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The effect of hepatotropic virus (HBV-HCV) infections on tuberculin skin test in patients with cirrhosis

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Background/aims: *To optimize the detection of infected persons and to treat latent tuberculosis infection are essential in patients taking part in a liver transplantation program. The tuberculin skin test is currently used to detect Mycobacterium tuberculosis infection and has widespread usage. This study aimed to evaluate the effect of hepatitis B and C viruses on tuberculin skin test results in patients taking part in the liver transplantation program in our center.* ***Material and Methods:*** *A total of 90 cirrhosis patients who participated in the liver transplantation program between January 2004 and February 2005 were included in this study. None of the patients displayed signs of active tuberculosis during the follow-up.* ***Results:*** *The mean indurations of the tuberculin skin test were found to be 14.7±6.9 mm in patients with a viral etiology and 6.1±5.4 mm in those with a non-viral etiology. The tuberculin skin test findings were significantly higher in end-stage liver disease caused by viruses than those with a non-viral etiology (p<0.05). The mean induration of the tuberculin skin test in Child B patients was found to be 7.15±6.4 mm, while in Child C patients it was 12.64±7.5 mm. In Child C patients, the tuberculin skin test scores were significantly higher than in Child B patients (p<0.05).* ***Conclusions:*** *These observations show the need for new methods to detect latent Mycobacterium tuberculosis infection in liver transplant candidates. Further research is needed to understand the reasons for the higher tuberculin skin test results with a viral etiology and in Child C patients.*

Key words: Tuberculin skin test, purified protein derivative (PPD), liver, hepatotropic viruses, liver transplant recipients

Siroz hastalarında hepatotropik virüs (HBV-HCV) infeksiyonlarının tüberkülin deri testi üzerine etkisi

Amaç: *Karaciğer transplantasyon programına başvuran hastalarda latent tüberküloz enfeksiyonunu tedavi etmek ve enfekte kişileri saptamak önem arzetmektedir. Yaygın bir kullanıma sahip olan tüberkülin deri testi Mycobacterium tuberculosis enfeksiyonunu saptamada kullanılır. Bu çalışmanın amacı merkezimizde karaciğer transplantasyon programında olan hastalarda hepatit B ve C virüslerinin tüberkülin deri testi sonuçlarına etkisini değerlendirmektir.* ***Yöntem ve Gereç:*** *Çalışmaya Ocak 2004 ve Şubat 2005 arasında karaciğer transplantasyon programına alınan 90 siroz hastası dahil edilmiştir. Takipleri boyunca hastalar aktif tüberküloz bulgusu göstermemişlerdir.* ***Bulgular:*** *Tüberkülin deri testi endurasyonu viral etyolojiye bağlı siroz hastalarında ortalama 14.7±6.9 mm, viral olmayan nedenlere bağlı siroz hastalarında ise 6.1±5.4 mm saptanmıştır. Tüberkülin deri testi sonuçları viral nedenli siroz hastalarında, viral olmayan nedenlere bağlı siroz hastalarına göre anlamlı yüksek bulunmuştur (P<0.05). Ortalama tüberkülin deri testi endurasyonu Child B hastalarda 7.15±6.4 mm, Child C hastalarda 12.64±7.5 mm olarak saptanmıştır. Child C hastalarda Child B hastalara gore tüberkülin deri testi anlamlı yüksek bulunmuştur (P<0.05).* ***Sonuç:*** *Bu bulgular karaciğer transplantasyon adaylarında latent Mycobacterium tuberculosis enfeksiyonunu saptamak için yeni yöntemlere gereksinim olduğunu göstermektedir. Viral etyolojiye bağlı hastalarda ve Child C hastalarında yüksek tüberkülin deri testi sonuçlarının nedenlerini anlamak için ileri çalışmalara gerek vardır.*

Anahtar kelimeler: Tüberkülin deri testi, PPD, karaciğer, hepatotropik virüsler, karaciğer transplantasyon alıcıları

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INTRODUCTION

The immune system is depressed in chronic liver disease (1-4). As a result, infection in these patients, especially tuberculosis (TB), is an important problem in areas where TB is endemic. The TB notification rate is 25 cases / 100,000 population / year in Turkey (5). The frequency of TB in liver transplant recipients ranges from 0.9-2.3% in Europe and the United States (6). The absence of TB infection in a high-risk population, especially patients in a liver transplantation program, is crucial, and in this group, early diagnosis of TB is essential. The guidelines of the American Society of Transplantation advise that all candidates for organ transplantation should undergo tuberculin skin test (TST) regardless of Bacillus Calmette-Guérin (BCG) vaccination status (7).

