PULMONARY TUBERCULOSIS IN A BCG VACCINATED AREA: RELATIONSHIP OF DISEASE SEVERITY WITH IMMUNOLOGICAL AND HEMATOLOGICAL PARAMETERS AND DRUG RESISTANCE PATTERNS

Rabia Hussain[1], Rumina Hasan[1], Mohd Khurshid[1], A Willem Sturm[1], Jerrold J Ellner[2] and Ghaffar Dawood[3]

[1]The Department of Microbiology, Aga Khan University, Karachi, Pakistan, Current address, University of Durban, South Africa; [2]Division of Infectious Diseases, Case Western University, Cleveland, Ohio, USA; [3]The Masoomeen Trust Hospital, Karachi, Pakistan

Abstract. Clinical hematological and immunological parameters were studied in a group of 145 pulmonary patients with active tuberculosis, from a defined area of Karachi (Kharadar) belonging to the lower socioeconomic strata. Although clinical symptomatology could not differentiate the extent of lung involvement, a majority (69.6%) of the patients were diagnosed radiologically as having moderately advanced pulmonary disease. The peak number of patients were in their second decade of life. No differences were observed in the extent of disease based on age or gender. All hematological parameters for the group were in the normal ranges except for low levels of hemoglobin (9.58 ± 1.55 SD; normal range 12-14 mg/dl) and a high ESR (90 ± 31 SD; normal range 0-13 mm/hour). A negative correlation of PPD skin test induration ($r = 0.21$, $p = 0.02$), and a positive correlation of total white blood cell ($r = 0.20$; $p = 0.015$) was observed with the amount of lung tissue involved. The resistance amongst the strains for the four first line anti-tuberculosis agents was found to be: isoniazid = 27.4%; ethambutol = 14.5%; rifampicin = 11.29% and streptomycin = 12.9%. Multi-drug resistance to the most commonly prescribed combination (rifampicin and ethambutol) was 8.06%. Drug resistance patterns to individual drugs were comparable with resistance patterns observed in strains from greater Karachi at The Aga Khan Hospital during the same period. Such studies should provide improved rationale for patients diagnosis and treatment.

INTRODUCTION

The emergence of tuberculosis as one of the most important AIDS-related bacterial infections has revived interest in this old disease (Reidler *et al*, 1989). The decrease in incidence around 1970 in Western Europe was thought to be the result of strict case findings programs, early effective treatment and prophylactic regimens in contacts. In the last decade, an increase in tuberculosis was seen around the world with AIDS and drug resistance being two of the most apparent contributing factors (Thuer *et al*, 1990). For many reasons, tuberculosis was never controlled in developing countries where most of the world's population lives under conditions that favor spread of *Mycobacterium tuberculosis*. Development of resistance to the limited number of drugs available for the treatment is a real concern given the outbreaks of multi-drug resistant tuberculosis that have occurred in the US. Therefore, new strategies for control have to be developed. Prevention of spread of the bacteria, development of an effective vaccine and the synthesis of new drugs are the tools which could be used to achieve the goal of control of tuberculosis. All of these areas will benefit from a better understanding of the pathophysiology of tuberculosis.

Pakistan is a highly endemic area for tuberculosis with an estimated incidence of 150 cases per 100,000 population. The indicence of infection with HIV-1 is still very low as compared to international figures. There has been wide coverage with BCG vaccination since 1960 in Karachi, Pakistan. However, very little is known regarding the pathophysiology of the disease post BCG vaccination era. We have initiated these studies to understand the pathophysiology and immunology of the disease which may be modulated due to vaccine intervention. This paper focuses on the clinical characteristics and drug resistance to the most commonly prescribed regimens in a group of patients presenting with pulmonary tuberculosis at a charity hospital located in a densely populated and low

Correspondence: Dr Rabia Hussain, Department of Microbiology, Aga Khan University, Stadium Road, PO Box 3500, Karachi 74800, Pakistan.
Tel: 092-21-4930051 Ext 2149, Fax: 092-21-4934294.

income community of Karachi, Pakistan.

