Frequency and antibiotic susceptibility pattern of Mycobacterial Isolates from extra-pulmonary Tuberculosis Cases

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Abstract

Objective: To determine the frequency and antimicrobial susceptibility pattern of extra-pulmonary tuberculosis in Rawalpindi.

Setting: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi.

Methods: Between September 2000 and August 2002, 899 pulmonary and 460 extra-pulmonary specimens from suspected cases of tuberculosis were examined. The radiometric BACTEC 460 TB system was used for culture and antimicrobial susceptibility testing.

Results: Mycobacteria were isolated from 291 pulmonary specimens and 98 extra-pulmonary specimens. The frequency of extra-pulmonary tuberculosis was 25.2%. The commonest source of isolation was pus (44.9%, frequency 11.3%), followed by lymph nodes (13.3%, frequency 3.3%) and pleural fluid (13.3%, frequency 3.3%). We tested the anti-microbial susceptibility of the isolates to the four first line anti-tuberculous drugs, rifampicin, isoniazid, streptomycin and ethambutol. Of the extra-pulmonary isolates 13.3% were resistant to a single drug, 21.4% were multi-drug resistant and 9.2% were resistant to all the four drugs.

Conclusion: Increased awareness of the magnitude of the problem posed by extra-pulmonary tuberculosis is required so that appropriate control measures can be adopted (JPMA 53:328;2003).

Introduction

Tuberculosis (TB) continues to be a major health concern, especially in the developing world with more than 8 million new cases each year.[1] In Pakistan, its prevalence is estimated to be 175 per 100,000 of population.[2] Tuberculosis has the potential to infect any organ in the body due to dissemination via lympho-haematogenous route in the early period of the pulmonary infection and a significant number of tuberculosis cases occur in extra-pulmonary sites especially among the immunocompromised.[3-5] Extra-pulmonary tuberculosis is defined as the disease that is not associated with lung involvement with the exception of miliary tuberculosis.[6] Among the various extra-pulmonary sites, tuberculous lymphadenitis is the most common form that occurs in younger age group less than 15 years. The other sites commonly involved are the skeletal and genitourinary systems. At the extremes of age, miliary and meningeal tuberculosis are more common.[3,7] The diagnosis of extra-pulmonary tuberculosis poses a special challenge as it is often missed or misdiagnosed due to its atypical presentations. The first step in its diagnosis is its awareness and a high index of suspicion by the physicians.[5,7] Even then, it is difficult to isolate Mycobacterium tuberculosis due the small number of organisms present at these sites.[3] Various international studies in the world have focused on the problem of extra-pulmonary tuberculosis, reporting a high frequency.[3,4,6-10] However, this aspect of tuberculosis has not been properly and adequately investigated in Pakistan. We planned our study with the objective of determining the frequency and susceptibility of extra-pulmonary AFB culture positive cases at the Armed Forces Institute of Pathology, Rawalpindi.

Materials and Methods

Study design and setting

A non-interventional descriptive study of two years duration from September 2000 to August 2002. The study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi.

Patients and specimens

A total of 1359 (non duplicate) specimens were collected for mycobacterial culture and susceptibility testing from patients clinically suspected of pulmonary and extra-pulmonary tuberculosis referred from various civil and military hospitals in Rawalpindi-Islamabad. Of these, 899 specimens were from suspected cases of pulmonary tuberculosis and 460 were from extra-pulmonary sites. The sampling technique was convenient non-probability.

Specimens from suspected cases of pulmonary tuberculosis consisted of 2-5 ml early morning sputa and bronchial washings. Specimens from extra-pulmonary sites chiefly consisted of pus, lymph nodes, endometrium, pleural fluid and urine. Pus was obtained from various sites including psoas abscess, caries spine, retropharyngeal abscess, discharging sinuses from abdominal, thoracic cavities, neck and axillae. Pus swabs were not processed. Enlarged cervical, axillary and inguinal lymph nodes as a whole and endometrial curetting were included. Cerebrospinal fluid (CSF) was collected in sterile containers without anticoagulant. Urinary specimens consisted of three consecutive early morning urines without preservatives. Various fluids (ascitic fluid, pleural fluid, synovial fluid) were collected in sterile containers containing tri-sodium

citrate to prevent clotting. Tissue specimens (bone marrow, bone, meninges, skin and liver biopsies) were collected in universal sterile containers without fixatives.

