Value of ELISA IgG and IgM Antibodies using A60 Antigen in the Diagnosis of Tuberculosis

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Abstract

Objectives: To evaluate a new Modified Anda-TB IgG ELISA test for the serodiagnosis of Mycobacterium tuberculosis in patients and controls.

Settings and duration: The study was carried out between January to December 2010 at the Microbiology Department, Umm Al-Qura University, Makkah.

Patients and Methods: The Anda IgG and IgM ELISA tests were run on 674 patient’s sera which included 378 active cases of TB, 196 patients suffering from lower respiratory tract infections and 100 healthy blood donors. The ages of the patients (active cases of pulmonary tuberculosis and lower respiratory tract infections) and controls, ranged from 15-85 years with a mean age of 37 years. Smear and culture examinations were done according to standard procedures. Sera were obtained from the patients before receiving anti-tuberculosis chemotherapy and were stored at -70°C until they were tested. Patients were excluded from the study if they had received anti-tuberculosis drugs within the preceding 12 months.

Results: Anda IgG antibodies were found in 230/378 (61%) patients with active pulmonary tuberculosis, in lower respiratory tract infection (LRT) group, 24/196 (12.2%) patients were positive, while 6% were positive in the healthy blood donor group. Anda IgM was positive in 29/378 (7.7%) patients with active TB, 5.6% with LRT and 1% blood donors.

Conclusion: This study showed that about 61% active TB patients had Anda IgG antibodies against A 60 antigen while, only 8% had Anda IgM.

Key words: Anda-TB, IgG ELISA, A60 antigen.

Introduction

Tuberculosis (TB) is, globally, one of the most important infectious diseases. In 1993, the World Health Organization (WHO) declared tuberculosis (TB) to be a global health emergency. Until this day, TB remains one of the world’s major causes of illness and death. About one third of the world’s population, or two billion people, carry the TB bacteria, although most never develop the active disease TB. Pulmonary tuberculosis is still a major health hazard in both developed and developing countries. According to WHO data, its worldwide prevalence is estimated around 30 million cases with approximately 10 million new cases occurring annually. There were an estimated 8.8 million incident rate of TB (range, 8.5 million–9.2 million) globally in 2010, 1.1 million deaths (range, 0.9 million–1.2 million) among HIV negative cases of TB and an additional 0.35 million deaths (range, 0.32 million–0.39 million) among people who were HIV-positive. It is thought that in 2005 around 1.6 million people died as a result of TB. TB remains an endemic disease in Saudi Arabia also. There has been an increase in the incidence of TB in recent years, mainly due to its association with the Human Immunodeficiency Virus (HIV) and also due to occurrence of multi-drug resistance. Despite recent advances in Mycobacteriology, microscopy remains the primary laboratory tool supporting case detection. It is inexpensive to perform, very specific in high prevalence settings and detects most infectious subsets of patients.

Several new techniques have been developed to improve the diagnosis of tuberculosis, including newer radiometric methods, DNA probes, chromatography of mycolic acid, polymerase chain reaction (PCR) and serological tests. These diagnostic approaches have had a dramatic effect on the ability to diagnose disease accurately and expeditiously. While, molecular methods overcome the insensitivity of the smear method and the time required for culture, they depend upon retrieval of a specimen from the site of infection. Numerous serological tests that use various antigens, such as secreted and heat shock proteins, lipopolysaccharides, and peptides, have been developed.

In the absence of good diagnostic methods for tuberculosis, a lot of interest has been generated in

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serodiagnosis. Compared to other diagnostic methods, serological tests are faster and do not necessarily require samples that contain the tubercle bacilli. Sensitivity and specificity depend on the antigen used, gold standard for the diagnosis of tuberculosis and the type of tubercular infection. Though most of these tests have high specificity, their sensitivity is poor. There is, however, promise in serodiagnostic tests such as ELISA tests which, are of value in early diagnosis of the disease because of their easy performance. These are among the rapid and reliable diagnostic methods for the detection of pulmonary tuberculosis. The aim of the present study was to evaluate the Anda TB IgG and IgM ELISA test using A60 antigen for the serodiagnosis of MTB in three different groups of patients.