MATERIALS AND METHODS

Patient selection

Patients were recruited at a general hospital (Masoomeen Trust Hospital) operated by a charitable trust. The hospital has 86-bed inpatient facility and is located in a low income, densely populated area with an average household size of 6, living mostly in apartment complexes. Patients presenting at Masoomeen between January 1, 1991 and April 1, 1994 were enrolled in the study if they had not been formerly diagnosed at Masoomeen with this disease and if there was microbiological support for this diagnosis. Clinical information at presentation was obtained by means of a standardized questionnaire. Chest x-rays were performed on all subjects and evaluated by one of us (GD) using the classification of the National Tuberculosis Association of the USA into minimal, moderate and advanced lung tissue involvement (Crofton, 1990): Extent of lung involvement was further defined within the above three categories by assigning a score from 1-7 for extent of lung tissue involved with 1 being the least and 7 the most extensive lung tissue involvement.

Laboratory methods

Hematological data were collected on all patients on the day of enrollment. Sputum samples for microscopy were available from 139/145 patients. Microscopy was performed using Ziehl-Neelsen staining. Cultures were done by means of a set of Lowenstein-Jensen slants with and without glycerol. Susceptibility tests were performed by the proportion method (Griffith *et al*, 1967) on Middlebrook 7H10 agar. Two dilutions of each of the following drugs : Streptomycin, isoniazide, ethambutol and rifampicin. Acid fast bacilli were seen in all but 3 sputum samples. These were subsequently found to be positive by culture. PPD (5TU) (Connaught Labs, Ontario, Canada) was administered to all patients and read at 72 hours by the same individual. Pyrazinamide susceptibility was assessed by means of a bactericidal test in 7H9 broth pH 6.2 at concentrations of 50 and 25 mg/l as well as by means of a pyrazinamidase test (Buttler *et al*, 1983).

RESULTS

Patient characteristics

Tuberculosis was diagnosed in 145 patients. A total of 108 patients (74.4%) were newly diagnosed at enrollment. The ratio of males to females was 1 : 1 with no significant difference between age in the two groups (males; mean age = 40 ± SD 16, females mean age = 29.29 ± SD 16.68). Chronic diseases were documented in 26/145 (17.9%) of the patients. No differences in chronic disease were observed with respect to gender. The most common associated disease was diabetes mellitus (N = 14) followed by chronic lung disease (N = 9). Seven used or had used corticosteroids for lung disease and five were steroid users for another reason. The clinical signs and symptoms at presentation are given in Table 1. No statistically significant differences were found in the clinical characteristics of the newly diagnosed group compared to the group with poor treatment compliance or antituberculous treatment greater than three months before the time of study. It was interesting to note that anergy to PPD skin test defined as no induration after 72 hours was observed in 15/132 (11%) of the study group while an additional 7 patients (5.3%) were skin test negative (< 10 mm) despite microbiological and radiological evidence of established disease.

The age distribution is shown in Fig 1. Whereas males showed a random distribution in the differ-

Table 1

Clinical characteristics of study subjects.

Characteristic	%
Fever	100
Cough	98.6
Hemoptysis	11.8
Weight loss	92.4
Night sweat	48.9
Lymphadenopathy	9.6
Associated diseases	17.9
*PPD skin anergy	11.0

* no induration after 48 hours. The number of patients tested was 132

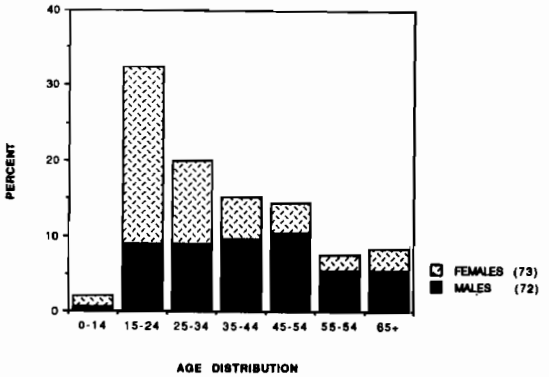

Fig 1–Age and sex distribution in 145 patients with active tuberculosis.

ent age categories, the greatest number of females with tuberculosis were between 15 and 24 years of age with a decreasing number with increasing age. The incidence of associated chronic diseases was higher (34%) in the older age group (> 45 years).

