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Original Article

# Diagnostic value of serum IgG by ELISA to detect Mycobacterium tuberculosis

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**Abstract**

**Introduction:** In developing countries, which is an endemic region in terms of tuberculosis, there is an urgent need for fast, accurate and inexpensive serological tests. The aim of this study was to determine the diagnostic value of serum immunoglobulin G (IgG) antibody by ELISA to detect Mycobacterium tuberculosis.

**Method:** This study was performed on patients with pulmonary tuberculosis in 2017-2020. After selecting the 2 case (Pulmonary Tuberculosis and latent tuberculosis, each had 30 patients, total 60 patients) and control (n = 30) subjects according to inclusion criteria, their blood samples were obtained and analyzed in the reference laboratory by standard kits for IgG against 16, 36 and 40 kDa antigens of Mycobacterium tuberculosis.

**Results:** The mean age of the subjects was 50.60 years old. The majority of participants were 46 (51.1%) women. There was no significant difference between the two groups regarding sex and age. Serological examination of patients with pulmonary tuberculosis showed 25 positive results and only 4 of the control group had a positive result. Sensitivity, specificity, positive and negative predictive values of serology test were 83.3%, 86.67%, 86.20%, and 87.88%, respectively.

**Conclusion:** Despite the acceptable sensitivity of the serologic IgG test, according to the statement of World health organization (WHO), it did not possess an acceptable specificity. It is recommended that a wider range of different antigens to be studied. Also, it is essential to evaluate the diagnostic value of the other immunoglobulins in patient in different stages of disease.

## Introduction

Although tuberculosis is a well-known disease with known epidemiology, it is still a public health problem worldwide, with 8.9 to 9.9 million new cases and 1.3 million deaths reported annually worldwide[1] and it is estimated that about one-third of the world's population is infected with Mycobacterium tuberculosis.[2]

In Iran, at the end of 2012, according to the Center for Infectious Diseases Management, there were 10,987 patients with 69% pathogenicity.[3]

In many parts of the world, including our country, some patients with tuberculosis are not diagnosed and as a result, are not treated properly. Unfortunately, we are currently witnessing the emergence and spread of multidrug-resistant TB bacilli due to some diagnostic problems and an increase in the number of HIV-infected people.[4]

One of the basic ways to solve the above problem and control the disease is to quickly identify infected patients and start appropriate antibiotics.[5]

Sputum sample testing is a quick and easy method which has a sensitivity of 80 to 82% with Zill Nelson staining, but there must be at least 5000 to 10 000 bacilli per milliliter of a sputum sample to get positive results. On the other hand, most of the patients cannot prepare proper sputum sample and sputum smear shows false-negative results which makes the diagnosis difficult.

Mycobacterium culture, although the gold standard of diagnosis, often takes 4 weeks or more.[7]

Radiological manifestations do not play a significant role in the diagnosis of tuberculosis because pulmonary tuberculosis has various radiological manifestations and on the other hand, chest radiography cannot differentiate active tuberculosis from old inactivated tuberculosis.[7-9]

The tuberculin skin test is also of limited clinical value and its negative result does not rule out the diagnosis of tuberculosis. In addition, its positivity is not always associated with active tuberculosis, and even when inoculated with the BCG vaccine with other non-tuberculous mycobacteria, it gives positive results. Measuring interferon-gamma levels is also not cost-effective and is questionable in poor countries. Also, co-

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infection with AIDS alters the clinical manifestations of tuberculosis and reduces the sensitivity of classical TB diagnostic methods. So, there is a diagnostic problem in most cases and invasive methods such as bronchoscopy are needed to prepare the sputum.[10]

Therefore, in developing countries and countries that are an endemic region in terms of tuberculosis, a serological test that is fast, cheap and accurate, does not depend on the individual, and can be measured in laboratories with low equipment and different populations can be useful.[11]

This study aimed to determine the diagnostic value of serum IgG antibody by ELISA in the diagnosis of Mycobacterium tuberculosis.

