from different study

Stain	Mani et al	Madhusudhan et al	Present Study
ZN	16.16%	27.65%	29.72%
Pap	33.35%	24.90%	37.83%
Auramine-O	31.34%	27.44%	32.43%

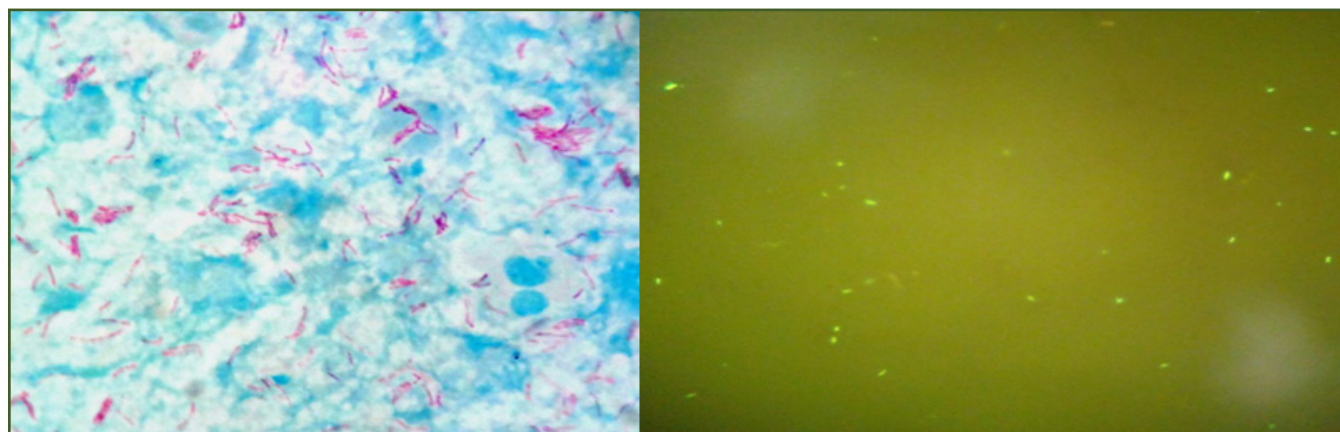


Fig. I: AFB Positive (ZN stain 100X), Fig.IIAuramine-O (FMP*) 40X.

Positive control smears were prepared from cultured colonies of *Mycobacterium tuberculosis* (MTB) and negative control salivary smears were prepared from saliva sediment of a healthy individual for both the staining methods.

ZN smears were graded using 100x oil immersion objective as per RNTCP guidelines. ^[13] Scanty = 1-9 AFB in 100 oil immersion fields; Grade 1+ = 10-99 AFB in 100 oil immersion fields; Grade 2+ = 1-10 per oil immersion field at least 50 fields; Grade 3+ = 10 or more AFB per field in at least 20 fields; Negative = no AFB in 100 fields.

Result

Among clinically suspected 150 patients. Tuberculosis was diagnosed in 74 patients. Males are more infected than female. PAP shows 49 (66.21%), Auramine-O shows 42 (56.75%); ZN shows 35 (47.29%) cases positive. Of the 150 cases evaluated all were above 16 years of age with a range of 16-80 years. Among clinically suspected 150 patients. Tuberculosis was diagnosed in 74 patients. The peak incidence was seen in the second-third decades.

