INVESTIGATION OF THE LEVEL OF IgG, IgM AND IgA ANTIBODIES AGAINST A60 ANTIGEN IN TUBERCULOSIS PATIENTS REFERRED TO PHLS, AHVAZ, IRAN

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ABSTRACT:

***Objective*:** Evaluation of ELISA test using A60 antigen of mycobacteria in rapid diagnosis of tuberculosis in early stage.

Study: A cross sectional descriptive study

Place and duration of study: TB Reference Centre, PHLS, and Department of Microbiology, Ahvaz, Iran, from January 2000 to December 2001.

***Patients and Methods*:** A total of 180 sera were examined. It consisted of 90 sera which belonged

to patients with clinical and radiological signs and microscopic examination of sputum for acid fast bacilli, suggesting active tuberculosis, who were referred to TB Reference Centre, PHLS, Ahvaz and 90 sera belonged to healthy subjects. ELISA test was used to determine the IgG, IgM and IgA antibodies activity against the A60 specific antigen of mycobacteria.

Results: Antibody levels were raised in both tuberculosis and non-tuberculosis patients, BCG vaccinated or non-vaccinated group, however, the antibodies, especially IgG and IgA were much higher in tuberculosis patients compared to healthy subjects.There was no relationship between antibody titer and sex or previous BCG vaccination, but the relevance with age and the degree of smear positivity was significant. The levels of IgG and IgA were higher in patients under 50 years old and patients with 3+ sputum smear had significant increased IgG titer. Besides, the IgG and IgA levels were higher in culture positive patients compared to those with a negative culture result and was statistically significant. The level of IgM was slightly higher in patients with no history of TB in the last few years, but in the rest of the patients, the raised IgM level was not significant.

Conclusion: The ELISA using A60 antigen can not alone represent active tuberculosis at early stage, but it may facilitate to asses the active disease, when combined by clinical evaluation and other laboratory diagnostic tests.

KEY WORDS: *M. tuberculosis*, ELISA, Antibody level, A60 antigen

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INTRODUCTION

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.[1] This contagious disease, though preventable, has had an increased incidence in recent years, mainly due to its association with Human Immuno-deficiency Virus[2] and also due to occurrence of multi-drug resistance.[3] In spite of availability of an adequate treatment, attempts to restrict and eradicate the disease has failed.[4]

The alarming increase in morbidity and mortality due to tuberculosis indicates the need to strength control measures. Control of the disease depends largely on early detection and treatment of active cases.

The detection of TB, especially in its early stages is difficult. The culture and microbiological tests despite being specific are time-consuming, while laborious and the clinical features of the disease are not specific.[5] To eradicate the disease, it is important to improve diagnostic techniques, so that the disease can be diagnosed and treated at early stage.

During the active phase of tuberculosis, antibodies especially IgM and IgG are developed against different mycobacterial antigens and these can be detected in patients' sera within a month after the development of the disease.[6] One of these antigens is, A60 antigen complex, which is an interspecific antigen found in the cytosol of mycobacteria. This is a member of the thermo stable macromolecular antigen family and is the main component of old tuberculin and Purified Protein Derivative (PPD). It reacts with antibodies created during mycobacterial infections.[7, 8]

There is promise in serodiagnostic tests such as Enzyme-Linked Immuno Sorbent Assay (ELISA) which are of value in early diagnosis of the disease because of their easy performance.[4] These are among the rapid, reliable and less costly diagnostic methods for the detection of pulmonary tuberculosis.[9] The goal of present study was, to determine the sensitivity and specificity of serological tests compared to traditional culture method.

PATIENTS AND METHODS

A total of 180 sera were analyzed for the presence of anti-mycobacterial antibodies. This group consisted of 90 serum samples belonging to patients with clinical and radiological signs and microscopic examination of sputum suggesting active tuberculosis who referred to TB reference centre between winter 2000 to the end of year 2001, and 90 serum samples were obtained from healthy subjects including the laboratory staff and volunteers referred to PHLS for periodical occupational check up.

