Research Article

# Incidence of various clinico-morphological variants of cutaneous tuberculosis and its drug susceptibility pattern-Delhi based study

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**Abstract**

**Background**: Tuberculosis was declared global emergency in year 1993. The global spread of the disease is complicated by the ubiquitous appearance of drug-resistant strains, and particularly multidrug-resistant (MDR) strains. MDR is defined as resistant to isoniazid (INH) and rifampicin4.

**Objective:** To identify Mycobacterium in patients of cutaneous tuberculosis, to study clinical variants of cutaneous tuberculosis and to determine the pattern of anti mycobacterial drug susceptibility in these isolates.

**Materials and Methods**: The present study was performed on 30 consecutive patients of cutaneous tuberculosis attended skin OPD at UCMS and GTB Hospital, Delhi. Biopsy samples were taken from Dermatology department in 0.85% NACL. Samples were processed in Microbiology department for microscopy, LJ culture, and drug susceptibility methods.

**Result:** Out of 31 specimens, only 6 (19.3) were culture positives. Out of 6 isolates, only 4 isolates were positive for niacin and nitrate. Indirect proportion method was done on the 6 isolates of tuberculosis for susceptibility against isoniazid, rifampicin, ethambutol, and streptomycin. 16.6% (1) of the *M. tuberculosis* isolates showed resistance to isonazid and streptomycin by IPM. Interpretation was made by 28 days. Rest 4 isolates were sensitive. No multidrug resistant isolate was reported in the present study .

**Conclusion**: Cutaneous tuberculosis is increasing rapidly in era of HIV. However, there have been studies on the pattern of anti mycobacterial drug susceptibility but this can change with time and with particular geographical area. Although the drug-resistant strains causing cutaneous tuberculosis is not yet a significant issue but this situation may change. So, cutaneous tuberculosis should be diagnosed early and accurately

**KEY WORDS:** TB (Tuberculosis), LJ (Lowenstein Jensen), MDR, indirect proportion method

## Introduction

Received. Moschella and Cropley[1] divided tuberculous infection of the skin into primary or secondary depending respectively, on whether the host is nonsensitized or presensitiized to mycobacterial antigen. Primary infection involves first interaction with the tubercle bacillus whereas secondary tuberculosis develops in BCG immunized or people with history of primary TB infection and are sensitized to the mycobacterial antigens.[2] The various modes of reaction of the skin to the presence of tubercle bacilli are determined by the sensitization status of the host to the mycobacterial antigens, cellular immunity, route of infection, the bacillary inoculums and the pathogenicity of the infecting mycobacterial strain.[3]

WHO declared TB as global emergency in 1993. The global spread of the disease is complicated by the ubiquitous appearance of drug-resistant strains, and particularly multidrug-resistant (MDR) strains. MDR is defined as resistant to antimycobacterials like isoniazid (INH) and rifampicin.[4] Early diagnosis of MDR-TB patients is therefore essential to initiate appropriate treatment. MDR was reported in 3.5% of newly

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diagnosed TB patients and in 20.5% of retreatment patients. Whereas, 9% of MDR-TB patients isolates showed evidence of additional resistance to one of the fluoroquinolones (FQs) and one of the second-line injectables, classified as XDR-TB.[5] In 1994, WHO AND International union against tuberculosis and lung disease (IUATLD) conducted the global project on drug-resistance surveillance with the goal to monitor the trends of resistance. The first report was published in 1997 and contained data from 35 geographical settings for the period 1994–1996.[6] The report showed that drug resistance was present globally, and that MDR-TB ranged from 0 to 14% in new cases and 0 to 54% in previously treated cases. A second report for the period 1996–1999, followed in 2000 and included surveillance data from 58 geographical sites.[7] This report confirmed that drug resistant TB was a sufficient problem since MDRTB ranged from 0 to 16% among new cases and from 0 to 48% in previously treated cases.[8]

Cutaneous tuberculosis is increasing rapidly in era of HIV.[14,15] However, there have been studies on the pattern of anti mycobacterial drug susceptibility but this can change with time and with particular geographical area. Although the drug-resistant strains causing cutaneous tuberculosis is not yet a significant issue but this situation may change. So, cutaneous tuberculosis should be diagnosed early and accurately. The newer diagnostic technique involving polymerase chain reaction (PCR) when applied to pulmonary tuberculosis increased not only the yields but also decreased the time duration of detection of mycobacteria significantly.[17] Studies on the pattern of antimycobacterial drug susceptibility exists but this pattern may change with time and with particular geographical area. There is paucity of literature comparing the conventional diagnostic methods with PCR in cutaneous samples. Keeping this in mind this study is designed to evaluate the role of PCR in the diagnosis of cutaneous tuberculosis.

## Materials and Methods

A cross-sectional study was conducted on 30 consecutive patients of cutaneous tuberculosis and in the departments of Microbiology and Dermatology and STD UCMS and Guru Teg Bahadur Hospital Delhi, from November 2010–March 2012.

