SEROLOGICAL STUDIES OF MYCOBACTERIUM TUBERCULOSIS INFECTION IN NORTH INDIAN POPULATION

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SUMMARY

The per cent rate of antibody to Mycobacterium tuberculosis in different socio-economic groups of north Indian population was evaluated by enzyme linked immunosorbent assay and analysed for possible association with environmental factors. The overall positivity rate of antibody to M. tuberculosis was 23.9%. Alcohol, diabetes mellitus, mild malnutrition, minor environmental hazards and strenuous physical work did not increase the chances of invasive tuberculosis. Rural population and pregnant women from low socio-economic status families are at a high risk and staff nurses working in the hospitals have the highest risk of getting infections through close contact with patients. A clean environment of tribal population protects them from tuberculous infections. There is a high cross reactivity between M. tuberculosis and M. leprae — proteins.

INTRODUCTION

There are estimated to be about 20 million active cases of tuberculosis which infect between 50 and 100 million people annually in areas of highest prevalence[1]. India alone has over 10 million patients with radiologically active pulmonary TB, one fourth of which are sputum positive[1]. An estimated 50 per cent of our total population is infected with tubercle bacilli and they remain asymptomatic. Most surveys on tuberculosis carried out in India and abroad are based on either tuberculin skin testing[2,3] or positive bacteriology (smear and/or culture)[4-6]. These studies emphasize an important problem in tuberculin testing i.e., variability. Importance of bacteriological findings also remains controversial[7].

Natural and experimental infection due to *M. tuberculosis* produces both cell mediated and humoral responses, and specific antibody can be detected in the blood for a long period[8]. A number of serologic tests are available to detect antibodies to *M. tuberculosis* including the recently used soluble antigen fluorescent antibody (SAFA) test[9], and enzyme linked immunosorbent assay (ELISA)[10]. However, ELISA is the technique of choice for epidemiological studies because of its higher sensitivity and specificity[9-13], besides being simple and useful for large number of samples.

The prevalence of antibody to *M.tuberculosis* in different socio-economic groups in north Indian population was studied and analysed to find out their possible association with environmental factors, and cross reaction with other mycobacterium species.

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MATERIAL AND METHODS

The following groups of subjects (total 1629) were selected viz., Norwegian normal controls (66), voluntary blood donors (135), Central Reserve Police Force (CRPF) jawans (190), factory workers (117), healthy tribal population (76), fresh recruits (61), pregnant women (202), cord blood (119), student nurses (171), staff nurses (92), alcoholics (103), diabetics (156) and leprotics (141).

Norwegian normal controls: included male and female students and healthy blood donors of different socio-economic status from the University of Bergen, Norway (Samples courtesy). There was no recent or past history of tuberculosis among these subjects.

Voluntary blood donors: were men and women belonging to middle or high socio-economic status, and provided homogenous sample of Delhi population.

CRPF Jawans: represent a good sample of the country. Their living conditions are good with excellent nutrition of 3500-4000 calories per day. Fresh recruits mostly from neighbouring villages were newcomers to the army and were in the initial training period of 6-9 months.

Healthy factory workers: from a paper mill, in a suburban state 30-45 km from Delhi were included. Most of the workers were from a local semi-urban area, belonged to low socio-economic class and lived in poor sanitary conditions, although their clinical and nutritional status was good. They were exposed to an industrial environment and heavy physical work for the past 10-12 years.

Tribal population: belonging to Palaman district of Bihar was included because (i) the tribals live in interior parts of the rural and forest regions about 100 km away from modern civilization (ii) they drink water from wells and fresh streams and (iii) their environment is not polluted.

Pregnant women and cord blood: samples were obtained from local hospitals to see the revalence of *M. tuberculosis* antibodies in pregnant women and possible perinatal transmission through cord blood.

Students and staff nurses: fresh recruits to nursing courses (before exposure to hospital environment), and staff nurses working in the hospital for over 5 years were included to reveal possible environmental factors influencing infection due to *M. tuberculosis*.

Alcoholics: included young truck drivers, whose working hours ranged from 100 to 120 hours per week. They consumed 200 to 350 g. of alcohol every day and their daily calorie intake was 3500 to 4000 calories. However, living conditions were unhygienic, in crowded areas of the city, with poor sanitation.

Diabetics: Only clinically and biochemically overt diabetics were included, because (i) diabetics are more prone to infection and tissue invasion (ii) their total calorie intake being variably restricted, under nutrition is common in them.

Leprotics: This group was included to examine the presence of cross reacting proteins between *M. leprae* and *M. tuberculosis*.

Technique: Blood samples were collected and the serum separated and kept at -75 °C till the test was carried out. The samples were tested for IgG antibodies against *M. tuberculosis* by our own technique published elsewhere [11], using enzyme linked immunosorbent assay.

RESULTS AND DISCUSSION

Table-1 shows the presence of specific IgG antibodies to *M. tuberculosis* in different groups of population. Among the healthy controls (1422) tested, 340 showed a positive ELISA test thereby giving an overall infection rate of 23.9%.

