

# Screening of specimens by Ziehl-Neelsen staining technique for the diagnosis of extra spinal musculoskeletal tuberculosis: A retrospective study

Sana Jamali<sup>1</sup>, Anuj Rastogi<sup>2</sup>, Rajat Ranjan<sup>2</sup>, Amit Pal<sup>3</sup>, Shameem Ahmad Khan<sup>2</sup>, Ashish Jaiman<sup>4</sup>

<sup>1</sup>Department of Microbiology, Autonomous State Medical College, Siddharthnagar, India

<sup>2</sup>Department of Orthopaedics, Integral Institute of Medical Sciences & Research, Integral University, Uttar Pradesh, India

<sup>3</sup>Department of Medical Microbiology, Maharana Pratap Combined District Hospital, Bareilly, India

<sup>4</sup>Department of Orthopaedics, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India

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Corresponding author:

Ashish Jaiman.

E-mail: [drashishjaiman@gmail.com](mailto:drashishjaiman@gmail.com);

ORCID: 0000-0002-4625-0107

## Abstract

**Introduction:** Musculoskeletal tuberculosis is a significant form of extra pulmonary tuberculosis (EPTB) which has substantial consequences if not diagnosed and treated early.

Reliable and rapid confirmation of the diagnosis is now possible by advent of Polymerase Chain Reaction (PCR), Cartridge Based Nucleic Acid Amplification Test (CB-NAAT/Gene-Xpert) and radiometric method (BACTEC)

However, most of these sensitive technologies are not available at places with constrained resources. Hence, diagnosis of tuberculosis at these places, continues to rely on smear microscopy using Ziehl-Neelsen (ZN) staining. Specificity of ZN staining and microscopy is high, but its overall sensitivity is variable (20-53%). Despite of low sensitivity, it is still an easy, cheap, efficient and accessible tool due to its high specificity.

**Aim:** Aim of the present study was to assess the role of ZN staining in diagnosis of extra-spinal musculoskeletal tuberculosis in resource limited settings.

**Material and methods:** A retrospective study was done from 1st January 2016 to 31st December 2019 on specimens received in microbiology laboratory for ZN staining from clinico-radiologically suspected cases of extra-spinal musculoskeletal tuberculosis. The clinical information of the cases was noted from the Orthopaedics Department case files, while results of ZN microscopy were retrieved from the records maintained in Microbiology Department.

**Results:** Specimens from 95 patients with clinico-radiologically suspected extra-spinal musculoskeletal tuberculosis were examined for Acid Fast Bacillus (AFB) by ZN staining technique. Out of 95 patients; 11 patients (11.58 %) were found to be AFB positive.

**Conclusion:** Owing to low yield percentage of ZN staining in extra spinal musculoskeletal tuberculosis; ZN stain alone cannot be used as a tool for diagnosis of this form of tuberculosis.

**Key words:** extra-spinal musculoskeletal tuberculosis, ZN staining, diagnostic tool

## Introduction

Tuberculosis is a disease known to mankind since ancient times. The disease is more common among the poor and marginalized sections of the community. According to the World Health Organization (WHO),

there were 9.6 million cases of tuberculosis in the world in 2014 and 1.5 million died from the disease. In addition, most of the deaths (>90%) occurred in developing countries [1]. Untreated tuberculosis cases infect 10 to 15 people every year [2, 3]. In 2010, the incidence

of tuberculosis was estimated as 2.15 million in Central Asia, which is expected to be tripled by 2030 [4].

Extra pulmonary tuberculosis (EPTB) accounts for 10-15% of all the forms of tuberculosis. The most common site of EPTB are lymph nodes, central nervous system, abdomen, skeletal system, pleura, pericardium, genitourinary system, skin and others [5,6].

Musculoskeletal tuberculosis includes tuberculous myositis and osteoarticular tuberculosis. Osteoarticular (spinal and extra-spinal) tuberculosis represents 1-5% of all cases of tuberculosis and 10-18% of EPTB, with spine being the most common site accounting for 50% of osteoarticular tuberculosis [7-9].

Early diagnosis and prompt initiation of antitubercular treatment is imperative for successful management of musculoskeletal tuberculosis, thereby decreasing the morbidity/disability. A good clinic-radiological assessment along with proper investigations with highly sensitive/specific tools is key for early diagnosis.

