



ROLE OF Z N STAINING AND MICROSCOPY IN DETECTION OF ACID FAST BACILLI IN SPUTUM

Pathology

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ABSTRACT

This study was done on 2401 patients suspecting to suffer from Tuberculosis. Study included prospective analysis. This study was done for 1 year and 8 months. Sputum examination was done in all cases and sputum staining for AFB is done by Z.N stain.

RESULTS: Total 2401 cases were included in this study. Out of 2401 Cases included, 190 cases were categorized as positive and 2211 were categorised as negative.

CONCLUSION: Z N staining is a rapid, easily available technique for evaluation of AFB. It helps in making diagnosis and management plan. Z N staining is a simple and quick procedure. Microscopy continues to be indispensable for detection of AFB. It is rapid, cost effective, easy to use with high specificity.

KEYWORDS

TB, AFB, MICROSCOPY, ZN staining

INTRODUCTION:

Tuberculosis (TB) continues to affect the human race since time immemorial. Tuberculosis is an infectious disease caused predominantly by *Mycobacterium Tuberculosis*. *Mycobacterium Tuberculosis* was discovered in the 19th century in Eastern Germany, physician and scientist Robert Koch (1843–1910). The burden of TB has been the subject of concern, and major efforts are under way to try to achieve its control. TB is most commonly transmitted by inhalation of infected droplet which are discharged in the air when a patient with untreated TB coughs or sneezes. TB usually affects the lungs but can involve any part of the body. Tuberculosis is a major health problem in most developing countries. Approximately, one third of the world's population has been infected with *Mycobacterium tuberculosis*. India accounts for one fourth of the global TB burden i.e. 2.2 million out of 9.6 million new cases annually (WHO 2014). It is also estimated that about 2.2 lakh people die due to TB annually. The spread of human immunodeficiency virus (HIV) has further aggravated the situation. The number of patients infected with both HIV and tuberculosis is estimated to be 3.8 million (Porter, 1995). In 1997, the RNTCP adopted the internationally recommended Directly Observed Treatment Strategy (DOTS) and involved documentation and surveillance of TB and brought about a degree of control. Here, emphasis is given to TB diagnosis by identification of acid fast bacilli (AFB) (direct smears) with Ziehl–Neelsen (ZN) staining.

MATERIALS AND METHODS: A prospective analysis of 2401 cases of chest infection was conducted for a duration of one year and eight months from 1-1-2017 to 31-8-2018 in Block Akhnoor Jammu. Detailed clinical work up of all patients was done including local and general physical examination including, detailed history of cough > 2 weeks, fever > 2 weeks, significant weight loss, haemoptysis, any abnormality in chest radiography, family history etc. All other routine and special tests were performed. Sputum examination was performed in all cases. Slides for the direct smears were prepared from purulent part of the sputum. The sputum was expressed on the slides to make 2-3 smears. The smears were then stained by Z.N.stain for microscopic examination.

SAMPLE COLLECTION: Main problem in sample collection is to obtain adequate good quality sample mostly in peripheral hospitals. In order to obtain good quality sample concerned technician needs to explain the process of collecting a good quality sputum sample to the concerned patient. Good quality sample consists mainly of mucoid or mucopurulent material obtained from bronchial tree with minimal amount of oral and nasopharyngeal material. 3 -5 ml of sample should be collected in a sterile container. Two samples are collected within a day or two consecutive days. One sample should be collected on the spot under supervision and other is collected early in the morning. Sample should be transported to the laboratory as soon as possible or otherwise it should be refrigerated to avoid micro organism growth.

RESULTS: Smear reading is an important step in the detection of acid fast bacilli. Manner and quality of smear reading has a major impact on the result of sputum smear microscopy. A prospective study over a period of 20 months was done with effect from January 2017 to August

2018. Total number of cases examined during this period is 2401, out of which 190 are positive and 2211 are negative for AFB on Z.N staining (Table 1). The smears were examined very carefully for the recommended duration, atleast 5 min or 100 fields which improves case detection. The results of sputum AFB microscopy are known to be influenced by various factors, like efficiency to read smears by microscopist.