Hepatitis B virus (HBV) infection is one of the most common causes of liver disease. It is known that antigen-specific cytotoxic lymphocytes and T-helper cell responses are responsible for the elimination or the control of HBV infection (8,9). Virus-specific CD4 –CD8 T lymphocytes and the liver are responsible in hepatitis C virus (HCV) infection for immune system response (10-13). A wide range of clinical manifestations are associated with HCV. It can cause various immune-mediated disorders. However, the precise effect of HCV infection on the host's immune system has not been well-described.

The TST, which measures the *in vivo* cellular immune response to *Mycobacterium tuberculosis* purified protein derivative (PPD), is currently used to detect *M. tuberculosis* infection in humans. The cellular mechanisms responsible for PPD reactivity are related mainly to previously sensitized CD4 (+) T cells, which are attracted to the skin test site (14).

It is important to evaluate the TST, to optimize the detection of infected persons and to treat latent tuberculosis infection (LTBI) in the cirrhosis population awaiting organ transplantation. TST is currently the only widely used method for identifying LTBI. Studies have implicated that there were no significant differences between the percentages of TB in TST-positive and -negative patients and that TST could be an imperfect LTBI identifier in transplant candidates (15-18). False-negative results, mainly due to immunosuppression, preclude the treatment of truly infected persons, and the treatment of individuals with false-positive results, often due to M. bovis BCG vaccination, reduces the cost-effectiveness of preventive interventions. The immunosuppression setting is suspected of enhancing the anergy rate in cirrhotic patients.

The aim of this study was to evaluate the effect of viral hepatitides on the host response to TST results and to evaluate the value of TST as a screening method for detection of latent *M. tuberculosis* infection in patients with cirrhosis who are in a liver transplantation program in a TB-endemic area.

MATERIALS AND METHODS

Study Population

A total of 90 cirrhosis patients who took part in the liver transplantation program in Başkent University Medical Center between January 2004 and February 2005 were included in this study and followed up until November 2008. These patients were classified into two groups. The first group included those with a viral etiology caused by hepatitis B (n=30), hepatitis C (n=9), hepatitis B+C (n=3), and hepatitis B+D (n=2). The second group included patients with a non-viral etiology: cryptogenic (n=17), alcohol (n=10), sclerosing cholangitis (n=3), autoimmune hepatitis (n=2), Wilson's disease (n=6), Wilson's disease + Alcohol (n=1), oxalosis (n=1), primary biliary cirrhosis (n=2), biliary atresia (n=1), Caroli disease (n=1), Budd-Chiari syndrome (n=1), and alpha-1 antitrypsin deficiency (n=1). All the patients' Child scores were calculated and the following parameters considered: albumin, bilirubin, prothrombin time, encephalopathy, and assit. The study protocol was approved by the ethical committee for clinical trials in Başkent University.

TST Method

Patients with end-stage liver disease undergoing evaluation for liver transplant candidacy at Başkent University Medical Center routinely received TST as part of the pre-transplantation work-up. The TST was administered via the Mantoux method, made on the volar side of the forearm, and 72 hours after intradermal challenge, the injection site was examined and documented by infection control practitioners.

Tuberculosis Screening

All patients were monitored for previous TB history and TB contact and examined by means of a chest X-ray, serum C-reactive protein (CRP) and sedimentation rate. None of the patients included were found to have active TB.

Statistical Analysis

Statistical analysis was performed with SPSS version 13.0 for Windows. The differences between the continuous variables were expressed as mean±SD. Comparisons between continuous variables were performed with a non-parametric Mann–Whitney U test, whereas a chi-square test was performed for the comparison of the proportions of each categorical variable between the patients and controls. A probability value <0.05 was considered significant.

RESULTS

Ninety subjects were included in the analysis (67 males, 23 females; age range: 3-81 years; mean: 38.27±17.3 years). None of the patients displayed signs of active TB during the follow-up. Thirty-six patients underwent transplantation, and 8 of these patients died after transplantation follow-up.

