ORIGINAL ARTICLE

Comparison of AFB Smear Microscopy and Culture from Specimens Received for the Diagnosis of Extra Pulmonary Tuberculosis

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ABSTRACT

Objectives: This study was undertaken to see the role of AFB microscopy and culture for the diagnosis of extra pulmonary tuberculosis.
Design and Setting: This descriptive study was carried out at PMRC TB Research Centre King Edward Medical University/Mayo Hospital Lahore.
Subject and Methods: Symptomatic suspects of extra-pulmonary TB were asked to submit their respective samples for AFB smear and culture. Smears were prepared and stained by Zheil Neelsen method and were subjected to culture on Lowenstein Jensen media.
Results: The smear and culture results of 260 extra-pulmonary specimens were analyzed. Smear positivity was seen only in 3.85%, and the culture on Lowenstein Jensen media showed positivity of 18.45 %. Microscopy when compared with culture showed sensitivity of 20.84% and specificity 100%, positive predictive value 100%, negative predictive value of 84% with false positive rate of 0 and false negative rate of 79.17%.
Conclusion: Smear microscopy is not found to be a sensitive tool, giving a high false negative rate when compared with culture. Thus culture is reliable and gold standard for bacteriological diagnosis of extra pulmonary tuberculosis.
Key words: Extra-pulmonary TB, Mycobacterium tuberculosis, ZN staining, AFB culture.

INTRODUCTION

Tuberculosis (TB) like other infectious diseases remains the single greatest contributor to the world's morbidity and mortality. WHO has estimated incidence of all new TB cases in Pakistan to be 291307. Out of which 54721 die due to this disease every year[1]. Global mortality rate of this disease ranged from 1.6 to 2.2 million each year in 2003, situation has further worsened with increasing incidence of multi drug resistance TB (MDR)[2].

Tuberculosis is a disease of protean nature and involves lungs and other organs of the body as well [3]. Pulmonary Tuberculosis infects lungs mainly while extra-pulmonary tuberculosis can present as pleural effusions, tuberculous lymphadenitis, tuberculous meningitis, abdominal tuberculosis and tuberculosis of bones and joints. Rapid, sensitive and specific method is needed for its diagnosis in order to provide appropriate treatment to the patient[4,5]. Initial diagnosis is dependent on the smear microscopy for acid fast bacilli (AFB) by Zeihl Neelsen (ZN) staining and culture is documented as gold standard for isolation and identification of mycobacterium tuberculosis (MTB). The Z N smear is rapid and inexpensive and is widely used in developing countries for both pulmonary and extra-pulmonary specimens. There are various reports regarding the sensitivity of ZN smear for extra-pulmonary specimen ranging from as low as o% to as high as 75%[6]. This limitation has been reported to be due to inadequacy and paucibacillary nature of specimen[7, 8]. This study was carried out to see the role of AFB smear microscopy and culture in detection of extra-pulmonary tuberculosis from clinical samples in our setting.

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MATERIALS AND METHODS

This descriptive study was carried out at PMRC TB Research Centre King Edward Medical University Mayo Hospital Lahore during the year 2007. A total of 271 specimens from extra-pulmonary TB suspects.

Study subjects included patients visiting TB OPD Clinic and Wards of Mayo Hospital and other leading hospitals of Lahore. Symptomatic suspects of extra-pulmonary TB with fever, fatigue, anorexia and weight loss were asked to submit their respective samples.

Direct and concentrated smears were prepared from clinical specimens after treating with 4 % Noah

(sodium hydroxide) for decontamination and digestion of clinical specimens. Sterile phosphate buffer PH 6.8 is added to neutralize the effect of Noah and the samples were concentrated by centrifugation at 3000 g for 15 minutes. Supernatant was discarded and sediment was re suspended in small amount (1-2 ml) of phosphate buffer. In case of tissues they were ground in sterilized mortar and pastel before decontamination and digestion. Body fluids like CSF, Synovial fluid, Pleural fluid, Ascitic fluid and bone marrow that are collected aseptically and were expected to have no contaminants, inoculated without decontamination. The smears were stained with ZN method using 1% Carbol Fuchsin, 25% Sulphuric Acid and 0.3% Methylene Blue. A minimum of 100 oil fields was observed to declare negative smear. Smear is considered positive if it contains at least 3 AFB[7] in observed 100 oil fields for this study. The results were reported according to WHO/International Union Against Tuberculosis and Lung Diseases (IUATLD) as no AFB per 100 high power fields reported as negative, 1-9 AFB per 100 high power fields reported as actual count per 100 high power field, 10-99 per 100 high power fields reported as 1^+, 1-10 AFB per high power field in at least 50 fields reported as 2^+ and more than 10 AFB per high power field in at least 20 fields is reported as 3^+. Culture is considered positive if it contains only 1 colony, however results were reported as less then 50 colonies, reported exact number of colonies, more than 50 and less then 100 colonies 1^+, 100 to 200 colonies 2^+ and more then 200 colonies were reported as 3^+. A known positive and a known negative slide were included with each run and each batch of staining. An experienced microbiologist rechecked the random positive and negative smears for Quality Assurance. Lowenstein Jensen (LJ) media were tested by inoculation of known ATCC strain of $H_{37}Rv$. Random slants of LJ media inoculated with sterile distilled water were also incubated from each batch as negative controls Sensitivity, specificity, positive predictive value and negative predictive of AFB smear microscopy were calculated taking culture as gold standard.

