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Zeihl Neelsen Stain: Still a Reliable Option for EPTB Diagnosis in Resource Constraint Settings

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Abstract

Background: Endemic countries like India are burdened with the bludgeoning effects of tuberculosis. While pulmonary tuberculosis is more prevalent and communicable, diagnosis of extra pulmonary tuberculosis (EPTB) is more challenging.

Methods: This was a retrospective study done for three consecutive years 2013 to 2015. The extra-pulmonary smear samples from patients suspected of tuberculosis were stained with Zeihl Neelsen stain and examined. Results: Based on ZN stain, EPTB was diagnosed in 13.4%, 9.5%, 10.6% suspected cases between 2013 to 2015 respectively. Highest positivity was seen among pus aspirates from lymph nodes in 13-25 years age group.

Conclusion: New technological advances are being made all across the globe for faster and specific tuberculosis diagnosis. However in resource limited settings, reliance is often on the smear microscopy findings. Diligently observed smears increase chances of diagnosis and is often rewarding.

Keywords: Extra pulmonary tuberculosis; Pulmonary tuberculosis; Ziehl Neelsen staining

Introduction

Tuberculosis is a major health problem even after more than 100 years of discovery of this bacillus. Though effective treatment is available especially for drug sensitive strains, gaps exist in knowledge about EPTB clinical and laboratory diagnosis, prevention and control programs. In 2016, the incidence of tuberculosis was 6.3 million with an estimated 1.3 million deaths among HIV negative individuals [1]. India ranks among the top ten countries in terms of global TB cases, accounting for approximately 25% of all TB cases [2]. Primary site of infection is lung but can affect any other area in the body. Extra pulmonary tuberculosis (EPTB) which means occurrence of TB in any parts of the body except lungs has become an important clinical problem accounting for around 15%-20% of all TB cases. These include pleural, genitourinary, endometrial, lymphatic, skeletal, ocular, central nervous system, pancreatic, miliary tuberculosis. Prevalence of extra pulmonary tuberculosis is higher in patients co-infected with HIV, especially in endemic countries though it is less infective than pulmonary tuberculosis [3]. In 2006, smear positive pulmonary TB cases were 5, 55,660 compared to 1, 83,180 EPTB cases (Ratio 3:1) while in 2016, the ratio was 5.6:1 [1,4]. Though the annual case detection rates for pulmonary and EPTB have increased [1,5]

Material and Methods

All specimens from suspected cases of MTB were collected with utmost care and processed as per universal guidelines. The smear from patient sample with suspected EPTB was prepared in Biosafety cabinet using new, clean and unscratched microscopic slide. Smear was air dried and then heat fixed it by passing slide over the flame 3 times. It was then flooded with 0.3% carbol fushin stain reagent and the slides were steamed gently for 1 minute by flaming from below the rack with a gas burner. Slides were not permitted to boil or dry out. The stain was allowed to remain on the slides for an additional 4 to 5 minutes without heat. The slide was rinsed with deionized water and decolourized with 3.0% acid-alcohol (95% ethanol and 3% hydrochloric acid) for two minutes. Methylene blue (0.3%) was used as a counterstain for 2 minutes. Finally the slide was rinsed with deionized water and air dried. It was examined under oil immersion lens (100X) for presence of acid fast bacilli. The slides were examined by two observers before declaring it as negative for acid fast bacilli.

Results

Acid Fast bacilli appeared approximately 0.3 to 3 um long, slender, beaded and pink colored slender rods against blue background. The extra-pulmonary samples received for ZN staining in the three consecutive years from 2013 to 2015 were 989, 967 and 1148 respectively. Percentage positivity was 13.4%, 9.5%, 10.6% respectively (Figure 1). The highest percent positivity was found in 13 to 25 years age group (Figure 2). The sex ratio of patients with EPTB was 0.6 in 2013, 1.28 in 2014 and 1.2 in 2015 (Figure 3). The highest positivity was seen among pus aspirates from lymph nodes. The sticky cheesy appearance of pus was found to be a major clue towards diagnosis of tuberculosis. Other samples included pleural fluid, cerebrospinal fluid, synovial fluid, urine, menstrual blood; endometrial curettage samples (Figure 4).

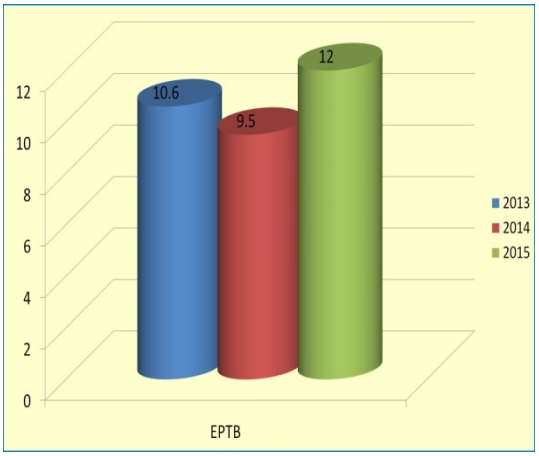

Figure 1: Percentage of EPTB smear positive cases.

