

Tanaffos (2002) 1(2),15-19

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Serodiagnosis of Tuberculosis in Children Using A60 Antigen

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ABSTRACT

Background: Measurement of antimycobacterial antibody may be used as an accurate diagnostic tool in childhood tuberculosis.

Materials and Methods: This study was conducted to evaluate the value of IgG, IgA, and IgM levels against A60 antigen by using ELISA method for diagnosis of tuberculosis. 238 children aged from 6 months to 18 years old, divided into 4 groups; group A(n=51), patients with documented diagnosis of tuberculosis ;group B (n=75), patients who had close contact with TB patients; group C (n=51), patients with lung diseases other than tuberculosis; group D(n=40), healthy children .Serums of 4 different groups of patients were studied.

Results: Group A consisted of 22 smear positive, 24 smear negative, and 5 extrapulmonary tuberculosis patients. Sensitivity for bacteriologic study in smear positive cases was 51.2%. The mean levels of IgG, IgA, and IgM were significantly high in group A compared to other groups. IgM anti-A60 antibody had 70.6% sensitivity and 62% specificity for tuberculosis. Among the smear negative tuberculosis patients IgM anti-A60 had a sensitivity of 62.1%. Sensitivity of IgM anti-A60 in group B was 53.3% .The sensitivity of combined use of IgG and IgM was 70.6% in group A.

Conclusion: The sensitivity of serologic diagnosis of A60 antigen is higher than bacteriologic findings (77.3% vs. 51.2%). A60-ELISA test is a simple and rapid diagnostic method, which can facilitate the diagnosis of tuberculosis. (Tanaffos 2002; 1(2): 15-19)

Key words: A60 Antigen, Tuberculosis (TB), ELISA, Children

INTRODUCTION

Unspecified clinical presentation of tuberculosis in children causes many difficulties for early detection of the disease. For early diagnosis of TB, several

serological tests with high sensitivity and specificity have been developed. Serologic techniques are initially based on agglutination tests; one of the antibodies used in these tests was antibody against mycobacterial component named as A60.

A60 is a macromolecule ($10^6 - 10^9$ Dalton) prepared from M.bovis and it consists of protein,

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polysaccharide, and lipid. Due to its molecular characteristics, this antigen has high immunogenicity (1,2,3,4,5). Antibodies against A60 antigen were found in body secretions especially in the cerebrospinal fluid in 85% of patients; approximately 87.5% of these antibodies were formed in the sera of tuberculosis patients (6).

In 1999, titers of IgG anti-A60 antibody were detected in 110 patients with active TB, and higher titers were obtained among patients with positive smear results(7).

Alifano et al. reported a sensitivity of 98% and a specificity of 68% for A60-ELISA test. In this study 31 children with tuberculosis and 198 healthy children had been entered(8).

Since sample-obtaining from children is difficult and childhood tuberculosis is usually a paucibacillar disease, diagnostic methods such as A60-ELISA which don't necessarily need specimens containing mycobacterium are of great value(9).

This study was performed to answer the questions are as follows:

What is the contribution of a serological diagnosis in abacillary or paucibacillary cases in children? What is the use of serological measurements in the screening of TB in children with close contacts?

MATERIALS AND METHODS

This study was carried out for assessing sensitivity and specificity of A60-ELISA test.

Blood samples were collected from 238 children aged from 6 months to 15 years old. The titers of IgG, IgA, and IgM antibodies against A60, measured before treatment initiation by combined ELISA kit (Anda Biological, France).

The volunteers were categorized in four groups as follows:

- Group A (patient group): 51 children with pulmonary and extra-pulmonary TB who had at least three diagnostic criteria including positive pathology/bacteriology test, history of close

contacts with TB patients, positive PPD, clinical signs, and radiological findings compatible with tuberculosis.

- Group B: 75 children who were in close contact with smear positive patients.
- Group C: 72 children with lung diseases other than TB.
- Group D: 40 healthy children which considered as healthy control group.

Demographic data, history of close contact with smear-positive tuberculosis patients; BCG vaccination, and PPD reactivity as well as the results of laboratory tests including three-time gastric lavage smear for acid fast bacillus were collected. Also, IgG, IgA, and IgM titers were measured. The data were analyzed using Epi-Info (version 6.04).

Cut-off values for setting diagnostic threshold for each antibody in the patient group were 2 SD higher than mean of antibodies in the control group, which were equal to cut-off values of 0.08 IU for IgM, 200 IU for IgG, and 125 IU for IgA which were suggested by Anda (Manufacturing company).

RESULTS

Among 51 children in the first group, smear of gastric lavage was positive for AFB (acid fast bacillus) in 22 patients (43.1%).

Bacteriology result was negative in children of contact group. The induration of PPD test was more than 14 mm in 34 children. The titer of IgG, IgA, and IgM were shown in figure 1. Table 1 showed the sensitivity and specificity of antibodies.

