MYCOBACTERIURIA IN PULMONARY TUBERCULOSIS PATIENTS IN MADRAS, SOUTH INDIA

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Summary. **Three consecutive, entire, early morning urine specimens, collected from each of 137 bacteriologically confirmed pulmonary tuberculosis patients aged more than 12 years were processed for culture of *M. tuberculosis* by the usual centrifugation method. Of the 411 urine specimens, 5 yielded *M. tuberculosis*. About 50 ml each from 405 of the above specimens, from 135 patients, was also processed for culture by a filtration method and *M. tuberculosis* was isolated from only one of them. In all, mycobacteriuria was present in 5 (3.6%) of 137 patients (95% confidence interval being 1.2% to 8.4%). Of these patients, 92 had no history of previous chemotherapy and 3 (3.3%) excreted tubercle bacilli in urine (95% confidence interval being 0.6% to 9.3%).**

Introduction

In patients with pulmonary tuberculosis (PT), mycobacteriuria has been reported to vary from 3.6% to 14.8%.[1-4] In USA, Bentz et al[1] observed mycobacteriuria in 4.7% of 275 untreated PT patients; in India, Chadha and Shahi[2] recorded it in 3.6% of 55 patients with unspecified period of treatment for PT; Agarwal et al[3] reported it in 6.4% of 110 patients without giving any information about previous therapy; and Challu et al[4] noted a higher rate of 14.8% in 236 untreated PT patients. These reports from India indicate a variation in the estimates of mycobacteriuria in PT patients and this may perhaps reflect lack of a systematic approach.

In mycobacteriology laboratories, urine specimen is usually centrifuged and the deposit is processed for culture. Filtration of urine and processing of the filter membrane for culture could yield a better recovery of tubercle bacilli which are likely to float during centrifugation. So, the objectives of the present study were : (1) a systematic investigation for the presence of mycobacteriuria in PT patients in Madras to get an estimate of renal involvement in them, and (2) to employ a filtration method on an experimental basis, in addition to the established centrifugation method, to recover tubercle bacilli from urine.

Material and Methods

One hundred and thirty-seven bacteriologically confirmed PT patients aged more than 12 years, admitted (except one) consecutively in a clinical trial were included in the study. From each of these patients, before starting chemotherapy, three consecutive, entire, early morning urine specimens were collected and processed for culture of *M. tuberculosis* by the centrifugation method using multiple media.[5] The entire left over centrifuged deposit was inoculated into Selective Kirchner's liquid medium (SKLM). A total of 411 specimens were processed.

Of the 411 specimens, 405 from 135 patients were also processed by a filtration method as described below :

To about 50 ml of the urine, sodium dodecyl sulphate was added to give a final concentration of 2 mg/ml and then incubated at 37°C in a water bath for 30 minutes. This was filtered, using a syringe, through a filter membrane (Pore size : 0.45 micron; diameter : 25 mm; from Microdevices Pvt Ltd., Ambala, India) which had been treated with 0.028% Malachite Green solution and assembled in a filter holder (Laxbro, India). Then, through the same assembly, 10 ml of 1% sodium hydroxide was filtered slowly (in about 10 minutes) followed by 20 ml of sterile

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distilled water. The filter membrane, in the first 120 specimens, was implanted on a selective 7H11 (S7H11) agar plate and incubated. Since plate contamination was encountered frequently, the membranes in next 84 specimens were implanted on S7H11 agar slopes. As selective Kirchner's liquid medium supports the growth of even paucibacillary inoculum,[5] the filter membrane in last 201 specimens was cut into two halves, one half being implanted on a S7H11 agar slope and the other half transferred to about 7 ml of SKLM. The media were examined weekly for visible growth of tubercle bacilli and subcultured for identification by the standard procedures followed in this laboratory.

For computing the confidence intervals, the ''square root transformation method'' as recommended by Radhakrishna et al[6] was followed.

Results and Discussion

Of the 137 patient (irrespective of previons treatment), 5 (3.6%) excreted tubercle bacilli in the urine (95% confidence interval being 1.2% to 8.4%). History of previous treatment was available for 130 patients and 92 of them (70.8%) had denied previous anti-TB treatment. Three (3.3%) of the 92 untreated patients excreted tubercle bacilli in nrine (95% confidence interval being (0.6% to 9.3%).

