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A COMPARISON OF ZIEHL-NEELSEN STAINING AND FLUORESCENT MICROSCOPY FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT

BACKGROUND : Tuberculosis continues to be significant ill-health in our country and is the largest reason for loss in healthy life within the productive age bracket. We need rapid methods for bacteriological diagnosis of tuberculosis. Hence the present prospective study was undertaken to see the efficacy of zeihl-neilsen staining in the detection of mycobacterium in sputum sample.

METHODS : Early morning sputum samples were collected from patients who were suspected of having tuberculosis at Saveetha Medical College and Hospital,Thandalam.Each sample was processed and subjected to zeihl neilsen and flourescent auramine-o staining.

RESULTS: The smear positivity for AFB on the conventional ZN method was 7.47% (12/152) while the positivity increased to 14.69% (23/152) on the modified fluorescent method. Some samples that resulted negative in ZN staining were found to be positive in fluorescent staining.

CONCLUSION: Fluorescent staining is found to be more sensitive and easily usable without much strain. The fluorescing bacilli are easily identifiable and less time consuming and hence, it has been advocated to be methods of choice where there are large numbers of sputum smears are to be examined.

KEYWORDS : Tuberculosois, staining, sensitivity, acid-fast bacilli ,sputum samples.

INTRODUCTION

Pulmonary tuberculosis is principally an unwellness of the systema respiratorium, caused by tubercle bacillus .Tuberculosis may be a predominant infectious reason for mortality these days [2, 5].According to World Health Organization (WHO), tuberculosis infections are currently spreading at the rate of one person per second per million people. With three millions dying from it [1,2].Tuberculosis continues to be a significant ill health in our country and is that the single largest reason for loss in healthy life year within the productive age bracket. There are various methods for bacteriological diagnosis of tuberculosis [21].Currently, radiometric assay permits detection of tubercle bacillus growth and provides antibiotic sensitivity with results quicker typically at intervals of ten days. However use of the technique is restricted as a result of matter contains radioactive carbon. Genetic probes are on the opposite hand quite straightforward to use and permit identification of culture bacterium in barely a couple of hours by polymerase chain reactions.
Mycobacterium tuberculosis strains may be detected directly within the mucous secretion specimen at intervals a pair of or three hours, however in follow, this method has not become a routine laboratory technique, particularly is due to lack of sufficient specificity and sensitivity.

Serological tests are presently not reliable enough for the designation of T.B. [2].Microscopic examination and culture are still essential components of the microscopic examination; the designation of T.B. is confirmed on the premise of demonstration of tubercle bacilli in the sputum or any other pathological material [1,2, 4, 6]. Smear examination is believed to be simple, cheap, quick and practicable and effective case finding method for developing countries.

As T.B. bacilli are terribly slow growing organisms, culture results are on the market once a amount of 3 or six weeks. So, Microscopic examination has the advantage of the giving a result quickly. The specimen most commonly examined is sputum and mucous secretion coughed up from the lungs [5]. Microscopic examination of Ziehl-Neelson or auramine stained specimen allows detection of most strains in less than an hour.

Ziehl-Neelson is the most extensively used procedure for the demonstration of mycobacterium tuberculosis in smear [7, 8]. The requisites for the staining procedures are; basic fuchsin, phenol, absolute alcohol; sulphuric acid and methylene blue.

Microscopic examination below oil immersion objective reveals eubacteria are red bacilli.

Fluorescent staining by Auramine is different strategies of staining. In this a smear is made from the specimen and stained with fluorescent stain known as auramine .The auramine stain enters the wall of mycobacterium tuberculosis microorganism cell and makes them glow against dark background below ultraviolet illumination light[4].Microscopic examination below low power objective can reveal mycobacteria as glowing yellow white, rice like bacterium in the smear.

Therefore the present prospective study was under taken to see the efficacy of Ziehl-Neelson method versus fluorescent staining in the detection of mycobacterium in sputum sample.

MATERIALS AND METHOD

The comparative study was conducted at Saveetha Medical College and Hospital, Tamil Nadu from 19.9.2017 to 19.8.2018. Total 150 (negative, scanty, 1+) sputum samples were collected from the Microbiology department at Saveetha Medical College and Hospital .

PATIENTS INCLUSION CRITERIA:

Patients testing at the Microbiology department at Saveetha Medical College and Hospital , having fever, night sweats, cough for more than 3 weeks with sputum, loss of appetite, loss of weight, chest pain, heamoptysis and/or radiological evidence of tuberculosis were included.