The mean age of the 43 (47%) patients with a viral etiology was 44.4±11.8 years, while that of the 47 (53%) patients with a non-viral etiology was 32.5±19.6 years. Of those with a viral etiology, there were 19 Child B and 24 Child C patients. Those with a non-viral etiology included 20 Child B and 27 Child C patients. The mean indurations of the TST were found to be 14.7±6.9 mm in the first group with a viral etiology and 6.1±5.4 mm in the second group with a non-viral etiology. The TST findings were significantly higher in end-stage liver disease caused by viruses than in those with a non-viral etiology ($p<0.05$).

There were 39 Child B and 51 Child C patients. The mean TST induration was found to be 7.15±6.4 mm in Child B patients and 12.64±7.5 mm in Child C patients, and the difference in PPD scores between the groups was statistically significant ($p<0.05$).

A comparison of end-stage liver disease patients' TST scores with Child scores caused by viral and non-viral etiology can be seen in Table 1.

Table 1. PPD results in viral and non-viral groups according to Child scores. Values are expressed as mean±SD.

	Child B	Child C
Viral	10.05±6.8 mm*	18.45±4.41 mm*
Non-Viral	4.4±4.69 mm	7.4±5.68 mm
P	p<0.05	p<0.05

*p<0.05 compared to non-viral PPD results.

DISCUSSION

The salient findings of the present study are: i- TST findings were significantly higher in end-stage liver disease caused by viruses than that with a non-viral etiology, and ii- TST scores were significantly higher in Child C patients than in Child B patients.

Patients with cirrhosis of the liver have an impaired immune response. They have an increased incidence of infection (2,3,4,19). These patients have a reduced response to delayed-type hypersensitivity skin tests compared with age-matched controls (1,20). The reasons for impaired immune response are not well known. Respectively, immune suppression in chronic liver disease was associated with the soluble form of intercellular adhesion molecule-1 (sICAM-1) and alkaline phosphatase levels (20). In clinical observations, HCV infections were associated with low interleukin (IL)-2 and high IL-10 production (21-23), whereas IL-2 promotes Th1 cell proliferation and is required for sustained CD8+ T cell responses (24). IL-10 acts as a general suppressor of inflammatory responses and induces an anergic state in Th1 and Th2 cells (25). IL-10 is increased in the CD4+ lymphocytes of HBV chronically infected patients. An altered Th cell costimulation and a predominantly inhibitory environment have been demonstrated in chronic hepatitis B infection (26). Patients with liver cirrhosis often failed to respond to vaccinations such as HBV vaccine (27). With this immune suppression setting in cirrhosis, especially of a viral etiology, higher anergy rates are expected, but this was not observed in this study.

Contrary to expectations, the TST values in our study were found to be higher in cirrhosis with a viral etiology than in that with a non-viral etiology. Larke et al. (28) found that a significantly greater proportion (76.2%) of HBsAg-positive patients were PPD-reactive in comparison to those who were positive for anti-HBs (67.2%) or who had no HBV markers (43.2%). The inverse association of HBeAg and PPD reactivity was confirmed across all age groups in both BCG-vaccinated and non-vaccinated groups (29). In light of these studies, we may speculate that viral antigens and viral load may influence TST results. Every country has its own virological and environmental characteristics, and HBV behavior can show differences according to the studied population. As HBeAg-negative patients represent the majority of HBV-positive patients with chronic liver disease in Turkey, this can partly exp-

lain the higher TST values in viral cirrhotic patients. Our results showed that TST is not a reliable method to detect latent TB in cirrhosis patients caused by HBV and HCV. In addition, TST values were found to be higher in Child C liver cirrhosis than in Child B liver cirrhosis. These observations show the need for new methods to detect latent *M. tuberculosis* infection in patients with cirrhosis. Interferon-γ (IFN-γ) release assay tests may allow specific and sensitive diagnosis of *M. tuberculosis* infection (30-32). However, there are limited data comparing TST and IFN-γ release assay tests in liver transplant candidates (7,33). Further researches may investigate IFN-γ release assay and its relation with viral etiology in cirrhotic patients.

Limitations existed in our study. Age and BCG vaccination status can affect TST results. Our patient population included all age groups. In Turkey, BCG vaccination has been included in the national immunization program for newborn children since 1953. Some of the patients included in this study were born before routine TB vaccination. We did not evaluate age and BCG vaccination status. Secondly, we did not present the information about the viral profiles - genotype, viral load and past treatments.

In conclusion, these results indicated that TST is not reliable in detecting LTBI in chronic liver disease. To understand the reasons for the higher TST results of a viral etiology and Child C cirrhosis patients requires further research. In liver transplant candidates, new methods should be performed for the detection of latent *M. tuberculosis* infection.

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