RESULTS

A Total 271 extra pulmonary were processed for AFB smear and culture out of which 11 (4.05%) get contaminated and were excluded from the study. Of the remaining 260 specimens 10 (3.85%) were smear positive and 48 (18.46%) were culture positive.

Table 1 shows the frequency of different types of extra-pulmonary specimens and positivity of respective AFB smear and culture results.

Table 1:

Type of Specimen	No of Specimen	AFB Smear +ve	Culture Positive
Lymph Nodes	92	6(8.5)	34(36.96)
Pleural Fluid	74	1(1.3)	4 (5.40)
Peritoneal Fluid	27	1(3.71)	2 (7.40)
Synovial Fluid	21	-	1 (4.76)
Pericardial Fluid	14	-	1(7.15)
CSF	13	-	1 (7.69)
Bone Marrow	2	-	-
FNA	1	-	-
Endometrial Curetting	2	-	-
Gastric Aspirate	11	1(9.09)	4 (36.36)
Urine	3	1(33.34)	1 (33.34)
Total	260	10(3.85)	48 (18.46)

Table 2: Sensitivity, specificity, positive predictive value (PPV), Negative predictive value (NPV), false positive rate (FPR) and false negative rate (FNR) of AFB smear taking culture as gold standard.

Table 2:

	%age
Sensitivity	20.84
Specificity	100
PPV	100
NPV	85.80
FPR	0
FNR	79.17

Table 2 shows that sensitivity and specificity of AFB smear microscopy for extra pulmonary specimen is 20.84%.and 100%. A high false negative rate of 79.17% specimen is also seen.

DISCUSSION

Diagnosis of extra-pulmonary tuberculosis is challenging, however, many factors attribute to make its diagnosis difficult, includes paucibacillary nature of specimens, lack of adequate sample amounts or volumes, the apportioning of the sample for various diagnostic tests (histology/cytology, biochemical analysis, microbiology and PCR) resulting in non uniform distribution of micro-organism[9].

Specimens which are fluids require large quantity of up to 1 litre to be centrifuged before subjecting to AFB smear and culture[10]. However it becomes very difficult to retain high quantity of specimen under the circumstances when distribution of the specimen for the differential diagnosis is required[11]. The smear positivity of extra-pulmonary specimens in present study is 3.85% and is in agreement with the study reporting smear positivity of

3.9% in extra-pulmonary specimen[12]. The study is also in agreement with Yam, s statement that AFB smear of fluids are rarely positive[8]. Others have shown the positivity of ZN smear 0-6 % and culture is less than 20% in extra-pulmonary TB suspects[10]. However smear and culture positivity in this study is not in agreement with a study that reporting smears and culture positivity of 20.25% and 46.83% respectively in extra-pulmonary specimens[2]. False negative rate (FNR) of 79.17% in extra-pulmonary specimens is matter of great concern and indicates that extra-pulmonary TB is difficult to diagnose by AFB smear microscopy. In a study FNR of 17% in pleural effusions and 33% in lymph node biopsies has also been reported[12].

Culture positivity of extra-pulmonary specimens is 18.46% in the present study and is considerably high as compared to the ZN smear 3.85%, thus proving that culture is a sensitive tool in the diagnosis of extra-pulmonary TB.

It is concluded that AFB smear although rapid but insensitive for extra-pulmonary specimens, MTB culture on LJ media is the gold standard for the diagnosis of typical Mycobacterium tuberculosis. Cytological criteria for diagnosis of tuberculosis are dependent on the confirmation by AFB smear and culture of MTB[13] and could not be used as confirmatory diagnostic tool. However if there is no bacteriologic evidence, existence of extra pulmonary TB could not be denied and observation of clinico pathological correlation, radiographic and clinical response to ATT with provision of appropriate specimen for microbiological tests should be observed and can be an ideal approach to diagnose extra-pulmonary TB in our settings. Moreover for definite and earlier bacterial diagnosis liquid culture media Bactec should be promoted.

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