ORIGINAL REPORT

Incidence of Isolation of Mycobacterium Tuberculosis from Blood Samples in Tuberculosis Patients in Imam Khomeini Hospital, Tehran, Iran

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Received: 16 Jun. 2010; Received in revised form: 4 Dec. 2010; Accepted: 8 Jan. 2011

Abstract- It is estimated that one third of the world's population is latently infected with tuberculosis (TB). The HIV epidemic fuels the TB epidemic by increasing the risk of reactivation of latent TB infection and by facilitating a more rapid progression of TB disease. Although the incidence of TB is constant or decreasing in many regions of the world, rates remain high in developing countries as a consequence of the HIV epidemic. This study was conducted as a collaboration of the Infectious Diseases department of Imam Khomeini Hospital with the Microbiology department of Tehran University of Medical Sciences. The hospital dataset of 94 patients admitted with TB during 2003-2005 was reviewed. We aimed to study factors correlating with positive blood culture including age, sex, immune deficiency status, HIV serology and SIRS (Systemic Inflammatory Response Syndrome) status. In this study, we found that positive blood cultures are more frequent in patients less than 45 years old. Positive blood cultures were also more frequent in HIV infected patients and there was a significant correlation between blood culture and SIRS status. Therefore, we recommend that we obtain blood cultures from these high-risk groups in order to increase early detection of TB.

Acta Medica Iranica, 2011; 49 (8): 556-559.

Keywords: Incidence; Tuberculosis; Immunity status

Introduction

Tuberculosis (TB) is a major cause of illness and death worldwide, especially in Asia and Africa. Globally, 9.2 million new cases and 1.7 million deaths from TB occurred in 2006, of which 0.7 million cases and 0.2 million deaths were in HIV-positive patients.

According to the latest WHO report on global tuberculosis control, the incidence of TB in Iran has declined from 20,308 cases in 1990 to 15,545 cases in 2006, and its mortality has declined from 2,358 cases to 1,839 cases per year. The prevalence of HIV in incident TB cases is reported to be 2.2% and the TB incidence and mortality among HIV infected patients is less than one percent (1, 2).

TB is the leading cause of death among persons with AIDS, killing one out of every three people with AIDS. The increasing incidence of TB due to its association with AIDS is causing global concern (3). The risk of tuberculosis increases shortly after HIV seroconversion, with the risk doubling within the first year. The annual incidence of tuberculosis is about 10% in HIV-infected individuals from high-burden communities in both industrialized and developing countries. This risk increases further with serious immunosuppression; an annual incidence as high as 30% has been reported in South African patients with clinically advanced HIV (4, 5).

In adults with normal immune systems, TB disease is usually confined to the lungs. However, in persons with immune suppression, tubercle bacilli frequently disseminate beyond the lungs and cause disease in other organ systems (6).

In the past 20 years, countries with HIV epidemics have seen a dramatic increase in extrapulmonary TB cases and deaths. About one in five TB cases worldwide is considered extrapulmonary, with the most common sites of disease including the lymph nodes, meninges, pericardium, peritoneal cavity, and intra-abdominal organs (7-9).

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Successful control of TB depends on rapid detection of the *M. tuberculosis* complex to allow for early treatment and proper isolation of patients, thereby decreasing the likelihood of spread to others. The simplest rapid method is the detection of acid-fast bacilli by microscopy. However, 40 to 60% of patients with pulmonary disease and ~75% of patients with extra pulmonary disease are smear negative, and in this situation even contemporary culture methods take several weeks to become positive. Detection of *M. tuberculosis* in culture and/or characteristic histological features is generally required to establish the diagnosis. Sensitivity of these methods is moderately good (71%) (6,10). TB is associated with male sex, HIV infection, smoking (with a dose–effect relationship), history of asthma, family history of TB, and adult crowding (11).

In this study we evaluated the lowered immunity status and demographic factors that influence the burden of disease among TB patients, including age, sex, immune deficiency status, HIV serology and SIRS (Systemic Inflammatory Response Syndrome) status. We aimed to evaluate whether these factors correlate with positive blood cultures for *M. tuberculosis* in patients from Imam Khomeini referral hospital in Tehran, Iran in 2004.

