

**Original Article**

# SCREENING OF EXTRAPULMONARY TUBERCULOSIS SAMPLES BY ZEHL NEELSEN STAINING IN PATIENTS PRESENTING AT TERTIARY CARE HOSPITAL AHMEDABAD

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**Financial Support:** None declared

**Conflict of interest:** None declared

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**How to cite this article:**

Patel LR, Panchal JA, Pethani JD, Rathod SD, Patel PH, Shah PD. Screening of Extrapulmonary Tuberculosis Samples by Zeihl Neelsen Staining in Patients Presenting at Tertiary Care Hospital Ahmedabad. Natl J Community Med 2013; 4(1): 165-7.

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**Date of Submission:** 23-11-12

**Date of Acceptance:** 18-02-13

**Date of Publication:** 31-03-13

## ABSTRACT

**Context:** Along with pulmonary tuberculosis now extrapulmonary variety continues to be a major health problem in our country & is emerging from the shadows of its senior cousin. Diagnosis of EPTB has always been a challenge. The aims of the present study was to detect the acid fast bacilli by zeihl neelsen and positive samples were confirmed by fluorescent staining from various unsuspected extrapulmonary specimens at our hospital, and to investigate their demographic characteristics.

**Objectives:** To detect the acid fast bacilli by zeihl neelsen staining in extrapulmonary samples and to investigate their demographic characteristics.

**Methods and Material:** A retrospective analysis was carried out during January 2011 to June 2011. All extrapulmonary samples were screened for acid fast bacilli by 20% Z-N stain and positives were confirmed by fluorescence microscopy.

**Results:** Total 793 extrapulmonary samples received during 1<sup>st</sup> January 2011 to 30<sup>th</sup> June 2011, from which 18 (2.26%) samples were found to be positive including 14(1.76 %) pus, 3(0.37%) pleural fluid & 1(0.13%) ascitic fluid. Common age group was 10 to 30 yrs. and Female : male ratio was 1.5:1. From total positive, 11% were HIV positive patients.

**Conclusions:** However, based on our results TB Control Programme might usefully target young populations for early diagnosis of EPTB to decrease TB morbidity and mortality.

**Key-words:** Extrapulmonary tuberculosis, Acid Fast Bacilli, HIV

## INTRODUCTION

Tuberculosis is a common, and in many cases (i.e. extrapulmonary) lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*. Extrapulmonary tuberculosis (EPTB) refers to disease outside the lungs. It is sometimes confused with non-respiratory disease. Disease of the larynx for example, which is part of the respiratory system,

is respiratory but extra-pulmonary. Extrapulmonary TB may be characterized by swelling of the particular site infected (lymph node), mobility impairment (spine), or severe headache and neurological dysfunction (TB meningitis) etc. Extrapulmonary TB is not accompanied by a cough. It is equally important that both the infectious and non-infectious forms of TB are diagnosed and treated as both can be fatal<sup>1</sup>.

Extra Pulmonary Tuberculosis (EPTB) has existed for centuries. It is a milder form of disease in terms of infectivity as compared to pulmonary tuberculosis. Diagnosis of EPTB has always been a challenge. It is a protean disease affecting virtually all the organs and has a wide spectrum of clinical presentation depending on the anatomical site involved and presents a diagnostic dilemma even for physicians with a great deal of experience. For an unsuspecting physician, the tuberculosis etiology may not even figure in the list of probable diagnosis<sup>2</sup>. Extrapulmonary TB often goes undetected as acid fast bacilli load in extrapulmonary specimens are scanty. To overcome this diagnostic problem we need to establish a substantial diagnostic method by introducing newer, faster & more sensitive methods. The bacilli in the received extrapulmonary specimens can be detected by ZN stain and positives were confirmed by fluorescent stain. ZN stain is commonly used throughout the world and still remains the standard method against which new tests must be measured. Fluorescent stain is regarded as a more reliable method due to more intensive binding of mycolic acids of the bacilli to phenol auramine, and so that the bacilli stand out sharply against black background to allow rapid and accurate screening under low power objective<sup>3</sup>. The aim of the present study was to detect the acid fast bacilli by zeihl neelsen staining in extrapulmonary samples and to investigate their demographic characteristics.

## METHODS

A total no. of 793 extrapulmonary samples were received during 1<sup>st</sup> January 2011 to 30<sup>th</sup> June 2011 from Sheth Vadilal Sarabhai General Hospital, Ahmedabad, Gujarat. Out of these, 304 samples were pus (including pus from lymph node and other sites), 183 ascitic fluid, 230 pleural fluid & 76 CSF. All the samples were received in sterile containers. From each samples smear were made on new, clean, unscratched glass slide. Smear was allowed to air dried and fixed by heat then slide was stain by Z-N stain and examined under oil -immersion(x100) while positive smear was confirmed by Auramine Phenol stain and examined using 10 x and then 40x objective under fluorescent microscope.