Smear and culture examination were done according to standard procedure.[10] Stained direct smears were checked for the presence of acid fast bacilli and the prepared sputa were inoculated into Lowenstein Jensen (LJ) medium. For all culture positive isolates, subsequent biochemical tests, including the niacin accumulation test, the nitrate reduction test, and heat-labile catalase test were performed for final identification of *M. tuberculosis*. Data regarding medical history and clinical status were obtained from each patient on special forms. The disease was considerered 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 the patients before receiving anti-tuberculosis chemotherapy and were kept at –70°C. Few of the patients had a history of TB in the past few years but they were not under anti-TB treatment recently. 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 immuno-enzymatic assay utilizing microtitration plates based on the A60 antigen extracted and purified from *Mycobacterium bovis* BCG.[11] ELISA test was performed according to manufacturer's instruction (Anda biologicals, Strasburg, France).[12] Accordingly, IgG, IgM and IgA antibodies activity was determined by adding 1:100 dilution of serum to microtiter plates coated with A60 antigen. After addition of peroxidase conjugated antihuman IgG, IgM or IgA, and color development, the reaction was stopped with H_2So_4 and A492 nm values were recorded by an automatic microplate reader.

According to the manufacturer's instructions, optical density absorbance values were transformed into relative sero unit by using the standard reference sera included in the kit. Briefly, three standard sera containing certain units / ml of IgG, IgM and IgA against A60 antigen were assayed for each series of analysis to construct a reference curve by bringing their optical densities on the logarithmic axis

Table-I: Distribution of antibody levels among patients and healthy subjects based on Anda cut off point

Aby (RTSU)	*IgG*			*IgA*			*IgM*		
	+	w+	-	+	w+	-	+	w+	-
Patients	76	8	6	54	26	10	16	4	70
Control group	58	22	10	18	32	40	4	8	78

W+: weak positive ;
IgG: +:>225 w+: 125-225 - :<124.9 ; *IgA:* +: >350 w+:200-350 - :<200 ; *IgM:* +: >1 w+: 0.8-1 - :<0.8

of the ordinate and the corresponding concentrations on the logarithmic axis of the abscissa. The normalized values for positive sera according to Anda biological kit limitation were as below: IgG: >225 RTSU; IgA: > 350 RTSU and IgM: >1.0 RTSU. *RTSU: Relative Tuberculosis Sero Unit

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.[14] *P* values with confidence coefficient of 95% for significance were calculated <0.05.

RESULTS

Based on the results shown in Table-I, in general antibodies especially IgG and IgA were higher in tuberculosis patients compared to healthy subjects ($P< 0.001$). From 180 study cases, 64.4% and 51.1% were men in patient and control groups respectively, which no correlation was seen between antibody levels and sex of the patients or healthy subjects ($P= 0.41$). Similarly, from BCG vaccination point of view, 84.4% of patients and 88.9% of controls had BCG scar. The difference between RTSU measurements in patients with previous BCG vaccination and unvaccinated patients was not statistically significant ($P= 0.08$).

The mean age for patient and control groups were 34.8 and 36.2 years old respectively. Based on the results, IgG and IgA levels were significantly higher ($P<0.001$) in patients under 30 years old and in age group of 41-50 years (Table-II).

The patients were divided into three groups according to the results of acid fast staining of their sputum smears.[14] The relevance of antibody levels especially IgG as seen in table-III, was significant by increasing the rate of smear positivity, but it was not statistically significant for IgG and IgM. According to the results from culture, 64 cases were culture positive (71.1%) and were identified as MTB based on biochemical tests, 23 cases were culture negative and 3 cases despite being positive in culture, but they were identified as non-tuberculous mycobacteria in subsequent biochemical tests. Hence they were excluded from the study. Table-III represents values for antibody measurements according to culture and smear results. In patients with positive culture the mean (± SD) level of IgG, IgM and IgA expressed in RTSU were 1219 ± 582, 0.74 ± 0.47 and 888 ± 606 respectively. In patients with negative culture these levels were 525 ± 507,

