Incidence of Typical and a Typical Mycobacteria in Pulmonary Infections at Lahore - A Study of 500 Cases

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Abstract

Five hundred sputum samples of clinically diagnosed or suspected tuberculous patients were subjected for the isolation of mycobacteria. Of these 239 (47.8 percent) were culture positive and 204 (40.8 percent) smear positive. Of the smear positive specimens 39 (7.8 percent) could not propagate on Lowenstein jensen medium. From total isolates, 235 strains were of Myco. tuberculosis and remaining four were atypical type. The atypical strains were classified on the basis of Runyon's classification. Two strains were of Myco. serofulaceurn: Group II, one Myco. intracellulare: Group III, and one Myco. smegmatis: Group IV. On animal pathogenicity test and Myco. serofulaceum less pathogenic for guinae lulare (Battey bacilli) was very low, whereas Myco.smegmatis did not cause any lesions or disease (JPMA) 34 : 84, 1984).

Introduction

Although the incidence of tuberculosis is not precisely known but it has been estimated that nearly as high as five percent of the human population in Pakistan is suffering from this disease (Report of Tuberculosis Survey, 1962). Even in many technically advanced countries deaths due to pulmonary tuberculosis and its socioeconomic losses are still important (W.H.O. Tech. Report. series, 1974).
The role of bacteriological examination of sputum in the diagnosis of tuberculosis and in effective control of the disease by chemotherapy has been high lighted during the past decade. Different species of mycobacteria are found in human pulmonary tuberculosis, the mostly encountered type is myco. tuberculosis while the others are Myco. Kansasii.
Myco. zenopi, Myco. cheloni, Myco. fortuitum, Myco. Smegmati c and Myco. intracellulare (Battey bacilli). Myco. serofulaceum and Myco. tuberculosis were found to be highly pathogenic pigs. The degree of virulence in case of Myco intracelsmegmatis Myco. simiae, which can also cause tuberculosis but are of rare occurrence (Dubos and Hirsch, 1965). Many workers reported the isolation of atypical mycobacteria from pulmonary tuberculous patients (Nel at al., 1977; Fonseca and Filho , 1978; Cooperative Study of Japanese National Sanatoria, 1970; Middle brook et al., 1955).
The number of prevalent mycobacteria varied from place to place and in some cases from hospital to hospital. Similar variations have also been reported in atypical myco -bacteria (Hobby et al., 1967).
The present study was undertaken to assess the occurrence of typical and atypical mycobacteria in pulmonary infections.

Material and Methods

Specimens of sputa were collected from ward and out patients of Institute of Tuberculosis and Chest Diseases, Mayo Hospital Lahore. A total of 500 patients diagnosed or suspected to be tuberculous on the basis of clinical or radiological findings were subjected to the study. Patients were given a sterile disposable plastic container and were asked to collect 5 10 ml of first morning sputa.
For processing of sputum samples, the concentration method of Kubica and Dye (1967), Vestal (1975) was followed and cultured on L.J. Media. The inoculated bottles were checked for the growth of

tubercie bacilli, Ziehl-Neelsen method was adopted for staining the smears. Pure cultures of the isolates were classified on the basis of Runyons classification (Runyon, 1959) and identified by morphological, cultural and bio-chemical tests. Two cultures of Myco. tuberculosis and all other four cultures of atypical mycobacteria were used for pathogenicity in guinea-pigs. The guinea-pigs weighing 450 gm each were selected and inoculated 1.0 mg intraparitonially and were housed singly in wooden cages as recommended by Royal commission on tuberculosis. On post-mortem the involvement of each organ was assessed by its enlargement, presence of tubercules (their numbers and size) presence of necrosis and othr characteristics and was quantitated roughly as heavy, moderate, scanty and minimal involvement in each organ (Mitchinson, 1964).

