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Comparative Study on Detection of Mycobacterium Tuberculosis In Sputum Samples Before And After Sputum Concentration Technique By Using Trisodium Phosphate Solution In A Tertiary Care Hospital, Thanjavur

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Abstract: Tuberculosis, inspite of advances in diagnosis and therapeutics still continues to be the leading cause of mortality and morbidity in many developing countries even today. It is one of the major killer infectious disease. In recent years, two important factors which heavily complicate the treatment outcome of tuberculosis patients are MDR-TB and XDR-TB.The mortality in Tuberculosis mainly due to delay in diagnosis. India has the highest TB burden accounting for 1/5 th of Global incidence. The most common form of TB is pulmonary tuberculosis. The sputum microscopy and culture both of them are backbone for the diagnosis of Tuberculosis. The present study aimed to compare and evaluate the of Trisodium Phosphate solution as sputum concentration technique by smear microscopy (Zeihl Neelsen Technique), to compare the smear microscopy. Sputum samples of suspected 150 cases of pulmonary tuberculosis attending Thoracic Medicine and General Medicine OPD at Thanjavur Medical College, (Tamil Nadu) were collected and processed for smear microcopy. Among the 150 sputum samples, AFB positivity in unconcentrated sputum microscopy by Zeihl Neelsen Technique-33(22%),AFB positivity in concentrated sputum microscopy by Zeihl Neelsen Technique-37(25%).The present study confirms that the importance of Trisodium Phosphate solution for sputum concentration technique for the smear microscopy by Zeihl Neelsen Technique.

Keywords: Mycobacterium tuberculosis, Acid fast bacilli, Zeihl Neelsen Technique, Trisodium Phosphate solution for sputum concentration technique.

INTRODUCTION

Tuberculosis one of the major seven killer infectious disease [1], is caused by Mycobacterium tuberculosis (tubercle bacilli), an acid fast bacillus. Tuberculosis is one of the highly infectious diseases in humans' beings for many centuries and now major public health problem particularly in resource poor developing countries like India [2].

It has been estimated that approximately about one third of world population is infected with tubercle bacilli. In Worldwide, more than 9.2 million new cases and 3 million deaths due to Tuberculosis occur annually. Tuberculosis in developing countries contributes to 3/4 th of Worldwide infected cases [3]. Now the emergence of Multi Drug Resistant-Tuberculosis (MDR-TB) and Extensively Drug Resistant –Tuberculosis (XDR-TB) are more common in many parts of the World.

India has the highest TB burden accounting for 1/5 th of Global incidence. 15 million people is suffering from Tuberculosis and a half a million cases die due to TB every year. So in India, 40% of population infected with TB and the coinfection with HIV is about 4.85% [4].

Pulmonary Tuberculosis is more common, affecting lungs. Involvement of lungs leads to Primary Tuberculosis (Primary Complex, Ghon's Focus) and Secondary Tuberculosis (Casseous necrosis, cavitatory lesion, fibrosis etc.).

Delay in diagnosis of Tuberculosis leads to delay in starting treatment leading to increase in infectivity in the community which in turn increases the morbidity, mortality and emergence of MDR-TB & XDR-TB.

As there is an increased prevalence of MDR-TB & XDR-TB in our country, the present study

is undertaken to find out the earlier [5] and reliable microscopical [6] diagnostic method by Zeihl Neelsen Staining method for detection of Tuberculosis.

AIM AND OBJECTIVES

- To detect the Mycobacterium tuberculosis infection in the sputum of clinically suspected cases of Pulmonary Tuberculosis by microscopy by Zeihl Neelsen Staining method.
- To compare microscopy for detection of Mycobacterium Tuberculosis in sputum samples before and after sputum concentration technique using Trisodium Phosphate solution [7].

MATERIALS AND METHODS

This study was conducted in Thanjavur Medical College Hospital, Thanjavur from the period of December 2012and November 2013. (One year) collaborating with Department of Medicine and Thoracic Medicine. The study Design is Simple Random Cluster Sampling Study. Ethical clearance certificate was received from Ethical Committee of Thanjavur Medical College and Informed consent is also obtained from each patient.

Inclusion criteria

- Patients those who are having cough for more than 2weeks duration.
- History of cough for more than 2weeks duration,associated with fever,weight loss, loss of appetite with or without haemoptysis.
- Adults – more than 12 -80 years of age group.
- History suggestive of recurrent Lower Respiratory Tract Infection not responding to routine antibiotics therapy.

Exclusion criteria

- Patients those who are having cough less than 2 weeks duration.
- Old treated and cured cases of Tuberculosis (either Pulmonary or Extrapulmonary Tuberculosis).
- Patients on Anti Tuberculous Therapy at present.
- Paediatric age group (newborn to 12 years).
- Patients of Chronic Obstructive Pulmonary Diseases like Chronic bronchitis and Bronchial Asthma.

WORK PLAN

Sputum samples were received from of 150 persons with history suggestive of Pulmonary tuberculosis (symptoms of cough more than 2 weeks duration, fever with loss of weight and appetite with or without haemoptysis).