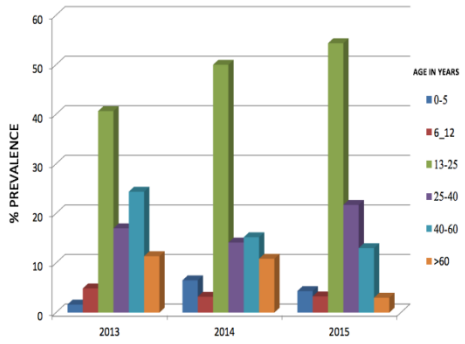

Figure 2: Percent prevalence of EPTB related to age at presentation.

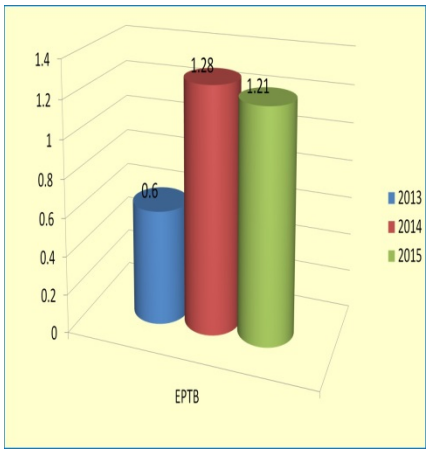

Figure 3: Gender ratio of EPTB cases in 2013- 2015.

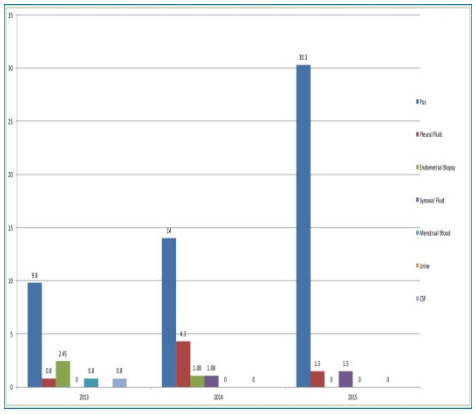

Figure 4: Percentage of EPTB-sample distribution of positive cases.

Discussion

A lot of emphasis has been laid on TB control programs but TB incidence still remains high. The reason could be delayed or improper diagnosis or compliance related issues during treatment. EPTB involves a variety of presentations related to different organ systems involved, hence in an endemic country like ours, there should be a high index of suspicion for tuberculosis. Suspected site specimens should be selected for EPTB diagnosis rather than reliance on serology. The practice of performing blood tests like serology for TB or PCR on blood samples should be discouraged due to unscientific approach [6]. The exception would be use of blood culture or PCR for the diagnosis of disseminated TB in children or immunosuppressed patients. The more recent use of IGRAs (Interferon Gamma Assays) like TB Gold for active TB diagnosis is another cause of alarm in endemic countries that will need to be addressed to the clinicians and laboratory professionals [7]. The International standards for TB care (ISTC) also recommends appropriate specimens from suspected sites for microbiological, microscopy and histological examination [8]. Even with limited sensitivity, smear microscopy using Ziehl Neelson stain is a very useful and cheap diagnostic method owing to its rapidity in infectious pulmonary tuberculosis and is simple enough to be undertaken in peripheral laboratories. Still, in private sector microscopy is underused and reliance is placed on more sensitive but less specific serological tests. In resource constraint settings, conventional ZN staining can be used for screening EPTB with lower yield but better results. One needs to examine at least 300 fields under oil immersion microscope before declaring negative for acid fast bacilli. Due to pauci-bacillary nature of extra pulmonary samples, it requires a lot of patience, nevertheless the results are rewarding. To overcome the problem of false negative smear results, World Health Organization has endorsed use of a cartridge based nucleic acid amplification (NAAT)- Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, California), in 2013 [9]. Recently a robust, rapid DNA-based LAMP assay test has been recommended as an alternative to sputum smear microscopy by WHO [10]. This may also replace Xpert MTB/RIF assay for detecting pulmonary TB in adults in areas with low multidrug resistant tuberculosis.

The percentage positivity of EPTB in our study was highest in pus aspirates from lymph nodes, comprising the major burden of EPTB in line with the overall national data [11]. The bacterial load of a clinical specimen has direct association with the sensitivity of ZN stain. Smear

positivity is generally achieved at a concentration of ≥ 10^4 organisms/ml. In fine needle aspiration cytology, sensitivity can vary between 37.4% to 59.4% [12]. Increased prevalence of EPTB among males of the economically productive age group may signify exposure due to outdoor activities. It may also indicate the need for re-vaccination against tuberculosis in these individuals.

Limitations

In Paucibacillary (Concentration of AFB in samples <10^4 / ml) cases the smear results might be negative for AFB.

Conclusion

The screening of EPTB by conventional staining method in resource constraint settings can provide good results despite low yield. It requires sincere examination of smears from extra pulmonary sites. Where Culture and molecular facilities are not available, commitment and thorough search for acid fast bacilli by a Microscopist may benefit especially against a diagnosis based on presumptive or circumstantial evidence.

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