

Original Research

To compare the Modified Ziehl Neelsen Staining and Ziehl Neelsen Staining in the Diagnosis of Osteoarticular Tuberculosis Patients

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ABSTRACT:

Aim: To compare the Modified Ziehl Neelsen Staining and Ziehl Neelsen Staining in the Diagnosis of Osteoarticular Tuberculosis Patients. **Materials and Methods:** This present study was conducted in Department of Microbiology. Entire subjects were signed an informed consent approved by institutional ethical committee. A total of 100 patients of clinically confirmed osteoarticular tuberculosis (OTB) with age group of 16 years to >70 years were enrolled in this study. All the patients were categorised into two groups (group A&B). Each group had 50 OTB patients. ZN technique was used in group A patients. And MZN technique was used in group B patients. **Results:** 60% and 64% patients of ZN and MZN staining were males respectively. Among ZN-staining, 18(36%) males and 12(24%) females had smear positive. Among MZN staining group, 20(40%) males and 15(30%) females had smear positive. In this present study, smear positivity was seen in 28(56%) ZN- staining and 35(70%) MZN staining OTB patients. **Conclusions:** The current investigation found that the occurrence of OTB was higher in the male population of middle age. The Modified Ziehl Neelsen staining approach demonstrated a higher level of positive for osteoarticular TB compared to the Ziehl Neelsen technique. Therefore, MZN is a superior method for diagnosing osteoarticular tuberculosis.

Keywords: Modified Ziehl Neelsen Staining, Diagnosis, Osteoarticular Tuberculosis

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INTRODUCTION

Tuberculosis, a long-standing bacterial illness that has been recognized for the last 5000 years, continues to affect around one-third of the global population. Each day, there are 5000 new cases reported and two lives lost every three minutes [1]. In India, 1.9 million new cases are reported every year, of which 0.8 million are 'infectious smear positive TB cases'. According to WHO, death rate due to TB in India is nearly 28 per 1,00,000 population, which is the highest death rate among all other communicable diseases and accounts for 26 per cent of all avoidable adult deaths [2]. Bone and joint TB (BJTB)/ osteoarticular TB is a secondary form of TB occurring most commonly due to hematogenous seeding [3]. Retrograde lymphatic and contiguous dissemination are the other less common modes of dissemination [4]. Several studies have demonstrated spinal involvement in a significant number of cases [5]. However contradictory findings have been found in some other studies [4]. Watts and Lifeso, in a review of current concepts of Bone and

Joint TB mentioned that spinal tuberculosis has existed for at least 5000 years, and mummified remains from northern Egypt dating 3400 B.C. provide strong evidence of its presence. The authors also mention that the first known description of tuberculous spondylitis was written in Sanskrit, sometime between 1500 and 700 B.C [6]. In the late Section eighteenth century, Pott provided the classic description of spinal TB and noted its association with paraplegia [6,7]. Osteoarticular tuberculosis can cause significant morbidity and a high index of suspicion is needed for early diagnosis so as to avoid destruction and disability [3,7]. Even with adequate medical and surgical treatment, osteoarticular TB can be associated with morbidity and mortality [8]. EPTB usually takes the form of arthritis or osteomyelitis. Rarely it can present as tenosynovitis or bursitis. The common sites of involvement are spine and weight bearing joints. Osteoarticular tuberculosis is commonly encountered in the elderly in developed countries but in developing countries like India it is common around 30 years of

age. Tuberculosis commonly affects lungs but can also be extrapulmonary. Microscopic examination of sputum for detection of acid fast bacilli is of utmost importance.⁴ For developing countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods like demonstration of AFB (acid fast bacilli) in smears is of great importance. No other diagnostic tool offers the affordable as well as efficiency in diagnosis of tuberculosis in public health set up, as sputum microscopy does. In sputum smear microscopy, ZN is the most commonly used technique, because of its simplicity and low cost^[9]. Objective of this present study was to evaluate the prevalence of osteoarticular tuberculosis and to compare the role of modified Ziehl Neelsen staining versus Ziehl Neelsen staining for the diagnosis of osteoarticular tuberculosis patients.

