Article

Evaluation of the Anda-TB IgG ELISA Test Using A60 Antigen for Serological Diagnosis of Tuberculosis

Hani S. Faidah[1], Mahomed Farouk Mahomed[1]*, Al-Sir H. Abu-Sabaah[1], Niraj Sharma[2]

1. *Department of Medical Microbiology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia.*
2. *TB hospital Jeddah.*

Accepted for publication: November 2008

Abstract: The World Health Organization (WHO) declared tuberculosis (TB) to be a global health emergency in 1993. Until this day, TB remains one of the world's major causes of illness and death. Microscopy remains the primary laboratory tool supporting case detection. A lot of interest has been generated in serodiagnosis. The aim of the present study was therefore to evaluate a new Modified Anda-TB IgG ELISA test for the serodiagnosis of *Mycobacterium tuberculosis* in two different groups of patients. The study was carried out between January and June 2007 on a total of 400 patients' sera from two different groups which included three hundred active cases of TB, and one hundred "healthy" blood donors. Smear and culture examinations were done according to standard procedures. 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. The Anda IgG ELISA test was carried out according to the manufacturer's instructions. Of the 300 patients with active TB 243, 81% were positive for IgG antibodies, while 74% were positive in the healthy blood donor group. Furthermore, the levels of IgG antibody were relevantly higher with increasing rate of smear positivity. In addition, the mean IgG levels were higher in culture positive patients than in culture negative patients. Our results are compatible with other studies in which A60 antigen was used to detect antibodies to tuberculosis. The diagnostic value of these tests depends on the context of their use.

Keywords: Anda-TB, IgG ELISA, A60 Antigen

SGH Med Jour (2009) 3 (1):

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*Corresponding Author: E-mail: drfarouk08@gmail.com

Introduction

In 1993, the World health Organization (WHO) declared tuberculosis (TB) to be a global health emergency (Pottumarthy *et al.* 2000). 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 (WHO 2005). 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 more than 9 million new cases of TB, and approximately 1.7 million deaths from this disease in 2006. One-third of the number of new TB cases occurs in Southeast Asia, but the estimated incidence per capita is highest in Africa. In contrast, TB remains an endemic disease in Saudi Arabia (Al-Hajjaj 2000). There has been an increase in the incidence of TB in recent years, mainly due to its association with the Human Immunodeficiency Virus (HIV) (Raviglione *et al.* 1995) and also due to occurrence of multi-drug resistance (WHO 1998).

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 (Perkins 2000). However, 40-60% of patients with pulmonary disease and approximately 75% of patients with extra-pulmonary disease are smear negative, and in this situation even contemporary culture methods take several weeks to become positive (Pottumarthy *et al.* 2000).

Therefore, a rapid diagnostic tool with both high sensitivity and specificity is needed to improve the conventional diagnostic methods (Chiang 1997). In the absence of good diagnostic methods for tuberculosis, a lot of interest has been generated in serodiagnosis (Lodha and Kabra 2004).

The aim of the present study was therefore to evaluate a new Modified Anda-TB IgG ELISA test for the serodiagnosis of *Mycobacterium tuberculosis* in two different groups of patients.

Materials and Methods

The study was carried out between January and June 2007 on a total of 400 patients' sera from two different groups which included three hundred active cases of TB, and one hundred "healthy" blood donors. The age range of the patients was 11-110 years with a mean age of 37 years. Smear and culture examinations were done according to standard procedures (Forbes *et al.* 2002). Stained direct 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. 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.

Serologic Test

Anti-mycobacterial antibodies were detected by enzyme immuno-assay utilizing micro-titration plates based on the A60 antigen extracted and purified from *Mycobacterium bovis* BCG (Cocito *et al.* 1987). The ELISA test was performed according to the manufacturer's instructions; Anda Biologicals, Strasburg, France (Maes *et al.* 1989). Accordingly IgG antibody activity was determined by adding 1:100 dilution of serum to microtitre plates coated with A60 antigen. After addition of peroxidase conjugated antihuman IgG, and colour development, the reaction was stopped with H_2SO_4 and read at 450nm using an automatic ELISA plate reader. According to manufacturer's instructions, optical density absorbance values were transformed into relative sero unit by using the standard reference sera included in the kit. Standard sera containing certain units/mL of IgG against A60 antigen were assayed for each

series of analysis to construct a reference curve by bringing their optical densities on the logarithmic axis of the ordinate and the corresponding concentration on the logarithmic axis of the abscissa. The normal values for positive sera according to Anda Biological kit limitation for IgG was >225 Relative Tuberculosis Sero Unit (RTSU).