TABLE 1: **Prevalence of antibodies to M. tuberculosis in different groups of population**

Groups	No. tested	No. positive	Percentage
Sarguja Tribes	76	3	3.9
Blood donors	135	19	14.1
Student nurses	171	27	15.8
Staff Nurses	92	45	48.9
Healthy Recruits (BHS)	61	23	37.7
CRPF Jawans	190	52	27.4
Pregnant Women	202	79	39.1
Cord Blood	119	24	20.1
Alcoholics	103	17	16.5
Diabetics	156	28	17.9
Factory Workers	117	23	19.7
Total	1422	340	23.9
Norwegian (normal)	66	2	3.0
Leprotics	141	87	61.7

Two of the 66 (3.0%) healthy controls among students and blood donors from Norway were positive for antibodies to *M. tuberculosis*. This group served as reference to our own normal healthy controls where 75% of them were exposed to tubercle bacilli as revealed by tuberculin test[14]. Illiteracy, overcrowding and unplanned urbanization are shown to be among the major factors contributing to the growth and spread of tuberculosis. The tribal population studied, though exposed to mild nutritional deficiencies, their environment is quite healthy. They live in interior parts of the rural and forest regions obtaining drinking water from well and fresh streams. They had antibodies to *M. tuberculosis* only in 3.9% subjects, demonstrating a very low rate of exposure to *M. tuberculosis*, comparing well with infection rate from European controls. However, in voluntary blood donors, which formed a homogenous sample of urbanised population, the infection rate of tuberculosis was 14.1%. This was taken as the baseline data for comparison with other groups.

Diabetes mellitus predisposes individuals to bacterial infections. However, in the present series of 156 patients only 28 (17.9%) gave positive serological test for IgG antibodies to *M. tuberculosis*. This prevalence rate was not significant in comparison to normal voluntary blood donors. Thus, in the present series diabetes mellitus did not have any increased exposure to *M. tuberculosis*, which is contrary to the earlier reports[15].

Alcoholics and factory workers showed infection rates of 16.5% and 19.7% respectively. Nutritional status of both groups was good. This difference was not significant in comparison to the homogenous blood donor group, suggesting that alcoholism and minor aberrations of the environment do not constitute a high risk to contact tuberculous infection. However, the present findings cannot rule out the role of malnutrition, bad personal hygiene and overcrowding in causing increased risk of tubercular infection. No environmental factors could be attributed as the possible cause of tubercular infection in earlier studies also[16].

In a comparison between low and high risk groups, the rate of incidence of infection due to tuberculosis was tested in 171 recently recruited nurse students and 92 staff nurses who had already spent over 10 to 15 years in the hospital environment. The infection rate was 15.8% and 48.9% in the two groups respectively. The difference between the two groups was significant ($p = < 0.01$) suggesting that a frequent exposure to hospital patients may be a very important predisposing factor for infection due to *M. tuberculosis*. A strong association between infection risk among contacts has already been demonstrated[4].

In pregnant women attending the antenatal clinic of Employees State Insurance hospital, the rate of infection with *M. tuberculosis* was found out to be 39.1% and, 20.1% of these women passed on the antibodies to their babies, as revealed from the testing of the cord blood ($p = < 0.10$). The antibodies in new born babies may be transient and whether they have any effect on their immune system, causing a delayed immune response to BCG vaccination is not known. Bad personal hygiene during pregnancy with frequent visits to hospital during pregnancy could be among the factors responsible for high rate of tuberculous infection in pregnant mothers in the present study. Anaemia is yet another factor[17], which was not studied by us.

Twenty three of the 61 healthy recruits (37.7%) representing the rural population and 27.4% of the paramilitary force i.e., Central Reserve Police Force (CRPF), which represents the mixed population, showed the presence of IgG antibodies to *M. tuberculosis*. CRPF personnel were selected from the group stationed at Jammu and the samples were collected during the waterborne epidemic of non-A non-B hepatitis. These patients had a sub-clinical infection of invasive amoebiasis as revealed by high per cent positivity rate of 73 per cent for antibodies to *E. histolytica*[18], suspected again to be due to waterborne infection. A non-significant difference between the normal healthy population and CRPF personnel suggests that infections of *M. tuberculosis* are not related to water borne disease.

Eightyseven of the 141 leprotic patients (61.7%) showed the presence of IgG antibodies to *M. tuberculosis* proteins, which shows a high cross reactivity between the mycobacterium proteins. The ELISA test cannot be specific due to cross-reacting proteins of mycobacterial species causing tuberculous lesions in different organs.

Our study of different groups of individuals suggests that alcohol, diabetes mellitus, mild malnutrition, minor environmental hazards and strenuous physical work did not increase the chances of invasive tuberculosis. Rural population and pregnant women from low socio-economic status families are at a high risk of acquiring infections due to *M. tuberculosis*. Staff nurses working in the hospitals, are at the highest risk of getting infections through close cantact with patients. The rate of infection varied from 3.1% to 48.9% in normal healthy groups and is comparable to the overall prevalence of tuberculous infection (past history of positive skin test) in the elderly of 35.5%[3] and varied between 10-41% (mean 25%) of elderly patients in a nursing home[19].

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