Materials and Methods

This study was conducted as collaboration between the Infectious Diseases department of Imam Khomeini Hospital with the Microbiology department of Tehran University of Medical Sciences. The hospital dataset included patients admitted with TB during 2003-2005. Patients' demographic data, clinical presentation and radiological and pathological findings were analyzed.

After reviewing the dataset, a total of 94 patients were included in the study. Demographic data was gathered by a questionnaire form completed by a physician, which acquired information such as age, sex, immune deficiency, HIV serology, SIRS status and blood culture categories.

SIRS scoring is used to indicate the body's reaction to injury or infection. Patients can be diagnosed as having SIRS if they present with two or more of the following criteria: body temperature over 38°C or less than 36 °C; heart rate greater than 90 beats per minute; respiratory rate greater than 20 breaths per minute or PCO_2 less than 32 mmHg; white blood cell count greater than 12 x $10^3/mm^3$ or less than 4 x $10^3/mm^3$, or the presence of more than 10% immature neutrophils. Informed consents were taken from patients before taking their blood samples for evaluation. This study was approved by the Institutional Review Board (IRB) of Tehran University of Medical Sciences.

At least 5 ml of blood were collected in BACTEC vials and were sent to the Microbiology department. Sediments were cultured on chocolate agar, and Lowenstein-Jensen cultures and Ziehl-Neelsen staining of the smears were performed.

After obtaining positive blood cultures for *M. tuberculosis*, smears were stained with Auramine and Ziehl-Neelsen method.

Low amounts of *M. tuberculosis* in samples could result in loss of detection with BACTEC method and false negative outcomes. In order to overcome this pitfall, the Lowenstein-Jensen cultures were prepared to show the growth of colonies on solid culture. Niacin tests could differentiate the TB from Non-Tuberculous Mycobacteria (NTM). Chocolate agar is used for detection of other contaminating pathogens.

In order to better evaluate the correlation between positive blood cultures and immune deficiency, HIV infected patients were excluded from the immune deficient group.

Data analysis was conducted by SPSS software. Chi-square test was used to evaluate positive blood culture and its correlation with different factors. *P* value less than 0.05 was considered significant.

Results

Age, sex, immune deficiency status, HIV serology, SIRS and blood cultures were evaluated in a total of 94 patients of which 60 were infected with pulmonary tuberculosis and 34 with extrapulmonary tuberculosis.

36 women (38.3%) and 58 (61.7%) men were studied in this survey. The youngest patient was 14 years old and the oldest was 88 years old, with an average age of 44.3 and mean age of 39.5 years old and standard deviation of 20.7.

84 patients (89.4%) were immune competent and 10 patients (10.6%) were immune deficient, consisting of 7 patients with diabetes mellitus and 3 patients on chemotherapeutic drugs. 20 patients were HIV positive and 33 patients were SIRS positive.

21 patients had positive blood cultures for *M. tuberculosis* while 73 had a negative culture. Among the 60 patients infected with pulmonary tuberculosis, there were 32 men and 28 women with an average age of 45 years old. Among these patients, 7 patients (11.6 %) were immune deficient, 15 patients (25%) were HIV positive, 27 patients (45%) were SIRS positive and 15

patients (25%) had a positive blood culture for *M. tuberculosis*. There were 34 extra pulmonary infected patients that comprised of 26 men and 8 women, with an average age of 41 years old. Three patients (8.8%) were immune deficient, 5 patients (14%) were HIV positive, 6 patients (17.6%) were SIRS positive and 6 patients (17.6%) had a positive blood culture for *M. tuberculosis*.

Extra pulmonary tuberculosis included spondylitis (6 cases), lymphadenitis (5 cases), soft tissue infection (4 cases), peritonitis (3 cases), pericarditis (3 cases), arthritis (2 cases), and 11 cases of diffuse organ involvement.