M. tuberculosis was isolated in 4 patients in one of the 3 specimens by the centrifugation method. In the 5th patient, tubercle bacilli were recovered from 2 different specimens, one by the centrifugation method and the other by the filtration method.

In all, of the 411 urine specimens from 137 patients, 5 yielded *M. tuberculosis* by the centrifugation method and all the isolates were obtained from the subcultures into SKLM which were inoculated with the entire left over centrifuged deposit after inoculating a loopful of it onto 3 media, namely, Lowenstein Jensen (LJ) medium, LJ with pyruvate (LJP) and S7H11 slopes. In one specimen, LJP gave a positive culture in addition to SKLM. However, Challu et al[4] reported isolation of *M. tuberculosis* in 11.6% of 233 specimens from LJ slopes inoculated with a loopful of centrifuged deposit.

Of the 405 specimens from 135 patients tested by the filtration method only, one was culture positive and that was obtained on S7H11 agar slope, whereas Challu et al[4] had reported isolation of *M. tuberculosis* in 12.6% of 95 specimens, collected one each from their patients.

Clogging of filter membranes was experienced in 10% of urines while Challu et al[4] excluded 141 (60%) of 236 specimens from analysis due to clogging and inadequate quantity of urine. This could perhaps be due to the larger volume (100 ml) of urine used for filtration in their study. In the present study, 14 of 285 (4.9%) cultures were contaminated (excluding the first 120 cultures), while Challu et al[4] observed it in 33.7% of 95 cultures using 5% oxalic acid for decontamination and LJ medium slopes for culture in their filtration method. In spite of lower rate of contamination by employing a milder treatment procedure (1% $NaOH_2$, examination of multiple urine specimens and nsing 2 media for culture, the filtration method did not yield additional positives in the present study.

It is to be mentioned that in a preliminary experiment filtration method was found to recover *M. tuberculosis* H37Rv from all of 13 normal urine specimens (about 20 ml) artifucially seeded with about 50 to 500 viable bacilli. The failure to recover bacilli from urine specimens in the present study may perhaps be attributable to the presence of such a low number of bacilli that the portion taken for filtration might not have contained any bacilli.

All the 5 patients with mycobacteriuria were male (out of 107) and were more than 23 years old (range 23-55). The pretreatment radiographic findings showed that 3 patients had extensive bilateral lesions while the other 2 had lesions in the right apical region. The Mantoux reaction was 10 mm or above in all of them. Two had received anti-TB treatment previously for 6-9 months and the other 3 had none. Smear and culture results of pretreatment sputum from 4 of them showed that they were ''heavy positives''-with confluent growth from at least one of the 4 specimens. However, the 5th patient produced only a single colony from one of the 3 sputum specimens examined. Before admission to the study, one of them had complaints of burning micturition, haematuria, oliguria, pain in lumbar region and

tenderness in renal angle; another patient complained of frequency of micturition and nocturia; the remaining 3 patients had no symptoms. Absence of urinary symptoms and normal intravenous pyelogram have been reported in patients with mycobacteriuria.[1]

Of these 5 patients, one was not followed up as his pretreatment sputum smears were negative for acid fast bacilli and, thus, he became ineligible for admission to the chemotherapy study. One patient refused hospitalisation and no information could be collected on his renal function. The remaining 3 patients were referred for further investigations, immediately after the urine culture results were known. Intravenous pyelogram was normal in these 3 patients. Utrasonogram was normal in 2 patients and in one suggestive of bilateral renal disease.

The isolates from urine and sputum specimens of 4 patients were sensitive to Streptomycin, Isoniazid and Rifampicin. In the fifth patient, while the urine isolate was sensitive to Streptomycin, Isoniazid and Rifampicin the sputum isolate was sensitive to Streptomycin and Rifampicin but resistant to Isoniazid.

Four patients received short course chemotherapy and 2 responded to treatment. One patient showed radiographic and clinical deterioration due to non-tuberculous etiology, at the end of the 24th month, and developed pneumothorax at the 36th month. Another patient with initial Isoniazid resistance developed resistance to Rifampicin at the end of chemotherapy and the treatment was changed. Later, he had a favourable response to treatment. Three urine specimens, each collected at the end of treatment from the above 4 patieuts, were all negative for culture of *M. tuberculosis*.

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