### Selection of Patient

Clinically diagnosed and histopathologically documented new cases of cutaneous tuberculosis of any sex and age group or untreated cutaneous tuberculosis patients, not taken antitubercular therapy in past 3 months or volunteers were enrolled for this study. Patients who had taken antitubercular therapy or its constituents drugs including aminoglycosides and quinolones in the past 3 months were excluded from the study. Negative control group was of patients who had diagnosis other than cutaneous tuberculosis. Biopsy specimen was taken from the Dermatology department in sterile universal container containing sterile 0.85% saline. Biopsy specimen was used for microbiological examinations like microscopic examination, culture for isolation and drug susceptibility pattern of mycobacterial isolates.

### Sample Processing

Biopsy specimens were homogenized in 0.85% saline. Smear was prepared and stained with ZN, acid fast bacilli appeared as pinkish red rods against the blue background.

Homogenized specimens were decontaminated with 4% NAOH method. Centrifugation was done and the sediments were inoculated on 2 Lowenstein Jensen (LJ) slopes screw. The tubes were incubated in slanted position with screw lightly loosen for a 1 week for even distribution of inoculums at 37ºC incubator. The growth was checked weekly till 8 weeks before declaring it as no growth. A positive culture was confirmed microscopically for acid fast bacilli after staining with Ziehl Neelsen stain. The absence of growth after 8 weeks on LJ medium was taken as negative. Rough, tough, buff irregular colonies were seen. Specimen were identified by biochemical tests.[9,16]

Drug susceptibilitytest was done on all isolates. Indirect proportion method was used, a quantitative test, in which degree of growth in antibiotic containing media and drug free media are compared. Stock solution of the drugs was prepared based on the potency of the drug in sterile distilled water for streptomycin, isoniazid and ethambutol and in absolute methanol for rifampicin.[9]

The concentrations are as follows:

| | |
|---|---|
| Isoniazid | 0.2 μg/ml |
| Ethambutol | 2 μg/ml |
| Streptomycin | 4 μg/ml |
| Rifampicin | 40 μg/ml |

## Result

Out of 31 specimens, 14 (45.1%) were male patients and 17 (54.8%) were female patients showing a marginal female preponderance. Cases of cutaneous tuberculosis were mainly observed in younger age groups <30 years. Majority of patients (18/31) were in the age group of 11–20 years. However, female patients were more in number in this group. The minimum age was 10 years, a female patient and maximum age was 53 years, a male patient (Table 1). Only 21 had lupus vulgaris (LV), 8 patients had scrofuloderma and

**Table 1:** Age and sex distribution of 31 patients

| Age (in years) | Males (%) | Females (%) | Total (%) |
|---|---|---|---|
| 0–10 | 0 | 2 (6.4) | 2 (6.4) |
| 11–20 | 8 (25.8) | 10 (32.3) | 18 (58 ) |
| 21–30 | 5 (16.1) | 3 (9.6) | 8 (25.8) |
| 31–40 | 0 | 1 (3.2) | 1 (3.2) |
| 41–50 | 0 | 1 (3.2) | 1 (3.2) |
| 51–60 | 1 (3.2) | 0 | 1 (3.2) |
| Total | 14 (45.1%) | 17 (54.8%) | 31 (100) |

**Table 2:** Age wise distribution of types of cutaneous tuberculosis

| Types of cutaneous tuberculosis | Age group (in Years) and No. of cases (%) | | | | | | Total (%) |
|---|---|---|---|---|---|---|---|
| | 0–10 | 11–20 | 21–30 | 31–40 | 41–50 | 51–60 | |
| Lupus vulgaris | 1 (4.76) | 12 (57.1) | 6 (28.5) | 1 (4.76) | 1 (4.76) | 0 | 21 (100) |
| Scrufuloder3ma | 1 (12.5) | 5 (62.5) | 2 (25) | 0 | 0 | 0 | 8 (100) |
| TBVC | 0 | 0 | 1 (50) | 0 | 0 | 1(50) | 2 (100) |
| Total | 2 (6.4) | 17 (54.8) | 9 (29.0) | 1 (3.2) | 1 (3.2) | 1(3.2) | 31 (100) |

**Table 3:** Sex wise distribution of types of cutaneous tuberculosis

| Types of skin tuberculosis | Males (%) | Females (%) | Total (%) |
|---|---|---|---|
| Lupus vulgaris | 10 (47.6) | 11 (52.3) | 21 (67.7) |
| Scrofuloderma | 3 (37.5) | 5 (62.5) | 8 (25.8) |
| TBVC | 01 (50) | 01 (50) | 02 (6.4) |
| Total | 14 | 17 | 31 |