MATERIALS AND METHODS

This present study was conducted in Department of Microbiology. Entire subjects were signed an informed consent approved by institutional ethical committee. A total of 100 patients of clinically confirmed osteoarticular tuberculosis (OTB) with age group of 16 years to >70 years were enrolled in this study. All the patients were categorised into two groups (group A&B). Each group had 50 OTB patients. ZN technique was used in group A patients. And MZN technique was used in group B patients.

METHODOLOGY

The clinical sample was collected by FNAC which was an outpatient procedure. Under sterile precautions, the sample was collected using 20-gauge needle. For the accuracy in the diagnosis, multiple clinical specimens were collected. Immediately after collection, specimens were transported to microbiology laboratory for smear preparation, staining and for reading the stained smears.

SMEAR PREPARATION

New unscratched slides were selected for smear preparation. Smear was prepared with sterile loop. A

good smear is spread evenly, over a size of 2 X 3 cm and is neither too thick nor too thin. This was allowed to air dry for 15 - 30 min and fixed by passing it over a blue flame 3- 4 times.

ZN STAINING

(1%) Carbol fuchsin, (25%) sulphuric acid, (0.1%) methylene blue was used according to RNTCP⁴. A minimum of 100 oil immersion fields was observed to declare negative smear. More than 3 AFB in observed 100 oil immersion fields were considered as positive.

MZN STAINING

This is very similar to that of standard ZN staining technique, except primary staining step with 1 % CF (Carbol Fuchsin) with higher phenol concentration (7.5%) as compared to 5% phenol concentration in standard CF solution was done for 15 min. Smears were flooded with filtered 1 % CF and heated until they were steamed and left to steam for 15 min. After rinsing the slides with a gentle stream of water, 25 % H₂SO₄ was used to decolorize the smears for 2 to 4 min, and if necessary, the decolorization step was repeated for another 1 – 3 min. The slides were rinsed as mentioned earlier and counterstained with 0.1 % methylene blue (MB) for 30 s. The slides were then washed, air dried, and examined under oil immersion.

STATISTICAL ANALYSIS

Data was analysed by using simple statistical methods with the help of MS-office software. All data was tabulated and percentages were calculated.

RESULTS

This present study was enrolled a total of 100 osteoarticular tuberculosis patients. Most of the patients 15(30%) of ZN staining were in age group of 25-35 years. 12(24%) patients were in age group of 35-45 years. Most of the patients 15(30%) of MZN group were belonged in age group of 25-35 years and 11(22%) patients were in age group of 35-45 years.

Table 1: Age wise distribution of osteoarticular tuberculosis patients

Age (Years)	ZN staining			MZN staining		
	Positive	Negative	Total	Positive	Negative	Total
Below 25	2	3	5	4	2	6
25-35	9	6	15	11	4	15
35-45	8	4	12	8	3	11
45-55	8	4	12	5	2	7
Above 55	3	3	6	7	4	11
Total	30	20	50	35	15	50

Table 2: Gender wise distribution of osteoarticular tuberculosis patients

Gender	ZN-staining			MZN-staining		
	Positive	Negative	Total	Positive	Negative	Total
Male	18	12	30	20	12	32
Female	12	8	20	15	3	18
Total	30	20	50	35	15	50

60% and 64% patients of ZN and MZN staining were males respectively. Among ZN-staining, 18(36%) males

and 12(24%) females had smear positive. Among MZN staining group, 20(40%) males and 15(30%) females had smear positive.

Table 3: Showing the smear results of osteoarticular tuberculosis patients

Staining	Positive	Negative	Total
ZN	28	22	50(100%)
MZN	35	15	50(100%)

In this present study, smear positivity was seen in 28(56%) ZN- staining and 35(70%) MZN staining OTB patients.