Results

In general IgG antibodies were higher in tuberculosis patients compared to "healthy" blood donors as shown in **Table 1**. From the total number of 400 samples tested, 64% were males and 51% were females. There was no correlation between antibody levels and the sex of the subjects tested. Similarly, from a BCG vaccination point of view 81% of patients and 87% of healthy blood donors had a BCG scar. There was no significant difference in RTSU measurements in patients with previous BCG vaccination and unvaccinated patients.

The age range of the patients was 11-110 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 **(Table 2).**

The patients were divided into three groups according to the results of acid fast staining of their sputum smears (Kaplan and Chase 1980). The levels of IgG antibody were relevantly higher with increasing rate of smear positivity **(Table 3).**

When analyzing results from culture, 254 cases were culture positive (84.7%) and were identified as MTB based on biochemical tests, 46 cases were culture negative (15.3%). In patients with positive culture the levels of IgG expressed in RTSU were from 857 to 1249. In patients with negative culture these levels were 540 for IgG. The mean IgG levels were higher in culture positive patients than in culture negative patients.

Table 1. Distribution of IgG antibody levels among patients and healthy blood donors based on Anda cut-off point.

Antibody (RTSU)	Positive IgG*	Weak Positive IgG**	Negative IgG***
300 Patients	243 (81%)	30 (10%)	27 (9%)
100 Healthy Blood Donors	74 (74%)	12 (12%)	14 (14%)

* >225; **125-225; ***<124.9

Table 2. Relevance of antibody levels to age among patients of tuberculosis.

Age	No. of Patients	IgG
< 30	153	1098
30-40	55	928
41-50	67	1480
>50	25	795

Table 3. Relevance of IgG antibody levels to degree of smear positivity and culture results.

Smear No. of Patients	Culture No. of Patients	IgG Antibody Levels
1+/50	+/37	857
2+/12	+/53	964
3+/192	+/164	1249
-/46	-/46	540

Discussion

On analyzing the results of the sera obtained from 300 active cases of TB and 100 "healthy" blood donors. A wide range of antibody levels were observed in TB patients. As many as 81% of patients showed high levels of IgG, 10% were weak positive, and 9% were negative for IgG antibodiues. In the healthy blood donors, 74% were positive for IgG, 12% weakly positive, and 14% were found to be negative. We found that patients with a 3+ smear positive and positive culture for MTB developed higher levels of IgG. However, IgG measurements alone cannot differentiate patients who have active disease from those with previous exposure to TB. We found that the IgG level is valuable in differentiating patients with positive cultures from those with negative cultures and no previous history of TB. This relationship between raised IgG levels and previous tuberculosis was also shown by Kaplan and Chase (1980) and Charpin *et al.* (1990).

From the data shown in **Table 3**, there was a significant correlation between antibody level and the degree of smear positivity. The highest levels of IgG were found in culture positive patients who were also 3+ smear positive, and were lowest in patients with a negative smear and no history of TB. Turneer *et al.* (1988) found a significant increase in IgG antibody levels in strongly smear positive patients. In the present study 86% of "healthy" blood donors showed raised levels of IgG antibodies. This could be partly due to environmental mycobacteria, since A60 antigen is a common antigen found in all mycobacteria including the environment. These findings are consistent with a study done by Gevaudan *et al.* (1992), which showed raised IgG levels in health workers.

Conclusion

Our results are similar to other studies in which A60 antigen was used to detect antibodies to tuberculosis. While a negative result would be useful in excluding disease in a population with a low prevalence of tuberculosis, a positive result could potentially aid in clinical decision making in sera from a group of selected symptomatic patients when there is a moderate to high degree of clinical suspicion of tuberculosis.

We do not recommend the use of this or any other ELISA test for routine use in any laboratory, especially for IgG antibodies alone, but in combination with IgM to detect current infection with MTB. Furthermore, we feel that serological tests should not be used alone for the diagnosis of tuberculosis, but adjunctive to other tests such as smear and culture. The diagnostic value of these tests depends on the context of their use.

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