METHODOLOGY

Patient was instructed to collect early morning deeply coughed out sputum samples (approximately 5ml of sputum sample- John R Warren *et al*.,) [8] in a sterile wide mouthed sputum collection container having 7-8ml of Trisodium Phosphate solution at home and it was transported to Microbiology Laboratory. Again patient was instructed to collect a spot sample in another sputum collection container without transport medium. Proper labelling was done after receiving sputum samples which had the patient name,age, sex, IP.No,date and including the time of sample collection. Then smear was made from sputum sample without transport medium and processed for acid fast bacilli staining by Zeihl Neelsen Technique (The specificity is slightly higher in Zeihl Neelsen staining method than other microscopical sputum acid fast bacilli staining method) [9, 10]. Under strict aseptic universal precautions (using personal protective equipments), sample in the transport medium was to be vortexed in a vortex shaker and left over the Sputum sample at room temperature for a overnight period. Then the sputum sample in the transport medium was processed under strict aseptic precautions with all universal precautions in BIOSAFETY CABINET- II-B, decant the supernatant solution using sterile pipette into the container having 5%Sodium Hypochlorite Solution. Using sterile nichrome loop of 5mm size, one loopful of inoculum from the deposit was taken, good quality smear was made for Acid Fast Bacilli Staining. Then AFB staining by Zeihl Neelsen Technique was done in the smear. Then the smear was examined under oil immersion field.The results were noted.

Good Quality Smear Preparation

A new unscratched slide was selected for smear preparation. Yellow purulent portion of the sputum was picked with a piece of clean broom stick and oval shaped smear of size about 2x3cm,should neither be too thick nor too thin (printed letters should be just readable through the smear) were prepared.

Preparation of Tri Sodium Phosphate Liquid Transport Medium (TSP) [7]:

The transport medium used was prepared by adding

- trisodium phosphate (Na3PO412H2O) 200 g
- ammonium sulphate 5 g,
- magnesium sulphate500 mg;
- ferric ammonium citrate 250 mg;
- mineralsalt solution 25 ml and
- Distilled water 975 ml.

Mineral salt solution [7]:

- Monopotassium phosphate-2.4gms.
- Magnesium sulphate-0.24gms.
- Magnesium citrate-0.6gms.
- Asparagin-3.6gms.
- Glycerol-12ml.
- Distilled water -642 ml.

As per the journal of J. Jena and B. N. Panda [7] the Tri Sodium Phosphate Liquid Transport Medium

(TSP) was prepared and dispersed in sterile McCartney bottle. The bottles were then stored at room temperature. Each bottle thus prepared, constituted a single unit of transport medium. Then under strict aseptic precaution, the TSP medium was transferred to sterile sputum container and supplied to patients to collect early morning deeply coughed out sputum sample.

Positive Control

Smear was made from known AFB positive sputum sample.

Negative Control

Smear was made from known AFB negative sputum sample.

Test sample

Smear was made from tested sample

Then AFB staining by Zeihl Neelsen Technique was done in the smear. Then the smear was examined under oil immersion field as per the RNTCP Guidelines(300 fields/15 minutes).The results were noted.

RESULTS AND DISCUSSION

Among the 150 persons of screening population, further analysis was done based on diagnostic method of direct smear microscopy for AFB detection by Zeihl Neelsen technique.

Zeihl Neelsen Staining in– UnConcentrated Sputum (Direct smear microscopy):

Table-1:

Grading of sputum (RNTCP)	No.of samples (n=150)	Percentage (100%)
Negative	117	78%
Positive-1+	13	8.6%
Positive-2+	17	11.3%
Positive-3+	3	2.0%

Among the 150 persons of screening population, Zeihl Neelsen staining technique in the unconcentrated sputum sample showed Positivity in 33(22%) sputum samples. About 78% of cases showed negative for sputum direct smear microscopy for AFB detection by Zeihl Neelsen technique. On statistical analysis using One Way ANOVA test, Zeihl Neelsen staining technique significantly detects positive cases by direct smear microscopy.

Among the 150 persons of screening population, further analysis was done based on diagnostic method of sputum smear microscopy after concentration method for AFB detection by Zeihl Neelsen technique.

Zeihl Neelsen Staining in–Concentrated Sputum-(smear microscopy)

Table-2:

Grading of sputum (RNTCP)	No. of samples (n=150)	Percentage (100%)
Negative	113	75.3%
Positive-1+	6	4.1%
Positive-2+	20	13.3%
Positive-3+	11	7.3%

Among the 150 persons of screening population, Zeihl Neelsen staining technique in the concentrated sputum sample showed Positivity in 37 samples (25%). About 75.3% of cases showed negative for sputum smear microscopy after concentration Method for AFB detection by Zeihl Neelsen technique. On statistical analysis using One Way ANOVA test, Zeihl Neelsen staining technique in the concentrated sputum sample significantly detects more positive cases by microscopy than the direct smear microscopy.

