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Full Length Research Paper

Serum antibody responses during treatment in tuberculosis patients from Henan, China

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Abstract

To investigate antibody responses during treatment in tuberculosis patients from Henan Province, China. Forty-five smear-positive pulmonary tuberculosis patients, 43 smear-negative pulmonary tuberculosis patients , 43 extra pulmonary tuberculosis patients were enrolled to collect blood samples at 0, 2, 4, 6, 8, 16, 24 weeks during tuberculosis treatment, totally 7 time points. Twenty-three healthy controls were enrolled to collect blood samples only once. Sera were applied to analyze TB IgG antibody by ELISA kit made in China. The basic levels of antibodies at 0 week in tuberculosis patients were significantly higher than those in healthy people. During treatment, the dynamic change of antibody was shown in different patterns. There was a significant decrease in antibody positivity with treatment for pulmonary tuberculosis patients. The present study signified that in resource-limited settings, antibody test may still be of some value in helping to diagnose tuberculosis and to monitor the treatment outcome.

Keywords: Tuberculosis, antibody, IgG, immunological diagnosis, chemotherapy.

INTRODUCTION

Tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis and is one of the leading causes of mortality due to infectious disease worldwide (Welch et al., 2012). Serum antibody tests remain attractive for use in resource-limited settings because they generally are simple, rapid and relatively inexpensive. They help to detect cases that are usually missed by routine sputum smear microscopy, such as extra pulmonary disease and pediatric TB (Welch et al., 2012). In this study, we tried to investigate serum antibody responses during treatment in tuberculosis patients from Henan Province, China by using a commercial TB-Ab ELISA kit made in China.

MATERIALS AND METHODS

Collection of blood samples

Blood samples were obtained from individual TB patients in Henan Provincial Chest Hospital from March 2010 to June 2011. Subjects all signed informed consent before enrolled. The whole study was approved by Henan Provincial Chest Hospital Ethical Committee. Group A: smear-positive pulmonary TB; Group B: smear-negative pulmonary TB. They were culture-confirmed cases. Group C: extra pulmonary TB. In this group, there were 37 pleural TB, 7 abdomen TB, 1 chest wall TB, 1 spine TB, 1 bone and joint TB, 1 kidney TB, 1 gynecological TB. There were 50 subjects in each group with the age of 18-65 years old. If presently on anti-TB chemotherapy, they should have a regimen which started not more than 14 days prior to enrollment. If previously treated for TB, they should have been off of anti-TB chemotherapy for at least 60 days before starting present regimen, or before enrollment if not presently on drugs. Blood samples were collected at 0, 2, 4, 6, 8, 16, 24 weeks during their TB treatment and sera were separated to store at -20℃. Group D: 23 control subjects were from TB doctors, nurses, lab technicians or administrative people in the same hospital. They were 18-65 years old with no signs or symptoms of an active TB infection. Blood samples were collected for one time when they were enrolled.

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ELISA for TB-Ab test

Tuberculosis Antibody (IgG) Diagnostic Kits (ELISA) was from Chengdu Yong'an Pharmaceutical Company (Chengdu, Si Chuan Province, China, www.cypco.cn). The ELISA kit was primarily based on an antigen of Mycobacterium tuberculosis (Rv3310) that was expressed and purified from E. coli (>95% purity). The product got the certificate from China FDA in the year 2009 (No.3400423). After all sera collection, TB-Ab value was tested in 96 microplates strictly followed the SOP instruction in the kits. OD value was tested at 450nm wave length by HALO MPR-96 Microplate Reader (Dynamica). Each plate was arranged with 3 positive controls, 3 negative controls and 4 intermediate controls. Samples were double tested. OD (sample) divided by OD (intermediate control) and times 100 was the result of TB-Ab level of the sample. The level of more than 120, less than 100, between 100 to 120, were judged as TB-Ab positive, negative, intermediate, respectively, according to the introduction sheet in the kit.

Data management and statistical analysis

All data were entered into a Microsoft Office Excel file, the mean and standard deviation (SD) of TB-Ab value of the four groups at 0 week (basic Ab level) were calculated. The differences among groups were analyzed by student's T tests. Positive rates at different time points (0, 2, 4, 6, 8, 16, 24 weeks) in group A, B, C were calculated. The differences among groups of TB-Ab value for each subject were applied to observe dynamically the responses during the half-year treatment. Analyze the graph types in each group.