## Methods

This study was performed on patients referred to the clinic of infectious and tropical diseases with suspected symptoms of tuberculosis. Patients suspicious of HIV infection, corticosteroids or other immunosuppressive drug users, and those receiving chemotherapy in the last three months were excluded. All patients underwent PPD testing, a chest x-ray, and clinical examinations. Also, three sputum sample smears, which were eventually diagnosed by a TB infectious disease specialist were drowned.

The control group was referred to the infectious disease clinic due to other complaints, which included individuals (n = 30) who had a normal chest X-ray, negative sputum smear and PPD skin test results.

The first case group with latent tuberculosis (n = 30) were those who were considered to have latent tuberculosis based on a normal chest X-ray and a positive skin test.

The second case group (n = 30) had pulmonary tuberculosis in addition to the initial inclusion criteria based on a positive skin test result (PPD), chest X-ray, and positive sputum smear samples (were diagnosed as having pulmonary tuberculosis).

After selecting the individuals based on the inclusion criteria, informed consent was obtained and chest radiography and PPD test were performed for all patients. Also, 3 sputum samples were received from patients for smear examination. Finally, radiographs of patients and test results were reviewed by an infectious disease specialist, and the final opinion was announced. After confirmation of tuberculosis, it was divided into active pulmonary tuberculosis and latent tuberculosis.

Demographic characteristics including sex and age were also recorded. To examine blood samples for antigen serology, an IMMUNOLAB TUB03-ILE kit containing 36, 16, and 40 kDa antigens were used. The samples were centrifuged at 3000 Rpm for 5 minutes to separate the serum. Thereafter, the samples were placed separately in the wells of the ELISA according to the instructions for 60 minutes. They were incubated at room temperature.

In the ELISA method, the surface antigen of Mycobacterium tuberculosis is attached to the bottom of the ELISA well. The serum samples were then washed 3 times with a buffer to remove excess antibodies that did not react with the antigen per unit time. Conjugated enzymes including peroxidase or alkaline phosphatase were then added to the medium. Samples were washed with buffer 3 times after 30 minutes of incubation at room temperature. The chromogen solution was then added to the medium and incubated for 20 minutes to change the color of the solution to blue. After adding the solution, the color of the solution stopped turning yellow. The present dye solution was then measured by spectrophotography at a wavelength of 620 nano 27 m. Since the concentration of IgG antibodies is directly related to the intensity and amount of soluble dye; wells that had a wavelength greater than or equal to 620 nm were considered as a positive test response and otherwise a negative test response.

*Ethical considerations:* This study is a research project approved by the ethics committee of Tabriz University of Medical Sciences with a no.: IR.TBZMED.REC.1397,379

*Statistical analysis:* After collecting the data, SPSS software version 22 was used for analysis. Descriptive statistics were used to determine the frequency, percentage, mean and standard deviation, and a two-by-two table was used to determine sensitivity, specificity, positive and negative predictive values.

*Tools of data collection:*

- A checklist for recording the patients' demographic characteristics which included questions about age and sex, previous and current diseases, the presence of high-risk behaviors, history of imprisonment and addiction was used
- ELISA model was TUB03-ILE of IMMUNOLAB company.

## Result

The data showed that the mean (standard deviation) age of the subjects was 50.60±8.63 and 25 (83.2%) of the people with pulmonary tuberculosis were in the age range of over 40 years. Also, the majority of participants were women [n=46 (51.1%)], and 18 persons (60%) in the case group were female. There was no statistically significant difference between the three (two case groups and control group) in terms of sex and age (Table 1).

Based on the results of the serological test, 25 persons with pulmonary tuberculosis had a positive result and only 4 people in the control group had a positive result. Also, only 6 cases with positive test results were reported in people with latent tuberculosis. (Table 2).

According to the results and the use of epidemiological table, sensitivity, specificity, positive predictive value, and negative predictive value of serology test for patients with pulmonary tuberculosis were 83.33%, 86.67%, 86.20% and 83.87%, respectively, and they were for latent tuberculosis 20%, 86.67%, 60%, 52%, respectively.