Blood culture correlations

Patients were divided into two groups according to their age. In the group aging less than 45 years, 17 patients (30.4%) had a positive culture for *M. tuberculosis* and 39 patients (69.6%) had a negative culture. Age had a significant correlation with positive blood culture (P=0.023) in patient older than 45 years old. Four patients (10.5 %) had a positive culture while 34 patients (89.5 %) had a negative culture. Therefore, positive blood cultures are more frequent in a younger population. Among the female patients, 4 (11.1%) had a positive blood culture and 32 (88.9%) had a negative one. Positive blood cultures were more frequent in men (p=0.039). 17 men (29.3%) had a positive culture while 41 (70.7%) had a negative one.

Among immune deficient patients, 40% had a positive blood culture while 6 (60%) had a negative one. In addition, 17 immune competent patients (20.2%) had a positive blood culture while 67 (87.8%) had a negative one. Positive blood cultures and its association with immune deficiency was not significant (P=0.16).

Among HIV positive patients, 12 (60%) had a positive blood culture and 8 (40%) had a negative one. On the other hand, 9 HIV negative patients (12.2%) had a positive culture and 65 (87.8%) had a negative culture (P<0.001). Positive blood cultures were more frequent in HIV infected patients.

Among SIRS positive patients, 13 (39.4%) had a positive culture and 20 (60.6%) had a negative one. In SIRS negative patients, 8 (13.1%) had a positive culture and 53 (86.9%) had a negative one (P=0.04). There is a significant correlation between positive blood cultures and the presence of SIRS.

Discussion

In our study, positive blood cultures were more frequent among men, similar to an estimated 2:1 male-to-female sex ratio of cases notified elsewhere in Europe and worldwide (12,13). Reasons for global sex differences in the epidemiology of tuberculosis remain largely unknown.

Positive blood cultures were also more frequent in HIV infected patients in our study. As mentioned above, HIV–infected patients have a higher risk of being infected by TB. HIV critically impairs cell-mediated host responses to *M. tuberculosis*. Numeric depletion of *M. tuberculosis*-specific CD4 lymphocytes and functional impairment of CD4 lymphocyte–macrophage interactions result in impaired granuloma formation, ultimately leading to failure to impede *M. tuberculosis* replication. The interaction between TB and HIV is bidirectional. Activation of mononuclear cells during the host response to TB leads to accelerated HIV replication, which may increase HIV load at anatomical sites involved with TB and systemically (14).

In our study blood cultures were positive in 22.3% of tuberculosis infected patients (25% in pulmonary tuberculosis and 17 % in extrapulmonary tuberculosis). Presentation of TB is often unmodified in HIV-infected individuals with well-preserved immunity. With progressive immunodeficiency, however, attenuation of host tissue-damaging responses and failure of mycobacterial containment result in an increased likelihood of atypical presentation. In general, the appearances tend to be more subtle and nonspecific, and chest radiography can be normal despite extensive pulmonary infection. The proportion of extrapulmonary tuberculosis is increased, most commonly involving the lymph nodes, pleura, pericardium and CNS. Disseminated and miliary TB are also more common (14). More than 50% of TB occurring in advanced AIDS is associated with extra pulmonary disease and approximately 20% of extrapulmonary TB in the U.S. is associated with HIV infection (15, 16).

We found a significant correlation between age and positive blood culture (P= 0.023) in patients older than 45 years old that could be due to more HIV positive patients in this age group. The increasing age of previously HIV-infected persons increases the risk of Mycobacterium tuberculosis reactivation due to advancing immunosuppression. Since the disproportionate increase in notification rates in London between 1987 and 1993, the TB incidence has increased most in men in the 15-34 year age group (17).

There was also a significant correlation between blood culture and SIRS positivity. In studies with fewer positive blood cultures, diffuse tuberculosis involvement was also lower. In our study, HIV positive patients and

SIRS positive patients had a higher rate of diffuse involvement and bacteremia and the blood culture was more often positive. In this study, there were not a significant correlation between positive blood cultures and immune deficiency due to inadequate samples.

In this study, the positive blood culture in HIV positive, SIRS positive, and in men under 45 years was more frequent. Therefore, we recommend that screening for TB with blood cultures be conducted in these groups in order to increase early detection of TB. We also suggest that when screening patients for active tuberculosis disease, irrespective of the pulmonary or extra-pulmonary involvement, a screen for HIV infection should be done as well to detect co-infections.

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