The detailed patients history, physical findings, chest radiographs and laboratory investigations were reviewed to obtain the necessary information about diagnosis of extrapulmonary TB. Testing of HIV infection is carried out to all

TB patients. Patients those suffering from MDR - TB (following the guideline of RNTCP), those specimens were sent for culture to IRL (Intermediate Reference Laboratory) at Civil Hospital, Ahmedabad. A limited number of medical centres are able to perform accurate and rapid culture and susceptibility testing.

## RESULTS

Between 1<sup>st</sup> January to 30<sup>th</sup> June - 2011, a total of 793 samples were screened for acid fast bacilli, 18 (2.26 %) samples were found to be positive for acid fast bacilli in which 5 patients belongs to age group 10 to 20 yrs. and 5 patients belongs 20 to 30 yrs shown on Table no.1. From total 18 positives of EPTB cases, 2 (11%) were HIV positive while 16(89%) were HIV negative.

**Table1: Case(s) of extrapulmonary tuberculosis according to age group.**

Age group in year	Cases
0 - 10	03
11 - 20	05
21 - 30	05
31 - 40	02
41 - 50	02
51 - 60	01
>60	00

In our study AFB positivity was highest in pus 14(7%) followed by pleural fluid 3(1.3%) and ascitic fluid 1(0.5%) and no acid fast bacilli were found from CSF. The females were more affected 11 (61.1%) than male 7 (38.9 %).

## DISCUSSION

Extrapulmonary TB should be diagnosed at the earliest to prevent life threatening complications. Use of more sensitive methods for diagnosis are very helpful (e.g. fluorescence microscopy) as workload is more in tertiary care hospitals so that every culture sample can be screened for AFB even if it is unsuspected for TB. As AFB load is scanty in extrapulmonary specimens newer techniques like PCR & fluorescence microscopy should be used for rapid diagnosis.

In present study showed AFB positivity was highest in pus (1.76 %) followed by pleural fluid (0.37 %) which was correlated with other studies<sup>4,5</sup> but rate of positivity for pleural fluid was different. In India and other developing countries LNTB continues to be the most common form of EPTB and lymphadenitis due to non-tuberculous mycobacteria (NTM) is seldom seen<sup>6,7,8</sup>. On the other hand, NTM are the most

common cause of lymphadenopathy in the developed world<sup>9</sup>

Results of our study suggest that younger may be independent risk factors for EPTB. So the incidence of extrapulmonary TB is more common in younger age group (72%) in below 30 year of age<sup>5</sup>. Demographic characteristics of EPTB cases have shown higher detection in females and in patients of young age. Similar observations have been made in past<sup>10</sup>.

In our study, females were preponderance, with an overall female : male was 1.5: 1, that is correlated with study done by Chandir Subhash *et al*<sup>11</sup> and Fawzia Al -Otaibi ,Malak M. El Hazm *et al*<sup>12</sup>. Female patients showed high incidence of EPTB in the younger age group (20-30 years). An explanation of this finding remains unclear, but it suggests that women of child bearing age seem to be most vulnerable for EPTB. This group should be targeted for further study to find the causes and intervention for disease prevention. And also endocrine factors might play a role. This is considered with studies from USA and Europe<sup>13</sup> which have found that younger age was independent risk factors for EPTB<sup>14</sup>.

In our study, lymphnodes EPTB (1.76%) cases were highest followed by pleural fluid (0.36%). Certain other studies<sup>10-12</sup> have also reported high number of cases with lymph node involvement. In our study EPTB in HIV positive patients were less as compare to other study<sup>5,15</sup> indicating that EPTB can occur in non immunocompromised patients.

In conclusion, there is an overall increase in the incidence of EPTB contributing to the overall burden of Tuberculosis in developing countries and the proportion of EPTB is relatively low and EPTB is less infectious than PTB. Therefore, EPTB is usually not prioritized for case finding strategies in TB control programs. However, based on our results TB control programs might usefully target young populations for early diagnosis of EPTB to decrease TB morbidity and mortality. High index of clinical suspicion, timely judicious use of invasive diagnostic methods and confirmation of the diagnosis by establishing more sensitive method like Fluorescent stain in comparison to Z- N stain and early institution of specific anti tuberculosis treatment and close clinical monitoring for

adverse drug reactions are the key to the successful management of EPTB.

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