SAMPLE COLLECTION:

Sputum samples were collected early morning in clean, sterile, leak proof, wide mouth containers. The processing of the sample were carried out in a biosafety cabinet. each sample collected was subjected to Ziehl-Neelson(ZN) staining and fluorescent Auramine-O (AO)staining.

RESULTS

Table 1-Result of smear examination by Ziehl-Neelson and Fluorescent staining

	ZN - Staining	Flourescent Staining
Negative	140 (92.53%)	129 (85.31%)
Positive	12 (7.47%)	23 (14.69)
Total	152 (100%)	152 (100%)

Table 2-Comparison of smear examination result by Ziehl-Neelson and Fluorescent staining

	ZN -Positive	ZN - Negative	Total
Flourescent positive	12	11	23
Flourescent negative	0	129	129
Total	12	140	152

A sum of 152 clinically diagnosed pulmonary tuberculosis patients were included in the study Out of 152 sputum samples, the smear positivity for AFB on the conventional ZN method was 7.47% (12/152) while the positivity increased to 14.69% (23/152) on the modified fluorescent method.

Table 2: shows 12 samples were both fluorescent and ZN positive,

where 11 samples were Fluorescent positive and ZN negative. Out of which 129 samples were both fluorescent and ZN negative. Where none of the stained sample was ZN positive and fluorescent negative respectively.

DISCUSSION

India contains a long history of analysis and demonstration comes on TB. The detection of Acid quick bacilli is commonly thought-about because the proof of the infected stage. Thus, the laboratory plays a crucial role within the diagnosis of TB [3]. In developing countries, research of the specimen is out and away the quickest, cheapest, and most reliable technique for the detection of AFB [5,6]. but fluorescent staining has been additional in Revised National infectious disease management Program (RNTCP) owing to a lot of sensitive and speedy results and might be employed in field areas.

In the gift study, the results showed that from mucus specimen of 388, twenty nine patients had smear positive by atomic number 30 staining and by fluorescent staining fifty seven patients gave positive result [9]. The results of gift study indicate that Auramine staining of mucus smears in could be a sensitive method of mucus research for demonstration of AFB in mucus specimen, compared to Ziehl-Neelson staining.

The use of Fluorescent research greatly improves the diagnostic price of smear particularly in patients with rarity of bacilli that area unit doubtless to be uncomprehensible on Zeihl Neelson stained smears. The method is economical in each time and expense and counseled for laboratories handling sizable amount of mucus specimens [7]. Fluorescent staining is superior thereto of atomic number 30 staining within the presence of a coffee microorganism load as seen in smears with diagnostic cytomorphologial featured infectious disease, in drawback areas like AIE (acute inflammatory exudates alone or with occasional granulomma, AFB positivism by atomic number 30 staining is sort of pretty much as good because the fluorescent technique as a result of microorganism load is high)[12,13]. Using fluorescent research, the tubercle bacilli once examined below radical violet illumination, the bacilli appeared as a bright rod against a dark back ground. Since there was a distinction, the bacilli were pronto seen and thus in terribly less time massive space may be examined. Images were then captured with the camera and enhance through imaging process techniques [14].

While in atomic number 30 staining acid quick bacilli appeared bright red rods in blue background. during this conjointly image were captured.

The potential advantages of automatic screening for tubercle bacilli are: speedy, acute, cheap diagnosis; the power to screen sizable amount of people; inflated resources to watch patients; and reduction in health risk to employees.

Thus the study reveals that mucus stained by the florescent technique is helpful and reliable for pulmonary infectious disease. Since the fluorescent research is dear some laboratories cannot afford to shop for florescent microscopy, therefore in these laboratories Ziehl-Neelson staining is most used [14,15].

CONCLUSION

Sputum examination for the tubercle bacilli is usually conducted for patients clinically and/radiologically suspected of pulmonary tuberculosis. However, the standard method of sputum examination, that is, range 30|metallic element|metal} staining isn't sensitive enough and an oversized number of the suspected cases miss identification. Moreover many cases remain unsuspected and don't seek treatment.

Fluorescent stain may be a additional economical over atomic number 30 stain in detection Tubercle bacilli in body fluid. Since screening is completed beneath low power of magnification (40X), fluorescence has been found to be less time consuming compared to atomic number 30 technique (100X) within the identification of T.B.

Hence, it has been advocated to be methods of choice where the large numbers of sputum smears are to be examined. The fluorescing bacilli are identifiable and cause less eye-strain.

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