**Table 4:** Site of involvement in 31 cases

| Site of skin TB | Lupus vulgaris (*n*) | SF (*n*) | TBVC (*n*) | No. of cases (%) |
|---|---|---|---|---|
| Face | 1 | 0 | 0 | 1 (3.2) |
| Neck | 0 | 2 | 0 | 2 (6.4) |
| Trunk | 1 | 0 | 0 | 1 (3.2) |
| Upper limb | 5 | 5 | 0 | 10 (32.2) |
| Lower limb | 14 | 1 | 2 | 17 (54.8) |
| Total | 21 | 8 | 2 | 31 (100) |

**Table 5:** Drug resistance by indirect proportion method (IPM) of 6 isolates

| Sample | Isoniazid % (*n*) | Rifampicin % (*n*) | Ethambutol % (*n*) | Strepomycin % (*n*) |
|---|---|---|---|---|
| Biopsy tissue | 1 | - | - | 1 |
| Total | 16.6 (1) | 0 | 0 | 16.6 (1) |

2 had tuberculosis verrucosa cutis. In the present study, the majority of LV cases were observed in patients below the age of 30 years. Among these, highest were occurred in (12/ 21) cases of LV occurred in the age group of 11–20 years, 6 cases were seen in age group 21–30 years and one case each of LV was in age groups 0–10, 31–40 and 41–50 years. In scrofuloderma cases 5 out of 8 cases were seen in the age groups 11–20 years, and one and 2 cases were seen in the 0–10 and 21–30 years age group, respectively. Only 2 cases of TBVC were found in the age group 21–30 and 51–60 years (Table 2). Out of 21 patients with LV, 11 (52.3%) were females and 10 (47.6%) were males, whereas in scrofuloderma there were 3 males (37.5) and 5 females (62.5), there were only 2 cases of TBVC, 1 male and 1 female, respectively (Table 3). Majority of the lesions were distributed in the limbs. Lower limb (54.8%) was the more frequently involved site, followed by upper limb (32.2%) and neck (6.4%). Trunk (3.2%) and face (3.2%) was the least affected site (Table 4).

Biopsy specimens of cutaneous tuberculosis were homogenized and decontaminated by NALC-NAOH method and were inoculated on 2 bottles of LJ media each for isolation. M. *tuberculosis* showed eugenic growth. All of them grew as dry, rough, and irregular with wrinkled surface. The growth was detected at 37 ºC at 4–6 weeks. Out of 31 specimens, only 6 (19.3) were culture positives. Out of 6 isolates, only 4 isolates were positive for niacin and nitrate. Indirect proportion method was done on the 6 isolates of M. *tuberculosis* for susceptibility against isoniazid, rifampicin, ethambutol, and streptomycin. 16.6% (1) of the M. *tuberculosis* isolates showed resistance to isonazid and streptomycin by IPM. Interpretation was made by 28 days. Rest 4 isolates were sensitive. No multidrug resistant isolate was reported in the present study (Table 5).

## Discussion

Out of 31 specimens, only 6 (19.3) were culture positives. Out of 6 isolates, only 4 isolates were positive for niacin and nitrate. Indirect proportion method was done on the 6 isolates of M. *tuberculosis* for susceptibility against isoniazid, rifampicin, ethambutol and streptomycin. 16.6% (1) of the M. *tuberculosis* isolates showed resistance to isonazid and streptomycin by IPM. Interpretation was made by 28 days. Rest 4 isolates were sensitive. No multidrug resistant isolate was reported in the present study (Table 5).

In present study, drug susceptibility was done by indirect proportion method (IPM) on 6 isolates. IPM was done for isoniazid (0.2 μg/ml), rifampicin (40 μg/ml), ethambutol (2 μg/ml) and streptomycin (4 μg/ml). By IPM, only one isolate (16.6%) showed resistance to isoniazid and streptomycin. That was a case of lupus vulgaris. In a single report on the resistance pattern in cutaneous TB, 3 of the 9 isolates (33.3%) were found to be resistant to one or more drugs,[10] whereas Aggarwal et al.[11] reported resistance to one or primary antitubercular drugs to be 46.2% of the isolates obtained, 23.1% being multidrug resistant. But in their study, for drug susceptibililty they used BACTEC system. Sharma et al.[12] carried out drug senstivity pattern of 9 isolates of

*Mycobacterium tuberculosis* isolates from cutaneous tuberculosis cases. Resistance to one or more drugs was present in 3 (33%) isolates. All the 3 of the 9 (33.3%) isolates were resistant to isoniazid, while 1 (11.1%) isolate was found to be resistant to streptomycin. Lal et al.[13] reported a case of lupus vulgaris resistant to isoniazid, streptomycin and thiacetazone.

## Conclusion

In cutaneous tuberculosis diagnosis, continuous surveillance is must for controlling the present infections and preventing future cases. Since the conventional drug susceptibility methods are cumbersome and time consuming. Rapid drug susceptibility methods like BACTEC and MGIT should be implied on a larger scale.

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