Table 1. Common clinical findings in cases of tuberculosis according to the site of infection, which were the criteria for collection of various specimens.

Sputum/BAL	Chronic productive cough and typical chest radiographic findings (hilar lymphadenopathy, pulmonary infiltrates or cavity formation in the upper lobes or the apical segments of lower lobes).
Pus	Cold abscess, routine culture negative, chronic discharging sinus.
Endometrium	Primary or secondary infertility, menstrual irregularities and abdominal pain. Clinical picture of pelvic inflammatory disease, unresponsive to treatment.
Pleural fluid	Pleuritic chest pain, cough, characteristic radiographic findings, exudative pleural aspirate with lymphocytes predominating.
Lymph node	Persistently enlarged lymph node despite antimicrobial therapy, fever, discharging sinus.
Urine	Persistent dysuria, frequency, abnormal intravenous pyelogram, sterile pyuria.
CSF	Prolonged low-grade fever, headache, meningismus and focal neurological signs. CSF examination showing clear fluid, cell count of 0-1500/mm3, predominantly lymphocytes, moderate elevation of proteins, low glucose, presence of AFB and absence of other organisms on microscopy.
Ascitic fluid	Ascites, fever, abdominal pain, weight loss. Lymphocytic exudate on routine examination of ascitic fluid.
Synovial fluid	Chronic arthritis, periarticular and cartilage destructive changes on x-rays, draining sinuses.
Semen	Chronic dysuria, scrotal mass, draining sinus, oligospermia.
Bone marrow	Pyrexia of undetermined origin.

All the clinical specimens included in the study were from patients with strong clinical suspicion of tuberculosis. Strong clinical suspicion included general findings suggestive of tuberculosis like low-grade fever, weight loss, night sweats, anaemia, raised ESR, a reactive tuberculin skin test (area of induration = 10 mm after intradermal dose of 5 TU11) and organ-specific findings[11] (Table 1).

Clinical history and investigations including imaging findings of each patient were reviewed. Isolation of mycobacteria from extra-pulmonary sites by culture was taken as the sole criterion for labeling the case as that of extra-pulmonary tuberculosis. Patients on anti-tuberculosis treatment were excluded from the study.

Specimen processing

All the clinical specimens were processed by the standard N-acetyl-L-cysteine sodium hydroxide digestion-decontamination technique[12] except those specimens which are normally sterile like cerebrospinal, pleural, synovial and ascitic fluids which were centrifuged at 2000g for 20 minutes[13] without decontamination. The specimens were then utilized for Ziehl Neelsen (ZN) staining and culture.

Culture

Culture was done on the radiometric BACTEC 460 TB mycobacterial broth culture system (Becton Dickinson Instrument Systems, Towson, Md. USA). The specimens were incubated aerobically at 37^0 Centigrade for up to 8 weeks. The BACTEC 460 TB vials were monitored by instrument twice weekly for the first 2 weeks and then weekly. Vials showing a growth index (GI) of 10 or more were monitored daily. Those vials in which the GI reached 100 were taken as positive.[14]

Mycobacterial growth was confirmed on microscopy by a ZN acid-fast smear. Contamination of the medium was checked by Gram stain smears and by inoculation on to Columbia agar (Oxoid, Basingstoke, UK) containing 5% horse blood.

Mycobacterial isolates were identified as either Mycobacterium tuberculosis or mycobacteria other than Mycobacterium tuberculosis (MOTT) by the radiometric p-nitro-a-acetylamino-b-hydroxy propiophenone (NAP) test[14,15] (Becton Dickinson Diagnostic Systems) and the niacin test.[16]

Antibiotic sensitivity testing was done by single concentration break point method using Bactec 460 TB system. Four first line anti-mycobacterial drugs were used. These were streptomycin (2.0 µg/ml), isoniazid (0.1 µg/ml), rifampicin (2.0 µg/ml) and ethambutol (2.5 µg/ml). A GI of 20 or more was considered positive and identification of M. tuberculosis complex was performed once the GI of the culture had reached 300 or more. Susceptibility testing by BACTEC method was performed at a GI of 500 or more. Some of the cultures had a GI of more than 800 then 1:2 dilution, was made before susceptibility testing was done. The susceptibility of each, M. tuberculosis was determined when the GI in control vial was 30 or more.[14]