Disease classification

Minimal, moderate and advanced pulmonary disease was further scored from 1-7 given in square brackets based on the extent of lung tissue involved. Minimal was scored as (1) where radiologically slight to moderate density was observed involving one apex or (2) if both apices were involved but restricted above the second chondrosternal junction and the spine of the fourth or the body of the fifth thoracic vertebra; moderate was scored as (3) if disseminated confluent lesions of moderate density without cavities were observed and not exceeding more than a total of one lung volume or (4) if in addition to the above features, a single cavity but less than 4 mm was also observed; advanced was scored as (5) if miliary mottling was also present in addition to the above involvement or as (6) when exceeding one lung volume, and (7) with multiple cavities.

Relationship of age with disease severity

Fig 2 shows the relationship between age, sex and the extent of pulmonary disease. The extent of lung disease was comparable in males and females and there was no relationship with age in either group. A surprisingly high percentage (69.6%) of patients had moderate disease (score 2-4) across

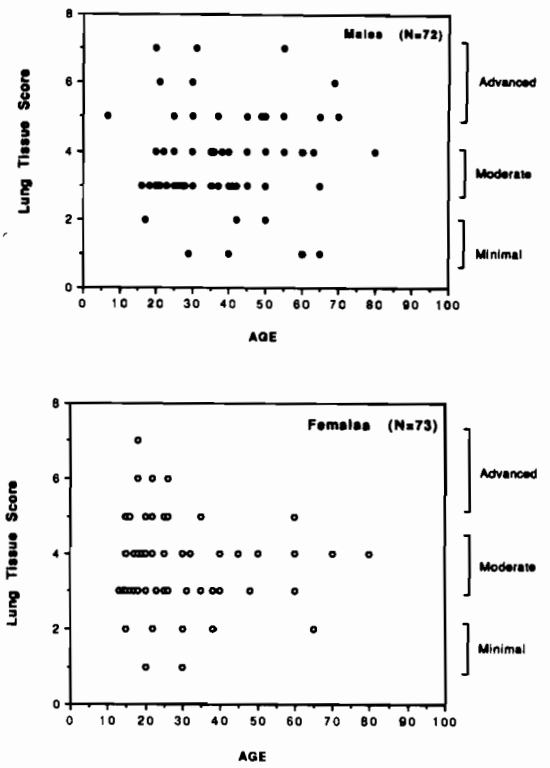

Fig 2–Relationship of severity of pulmonary disease with age and gender in patients with active tuberculosis.

the age spectrum in both males and females which may be related to previous BCG vaccination resulting in less severe disease.

Relationship of hematological parameters with disease severity

Group means for all hematological parameters were within normal ranges except for ESR which was raised (90 ± SD 31) and hemoglobin which was slightly decreased (9.58 ± SD 1.55) within the group. When hematological parameters were analysed in relation to disease severity (Fig 3) the only statistically significant relationship observed was with the white blood cell count which showed a significant positive correlation ($r = 0.2$; $p = 0.021$) with the extent of lung involvement and was due to an increase in neutrophils. ESR and hemoglobin showed no significant trend with the extent of lung disease (data not shown). Interestingly, PPD skin