Table 1. Sensitivity and specificity of anti-A60 antibodies in group A

Ig	Sensitivity(CI)*	Specificity(CI)*
IgA	29.4%(18.1-42.9)	97.5%(80.5-98.01)
IgG	37.3%(24.5-51.9)	95%(81.8-99.1)
IgM	70.6%(55.9-82.1)	62.5%(45.8-76.8)

*CI: Confidence Interval

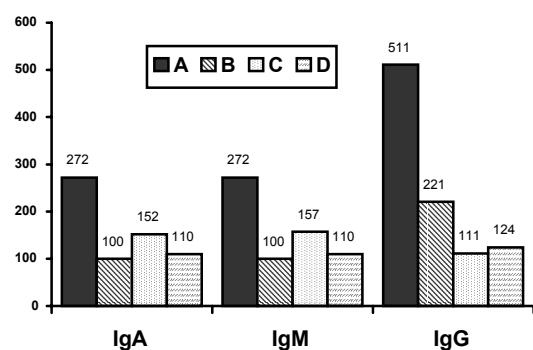


Fig 1 . Comparing the titers of IgA, IgM, IgG in the groups

Considering the cut-off value determined for IgM in the TB group, the sensitivity and specificity of anti-A60 were 70.6% and 92.5%, respectively.

Out of 51 children with TB, 22 were smear positive with a bacteriological sensitivity of 51.2% (95% CI: 35.7% - 66.4%). The sensitivity of A60 test in smear positive TB patients for IgG and IgM were 54.3%(95% CI:32.67%-74.93%) and 77.3% (95%CI:54.18%-91.3%), respectively.

The sensitivity of IgM in smear negative TB patients was 62.1% (95% CI: 42.36%-79.70%). The sensitivity of IgM in contact group was 53.3%.

When the results of IgG and IgM were considered, we found the sensitivity of 70.6% for combined use of tests (Table 2).

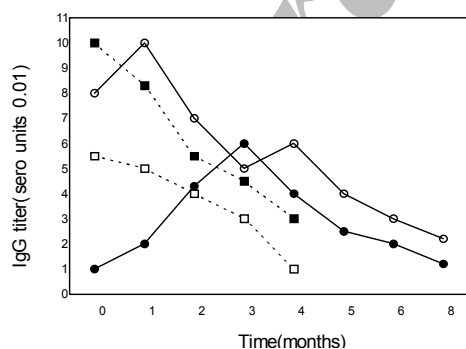


Fig2. Production of antibodies during a natural tuberculous infection. () IgM primo-infection () IgG primo-infection () IgM post-primary infection () IgG post-primary infection.

Table2 : Sensitivity of combined IgG and IgM anti-A60

(IgG+IgM)	Group A	Group C and D
+	36	84
-	15	28

With progression of primary TB infection, the level of IgM anti-A60 antibody in patients increased within the first 10-14 days and the level of IgG increased after few days.

In case of anergy, IgA increases in parallel to IgG in the serum of patients (8,10)(Figure2).

DISCUSSION

Since tuberculosis in children is difficult to diagnose and bacteriological documentation is not easy to obtain, serological methods and especially the titer of IgM, IgA, and IgG antibodies in different stages of tuberculosis are used for rapid diagnosis and early treatment of TB (10,11).

The mean IgG titer in TB patients was significantly higher than the mean score of the control group, this finding is due to stimulation of humoral immunity (12,13,14).

The mean titer of IgA in TB cases was significantly higher than the control and the contact groups.

The mean titer of IgM antibody in TB patients was higher than the control and the contact groups.

The positivity of IgM was probably due to recent BCG vaccination in control group and occurrence of primary infection in contact group.

The sensitivity of IgM anti-A60 antibody in the sera of TB patients was 70.6% and the specificity was 62.5%, this is indicated that A60-ELISA test is a useful diagnostic method in comparison to bacteriological studies. (52.1% sensitivity).

Mahajan et al. showed that the sensitivity and specificity of anti-A60 were higher than the bacteriologic studies, which was similar to our finding (11).

The sensitivity of IgG anti-A60 in the TB patients with positive sputum for AFB was 54.3%. The sensitivity of IgM anti-A60 in smear positive cases was 77.3%, which confirms the value of serologic test in TB patients.

IgM sensitivity in children with close contact was 53.3%. Therefore, IgM titer measurement can be used as a sensitive screening test in cases who are suspicious of tuberculosis disease. According to our study, combined use of IgM and IgG had an overall sensitivity of 70.6%. This finding helps to reach the correct diagnosis of TB in children. Al-Hajjaj et al. Reported that the sensitivity of combined use of IgM and IgG was 87%, which was higher than our study (7).

Our results like as other studies show that no single assay is available for the definite diagnosis of TB in children. Therefore, the diagnosis of childhood TB relies on a combination of clinical, radiological, mycobiological, and serological data.

In conclusion, we suggest that A60-ELISA test (Anda TB test) could be used as a reasonable method for early diagnosis of childhood TB.

Also, this method could be used as a sensitive screening test in cases suspected of having TB and the cases that are in close contact with TB patients. Therefore, A60-ELISA test is proposed to be a cost-effective, rapid, and highly sensitive and specific diagnostic test in childhood tuberculosis.

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