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Evaluation of IgG, IgM and IgA antibody responses for serodiagnosis of Tuberculosis

R. Gopinath[1*], Sriram Kamireddy Ravichandran[2], R.Balamurugan[3], S.Gowsalya[4] and M. Elanchezhiyan[5]

[1]Department of Microbiology, Govt Theni Medical College and Hospital, Theni, Tamilnadu- 625512, India.

[2]House surgeon, Pondicherry Institute of Medical Sciences, Puducherry.

[3]VRDL, Govt Villupuram Medical College and Hospital, Villupuram-605601

[4]Technical Assistant, National Institute for Research in Tuberculosis, Chetpet, Chennai - 600 031.

[5]Department of Microbiology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai 600113.

***Correspondence**

Dr. Gopinath Ramalingam

Department of Microbiology,

Govt Theni Medical College and Hospital,

Theni, Tamilnadu- 625512, India

Email: gopimicroo@gmail.com

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Abstract

Many serological methods for the diagnosis of active TB have been designed and commercialized. Serum samples obtained from the recruited groups of individuals were subjected to the evaluation of the commercial ELISAs for detection of antibody responses raised against Mycobacterial antigens. The aim of this study was to analyze the serum levels of all of three antibodies, IgG (IgG1 and IgG2), IgM and IgA on ultimatum by employing commercially available ELISA. In this study, a total of 300 TB patients and 100 healthy control subjects were recruited in the study. The 300 TB patients were further classified based on their medical status and constituted of 156 treatment naïve and 144 under therapy patient groups. Humoral responses (IgG, IgM and IgA) were determined for the Mycobacterium tuberculosis protein using indirect ELISA methods in all of serum samples. In overall tuberculosis, Of 300 TB patients, 271 (90.3%) were IgG positive. The sensitivity, specificity, PPV, NPV and accuracy for IgG were 93.8%, 100%, 100%, 79%, and 95.3%, respectively. IgG1 and IgG2 levels of patients were significantly higher ($p<0.01$) than both TB patients. The IgG antibody has high sensitivity and specificity for tuberculosis diagnosis, but IgM antibody should also be evaluated along with IgG antibody to increase specificity.

Keyword: Mycobacterium tuberculosis; Antibody; IgG; IgM; IgA

Introduction

Tuberculosis (TB) has been well documented throughout history as a nagging problem to humans. Antibodies are developed against various antigens of *Mycobacterium tuberculosis* (MTB). The efficacy of humoral immunity was inconsistent, the majority of studies in the literature ranging from the era of serum therapy to immunization with antigens indicated a protective role for antibody. Recently serological studies in animals have revealed species-specific antibody responses to TB (Koleske *et al.,* 2023).Thus, transfer of antibody preparations between the different species might be less effective than within the same species. Besides it is also difficult to conclude much about the efficacy of antibodies from negative studies (Li *et al.,* 2017). During the active phase of tuberculosis, antibodies especially the IgG, IgM and IgA are developed against different mycobacterial antigens and these can be detected in the patients' sera within a month after the development of the disease. They serve as one of the most rapid and reliable diagnostic methods for the detection of TB. The mechanisms by which antibodies function against TB are thought to be via the modulation of host immunity to more efficiently control infection, or through direct anti-mycobacterial properties. In the former case, antibodies may opsonize MTB, enhancing the phagolysome maturation and intracellular killing or stimulate an amplified cell-mediated response (Sheedy and Divangahi, 2021). MTB are certainly susceptible to the mechanisms of Ab-mediated immunity irrespective of whether they are in the intracellular or extracellular phase. In general, the IgM constitutes about 10% of total immunoglobulin (normal concentration is 0.5-2.0 mg/ml) and are presumed to be pro-inflammatory through their high complement-activating capacity (Tran *et al.,* 2019), whereas IgG can be pro- or anti-inflammatory depending on the complement-activating capacity and type of FcR receptor engaged (Achkar et al., 2015; Ballow, 2011). Humans produce as much IgA as IgG, especially at the mucosal sites. Mucosal IgA defence against the invading pathogens like