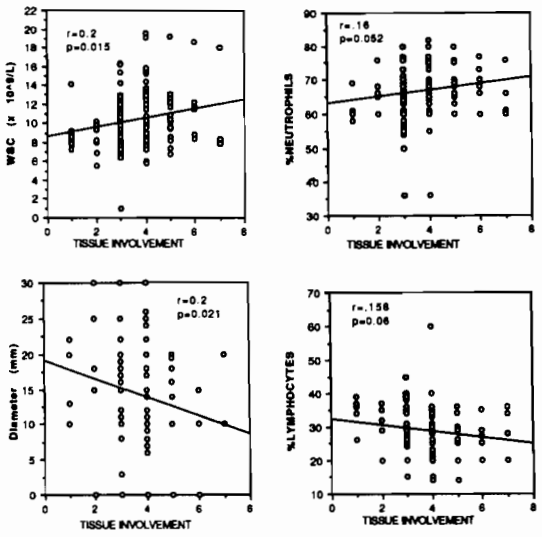

Fig 3–Relationship of hematological parameters and PPD skin test with severity of pulmonary disease. WBC = White blood cell. Linear regression analysis was carried out to determine the correlation between pulmonary disease and hematological parameters.

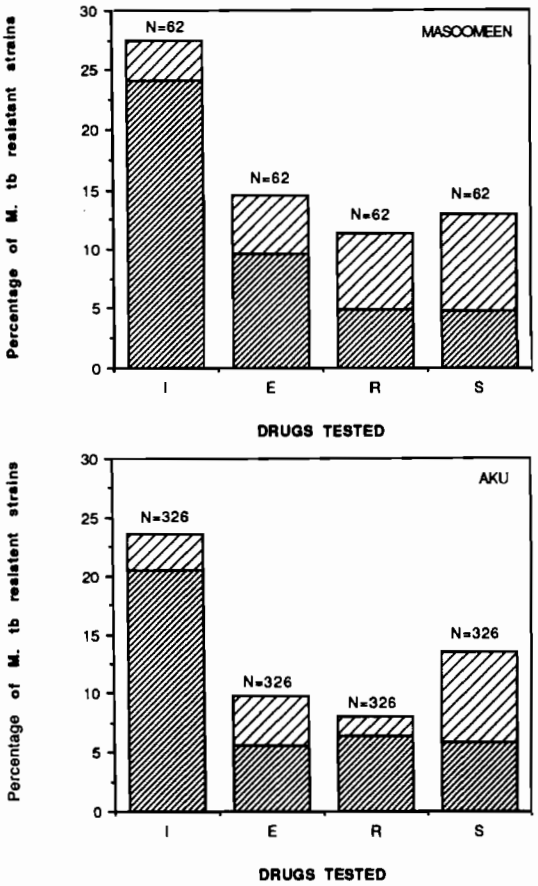

Fig 4–Percentage of isolates resistant to individual drugs. I = isoniazid; E = ethionamide; R = rifampicin; S= streptomycin.

test responsiveness showed a significant negative correlation with the extent of lung involvement ($r = 0.2$; $p = 0.021$) with a concomitant decrease in lymphocyte count. These results indicate that group means can mask the trends which exist in relation to disease severity.

Resistance to individual drugs

Strains for antibiotic susceptibility tests were available from 62 sputum samples from Masoomeen Hospital (Fig 4; top panel) and 326 samples from AKUH laboratory (Fig 4; bottom panel). All isolates were identified as *Mycobacterium tuberculosis*. Of the 62 strains tested, 54.5% were fully sensitive. The pattern of resistance to individual drugs at both intermediate and high drug concentrations is shown in Fig 4. Isoniazid resistance was seen in 27.4% of strains with a majority of strains showing resistant to the higher concentration of the drug also. Resistance to ethambutol and rifampicin was similar and was found in 14.5% and 11.29% respectively: streptomycin resistance was seen in 12.9%. AKUH strains which were from all sectors of Karachi showed slightly lower overall resistance.