Zeihl Neelsen Staining in– UnConcentrated Sputum (Direct smear microscopy):

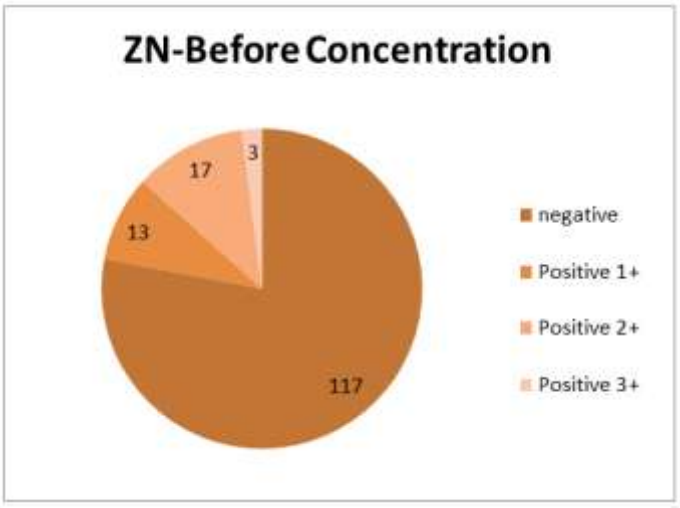

Chart-1:

Zeihl Neelsen Staining in–Concentrated Sputum-(smear microscopy)

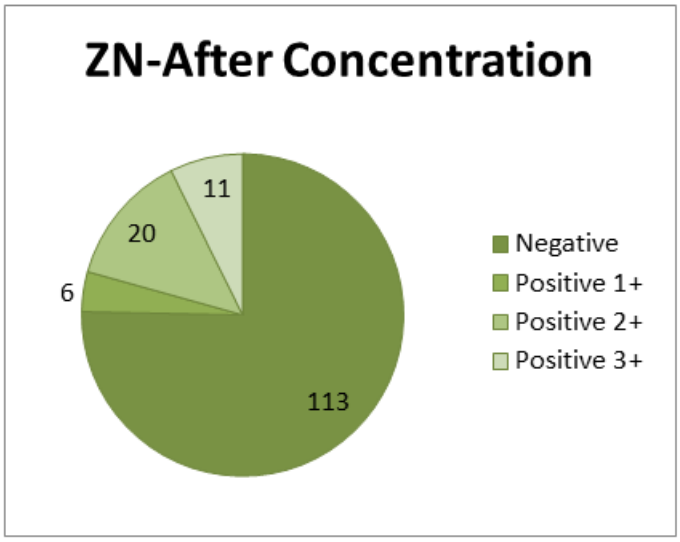

Chart-2:

Among the 150 persons, 33(22 %) samples showed AFB Positivity in Unconcentrated sputum samples and 37(25%) in concentrated sputum samples in microscopy by Zeihl Neelsen Technique.

- Among the 37 positive samples, Males (81%) were commonly affected than females (19%).
- Among the 37 positive samples, history of Smoking and Alcohol is 80% cases were smokers and 80% of cases were alcoholic.
- Among the 37 positive samples, age group of 31-50 years (82%) were more commonly affected.
- Persons of low socioeconomic status belonged to 51% were commonly affected followed by middle class persons 49%.

DISCUSSION

In this present study, 150 sputum samples are collected in the Trisodium phosphate transport medium container and another container without transport medium as spot sputum sample.Then the sample in trisodium phosphate transport medium was vortexed and allowed it to stand for overnight at room temperature. Then the supernatant was discarded and the deposit was processed for smear was prepared from the deposit for AFB microscopy by Zeihl Neelsen Technique.From the sputum sample collected in container without transport medium, smear was prepared for AFB staining by Zeihl Neelsen Technique. AFB Microscopy results were noted.

Among the 150 samples, 33(22 %) samples showed AFB Positivity in microscopy by Zeihl Neelsen

Technique in Unconcentrated sputum samples, 37(25%) in concentrated sputum samples.

SAARC- Journal of Tuberculosis Lung Diseases, HIV & AIDS [10], Yatin N. Dhlakia *et al*., [11], stated that HIV, Diabetes, smoking & alcoholism are common risk factors for occurrence of TB. This study coincides with the present study.

Anita Pandey [9] and Myrna T. Mendoza *et al*., [10] in their study, stated that the speficity is slightly higher in Zeihl Neelsen staining method than Cold staining and Fluorescent Microscopic method .

J. Jena & B. N. Panda *et al*., [7] Negussi Gebre [12], Negussi Gebre *et al*., [13], Gebre-Selassie S *et al*., [14], stated that the importance of sputum concentration method in a resource poor setting. Sputum concentration increases the rate of recovery of M. tuberculosis. As the developing countries mainly depends upon the sputum microscopy, the improvement of sputum microscopy by concentration method give more sensitive outcome in the diagnosis of pulmonary tuberculosis.

CONCLUSION

The present study confirms that the sputum concentration technique using Trisodium Phosphate solution significantly detects more positive cases by microscopy than the direct smear microscopy.

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