Clinically extra- spinal musculoskeletal tuberculosis is suspected in patients who present with pain, swollen joints with/without constitutional symptoms like night sweats, loss of appetite, weight loss and mild grade fever.

Tubercle bacilli do not stain readily, but once stained, they resist decolorization by acid or alcohol and are hence called acid-fast bacilli (AFB). They can be identified early and easily with ZN staining method in resource-limited settings [10, 11]. ZN staining is highly specific, but its overall sensitivity is variable (20-53%) and poor in EPTB [10, 12]. Viable and dead bacilli cannot be distinguished by smear microscopy, but can be distinguished by culture methods. They are obligate aerobes and take about 15-30 days to grow on culture in an enriched media with a moderately acid-base media at a temperature of 37 degree C.

Despite the recent development of more sensitive technologies like PCR, CB-NAAT/Gene-Xpert and radiometric method (BACTEC); diagnosis of tuberculosis in most low socioeconomic countries, continue to rely on smear microscopy for diagnosis of tuberculosis [2]. Although sensitivity of ZN staining is low/variable, still it is an easy, cheap, efficient and accessible tool due to its high specificity, for early diagnosis and prompt start of antitubercular treatment of ZN positive cases in resource limited settings. Literature on ZN staining for screening of extra-spinal musculoskeletal tuberculosis is scarce. Hence, the aim of the present study was to study the role of ZN staining in early diagnosis of extra-spinal musculoskeletal tuberculosis in resource constrained settings.

## Material and methods

Institutional research committee approval and ethical committee clearance were obtained for the study. A retrospective study was done between January, 2016 and December, 2019.

Inclusion criteria: Clinico-radiologically suspected cases of extra-spinal musculoskeletal tuberculosis; whose specimens were sent to the microbiology laboratory from Orthopaedics Department for ZN staining were included in the study.

Exclusion criteria: Cases with spine tuberculosis, multiple system involvement and/or disseminated miliary tuberculosis were excluded from the study

The demographic characteristics of the patients and clinical data were noted from the case files, while results of ZN microscopy were retrieved from the records maintained in Microbiology Department.

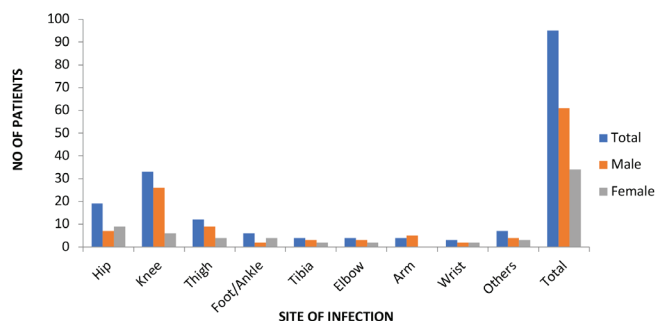
## Results

Specimens from 95 patients with suspected extra-spinal musculoskeletal tuberculosis were screened for AFB.

The mean age of patients was 31.07 years (range 4-70 years). Most of the patients were less than 40 years old (68.42%). There were 61 (64.21%) males and 34 (35.79%) females (male: female ratio 1.79:1).

The commonest symptom was pain with/without swelling. One patient presented with multiple sinuses over ankle/foot. Two cases of suspected knee joint tuberculosis presented with cold abscess. All the cases were human immunodeficiency virus (HIV) negative. Number of cases based on involvement of site is depicted in Figure 1. Knee was the most common site of involvement followed by hip and thigh.

**Figure 1** - Distribution of suspected cases according to site of involvement



Out of 95 patients, only 11 patients (11.58%) were found to be AFB positive, most being from pus specimens 9 (9.47%) while 2 (2.10%) from synovial fluid. Distribution of the 11 AFB positive cases based on age, gender and site of involvement is shown in Table 1. Maximum AFB positive cases were seen in specimens from knees 5 (45.45%) followed by 1 (9.09%) each from wrist, elbow, hip, thigh, tibia and ankle. Seven of the 11 AFB positive cases were males (63.63%) while 4 (36.36%) were females. Mean age of the AFB positive cases was 27.82 years (range 06-68 years).