Results

A total of 1359 clinical specimens were processed: 899 sputa/bronchial washings and 460 from extra-pulmonary sites. Mycobacteria were isolated from 291pulmonary and 98 extra-pulmonary specimens. Among the pulmonary isolates, 213 (73.19%) were M. tuberculosis and 78 (26.8%) were MOTT.

Table 2. Mycobacterial isolates from pulmonary and extra-pulmonary specimens (n=1359).

	Pulmonary (n =899)	Extra-pulmonary (n =460)
MTB	213 (73.2%)	88 (89.8%)
MOTT	78 (26.8%)	10 (10.2%)

MTB = Mycobacterium tuberculosis

MOTT = Mycobacteria other than Mycobacterium tuberculosis

Among the extra-pulmonary isolates, M. tuberculosis was isolated from 88 (89.8%) specimens while MOTT was isolated from 10 (10.2%) specimens (Table 2).

Four first line anti-tuberculosis drugs were used for antibiotic susceptibility testing against each isolate from extra-pulmonary sites. Nine isolates (9.2%) were resistant to all the four drugs tested. Single drug resistance was found in 13 cases (13.3%). Multi-drug resistance (defined as resistance of mycobacteria to INH and rifampicin[17]) was detected in 21 (21.4%) cases. Single-drug resistance in M. tuberculosis was 14.8%, multi-drug resistance was 15.9% while resistance to all the four drugs was 5.7%. Seventy percent MOTT were multi-drug resistant while 40% were resistant to all the four drugs (Table 4).

Table 3. Distribution of isolates of mycobacteria from extra-pulmonary sites (n=98) and frequency of extra-pulmonary tuberculosis (n=389)

		Pus n =100	LN n =34	Endometrium n =100	Urine n =49	Body fluids n =84	Semen n =9	CSF n =14	Others n=70	Total n =460
MTB	Number	41	11	7	5	12	5	4	3	88
	Frequency	10.5%	2.8%	1.8%	1.3%	3.1%	1.3%	1.0%	0.8%	22.6%
MOTT	Number	3	2	2	2	1	-	-	-	10
	Frequency	0.8%	0.5%	0.5%	0.5%	0.3%	0%	0%	0%	2.6%
Total	Number	44	13	9	7	13	5	4	3	98
		(44%)	(38.2%)	(9%)	(14.3%)	(16.0%)	(56.0%)	(29.0%	(4.3%)	(21.3%))
	Frequency	11.3%	3.3%	2.3%	1.8%	3.3%	1.3%	(1.0%)	0.8%	25.2%

MTB = Mycobacterium tuberculosis

MOTT = Mycobacteria other than Mycobacterium tuberculosis

LN = Lymph node

CSF = Cerebrospinal fluid

Body fluids = these included pleural, ascitic and synovial fluids

Others = these included tissue specimens (bone marrow, bone, meninges, skin and liver biopsies)

Out of the total 98 culture positive extra-pulmonary cases, although pus appears to have the highest yield of mycobacterial growth with 44 isolates (44.9%) followed by lymph nodes 13 (13.3%), pleural fluid 13 (13.3%), endometrium 9 (9.2%), urine 7 (7.1%), semen 5 (5.1%) and CSF 4 (4.1%), but if we consider the yield keeping in view the total number of specimens submitted, semen had the best yield of more than 55%, followed by pus (44%) (Table 3).

The frequency of extra-pulmonary tuberculosis was 25.2% (98 out of 460). Frequency of extra-pulmonary M. tuberculosis was 22.6% (88 out of 460) and of extra-pulmonary MOTT was 2.6% (10 out of 460). The break down of frequencies of organ-specific tuberculosis is given in Table 3.

Table 4. Resistance among the extra-pulmonary isolates of mycobacteria against four first line anti-tuberculosis drugs, streptomycin, rifampicin, isoniazid and ethambutol (n=98).