Table-II: Relevance of antibody levels to age among TB patients

Age	*No. of patients*	*IgG*	*IgA*	*IgM*
<30	46	1091*	736*	0.914
30-40	24	928	809	0.612
41-50	8	1330*	1525*	0.604
>50	12	715	708	0.446

* $P<0.001$; Aby values:RTSU

Table-III: Relevance of antibody level to degree of smear Positivity and culture results

Smear No of patients	*culture No of patients*	*IgG*	*IgM*	*IgA*
1^+ /60	**+/10	867	0.748	794
2^+ /16	+/ 22	928	0.623	732
3^+ /14	+/32	*1219	0.74	*888
-/0	-/26	525	0.75	658

* $P<0.001$; ** 3 were culture positive for nontuberculous mycobacteria

0.75 ± 0.42 and 658 ± 452 for IgG, IgM and IgA. According to the presented data, the mean RSTU values for IgG were significantly higher in the culture positive patients ($P<0.001$). The sensitivity of ELISA for IgG measurement in patients with a positive culture in this study was equal to 100%, the specificity to 88.5% and the positive predictive value to 96.2.%.

DISCUSSION

We analyzed the sera obtained from 90 control subjects & 90 diseased patients. All were found to be ELISA positive and showed raised IgG, IgM & IgA levels. Based on the results, a wide range of antibody levels were observed in TB patients. As many as 84.4% of the patients showed high level of IgG, 60% had raised IgA level & only 17.8% of them had slightly raised IgM level. We found that patients with a positive culture developed higher levels of IgG ($P<0.001$). Of course, IgG measurement alone, cannot differentiate patients with active disease from those who had TB in the preceding few years. However, the IgG level is valuable in differentiating patients with positive cultures from those with negative cultures and no prior history of TB. Kaplan & colleagues[13], Charpin & coworkers[15] previously showed this relationship between IgG levels and the previous TB disease. The minority of the patients showed slight raised IgM. These were the group that had no history of TB in the past. The level of IgM was not statistically significant in majority of TB patients who had a history of TB in the last few years. Based on our results, we may conclude that variation in IgG, IgA & IgM antibody levels could be an important index in determining the stage of tuberculosis, so raised IgG & low level of IgM, could represent as a feature in evaluation of secondary disease. Similar results have been reported by other investigators.[7,15] In a survey using Anda biological kit, high level of IgG & IgA antibodies has been shown in sera of more than 50% of tuberculosis patients, but only 41% of them had IgM titre slightly higher than normal level in the serum which was comparable to present study.[16]

Based on the data from table-III, there was a significant correlation between antibody level and degree of smear positivity. The highest levels of IgG ($P<0.02$), and IgA were encountered in culture positive patients with 3+ smear and the lowest in patients with a negative smear and no history of TB. In the latter, IgG antibody levels were close to values encountered in normal control subjects. Turneer and colleagues[17] previously pointed out the significant IgG elevation in highly smear positive patients.

About 64% of healthy subjects showed raised levels of IgG and IgA in their sera, which could be due to work, social or family contacts, exposure to infective source and more important, environmental mycobacteria. Since A60 antigen is a common antigen found in all mycobacteria including the environmentals, so it could be a fair explanation for raised antibody levels in healthy subjects in present study. Our findings were in agreement to Gevaudan and coworkers[7] who showed the raised IgG levels in health workers.

CONCLUSION

Antibody assay in tuberculosis patients using A60 antigen cannot solely represent the active tuberculosis unless it is combined by other clinical assessments and laboratory di-

agnostic tests. However, variation in IgG, IgA and IgM antibody levels, could be an important index in determining the stage of tuberculosis and high levels of IgG may represent active secondary illness. The test may be also useful for monitoring the effects of anti-tuberculosis chemotherapy and in patients suffering from both tuberculosis and HIV virus, as other investigators have been suggested.[9,18,19]

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