bacteria and virus via the mucosal surfaces of the respiratory, gastrointestinal and urogenital tracts, whereas systemic IgA is important in triggering potent inflammatory responses like the antibody-dependent cytotoxic clearance, endocytosis, phagocytosis, generation of superoxide radicals, cytokines, inflammatory mediators and complement activation, like IgG. Normal concentration of IgA is 1-4 mg/ml and it constitutes about 15% of total immunoglobulin. During the active phase of tuberculosis, antibodies especially IgG, IgM and IgA are developed against different mycobacterial antigens and these can be detected in patients' sera within a month after the development of the disease (Feng *et al.*, 2014). They serve as one of the most rapid and reliable diagnostic methods for the detection of TB. The aim of the present study was to evaluate the serum profile of all of the three isotype antibodies, IgG (IgG1 and IgG2), IgM and IgA serodiagnosis of MTB in three different groups namely TB treatment naïve, TB treated patients and controls.

Materials and Methods

Study population

This study was conducted on 300 TB patients (156 treatment naïve and 144 under therapy) and 100 age and sex matched control subjects. The TB samples were obtained from Govt. Hospital of Thoracic Medicine, Tambaram Sanatorium and IRT, Perundurai Medical College, Perundurai, Erode District, Tamil Nadu. Ethical approval for this study was obtained from the Institutional Human Ethics Committee Approval No: UM/IHEC/16-2013-I.

Blood Collection

Venous blood samples (3-5 ml) were collected in plain vacutainer from patients. Blood samples were centrifuged at 5,000 rpm for 10 minutes. A similar volume of blood was collected from healthy controls also. Serum was separated and stored at -20ºC until usage.

Antibody Assay

Commercially available Antibody kits were used for this study. Antibodies namely IgG, IgM and IgA were detected by using the kit obtained from MyBioSource, IgG and IgM (Cat. No MBS494171 and MBS494348). For the detection of IgA ELISA kits were received from Genway Biotech (Cat. No GWB-20EB90). For the detection of IgG (IgG1 and IgG2) subclasses

from the above demography the EIA kits were obtained from Invitrogen-Thermo Scientific, USA (Cat. No. 991000).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8 software using One-way ANOVA (GraphPad Software2365 Northside Dr.Suite 560San Diego, CA 92108). The diagnostic value of this ELISA could be evaluated in terms of sensitivity, specificity and positive predictive value, negative predictive value and accuracy test were evaluated by using online MedCalc Statistical Software (MedCalc Software bvba, Belgium; version 18.9; 2018). P values with confidence coefficient of 95% for significance were calculated <0.05.

Results

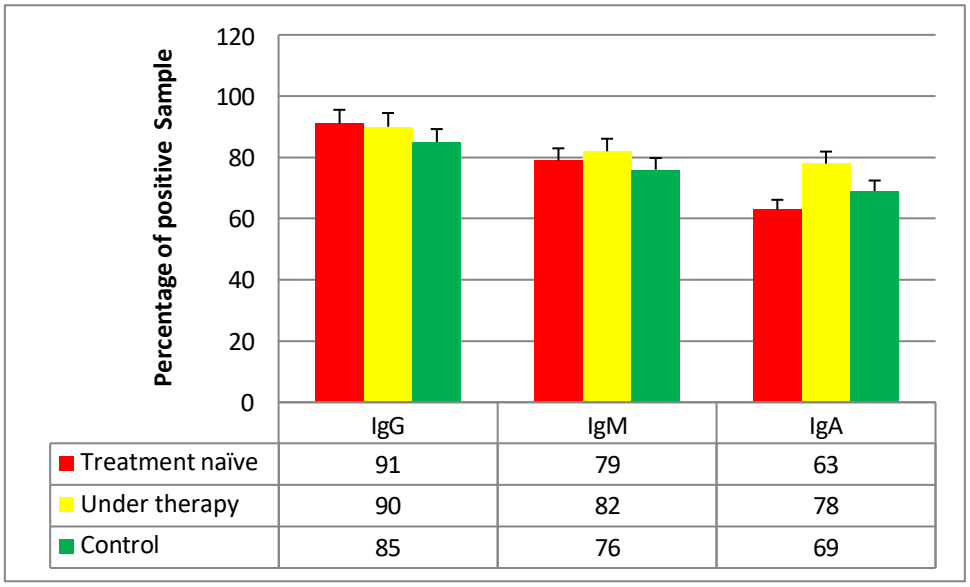
Characteristics of the study population

A total of 400 (156 treatment naïve, 144 under therapy and 100 controls) subjects were recruited in the study and they were divided into three categories which included: Treatment naïve, under therapy and control subjects. Out of the total 400 patients recruited in our study, the 156(39%) treatment naïve patients consisted of 79(50.6%) males and 77(49.3%) females. Similarly among 144(36%) under therapy individuals 75(52%) were males and 69(47.9%) were females and among the 100(25%) control samples 52(52%) were males and 48(48%) were females.

