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Original Research Article

A Hospital-Based Comparative Study of Modified Ziehl Neelsen Staining in The Diagnosis of Osteoarticular Tuberculosis Patients

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Abstract

Objectives: In this study, the prevalence of osteoarticular tuberculosis (OTB) was assessed and the diagnostic efficacy of modified Ziehl Neelsen staining (MZN) vs standard Ziehl Neelsen staining was compared.

Methods: In this cross sectional and comparative research, individuals with clinically proven cases of OTB who were older than 18years were included. Exclusion criteria included patients who are not willing to participate or less than 18years of age. As an outpatient procedure, FNAC is done to obtain the clinical sample following all the standard aseptic advisory. The sample was taken with a disposable syringe with 20-gauge needle, following all sterile procedures. Numerous clinical specimens were gathered to ensure the accuracy of the diagnosis. Specimens were transported to the Microbiology laboratory for smear preparation, staining, and reading of the stained smears immediately after collection. A minimum of 2 smears were prepared using the specimens from each patient. The group A specimens were stained using ZN staining while the group B specimens were stained using the MZN staining technique, Chi square test is performed for statistical analysis.

Results: Most of the smear positive patients i.e 35 (68.62%) of ZN staining and 37 (67.27%) of MZN staining were in age group of 28-47years. Majorities of patients in ZN staining 30(58.82%) and MZN staining 32 (58.18%) group were males..

Conclusions: In the population of middle-aged males, OTB was more prevalent. In comparison to the Ziehl Neelsen staining technique, the Modified Ziehl Neelsen staining approach had a higher rate of OTB smear positivity. Therefore, MZN is a more accurate method for detecting osteoarticular tuberculosis.

Keywords: Osteoarticular tuberculosis, Modified Ziehl Neelsen staining, Ziehl Neelsen staining, age group.

Introduction

A well-known bacterial disease for the past 5000 years, tuberculosis still affects around one-third of the world's population, adding 5000 new cases every day and claiming two lives every three minutes [1]. Every year, 1.9 million new cases of tuberculosis (TB) are recorded in India, 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 (OTB) 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].

Numerous investigations have shown that a sizable majority of patients show spinal involvement [5]. However, some other investigations have discovered contradictory results [4]. Spinal tuberculosis has been around for at least 5000 years, and mummified bones from northern Egypt dating to 3400 B.C. provide significant evidence of its presence, according to Watts and Lifeso in a review of current notions of bone and joint TB. The authors also mention that the first known description of tuberculous spondylitis was written in Sanskrit, sometime between 1500 and 700 B.C . In the late eighteenth century, Pott provided the classic description of spinal TB and noted its association with paraplegia [6,7].

A strong index of suspicion is required for an early diagnosis of osteoarticular TB in order to prevent damage and impairment [3,7]. Osteoarticular TB can be fatal and morbid even with appropriate surgical and medical treatment [8]. Osteomyelitis is the most common manifestations of Extrapulmonary Tuberculosis (EPTB). Rarely, it may manifest as bursitis or tenosynovitis. 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. Although tuberculosis frequently affects outside of the lungs, still it is always worth to examine stained smear of sputum under a microscope to look for acid fast bacilli. Evaluation of quick and affordable diagnostic techniques, such as the identification of AFB (acid fast bacilli) in smears, is crucial for developing nations with a higher case load and limited resources. No other diagnostic tool offers this much affordability as well as efficacy in diagnosis of tuberculosis in public health set up, as sputum microscopy does. In sputum smear microscopy, Ziehl Neelsen (ZN) staining is the most commonly used technique, because of its availability and cost effectiveness [9].

Objective of this present study was to evaluate the prevalence of osteoarticular tuberculosis and to compare the role of Ziehl Neelsen staining and modified Ziehl Neelsen staining method for the diagnosis of osteoarticular tuberculosis patients.

Materials and Methods

This present study was conducted in Icare Institute Of Medical Sciences And Research And Dr. Bidhan Chandra Roy Hospital, Haldia during a period from March 2018 to March 2022. Entire subjects were signed an informed consent approved by institutional ethical committee. A total of 82 patients of clinically confirmed osteoarticular tuberculosis (OTB) with age group of >18 years were enrolled in this study. All the patients were categorised into two groups (group A&B).. Atleast 2 smears were made from the samples of each patients. Smears were labelled and divided into two groups. Standard ZN technique was used in group A patients. And MZN technique was used in group B patients.

After keeping the approved proforma ready for relevant data collection, as an outpatient procedure, making the patient in comfortable position and explaining the detailed procedure, FNAC is done to obtain the clinical sample. The sample was taken with a 20-gauge needle following aseptic precautions. Multiple clinical specimens were gathered to ensure the accuracy of the diagnosis. Specimens were

taken to the Microbiology laboratory as soon as they were collected for smear preparation, staining, and reading of the stained smears. Using a sterile loop, standard smears were prepared. These were allowed to air dry for 15 - 30 min and heat fixed. ZN Staining 1% Carbol fuchsin, 20% sulphuric acid, 0.1% methylene blue was used as per the standard guidelines.