	Single-drug resistance	Multi-drug resistance*	Resistant to all four drugs
MTB (n =88)	13 (14.77%)	14 (15.9%)	5 (5.68%)
MOTT (n =10)	-	7 (70%)	4 (40%)
All cases (n =98)	13 (13.26%)	21 (21.42%)	9 (9.18%)

MTB = Mycobacterium tuberculosis

MOTT = Mycobacteria other than Mycobacterium tuberculosis

*Simultaneous resistance against isoniazid & rifampicin

Discussion

Extra-pulmonary tuberculosis is often associated with immunocompromised state and its association with HIV infection is well known.[3-5] The frequency of extra-pulmonary tuberculosis has been reported to be as high as 20% of all tuberculosis cases without HIV infection and 53-6% in HIV positive patients.[3] In Pakistan, although AIDS is not common, but widespread poverty and malnutrition means that the immune status of a large proportion of population is compromised.

The frequency of extra-pulmonary tuberculosis in our study was 25.2%. This is a significant number, especially since the incidence of tuberculosis in Pakistan is so high. The real figures might be even higher because we had taken a positive culture as the sole criterion for the diagnosis of extra-pulmonary tuberculosis. Ahmed and Aziz have reported a frequency of 33% from Karachi.[18] Similar high figures have been reported from all over the world. Cowie has reported a frequency as high as 46% from Canada[6] and Lado et al 40.2% from Chile.[19] However, figures from Malaysia[8] (11%) and Hong Kong[10] (22.3%) are much lesser. One of the reasons may be that tuberculosis was not associated with AIDS in these cases.

Although the best yield was noted in cases of semen specimens (55.6%) followed by pus from different sites, but this may be because of the very low number of semen specimens and also the possibility that only highly suspected cases for genital tract TB were referred. Among the culture positive specimens (n=98), pus yielded the maximum number of isolates ~ 44 (44.9%), followed by pleural fluid ~13 (13.3%), lymph nodes ~ 13 (13.3%) and endometrium ~ 9 (9.2%). Other studies have reported a higher incidence of lymph node involvement but by and large the distribution of organ systems is the same.[3,6,8,10,19,20]

We observed high level of resistance in our isolates. The frequency of single-drug resistance (13.3%) was similar to two other worldwide studies.[17,21] However multi-drug resistance at 21.42% for all isolates and 15.9% for Mycobacterium tuberculosis was much higher than that reported by the World Health Organization (1.8%).[17] However, the MDR referred to by the World Health Organization was based on different variable reports from different centres and it varies from 0 to 18.1%. Most of the time in our set up, extra-pulmonary tuberculosis is being diagnosed on clinical presentation or on therapeutic response of anti-tuberculosis drugs, rather than to identify it on the basis of culture isolation. This may result in inadvertent use of anti-tuberculosis drugs and subsequently development of resistance. Karamat et al, in 1999 had reported single-drug resistance among Mycobacterium tuberculosis from both pulmonary and extra-pulmonary isolates at 21.3%, multi-drug resistance at 13.66% and resistance to all four first-line drugs at 5.3% in Rawalpindi-Islamabad area[22], which are similar to the resistance pattern observed in our study. This may suggests that there is apparently no significant change in anti-tuberculosis resistance pattern of M. tuberculosis at least at Rawalpindi- Islamabad area.

The frequency of MOTT in our cases appeared quite low as compared to the previous reports for extra-pulmonary tuberculosis in cases of AIDS and other immunocompromised patients. The risk of developing extra-pulmonary TB in cases of AIDS and other immunocompromised states is high in countries where TB is not endemic as MOTT find place to infect as opportunistic microorganisms. In our population M. tuberculosis reactivates from latent TB infection[23] (highly tuberculin positive population) prevalent in our population and MOTT, therefore, may not find place to infect.

Conclusion

One-fourth of all tuberculosis cases are extra-pulmonary with high level of drug resistance. Their clinical presentations are often vague and confusing and diagnosis can be easily delayed or missed, increasing the morbidity and mortality among the patients. Increased awareness of the magnitude of the problem, a high index of suspicion, early case identification and treatment are required in order to prevent the emergence of multi-drug resistant strains and control of the disease.

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