DISCUSSIONS

The infrequent occurrence of osteoarticular tuberculosis poses a diagnostic challenge for the treating clinicians and often results in delayed recognition and treatment. Many a time, the patient has been subjected to unnecessary or even multiple surgeries pending correct diagnosis. Acute suppurative presentation is one of the atypical forms of osteoarticular tuberculosis and closely mimics acute pyogenic infection or septic arthritis. A total of 100 osteoarticular tuberculosis patients were included in this study. All the patients were categorized into two group (group A&B). Each group had 50 patients of osteoarticular tuberculosis. ZN-staining was performed in group A patients. And MZN staining was performed in group B patients. Most of the patients 15(30%) of ZN staining were in age group of 25-35 years. 12(24%) patients were in age group of 35-45 years. Most of the patients 15(30%) of MZN group were belonged in age group of 25-35years and 11(22%) patients were in age group of 35-45 years. 60% and 64% patients of ZN and MZN staining were males respectively. According to Arathi N et al. [10] the ages of patients were in a wide range of 6 to 60 years with an average being 23.6 years; maximum number of cases (81.25%) were seen between 11 and 30 years. Sex distribution revealed slight male predominance with male to female ratio of 1.3:1. In this present study, among ZN-staining, 18(36%) males and 12(24%) females had smear positive. Among MZN staining group, 20(40%) males and 15(30%) females had smear positive. In both group most of positive cases were males. Murray et al. [11] reported that TB is a common infection among the individuals aged 25 – 44 years. Chinnakali et al. [12] reported the SP TB cases were common among 20 – 54 years age. Even in the literature also male dominance was reported. Sharma *et al.* [13] conducted a study good quality found gender gap in the prevalence of TB with more occurrence in the rural areas compared to the urban part. Even one of the African studies also reported higher prevalence among the male [14]. With these, it is clear that even OTB is common among the male. But unlike PT, gender has no influence in the diagnosis of OTB. Because in PT, the sputum submission protocol surely influences the (SP) smear positive results because the sample had to be produced, as well as submitted by the patient. Unlike quality of sputum, in this study, there is no influence of specimen in the diagnosis of

SP OTB case because the specimen was collected by the specialist. Moreover, there was no significant financial burden with MZN technique compared to ZN technique. The minor difference in the reagents cost for MZN technique is negligible compared to the additional SP results [15]. The three different patterns of acute suppurative presentation associated with osteoarticular tuberculosis demand awareness of this atypical form, precise and methodical clinical assessment and support of dedicated laboratory services to aid in diagnosis. We suggest collection of samples for Ziehl–Neelsen staining prior to drainage procedure when suspicion for tuberculosis is high or areas where tuberculosis is in endemic proportions. The smear for acid fast bacteria reveals the diagnosis early in many cases. In patients with obvious radiological lesion in bone, FNAC/ trochar biopsy serves the dual purpose of abscess decompression and tissue for diagnosis. Synovial biopsy should always be taken if arthroscopic or open drainage of acute arthritis is performed when risk factors are present. The various risk factors in children described are recent tuberculosis contact, previous pulmonary tuberculosis, malnutrition, poor sanitation, overcrowding, exanthematous fevers, diabetes, trauma, previous steroid therapy, and immunodeficiency [16,17]. In this present study, smear positivity was much greater in MZN staining 35(70%) as compared to ZN staining group patients 28(56%). Several modifications of ZN were available in the literature. But in one of the study's Chandra et al. [18] mentioned that MZN is a better diagnosis in the diagnosis of PT. The sputum SPT was reported to be 9.43 % and 9.8 % respectively for ZN and MZN staining techniques.

CONCLUSIONS

The current investigation found that the occurrence of OTB was higher in the male population of middle age. The Modified Ziehl Neelsen staining approach demonstrated a higher level of positive for osteoarticular TB compared to the Ziehl Neelsen technique. Therefore, MZN is a superior method for diagnosing osteoarticular tuberculosis.

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