Patients and Methods

The study was carried out from January to December 2010 on a total of 674 patient’s sera. It included 378 active cases of TB, 196 patients with lower respiratory tract infections and 100 healthy blood donors. Their ages ranged from 15-85 years with a mean age of 37 years. Blood samples were collected from King AbulAziz Hospital, Makkah, Chest Disease Hospital, Taif, and Tuberculosis Hospital, Jeddah. Smear examination using Ziehl-Neelson (ZN) stain and culture on Lowenstein-Jensen (LJ) Media were done according to standard procedures. Smears were checked for the presence of acid fast bacilli. Data regarding medical history and clinical status were obtained from each patient on special forms. The disease was considered active if one or more of three sputum cultures obtained on different days were positive and inactive if they were negative. However, the 114 patients who were both smear and culture negative were regarded as active cases of TB on the bases of radiological findings. Sera were obtained from patients before receiving anti-tuberculosis chemotherapy and were stored at -70°C until they were tested. Patients were excluded from the study if they received anti-tuberculosis drugs within the preceding 12 months.

Anti-mycobacterial antibodies were detected by enzyme immunoassay utilizing microtitration plates based on the A60 antigen extracted and purified from Mycobacterium bovis BCG. The ELISA test was performed according to the manufacturer’s instructions (AndaBiologicals, Strasbourg, France). The values for positive sera for IgG were >225 Relative Tuberculosis Sero Unit (RTSU) and for IgM >1.0 RTSU.

All data were analyzed by SPSS software using Chi square, T test and analysis of variance. Considering the results of the culture as a "gold standard", the diagnostic value of this ELISA could be evaluated in terms of sensitivity, specificity and positive predictive value. p values with confidence coefficient of 95% for significance were calculated.

An informed consent was obtained from each individual before inclusion in the study. Every subject had been informed about the procedure before collecting the blood samples, making sure that they fully understood how it was to be carried out. The subjects were also made aware of the fact that they could refuse to enter the study and that it was not compulsory, but rather voluntary.

Results

Of the 378 cases of active pulmonary tuberculosis, 230 (61%) were ELISA IgG positive and 29 patients (7.7%) were ELISA IgM positive. When comparing the A60 ELISA test results to that of culture and smear reactivity, 120 (80%) of patients were IgG positive with both smear and culture positive. Eighty (71.4%) were IgG positive in the smear negative, culture positive group, while, 30 (26.3%) of patients were IgG positive, but both smear and culture were negative. Among the patients with lower respiratory tract infections, 24/196 (12.2%) were A60 ELISA IgG positive, while, in the healthy blood donor group 6% of patients were positive for A60 IgG antibodies. In the active pulmonary tuberculosis group, both IgG and IgM antibodies were present in 6.6% of patients. While, in the lower respiratory tract infection group of patients 3.6% were positive for both IgG and IgM antibodies (Table-1).

Table 1: Patient groups and diagnostic results.

<table>
<thead>
<tr>
<th>Patient Group and Diagnostic Result</th>
<th>No.</th>
<th>Percentage Positive for A60 ELISA Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgM</td>
</tr>
<tr>
<td>Active pulmonary tuberculosis</td>
<td>378</td>
<td>230</td>
</tr>
<tr>
<td>S+ C+</td>
<td>152</td>
<td>120</td>
</tr>
<tr>
<td>S- C+</td>
<td>112</td>
<td>80</td>
</tr>
<tr>
<td>S+ C-</td>
<td>114</td>
<td>30</td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>196</td>
<td>24</td>
</tr>
<tr>
<td>“Healthy” blood donors</td>
<td>100</td>
<td>6</td>
</tr>
</tbody>
</table>

The age range of the patients was 15-85 years with a mean age of 37 years. IgG levels were much higher in patients under 30 years old and in the age group 41-50 years. Based on the results, IgG levels were significantly higher (p<0.001) in patients under 30 years of age and in the age group 41-50 years (Table-2).
The sensitivity of the A60 ELISA test was 76% and the specificity 74%, while, the positive and negative predictive values were 87% and 57% respectively (Table-3).

Table 2: Active cases of tuberculosis: antibody levels in relation to age among TB patients.

<table>
<thead>
<tr>
<th>Age</th>
<th>N(%)</th>
<th>ELISA IgG # (RTSU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>196(51.9%)</td>
<td>1102*</td>
</tr>
<tr>
<td>30-40</td>
<td>84(22.2%)</td>
<td>956</td>
</tr>
<tr>
<td>41-50</td>
<td>33(8.7%)</td>
<td>1428*</td>
</tr>
<tr>
<td>&gt;50</td>
<td>65(17.2%)</td>
<td>723</td>
</tr>
</tbody>
</table>

*p<0.001 # Antibody values = RTSU: Relative Tuberculosis Sero Unit.

