Original Research Article

Modified Ziehl Neelsen Staining in the Diagnosis of Osteoarticular Tuberculosis Patients: A Hospital Based Comparative study

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Abstract

Objectives: Thisstudy was to evaluate the prevalence of osteoarticular tuberculosis and to compare the role of modified Ziehl Neelsen staining with Ziehl Neelsen staining in the diagnosis of osteoarticular tuberculosis patients. **Methods:** 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. With each group patient's specimen, smears was prepared. Group A specimen was stained by ZN staining and the group B specimens was stained by MZN staining technique. **Results:** Most of the patients 30(30%) of ZN staining and MZN staining 29(29%) group were in age group of 31-45 years. 62% and 63% patients of ZN and MZN staining were males respectively. Smear positivity was seen in 59(59%) ZN-staining and 67(67%) MZN staining OTB patients.**Conclusions:** OTB was more preponderance in middle age male population. Smear positivity for OTB was greater in Modified Ziehl Neelsen staining technique as compared to Ziehl Neelsen staining technique. Hence, MZN is a better technique for the diagnosis of osteoarticular tuberculosis.

Key words: Osteoarticular tuberculosis, Modified Ziehl Neelsen staining, Ziehl Neelsen staining, age group.

Introduction

Tuberculosis, a well-known bacterial disease forthe last 5000 years, is still infecting nearly one-third ofworld population with a daily addition of 5000 new casesand loss of two lives every third minute [1]. In India, 1.9million new cases are reported every year, of which 0.8 millions are 'infectious smear positive TB cases'.According to WHO, death rate due to TB in India is nearly28 per1,00,000 population, which is the highest deathrate among all other communicable diseases and accountsfor 26 per cent of all avoidable adult deaths [2]. Bone and joint TB (BJTB)/ osteoarticular TB is a secondary form of TB occuring 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].

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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 developingcountries 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 & Methods

This present study was conducted in Department of Microbiology, RIMS, Ranchi during a period from January 2020 to January 2021. Entire subjects were signed an informed consent approved by institutional ethical committee RIMS.A total of 200 patients of clinically confirmed osteoarticular tuberculosis (OTB) with age group of 15 years to >75 years were enrolled in this study. All the

patients were categorised into two groups (group A&B). Each group had 100 OTB patients. ZN technique was used in group A patients. And MZN technique was used in group B patients.

Procedures

The clinical sample wascollected by FNAC which was an outpatient procedure.Under sterileprecautions, 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 tomicrobiology laboratory for smear preparation, staining andfor reading the stained smears.

Smear Preparation

New unscratched slides were selected for smear preparation. Smear was prepared with sterile loop. A goodsmear is spread evenly, over a size of 2 X 3 cm and isneither too thick nor too thin. This was allowed to air dryfor 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 3AFB in observed 100 oil immersion fields were considered as positive.

MZN Staining

This is very similar to that of standard ZN stainingtechnique, 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 wereflooded with filtered 1 % CF and heated until they weresteamed and left to steam for 15 min. After rinsing theslides with a gentle stream of water, 25 % H2SO4 was usedto 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.

Observations

This present study was enrolled a total of 200 osteoarticular tuberculosis patients. Most of the patients 30(30%)of ZN staining were in age group of 31-45 years. 25(25%) patients were in age group of 46-50 years. Most of the patients 29(29%) of MZN group were belonged in age group of 31-45 years and 23(23%) patients were in age group of 46-50 years.

Table 1: Age wise distribution of osteoarticular tuberculosis patients

Age (Years)	ZN staining			MZN staining		
	Positive	Negative	Total	Positive	Negative	Total
15-30	5	6	11(11%)	7	5	12%
31-45	21	9	30(30%)	24	5	29%
46-50	16	9	25(25%)	16	7	23%
51-75	11	4	15(15%)	11	5	16%
>75	8	11	19(19%)	9	11	20%
Total	61%	39%	100%	67%	33%	100%

Table 2: Gender wise distribution of osteoarticular tuberculosis patients

Gender	ZN-staining			MZN-staining		
	Positive	Negative	Total	Positive	Negative	Total
Male	37(37%)	25(25%)	62%	40(40%)	23(23%)	63%
Female	26(26%)	12(12%)	38%	27(27%)	10(10%)	37%
Total	63%	37%	100	67%	33%	100

62% and 63% patients of ZN and MZN staining were males respectively. Among ZN-staining, 37(37%) males and 26(26%) females had smear positive. Among MZN staining group, 40(40%) males and 27(27%) females hadsmear positive.

Table 3: Showing the smear results of osteoarticular tuberculosis patients

Staining	Positive	Negative	Total
ZN	59(59%)	41(41%)	100(100%)
MZN	67(67%)	33(33%)	100(100%)

In this present study, smear positivity was seen in 59(59%) ZN-staining and 67(67%) MZN staining OTB patients.

Discussions

The infrequentoccurrence of osteoarticular tuberculosis poses adiagnostic challenge for the treating clinicians andoften results in delayed recognition and treatment.Many a time, the patient has been subjected tounnecessary or even multiple surgeries pendingcorrect diagnosis. Acute suppurative presentationis one of the atypical forms of osteoarticular tuberculosis and closely mimics acute pyogenic infection or septic arthritis. A total of 200 osteoarticular tuberculosis patients were included in this study. All the patients were categorized into two group (group A&B). Each group had 100 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 30(30%) of ZN staining and 29(29%) of MZN staining were in age group of 31-45 years. Majorities of patients in ZN staining 62(62%) and MZN staining (63%) group were males.

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 group, 37(37%) males and 26(26%) females hadsmear positive. Among MZN staining group, 40(40%) males and 27(27%) 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 maledominance was reported. Sharma *et al.* [13] conducted a studygood quality found gender gap in the prevalence of TB withmore occurrence in the rural areas compared to the urbanpart. Even one of the African studies also reported higherprevalence among the male [14]. With these, it is clear thateven 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 wellas submitted by the patient. Unlike quality of sputum, inthis study, there is no influence of specimen in thediagnosis of SP OTB case because the specimen wascollected by the specialist. Moreover, there was nosignificant financial burden with MZN technique comparedto ZN technique. The minor difference in the reagents costfor 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 abscessdecompression 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 67(67%) as compared to ZN staining group patients 59 (59%). 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.9 Thesputum SPT was reported to be 9.43 % and 9.8 % respectively for ZN and MZN staining techniques.

Conclusions

This present study concluded that the OTB was more preponderance in middle age male population. Smear positivity for osteoarticular tuberculosis was greater in Modified Ziehl Neelsen staining technique as compared to Ziehl Neelsen. Hence, MZN is a better technique for the diagnosis of osteoarticular tuberculosis.

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