## Discussion

In 2001, the world health organization stated that any

**Table 1.** Demographic situation of patients

| Variables | | Control Group (n=30) | latent tuberculosis (n=30) | pulmonary tuberculosis (n=30) | Pv |
|---|---|---|---|---|---|
| | | Mean ± SD | Mean ± SD | Mean ± SD | |
| Age | | 51.06±15.70 | 45.70±13.38 | 55.03±12.43 | 0.28 |
| | | Number(%) | Number(%) | Number(%) | |
| Gender | Male | 17(56.7%) | 15 (50%) | 12(40%) | 0.301 |
| | Female | 13 (43.3%) | 15 (50%) | 18 (60%) | |

**Table 2.** Antigen serology diagnostic value

| Test result | pulmonary tuberculosis | | latent tuberculosis | |
|---|---|---|---|---|
| | Yes | No | Yes | No |
| Positive | 25 | 4 | 6 | 4 |
| Negative | 5 | 26 | 24 | 26 |
| Total | 30 | 30 | 30 | 30 |

diagnostic tool for tuberculosis must be at least 80% sensitive and 95%.[12] Despite the urgent need for rapid diagnostic testing, serological tests face some limitations; for example, it must be able to isolate active pulmonary tuberculosis from other non-tuberculous lung diseases such as bronchitis, cancer, and pneumonia because this disease can mimic the radiological and clinical symptoms of tuberculosis. This study aimed to evaluate the diagnostic value of serum IgG antibody by ELISA in the diagnosis of Mycobacterium. The sensitivity and specificity of this test in patients with pulmonary tuberculosis were 83.33% and 86.67%, respectively. The standard set by the World Health Organization has an acceptable sensitivity for the diagnosis of pulmonary tuberculosis (low specificity). However, different percentages have been obtained in different studies. For example, Welch et al 2008 calculated sensitivity as 76% and specificity as 57.1%,[4] and Anderson et al at 2008 calculated sensitivity as 5.6% and specificity as 100% for the diagnosis of pulmonary tuberculosis.[13]

IBL *M. tuberculosis* IgG ELISA-Like the ELISA kit used in the present study measures the amount of IgG produced in response to Mycobacterium tuberculosis 36, 16, and 40 kDa antigens. Anderson calculated the sensitivity and specificity of the test as 5.6% and 100%, respectively, by surveying a population of 106 people.[2]

One of the kits used in this experiment was the IBL (IBL *M. tuberculosis* IgG ELISA (IBL-Hamburg)) kit, which used the same antigens as the Elisa kit. During the studies performed with this kit, the rate of positive serological tests in patients with latent tuberculosis was not effective. In contrast to Anderson's study, 20% of patients with latent tuberculosis were reported positive in the present study, which may be due to the differences in the type of kit used in the two studies. In this study, none of the used serological test kits could reach the ideal diagnostic threshold. Since the differentiation of active pulmonary tuberculosis from latent tuberculosis can be a great success in controlling this disease, further studies are needed to find the appropriate method.

## Conclusion

The diagnostic value of IgG antibody in patients with active pulmonary tuberculosis and latent tuberculosis was studied. Despite the appropriate sensitivity of serological IgG antibodies according to the statement of the World Health Organization, this test did not have an acceptable specificity. Also, IgG antibody was not sensitive and specific in detecting latent tuberculosis.

### Limitations of the study

1. The limitation of the present study was the low sample size of patients with tuberculosis
2. Antibody samples of Mycobacterium tuberculosis available in the laboratory during the study prevented the study of a wide range of antigens of this bacterium.
3. Investigation of a limited number of antigens
4. Checking only one type of immunoglobulin

### Conflict of Interest

The authors declare no conflict of interest in this study.

### Ethical Approval

This manuscript was approved by the regional ethic committee of Tabriz University of medical sciences with no.: IR.TBZMED.REC.1397.319. All patient information was confidential as indicated in the checklist. Patients were not charged for the study. The informed consent form was provided for all patients

### Author's Contribution

Study design and supervision, MH; study conduct, HOO; data gathering and writing, LA.

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### Study Highlights

***What is current knowledge?***

- There is a diagnostic problem in most cases depend on PPT or sputum and invasive methods such as bronchoscopy are needed to prepare the sputum

***What is new here?***

- Sensitivity of serological IgG antibodies did not have an acceptable specificity. Also, IgG antibody was not sensitive and specific in detecting latent tuberculosis

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