Table 3: Sensitivity and specificity of the Anda A60 ELISA test.

<table>
<thead>
<tr>
<th>Diagnostic Tests</th>
<th>Culture Total</th>
<th>ELISA Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>200</td>
<td>30</td>
</tr>
<tr>
<td>-</td>
<td>64</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>264</td>
<td>114</td>
</tr>
</tbody>
</table>

Sensitivity: 76% Positive Predictive Value: 87%
Specificity: 74% Negative Predictive Value: 57%

**Discussion**

Of the 378 cases of active pulmonary tuberculosis, 230(61%) were ELISA IgG positive and 29 patients (7.7%) were ELISA IgM positive. When comparing the A60 ELISA test results to that of culture and smear reactivity, 120(80%) of patients were IgG positive with both smear and culture positive group. Eighty (71.4%) were IgG positive (in smear negative and culture positive group), while, 30(26.3%) of patients were IgG positive (in both smear and culture negative group).

Among the patients with lower respiratory tract infections, 24/196 (12.2%) were A60 ELISA IgG positive, while, in the healthy blood donor group 6% of patients were positive for A60 IgG antibodies. We found that patients with a positive culture developed higher levels of IgG. Although IgG measurements alone, cannot differentiate patients with active disease from those who had TB in the past. However, the IgG level is valuable in differentiating patients with positive cultures from those with negative cultures and no prior history of TB. The findings of this study are very similar to studies done previously by others. Based on the data from table 2, IgG levels were significantly higher (p<0.001) in patients under 30 years of age and in the age group 41-50 years. Among the patients with lower respiratory tract infections, 24/196 (12.2%) were A60 ELISA IgG positive, while, in the healthy blood donor group 6% of patients were positive for A60 IgG antibodies. The 6% positive rate for IgG among the healthy blood donor group could be due to work, social or family contacts, or exposure to infective source and to environmental Mycobacteria, since A60 antigen is commonly found in all mycobacteria including the environmental. This is in agreement with a previous study in which similar findings were reported.

The sensitivity of the A60 ELISA test was 76% and the specificity 74%, while, the positive and negative predictive values were 87% and 57% respectively. Our results are in keeping with other studies done on evaluation of the Anda A60 antigen IgG ELISA test. In which sensitivities of between 67%-92%, and specificities of between 82%-96% have been reported. A study from Iran reported a sensitivity of 100% and a specificity of 88.5%. This was slightly higher than the results from this study.

In a previous study in Saudi Arabia, the sensitivity and specificity of the Anda-TB ELISA test was 87% and 95% respectively. Wu et al. reported a sensitivity of 49.4% and specificity of 68.4%. In another study anti A60 IgG levels in Taiwanese patients with abnormal chest radiography, the sensitivity was 80.77% and the specificity 88.4%. Thus, A60 IgG in combination with chest radiography could be useful in diagnosing tuberculosis. In different studies, in which IgG against A60 is considered a useful diagnostic tool in pulmonary tuberculosis, reported sensitivities ranging between 78% and 94% by different authors. Other studies in India, Spain, China, Poland and Italy demonstrated the usefulness of serologic tests in pediatric and adult cases with sensitivities of between 75% and 92%. However, the studies from Italy showed higher sensitivity and specificity (89% and 82.3% respectively) when using a combination of IgG and IgA.

ELISA test for routine use should include both IgG and IgM antibodies to detect current infection with MTB. The variation in IgG and IgM antibody levels could be an important index in determining the stage of tuberculosis. Therefore, raised IgG and low levels of IgM, could represent as a feature in evaluation of secondary disease. Furthermore, the IgM tracing immune response to A60 was shorter and lower during primary tuberculosis as compared to post-primary tuberculosis. The findings of this study point to the high prognostic value of the A60-ELISA test for tuberculosis. Anti-A60 IgM mark initial stages of the disease or reactivation processes whereas, anti-A60 IgG last longer than IgM and provide an evaluation of the intensity of the infectious process. Repeated serological tests allow monitoring of the course of the infection and the efficacy of therapy. The combined use of both IgG and IgM tests helps in the correct diagnosis of "false positive" cases.

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References