Table 2 shows multi-drug resistance rates to drug combinations in the study isolates for the drug regimens most commonly prescribed at Masoomeen Hospital. At least 10/145 of the patients were being prescribed drugs to which they were completely resistant. Very little drug resistance information is available for pyrazinamide since resistance to this drug is not being routinely determined in any service laboratory in Karachi. In these preliminary studies relatively high level of resistance was observed. However, the high level of pyrazinamide may be biased due to small group size but these initial findings warrants further studies in this neglected area.

Table 2

Resistance to prescribed combination of first line drugs.

Drug combination	Prescribed to	% Resistant*
RE	98/145	8.06
REI	12/145	8.06
REZ	29/145	17.39

* Resistant rates were determined in 62 isolates except for pyrazinamide which was determined in only 23 isolates. R = rifamipicin; E = ethambutol; I = isoniazid; Z = pyrazinamide

DISCUSSION

This study describes the clinical and laboratory data in a group of 145 patients with active pulmonary tuberculosis. All but 3 of the 145 pulmonary cases were smear positive. This reflects our intake criteria which was microbiological confirmation for tuberculosis as evidence by positive smear for AFB and or culture, resulting in an exclusion of paucibacillary patients who were negative for acid fast bacilli. Only 3 smear negative sputa were included since these were eventually shown by culture to be *M. tuberculosis*. This also resulted in exclusion of younger children who tend to have a higher incidence of paucibacillary disease.

The age distribution in our patient population showed a striking difference between males and females. Whereas females showed the same declining frequency with age as reported for smear positive tuberculosis in other developing countries (Cauthen *et al*, 1987), there was an equal distribution among the different age groups in males. If the disease becomes better controlled in a population, a shift to older age is seen which is thought to be the result of a higher proportion of reactivated cases (Cauthen *et al*, 1987). One could speculate that the observed differences in this study, are the result of social and cultural factors where females tend to stay at home and are more likely to be exposed to sick members in the family, leading to more effective transmission of *M. tuberculosis*, whereas the majority of males in this community of Karachi in which the study took place work outdoors as laborers mostly at the seaport and come home during the night. Even their meals are taken in open-air eating places and not with the family. This difference in behavior could result in the differences between the sexes in age distribution. Studies in other sectors of greater Karachi will shed further light on this problem.

The classification of lung involvement was originally developed for prognostic purposes. It is based on the amount of lung tissue involved and the presence or absence of cavitary lesions (Crofton, 1990). This is the first report in which definition of disease has been carried out beyond this level and has provided insights into parameters which relate to disease severity which was not evident by clinical symptomatology. The most interesting relationship was observed with the hematological parameters. There was a positive correlation between the peripheral white blood cell counts and the extent of lung involvement. The high level of WBCs (neutrophils rather than lymphocyte) in the peripheral circulation in patients with pulmonary tuberculosis is in contrast with the general opinion that the WBC response in tuberculosis is mainly a monocyte and lymphocyte response (Ellner *et al*, 1990). One possible explanation for the increase in neutrophils in our study group may be superimposed bacterial infections. In patients admitted at the Aga Khan Hospital, sputa of tuberculosis patients processed for bacterial infections do not show a high rate of positivity. However, this issue needs to be analysed further to understand if the relationship between WBC count and TB is due to other underlying or complicating diseases or due to ethnic differences in the population.

Our study population shows a low percentage (15.5%) of patients with advanced disease. The time between the onset of symptoms and presentation at the hospital may play a critical role. In the US, this is usually between 1-2 months. Our experience at The Masoomeen Trust Hospital is between 2-4 months which is typical of "passive case-finding" in other developing country populations. Thus early presentation is not the explanation for the low incidence of advanced disease seen in this group. An alternative explanation may be related to the high BCG vaccination rate in Pakistan which could limit progression of the disease. The BCG status in our patient population is unknown but in medical students between the ages of 17 to 25 where the highest incidence of disease is seen, 93% (57/61)

had BCG scars. It also is of note that 57% of medical students and 87% of household contacts of patients with active tuberculosis were tuberculin positive as reported by us earlier (Hussain *et al*, 1995). The high rate of tuberculin reactivity in the two control groups is not surprising since this is a BCG vaccinated population and high level of PPD positivity is likely to be maintained due to latent foci as well as recent exposure. Disease in this population will therefore tend be more localized and is the most likely cause for the high percentage of moderate lung tissue involvement.

