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UROGENITAL TUBERCULOSIS AND ITS APPLICATION BY ACID FAST STANING (ZN)

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ABSTRACT

Tuberculosis (TB) is believed to have been present in humans for thousands of years. Skeletal remains show that prehistoric humans (4000 BC) had tuberculosis, and tubercular decay has been found in the spine of Egyptian mummies (3000-2400 BC). During the 17th century, exact pathological and anatomical descriptions of tuberculosis began to appear. In 1679, Sylvius wrote his *Opera Medica*, in which he was the first to identify actual tubercles as a consistent and characteristic change in the lungs and other areas of consumptive patients. The earliest references to the infectious nature of tuberculosis also appeared in the 17th century Italian medical Literature. Due the variety of its symptoms, TB was not identified as a unified disease until the 1820s, and was named tuberculosis until 1839 by J. L. Schonlein. Tuberculosis kills over 1.7 million people worldwide every year and nearly 40% of patients with active tuberculosis remain undiagnosed because of the poor sensitivity of the current, century old diagnostic method. The situation is further exacerbated with the increasing incidence of drug resistant TB. Early diagnosis of TB remains an elusive challenge, especially in individuals with disseminated TB and HIV co-infection. The bacilli grows slowly, the generation time in vitro being 14-15 hours. Colonies appear in about two weeks and may sometimes take upto eight weeks. Optimum temperature is 37^0 C and growth does not occur below 25^0 C or above 40^0 C optimum pH is 6.4-7.0. M. tuberculosis is an obligate aerobe. Tubercle bacilli do not have exacting growth requirements but are highly susceptible even to traces of toxic substances like fatty acids in culture media or charcoal.

INTRODUCTION

The bacteria that cause TB are spread when an infected person coughs or sneezes.

Most people infected with the bacteria that cause tuberculosis don't have symptoms. When symptoms do occur, they usually include a cough (sometimes blood-tinged), weight loss, night sweats and fever.

Treatment isn't always required for those without symptoms. Patients with active symptoms will require a long course of treatment involving multiple antibiotics.

Tuberculosis (TB) is potentially fatal contagious disease that can affect almost any part of the body but is mainly an infection of the lungs. It is called by a bacterial microorganism, the tubercle bacillus or *Mycobacterium tuberculosis,* Although TB can be treated, cured, and can be prevented if persons at risk take certain drugs, Scientists have never come close to wiping it out. Few diseases have caused so much distressing illness for centuries and claimed so many lives.

Mycobacteria are slender rods that sometime show branching filamentous forms resembling fungal mycelium. In liquid cultures they form a mold -like pellicle, hence the name "mycobateria" meaning fungus like bacteria. They do not stain readily, but once stained, resist decolourisation with dilute mineral acids. Mycobacterium are therefore called acid fast bacilli or AFB. They are aerobic, nonsporing non capsulated and nonsporing. Several media both solid and liquid have been described for cultivation of tubercle bacilli. In men the site most commonly involved is the epididymis, followed by the prostate. Testicular involvement is less common and usually is the result of direct invasion from the epididymis.

The bacilli grows slowly, the generation time in vitro being 14-15 hours. Colonies appear in about two weeks and may sometimes take upto eight weeks. Optimum temperature is 37^0 C and growth does not occur below 25^0 C or above 40^0 C optimum pH is 6.4-7.0. M. tuberculosis is an obligate aerobe .Tubercle bacilli do not have exacting growth requirements but are highly susceptible even to traces of toxic substances like fatty acids in culture media or charcoal. Several media both solid and liquid have been described for cultivation of tubercle bacilli. In men the site most commonly involved is the epididymis, followed by the prostate. Testicular involvement is less common and usually is the result of direct invasion from the epididymis.

It is generally believed that tuberculous prostatits result from antegrade infection within the urinary tract, epididymitis however, probably is the result of blood- borne infection because it often is an isolated finding without urinary tract involvement.

MATERIALS AND METHODS

Sample Collection and Preparation

To study subjects (three hundred and fifty patients) were requested to submit two urine samples each in a clean, sterile, leak-proof, wide-mouth containers. In total, 350 samples were collected. One sample from each patient was taken on the spot and the subjects were provided with a second prelabeled container for a morning sample to be taken at home. Preparation of smear for staining was done as described elsewhere. For each sample, the smears were made in duplicate. Positive and negative control smears were also prepared.

Ziehl-Neelsen (ZN) Staining Procedure

The smears were arranged in serial order on staining bridge, with smear side up and flooded with filtered 0.1% Carbol Fuchsin. The smears were steamed and allowed to stain for 5 minutes, rinsed with water, and drained. They were decolorized with 25% sulphuric acid for 5 minutes, rinsed with water, and drained. They were then counterstained with 0.1% methylene blue solution for 1 minute and rinsed with water. The smear was allowed to air dry and examined microscopically using the oil immersion (100x) objective.

Fluorescence microscopy staining procedure

The smears were flooded with filtered 0.1% auramine for at least 20 minutes. They were then rinsed with water and drained. Acid alcohol decolorizing solution (0.5%) was applied on the smear for 30 to 60 seconds, rinsed with water, and drained. They were then flooded with 0.5% potassium permanganate counterstained for a maximum of 1 minute and rinsed with water. Te smears were allowed to air dry and examined microscopically using the dry (40x) objective lens of an LED illumination-based fluorescence microscope (Zeiss primo star ilED).

RESULT

Growth detection of AFB in culture considered the most sensitive method for diagnosis of TB is not routinely done in our health facilities mainly due to the slow growth of the bacteria and the lack of equipment required for the test. Prompt diagnosis of TB, therefore, is achieved by AFB smear microscopy, mostly by the ZN technique and recently by the fluorescence

microscopy and the Xpert MTB/RIF in few facilities that have them. We compared the results of ZN stain smear and fluorescence staining with Xpert MTB/RIF test for detection of AFB in urine samples. Results of this study demonstrated that Xpert MTB/RIF diagnosed approximately 15% more of the 200 samples screened than FM and as high as 45% more than ZN. The superior performance of Xpert MTB/RIF over fluorescence and ZN microscopy in the diagnosis of tuberculosis has been established in many studies. Also, FM produced a higher diagnostic yield compared to that of ZN staining technique among our study samples. This finding confirms the previously reported superior performance of FM over the conventional ZN technique for AFB detection.

Table no. 2: Percentage of positive patients.

S. no.	SEX/AGE	NO of Positive Samples	Positive Percentage
1	Male	05	29%
2	Male child	00	0%
3	Female	13	61%
4	Female child	02	11%
5	Total no of positive	20	4.95%

DISCUSSION

Total No of patients were three hundred fifty, out of which 69% were female and 29 % males were positive. It seems that urogenital tuberculosis is much more common in females than in males. The incidence is more common in age group of 35-65 years of age. In terms of diagnosis ZN and FM staining are rapid and simple techniques with quite low cost. Only ZN staining has so far been used for the diagnosis of TB in this region. However it has a low level of sensitivity compared to FM method.

CONCLUSION

The conclusion from this study is that when the conventional method of smear examination by Z-N is done alone, there are possibilities of false negative results which can be reduced by using Fluorescent staining along with Z-N and the rate of positivity may be improved by combining the conventional Z-N + Fluorescent staining. So, it may be concluded that Fluorescent Microscopy alone may be considered as sufficient microscopic technique for diagnosis of tuberculosis in any diagnostic set up along with culture which is the gold standard.

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