MZN Staining

Modified Ziehl Neelsen staining method is almost similar to that of standard ZN staining technique except the time duration. Here primary staining step with filtered 1% Carbol fuchsin which was kept for 15min. Otherwise, this is fairly similar to the usual ZN staining approach. Smears were heated to the point of steaming but not allowed to boil or dry. Then left to steam for 15 minutes after being filled with filtered 1% CF. After rinsing with water, 25 % H2SO4 was used as decolorizer, and if necessary, the decolorization step was repeated for another 1 – 3 min. The slides were again rinsed with water and counterstained with 0.1 % methylene blue (MB) for 30sec. The slides were then washed, air dried, and examined under oil immersion(100x) using light microscope. [10,11].

Statistical Analysis

Utilizing MS-Office software, simple statistical techniques were used to analyse the data.

Results

82 patients with osteoarticular TB participated in the current investigation. There was no statistically significant difference between the staining methods; of them, 62% (51) were smear positive (SP) by ZN staining and 67% (55), by MZN staining. The male to female ratio among the participants in the study was 1.56, with 61% (50) being men and 39% (32) being women. With ZN staining, the SPT for male and female was 30 (36.6%), 21 (25.60%) and with MZN staining, it was 32 (39%), 23 (28%), respectively. According to statistics, there was no discernible gender difference for either staining process. For ZN and MZN staining methods, the male to female ratio among SP cases was 1.42 and 1.39, respectively. The SPT was 7.2%, 30.9%, 36.36%, 9.09%, 12.72%, and 3.63% in the age groups of 18 to 27, 28 to 37, 38 to 47, 48 to 57, 58 to 67, and $\geq$ 68 years, respectively, with MZN staining. While with ZN staining, the SPT was 11.76%, 29.41%, 39.21%, 7.84%, 9.80%, and 1.96% in the age ranges of 18 to 27, 28 to 37, 48 to 57, 58 to 67, and $\geq$ 68 years, respectively.

Discussion

Osteoarticular TB does not have typical presentation, thus makes diagnosis difficult for treating professionals and frequently causes delayed diagnosis and therapy. The patient has frequently undergone pointless or even several procedures while waiting for the right diagnosis. One of the atypical presentations of osteoarticular TB is the acute suppurative presentation, which closely resembles an acute pyogenic infection or septic arthritis. A total of 82 osteoarticular tuberculosis patients were included in this study. Atleast 2 smears from all the patients were collected and stained using two different methods and put under two groups (group A&B) to compare and find out the better one. Each group had atleast one smear of each patients of osteoarticular tuberculosis. ZN-staining was performed in group A patients and MZN staining was performed in group B patients. Most of the smear positive patients i.e 35 (68.62%) of ZN staining and 37 (67.27%) of MZN staining were in age group of 28-47years.Majorities of patients in ZN staining 30(58.82%) and MZN staining 32 (58.18%) group were males. According to Arathi N et al. [12] 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 the current study, 30 (36.58%) and 22 (26.82%) males and females in the ZN-staining group showed positive smear results. 32 (39.02%) men and 23 (28.08%) women in the MZN staining group got smear positive results. The majority of positive cases in both groups were male. According to Murray et al. [13], TB is a prevalent illness among people between the ages of 25 and 44. Chinnakali et al. [14] reported the Smear positive TB cases were common among 20 – 54 years age. Even in the literature also male dominance was reported. In various literatures from India and Africa showed higher male prevalence [15,16]. With these, it is clear that even OTB is common among the male.

As the specimen in case of OTB is obtained by a specialist , there is a very little or no impact on the diagnosis related to specimen quality. When compared to more smear positive results, the slight reagent cost difference for the MZN approach is insignificant [15,17,18]. 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.

The FNAC/ trochar biopsy offers the dual purposes of abscess decompression and tissue for diagnosis in patients with evident radiological lesions in the bone. When risk factors are present, a synovial biopsy should always be collected before to open drainage of or arthroscopic acute arthritis. Recent tuberculosis contact, overcrowding, prior pulmonary tuberculosis, poor sanitation, exanthematous,fevers, malnutrition, traum a, diabetes, immunodeficiency and prior steroid medication are just a few of the risk factors for tuberculosis in children that have been discussed [16,17]. In this present study, smear positivity was more in MZN staining 55(67%) as compared to ZN staining group patients 51 (62%). Several modifications of ZN were available in the literature. In one of the studies by Chandra et al. [17] mentioned that MZN is a better for the diagnosis.

Conclusions

This study showed that middle-aged males were more likely to have OTB. In comparison to Ziehl Neelsen, the Modified Ziehl Neelsen staining approach helped us detecting a higher smear positive osteoarticular tuberculosis. Therefore, MZN is a more accurate method for detecting osteoarticular tuberculosis. Further continuation of this with more number of samples will help to get a statistically significant result thus establishing the method of choice.

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