RESULTS

Sample collection and ELISA test

In group A, 45 subjects finished sample collection with 5 subjects dropped out early (4 without 24 week point sample, 3 without 16 week and 24 week sample, other 38 subjects finished all.) In Group B, 43 subjects finished sample collection with 7 subjects dropped out early (4 without 24 week point sample, 3 without 16 week and 24 week point sample, 36 subjects finished all.) In group C, 43 subjects finished sample collection with 7 subjects dropped out early (2 without 24 week point sample, the other 41finished all.) In group D, 23 subjects all finished sample collection after they were enrolled. All samples of the above 154 subjects were analyzed TB-Ab value by ELISA successfully.

Basic TB-Ab value

At 0 week, the basic value in group A was 228±87, group B: 136±45, group C: 148±66, group D: 38±19. By two-tailed t test, group A, B, and C were significantly higher than that of group D ($P<0.001$ respectively). That is, TB-Ab basic value in all kinds of tuberculosis subjects, whether smear-positive, negative pulmonary TB, or extra pulmonary TB, were significantly higher than normal control subjects.

Changes of positive rates during treatment in different groups

The positive rates on each time point were calculated (results not shown). Compared to the corresponding rate of 0 week, differences in other time points were analyzed by CHITEST in each group. The results showed that TB-Ab positivity declined along with treatment. The decline made significant difference in 24 weeks in group A, and at 8 weeks point in group B ($P<0.05$, respectively), while in group C, no significant change ($P>0.05$).

Dynamic analysis of TB-Ab

TB-Ab value patterns were analyzed along with treatment time (0, 2, 4, 6, 8, 16, 24 weeks). Draw the graph for each subject in group A, B, C. They were divided into 6 types (Figure 1). Type 1 (subject C11 as an example), no change or minor change within 20%. Type 2 (subject A10 as an example), ascent continuously more than 20%. Type 3 (subject A8 as an example), descent continuously more than 20%. Type 4 (subject A45 as an example), first descent then ascent. Type 5 (subject B38 as an example), first ascent then descent. Type 6 (subject C21 as an example), fluctuation with the shape of the letter "W" or "M". Table 1 showed the distribution of the 6 types in the 3 groups.

DISCUSSION

In recent 15 years, it has been observed that during active tuberculosis disease, a humoral response occurs in the host, which can be measured using anti-M.tb antibodies. Immunoglobulin G antibodies directed against several M.tb antigens, such as 38-Kda, MTB 48, MPT64, Ag85b, CFP-10/ESAT-6, have been proposed as potential markers of tuberculosis (Welch et al., 2012; Selma et al., 2010; Feng et al., 2011; Min et al., 2011). It was found that no single serum sample reacted with all of the antigens tested and no single antigen was recognized

Figure 1. Six types of TB-Ab dynamic change during treatment

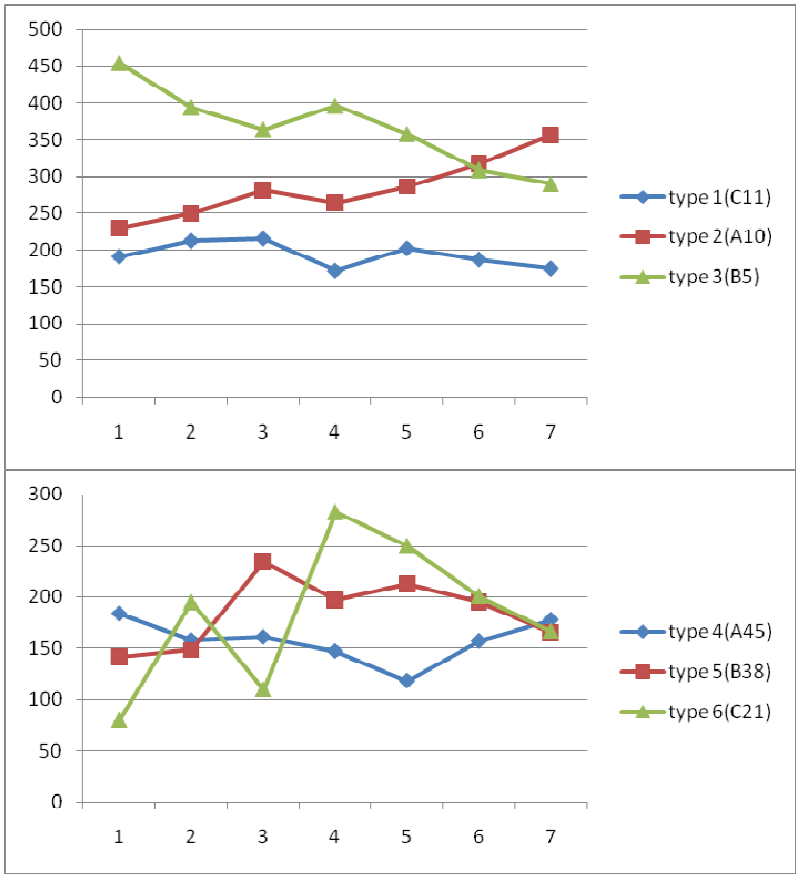

Table 1. Graph types in dynamic changes of TB-Ab in different groups

Group	Type 1	Type 2	Type 3	Type 4	Type 5	Type 6
A	4 (9%)	8 (18%)	24 (53%)	3 (7%)	3 (7%)	3 (7%)
B	5 (12%)	2 (5%)	23 (53%)	4 (9%)	6 (14%)	3 (7%)
C	7 (16%)	6 (14%)	20 (47%)	3 (7%)	4 (9%)	3 (7%)