The need for training laboratory technicians is very important to maintain the quality of sputum AFB microscopy. Laboratory technicians undergoes 15-day training in reading AFB sputum Smears and it helps them in screening of AFB smears. The quality of reagents and staining procedures is also an important quality determining factor which leads to increase in detection of positive cases. The sputum smears were examined under oil immersion lens of ordinary light microscope or fluorescent microscope. The number of acid-fast bacilli seen on the smears were recorded according to the guidelines of WHO (Table 2). The AFB positive slides are stored and then counter checked.

RESULTS OF Z.N STAINED SPUTUM SMEARS ON MICROSCOPY

TABLE 1

Total cases	Positive cases	Negative cases
2401	190	2211

Grading of AFB smears on microscopy

Examination finding	No. Of fields examined	Grading	Result
No AFB in 100 oil immersion fields	100	0	Negative
1-9 AFB per 100 oil immersion fields	100	Scanty	Positive
10-99 AFB per 100 oil immersion fields	100	1+	Positive
1-10 AFB per oil immersion field	50	2+	Positive
More than 10 AFB per oil immersion field	20	3+	Positive

DISCUSSION

In India the most reliable method for the diagnosis of pulmonary tuberculosis is microscopy of sputum which is the cheapest, fastest and with high specificity. A positive smear will alert the physician regarding AFB infection, but a false negative smear may lead to delayed detection and start of antitubercular therapy. Detection of AFB in sputum by Z N staining is an economical, simple and rapid method but the estimated detection limit of microscopy is 104 bacilli/ml of sputum. Many factors like type of specimen, efficiency of decontamination, concentration procedures, the type of staining procedures and experience of microscopist influence the sensitivity of the detection of AFB in sputum smears. The range of acid fast smear sensitivity is quite wide from 22% to 78% (Pfaller, 1994). Atleast two sputum smears are necessary to confirm the diagnosis of tuberculosis. Now a days due to modifications and different microscopic alternatives including fluorescent microscopy, Kinyoun staining and

modified carbol-fuchsin staining, sensitivity and specificity of AFB detection is improved. Fluorescent microscopy has been found to be on an average 10% more sensitive than conventional microscopy and almost 98% specific. However, in our peripheral hospital settings fluorescent microscopy was not widely accepted due to high cost, requirement of continuous power supply, frequent burn-out of expensive mercury vapor lamps and need of a dark room. With the advent and use of light emitting diode (LED)-based fluorescent microscopes have overcome these short-comings and are now being used widely. Recent study in the National Reference Laboratory (NRL), New Delhi, shows sensitivity and specificity of LED microscopy, mercury vapor fluorescence and light microscopy as 83.1% and 82.4%, 78.5% and 87.5%, 81.6% and 83.5%, respectively. Mean reading time of LED was three times faster than ZN. Now a days, Auramine O staining-based LED has replaced conventional ZN microscopy in many institutions in collaboration with India's Revised National Tuberculosis Control Programme (RNTCP) in 2012. Many improved Mycobacterium Tuberculer Bacilli detection methods have been attempted in immune-fluorescence technique also and the use of the fluorescent nano-particles w reveals amplified signal intensity and higher photo-stability than the direct use of conventional fluorescent dye which improves the detection of Mycobacterium Tuberculer Bacilli.

Complications

As such no complications are seen in sputum collection because it is very easy to collect the sample and the process is very simple and noninvasive.

Summary

In resource-limited countries like India, microscopy will remain the primary means of diagnostic tool for TB. Presently, the LED based fluorescent microscopy technique has been established as the best alternative to ZN-staining but still due to the nonavailability of newer techniques, ZN staining is still the mainstay. In conclusion, ZN staining and the microscopy are essential components in the management of AFB positive patients. ZN staining and microscopy are cost effective, simple procedure with high degree of accuracy. It also helps in the grading of patients according to the bacillary load and start of early therapy. It's ease of performance and no complications are factors that leads to it's popularity.

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