The overall rate of resistance in our study population and that seen in isolates form greater Karachi was not significantly different. In this study we have not addressed the issue of primary and secondary drug resistance which had been done in an earlier study in Karachi (Khan *et al*, 1993) where primary resistance was reported to be 17% and secondary resistance was reported in 36% of the isolates. Multi-drug resistant to the most commonly prescribed combination was unacceptably high. This combination was based on cost which is an important consideration in a charity hospital but would have disastrous consequence in the long term. Multi-drug resistance is an important problem in Pakistan (Aziz *et al*, 1986; Aziz *et al*, 1989) as it is in other countries. If resistance *in vitro* correlates with resistance *in vivo*, as many as 10% of patients were not treated optimally. The follow up study of our patient group which includes response to treatment should clarify this issue.

ACKNOWLEDGEMENTS

This work received financial support from the WHO/UNDP Program for Vaccine Development. We would like to thank Dr Bushra Jamil for helpful discussions, Miss Maqboola Dojki and Miss Farida Talat for technical assistance and Miss Regina Paul for excellent secretarial help.

REFERENCES

Aziz A, Ishaq M, Jaffer NA, *et al.* Clinical trial of two short-course (6 months) regimens and a standard regimen (12 months) chemotherapy in retreatment of pulmonary tuberculosis in Pakistan: results 18 months after completion of treatment (Lahore tuberculosis study). *Am Rev Respir Dis* 1986; 134 : 1056-61.

Aziz A, Siddiqui SH, Aziz K, *et al.* Drug resistance of *Mycobacterium tuberculosis* isolated from treated patients in Pakistan. *Tubercle* 1989; 70 : 45-51.

Buttler WR, Kilburn JO. Susceptibility of *Mycobacterium tuberculosis* to pyrazinamide and its relationship to pyrazinamidase activity. *Antimicrob Agents Chemother* 1983; 24 : 600-1.

Cauthen G, Pio A, ten Dam HG. Annual risk of tuberculosis infection in developing countries since 1975. CDC Atlanta/WHO International Document, 1987.

Crofton J, Clinical features of tuberculosis. In: Seton A, Seton D, Gordon A, eds. Crofton and Douglas Respiratory Diseases. 4th ed. London: Backwell Scientific, 1990; pp 395-421.

Ellner JJ, Boom WH, Edmonds KL, *et al.* Regulation of the immune response to *Mycobacterium tuberculosis*. In: Ayoub EM, Cassell GH, Branche Jr WC, Henry TJ, eds. Microbial Determinants of Virulence and Host Response. Washington DC, 1990; 77-91.

Griffith M, Barrett HL, Bodily HL, Wood RM. Drug susceptibility test for tuberculosis using drug impregnated disks. *Am J Clin Pathol* 1967; 47 : 812-7.

Hussain R, Dawood G, Abrar N, *et al.* Selective increases in antibody isotypes and IgG subclass responses to secreted antigens in tuberculosis patients and household contacts. *Clin Diagn Lab Immunol* 1995; 2 : 726-32.

Khan J, Islam N, Ajanee N, *et al.* Drug resistance of *Mycobacterium tuberculosis* in Karachi, Pakistan. *Trop Doctor* 1993; 23 : 13-4.

Reidler HL, Cauthen GM, Block AB. Tuberculosis and AIDS - Florida. *Arch Intern Med* 1989; 149 : 1268-73.

Thuer CP, Hopewell PC, Elias D, *et al.* Human immunodeficiency virus infection in tuberculosis patients. *J Infect Dis* 1990; 162 : 8-12.