Results and Discussion

Of 500 sputum samples only 204 (40.8 percent) were smear positive by concentration method. Two hundred and thirty nine (47.8 percent) samples yielded growth of different species of mycobacteria. Thirty five (7.8 %) smear positive specimens showed no growth on Lowen Stein-Jensen medium. Of 239 culture positive cases, 235 were of Myco. tuberculosis (typical) the remaining four (1 .67%) were recognised to be atypical type of mycobacteria. Further classification of atypical mycobacteria were based on Runyon's Classification. These were identified as Myco. serofulaceum, two strains Myco. intracellulare (Battey Baclli), one strain; and Myco. smegmatis, one strain.

It is well known that tuberculosis is a wide spread disease in human population in this country with large number of open cases causing infection to other people. Importance of culture facilities in the diagnosis of such infection is well proven by smear and culture results. Smear positivity was found to be lower than culture positivity and thus if culture is not done many cases may be wrongly diagnosed as negative.

The aim of this investigation was to determine the type of mycobacteria involved in pulmonary tuberculosis. It is shown that iq pulmonary tuberculosis the main organism involved was Myco. tuberculosis as has previously been reported by Siddiqui (1977): Atypical mycobacteria are rare in pulmonary infections. Cursory inspection of the atypical acid fast cultures obtained from our patients showed that they could readily be separated into three groups on the basis of Runyon's classification. The strains which gave rise to typical colonies which did not become pigmented on exposure to the light were included in group III (Myco. intra cellulare). The strain of which grew profusely on solid Media and produced an orange pigmentation in the dark (scoto chromigens) were included in group II (M. serofulaceum). The fourth strain formed confluent growth on solid Media within two days and showed no pigmentation and was classified as group IV (M. smegmatis). When present atypical mycobacteria are associated with progressive disease clinically indistinguishable from classical tuberculosis (Keltz et al., 1958).

The results of virulance on guinea pigs are summarised in the table.

Table

Tuberculous Autopsy Results of Guineapigs Inocculated With Myco. Tuberculosis and Atypical Mycobacteria Isolated From Pulmonary Tuberculous Patients.

Specimen	S.No.	Date of Injection	Total Survived	Date Animal died.	Inoccula-tion Site	Tuberculous Involvement: Spleen	Liver	Lung	Kidney	Viscera	Direct Smear
TYPICAL											
Myco.tube-rculosis.	95	3-11-80	22	29-11-80	++++	++	++	+++	++	++++	Highly + for Lung, Viscera and site of inocculation
Myco.tube-rculosis.	200	"	25	02-12-80	++++	+	++	++++	+++	++++	Lung highly positive.
ATYPICAL											
Myco.sero-fulaceum.	82	"	23	30-12-80	++++	+++	++++	++	++	+++	Lung + Liver highly +ve.
Myco.sero-fulaceum.	492	"	35	12-12-80	+++	–	++	+++	++	+++	Highly +ve for site of inocculation.
Myco.intr-acellulare.	202	..	35	..	++	+	+	+	–	+++	Positive for liver.
Myco.smeg-matis.	318	..	35	..	–	–	–	–	–	–	Negative
CONTROL.	–	"	35	"	–	–	–	–	–	–	Negative.

Highly Positive = ++++ , Moderate Positive = +++, Scanty Positive = ++ , Negative = –. Minimal Positive = +

Most of the guinea pigs died within four weeks from the time of inoculation. Lesions grossly characteristics of tuberculosis were seen in all except one of these guinea pigs at autopsy. The smear made of involved tissues were uniformly positive for acid fast bacilli in these cases. The remaining one inoculated by group IV culture did not show any disease symptoms in guinea pigs when guinea pigs sacrificed after 35 days every organ conclusively demonstrated a gradation in virulence among the acid fast organisms tested. The culture of Myco. tuberculosis and group II strains (atypical) found to be equally virulent for guinea pigs. However the group III appeared to be least capable of producing progressive fatal disease while group IV strains appeared non pathogenic for guinea pigs. Similar findings have been reported by Keltz et at. (1958) and Jafri (1967).

The findings of these studies are very encouraging and it would be of a great value to carry out further extensive invasive investigations at a National scale involving culture work, type of isolates, drugs sensitivity, guinea pigs virulence and histopathology in order to have some definite information about etiology, prevalence and pathogenicity of this disease.

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