by all sera. That is, antibody responses were heterogeneous and multi-antigen cocktail could achieve the highest possible test sensitivity (Wu et al., 2010; Wu et al., 2010; Ireton et al., 2010). Like Wu et al (2010) this work we also found that the levels of antibodies in smear-positive pulmonary TB patients were significantly higher than those in smear-negative patients, of which the antibody showed no significant difference from that of extra pulmonary tuberculosis patients. Importantly, the basic levels of antibodies before treatment in these three groups were significantly higher than those in healthy controls, indicating their values in the diagnosis of different kinds of tuberculosis. Furthermore, there was a significant decrease in antibody positivity with treatment for pulmonary tuberculosis. The decline mostly happened after treatment for 2 months when concurrently smear results and X-ray abnormalities obviously recovered, indicating their potent values in monitoring treatment outcome of pulmonary tuberculosis. In 2011, WHO published a policy statement regarding commercial serodiagnostic tests for diagnosis of tuberculosis. Based on a meta-analysis of commercially available tests, including 67 studies, the authors of the WHO statement concluded that M. tuberculosis antibody tests should not be used for the diagnosis of pulmonary and extra pulmonary M. tb infections (WHO, 2011). The present study revealed that in resource-limited settings antibody test may still be of some value in helping to diagnose tuberculosis and to monitor treatment outcome. In addition, little is known about the positive antibody responses in the phthogenesis and progression of TB in patients. There is much work to do.

CONCLUSION

The basic levels of antibodies before treatment in tuberculosis patients were significantly higher than those in healthy people. There was a significant decrease in antibody positivity with treatment for pulmonary tuberculosis. The present study indicated that in resource-limited settings antibody test may still be of some value in helping to diagnose tuberculosis and to monitor treatment outcome.

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REFERENCES

Feng T-T, Shou CM, Shen L, Qian Y, Wu ZG, Fan J, Zhang YZ, Tang YW, Wu NP, Lu HZ, Yao HP (2011). Novel monoclonal antibodies to ESAT-6 and CFP-10 antigens for ELISA-based diagnosis of pleural tuberculosis. Int J Tuberc Lung Dis, 2011; 15(6):804-810

Ireton GC, Greenwald R, Liang H, Esfandiari J, Lyashchenko KP, Reed SG (2010). Identification of Mycobacterium tuberculosis antigens of high serodiagnostic value. Clinical and Vaccine Immunology, 2010; 17(10):1539-1547

Min F, Zhang Yu, Huang R, Li W, Wu Y, Pan J, Zhao W, Liu X (2011). Serum antibody responses to 10 Mycobacterium tuberculosis proteins, purified protein derivative, and old tuberculin in natural and experimental tuberculosis in Rhesus monkeys. Clinical and Vaccine Immunology, 2011; 18(12):2154-2160

Selma W, Harizi H, Marzouk M, Ben KI, Ben LF, Ferjeni A, Harrabi I, Abdelghani A, Ben SM, Boukadida J. (2010) Rapid detection of immunoglobulin G against Mycobacterium tuberculosis antigens by two commercial ELISA kits. Int J Tuberc Lung Dis, 2010; 14(7):841-846

Welch RJ, Lawless KM, Litwin CM (2012). Antituberculosis IgG antibodies as a marker of active Mycobacterium tuberculosis disease. Clinical and Vaccine Immunology, 2012; 19(4):522-526

WHO (2011). Commercial serodiagnosis tests for diagnosis of tuberculosis: policy statement. World Health Organization, Geneva, Switzerland.

Wu X, Yang Y, Zhang J, Li B, Liang Y, Zhang C, Dong M (2010). Comparison of antibody responses to seventeen antigens from Mycobacterium tuberculosis. Clin Chim Acta 2010; 411:1520-1528

Wu X, Yang Y, Zhang J, Li B, Liang Y, Zhang C, Dong M, Cheng H, He J (2010). Humoral immune responses against the Mycobacterium tuberculosis 38-kilodalton, MTB48, and CFP-10/ESAT-6 antigens in tuberculosis. Clinical and Vaccine Immunology. 2010; 17(3):372-375