

ZN Stain versus Radiography in the Diagnosis of Bone Tuberculosis

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Abstract

Introduction: Mycobacterium tuberculosis (MTB) an acid fast bacilli (AFB) is the causative agent of tuberculosis (TB). Bone and joint TB (BTB) is an ancient disease with an incidence around 1 – 3%. However CT/MRI are extremely helpful in the diagnosis BTB. With this a study was conducted to find the utility of ZN stain in the diagnosis of BTB.

Methods: It was a prospective research conducted in the department of Microbiology, GSL Medical College between January to May 2022. Study protocol was approved by the Institutional ethical committee. Individuals of both gender, aged ≥ 18 years, with confirmed BTB were considered in this research. Non cooperative individuals were not considered. Demographic parameters and clinical findings were recorded. Bone marrow (BM) or biopsy was collected from the individuals as per the guidelines. Simultaneously sputum sample was collected. In the laboratory, smears were prepared and stained as per the ZN staining technique. Sputum collection, staining and screening the stained smears to detect acid fast bacilli AFB were carried as per the guidelines. Statistical analysis was carried by Chisquare test for; $P < 0.05$ was considered to be statistically significant.

Results: Total 43 members were included, the mean age was 49.2 years, maximum (10) study members were in 40 – 50 years age group and the male female ratio was 1.9. In the BM, AFB were detected in 36 (83.7%). Whereas it was 65.2% (28) only in the sputum specimen. Statistically there was no significant difference.

Conclusion: BTB infection is common among the male and ZN staining utility is limited in the diagnosis of the infection.

Keywords: Tuberculosis, bone tuberculosis, study, research

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Introduction

Mycobacterium tuberculosis (MTB) an acid fast bacilli (AFB) is the causative agent of tuberculosis (TB) worldwide. Lung infection or pulmonary TB is the common clinical condition caused by MTB. [1] Before CoVID pandemic, globally, TB was the ninth leading cause of death. [2] Ziehl Neelsen (ZN) staining is the common

technique used for the diagnosis of lung TB. High specificity, ease of technique, reporting results are the advantages with this.

The incidence of extra pulmonary TB (EPT) is around 15% only. Lymph nodes are the common organs that can effected in EPT infection. The incidence of bone and

joint TB (BTB) is around 1 – 3%. [2] BTB is an ancient disease, identified in the Egyptian mummies also. Nontuberculous mycobacterium (NTM) is also responsible for skeletal infections but the incidence is very rare. However due to HIV epidemic, there is increased incidence of NTM TB. [3]

PCR is the gold standard technique used for the diagnosis of BTB. [4] But the specificity of PCR may be limited. However CT/MRI are extremely helpful in the diagnosis of pulmonary as well as EPT. With this a study was conducted to find the utility of ZN stain in the diagnosis of BTB.

Methods:

It was a prospective research conducted in the department of Microbiology, GSL Medical College. Study was conducted between January to May 2022. Study protocol was approved by the Institutional ethical committee. Informed consent was taken from the study participants. Individuals of both gender, aged ≥ 18 years, with confirmed BTB were considered in this research. Non cooperative individuals were not considered in this research.

Various demographic parameters of the study participants along with clinical findings were recorded in the study proforma. Bone marrow (BM) or biopsy was collected from the required individuals as per the guidelines. Initially the skin was shaved to remove hairs and thoroughly cleaned. Sterile area was preferred for collecting BM, by aspiration. Needle was inserted into the bone through the skin using twisting motion. BM was aspirated

with the syringe, and another needle was used collect tissue.

Simultaneously sputum sample was collected. Collection of good quality sputum sample was explained and it was demonstrated practically also. The participants were provided with sterile sample containers and the specimen was collected in the open air. Immediately after collection, both specimen were transported to the microbiology laboratory. In the laboratory, smears were prepared and stained as per the ZN staining technique. Sputum collection, staining and screening the stained smears to detect acid fast bacilli AFB were carried as per the guidelines. [5]

Statistical Analysis:

The data were analysed using SPSS version 21. The data were presented in mean, median and percentage. Statistical analysis was carried by Chi-square test for; $P < 0.05$ was considered to be statistically significant.

Results:

During the study period total 43 members were included in this research. The mean age of the study participants was 49.2 years, maximum (10) study members were in 40 – 50 years age group. Gender wise 28 were male, 15 were female participants and the male female ratio was 1.9.

Out of the 43 specimen, in the BM, AFB were detected in 36 (83.7%). Whereas it was 65.2% (28) only in the sputum specimen. Out of the 11 (25.6%) BM sample which showed AFB didn't reveal AFB in sputum. Statistically there was no significant difference (Table 1).

Table 1: Comparison of sputum and BM results to find AFB by ZN staining; n (%)

Sputum	BM		
	Positive	Negative	Total
Positive	25 (58.2)	3 (7)	28 (65.2)
Negative	11 (25.6)	4 (9.3)	15 (34.9)
Total	36 (83.7)	7 (16.3)	43 (100)
Statistical analysis	χ^2 value = 0.8411; P value = 0.3590.		
	Statistically there was no significant difference		

Discussion

TB is a world pandemic, air borne communicable infection. BTB is a chronic infection of bone as well as BM. It can infect different joints such as knee, hip, feet, ankle, shoulder, elbow, hand and wrist. Different tools are available for the diagnosis of this white plague. Culture on LJ medium is considered to be the gold standard for the diagnosis. But requirement of prolonged time period is the major limitation. Because culture will take 6 – 8 weeks and additional time period for drug susceptibility test.

Out of the 43 (100%) BTB confirmed cases, AFB were detected in 36 (83.7%) of BM specimen and 28 (65.2%) sputum specimen; statistically there was no significant difference (Table 1). BTB is transmitted mainly through haematogenous route. As per our study it is very clear that there is no utility of sputum AFB test. When BM specimen is tested in 83.7% specimen the AFB was observed. This is because the bacteria infect bone and specimen is also collected from the same tissue. Hence there is high diagnostic yield. But the sensitivity of ZN staining is limited in the diagnosis of BTB. It was mentioned that ZN stain is 25 – 75% sensitive for the diagnosis of BTB. [6] As BTB is paucibacillary, culture technique also has limited sensitivity. [7] As per this study findings, radiology studies are better tools in the diagnosis of BTB.

The infection is high among the men and the male female ratio in this research was 1.9. As such in India there is high prevalence of TB among the men. The exact cause for this is not clear. But in one report it was mentioned that less number of women visit diagnostic or microscopy center. [8] In one of the previous reports from this center also high prevalence was reported among the male. [9]

MTB pathogen can infect individual at any age. ¹⁰ in this research majority of BTB cases were detected in 40 -50 years age group. But the exact reason is not clear. As per the literature, among the BTB infection, spine involvement is very common. But in

this research the area of infection was not analysed. This could be the limitation.

Conclusion

BTB infection is common among the male and ZN staining utility is limited in the diagnosis of the infection.

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