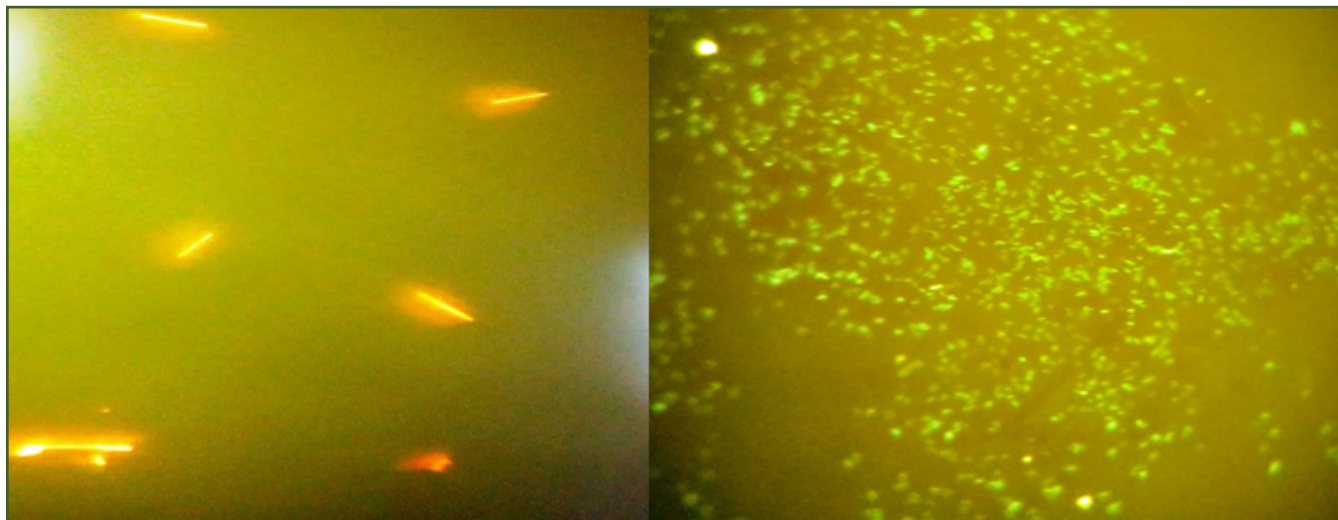


Fig. III Pap Stain (FMP*) 40X, Fig. IV Auramine-O (FMP*) 20X.

Discussion

Recent global statistics reveal 8.6 million incident cases of Tuberculosis (TB) with 1.3 million deaths.^[3] This TB crisis has further escalated with triggering of the HIV epidemic and emergence of multidrug-resistant TB.^[4]

Tuberculosis commonly affects lungs but can also be extra-pulmonary. Hence microscopic examination of sputum for detection of Acid Fast Bacilli (AFB) is of utmost importance. Early detection can prevent further complication.^[4]

An advantage of fluorescence microscopy lies in the ease with which the bacilli can be detected due to the color contrast, allowing for a larger area of the smear to be scanned at lower magnification when compared to conventional Z-N staining. Since the Pap stain is routinely used in cytology, it saves the time and material that would be involved in any extra staining and also avoids the use of toxic or carcinogenic substances such as phenol and rhodamine, which are used in A-R staining method.^[7]

In India prevalence of tuberculosis is 400 per 100,000 population.^[12]

Diagnostic utilization of UV-induced fluorescence for detection of infective pathogens was first described by Graham (1983)^[16] and later Mann (1983).^[17]

Ghali et al. (1984). first demonstrated auto fluorescence of pneumocystic carinii in Pap stained smears. Their results indicated eosin to be responsible for the auto fluorescence.^[9] Its value in the fluorescence microscopic diagnosis of various pulmonary infectious diseases is well established.^[3]

Küpper *et al.* reported significant difference between the sensitivities of Pap fluorescence (FMP), A-R fluorescence

(FMA) and Z-N staining respectively for their ability to detect atypical *Mycobacterium kansasii* in cytological samples.^[15]

Our results were similar with Kupper et al. and Lunawat PP et al. in their studies, which also found AF stain was more sensitive in comparison with AR and ZN stain for the diagnosis of tubercular bacilli.^[11]

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The Pap stain is routinely used in cytology. It saves time and material that would be involved in any extra staining and also avoids the use of toxic or carcinogenic substances such as phenol and Rhodamine, which are used in AR staining method. It also offers the advantage of simultaneous identification of other agents such as pneumocystic carinii, aspergilla and candida in the material obtained primarily for the diagnosis of TB, especially in immunocompromised patients.

Conclusion

Fluorescence microscopy is a useful, rapid, and reliable and is quite economical tool for the diagnosis of mycobacterium bacteria in specimens for AFB in comparison to ZN staining because of the higher sensitivity and rapidity of the fluorochromes technique compared to ZN.

The PAP stain is routinely used in cytology, it saves the time and material that would be involved in any extra staining and also avoids the use of toxic or carcinogenic substances, such as phenol and Rhodamine, which are used in AR staining method.

However, this method should be used primarily as a screening technique, and every diagnosis should be confirmed by microbiological culture.

The method used in the present study enables in rapid identification of these infective sources and hence prevent further transmission and is thus important in TB control.

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