**Table 1** Distribution of AFB positive cases

S.No.	Age (years)	Sex	Infection site	Sample
1.	12	M	Knee	Pus
2.	45	M	Wrist	Pus
3.	68	F	Ankle	Pus
4.	45	F	Elbow	Pus
5.	13	M	Knee	Synovial fluid
6.	22	M	Knee	Pus
7.	10	F	Thigh	Pus
8.	15	M	Knee	Synovial fluid
9.	06	M	Hip	Pus
10.	18	F	Knee	Pus
11.	52	M	Tibia	Pus

## Discussion

Tuberculosis is a worldwide public health problem, although 90% of cases being in the underdeveloped countries [13]. There has been a recent re-emergence of tuberculosis in the industrialized developed countries which has been attributed to an increase in life expectancy, immigration from endemic regions, emergence of multi-drug-resistant tubercular strains and HIV infection [14-16].

In a study by Patel et al., out of 18 AFB positive patients, maximum patients (5 each) were in age group 11-20 years and in 21-30 years (27.8%) [17]. In our study, out of 11 AFB positive patients, maximum patients (4) were in age group 11-20 years (36.36%). In another study by Prasad et al, mean age at presentation was 33.35 years (range 4-72) [18]. Mean age of patients in our study was 31.07 years (range 4-70 years).

In study by Patel et al, male to female ratio was 1.5:1 [17]. In another study by Prasad et al, it was 1.1:1 [18]. In our study, it was 1.79:1.

In a study by Patel et al, out of 793 EPTB samples screened for AFB, 18 (2.26%) samples were found to be AFB positive [17]. In another study by Prasad et al, 51 patients with extra-spinal musculoskeletal tuberculosis, 18 (35.3%) were AFB positive [18]. In our study, 11 (11.58%) patients were AFB positive out of 95 extra-spinal musculoskeletal tuberculosis patients.

In study by Prasad et al, commonest site (18 out of 51 patients) of articular involvement in musculoskeletal tuberculosis was knee joint (35.29%) [18]. Knee was most commonly (33 out of 95 patients) involved joint (34.74%) in our study too.

In study by Patel et al, 14 out of 18 (77.78%) AFB positive samples were received as pus [17]. In our study, 9 out of 11 (81.81%) samples were received as pus.

Anti-tubercular treatment was started immediately in the 11 AFB positive cases. The other 59 AFB negative cases were either advised more sensitive investigations like TB-PCR or histopathology while remaining were started on antitubercular treatment based on sufficient clinico-radiological evidence and blood investigations (erythrocyte sedimentation rate, C-reactive protein and liver function tests).

Extra-spinal musculoskeletal tuberculosis should be diagnosed at the earliest to prevent limb and life-threatening complications. AFB load is scanty in musculoskeletal specimens, hence difficult to diagnose. Although culture is considered as a gold standard method but it takes at least 4 weeks to come positive [19]. Newer and more sensitive techniques like PCR, fluorescence microscopy, CBNAAT/Gene-Xpert and

histopathology should be used for rapid diagnosis.

Gene-Xpert and AFB smear microscopy share almost same specificity but sensitivity of Gene-Xpert is much higher than AFB smear microscopy. Although ZN staining method for AFB plays a key role in the diagnosis of tuberculosis, its major disadvantage is its low sensitivity [20].

### Limitations:

There were certain limitations of the study: The study was performed retrospectively and results could not be correlated with radiological findings and histopathological reports. We could not comment on the disease status of the 59 patients who had AFB negative specimens as they were subjected to ZN staining only which has a low sensitivity.

### Conclusion

Extra-spinal musculoskeletal TB is one of the important forms of EPTB and has significant consequences if not recognized and treated early on. Involvement of weight-bearing joints like hip and knee is common. High index of clinical suspicion, timely judicious use of invasive diagnostic methods and confirmation of the diagnosis by more sensitive methods like fluorescent stain, CBNAAT, TB-PCR, biopsy, Gene X-pert in comparison to ZN stain and early institution of specific antitubercular treatment with close clinical monitoring for adverse drug reactions are the key to the successful management of extra-spinal musculoskeletal TB.

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