IgG, IgM and IgA antibody responses in TB patients and control subjects

ELISA was carried out for the TB patients (treatment naïve and under therapy) and controls subjects. The mean antibody levels for the three isotypes namely, IgG, IgM and IgA were compared. In general, IgG TB was found in 271 patients (142(91%) were treatment naïve and 129(90%) were under therapy), IgM TB was present in 242 patients (124(79%) were treatment naïve and 118(82%) were under therapy) and IgA antibodies alone were present in 200 patients (98(63%) were treatment naïve and 112(78%) patients were under therapy). This increment was statistically significant in IgG vs IgM ($p<0.01$); IgM vs IgA ($p<0.001$) and IgG vs IgA ($p<0.01$) when compared with healthy controls. Based on the results shown in Figure 1 antibodies especially IgG and IgM in general were higher in tuberculosis patients when

compared to the healthy subjects. Out of 300 TB patients, 271 (90.3%) were IgG positive; however, IgG was positive in 85 controls (85%). When we considered the IgG and IgM immunoglobulins' results, the sensitivity and specificity was improved. This improvement was more significant in IgG and IgM results. Around 91 % of the treatment naïve individuals were positive for IgG indicating active infection and around 82% of the under therapy individuals showed IgM positivity which was higher than the treatment naïve category indicating the treatment consequences and the percentage of IgA was also elevated in the under therapy individuals. Figure 2 shows the overall antibody levels in TB patients and control groups of IgG, IgA, and IgM antibody. This increment was statistically significant in IgG vs IgM ($p<0.001$); IgM vs IgA ($p<0.001$) and IgG vs IgA ($p<0.01$) when compared with healthy controls.



	IgG	IgM	IgA
Treatment naïve	91	79	63
Under therapy	90	82	78
Control	85	76	69

Figure 1: The antibody levels of percentage of positive sample with TB patients with Treatment naïve, under therapy and healthy controls.

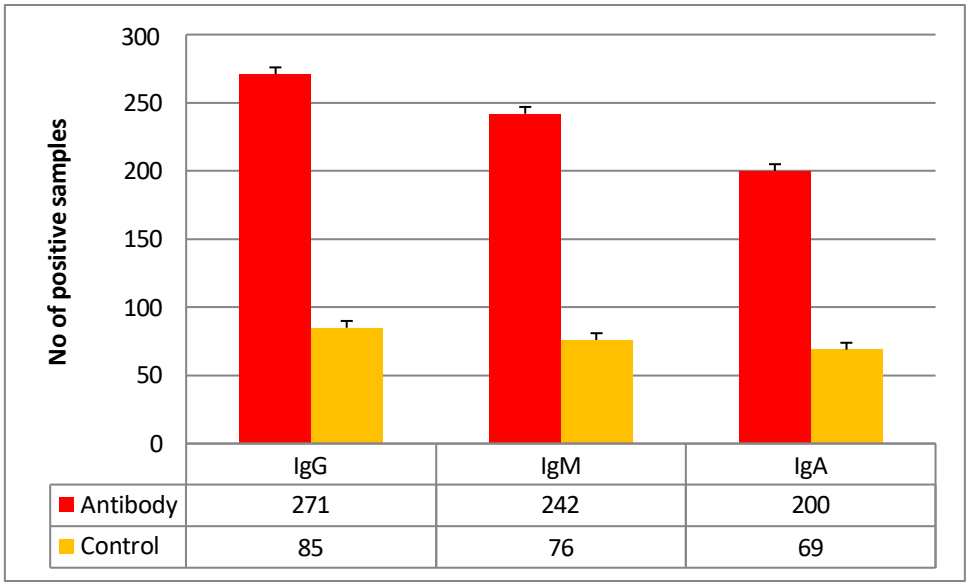


Figure 2: The overall antibody levels of TB patients and controls subjects.

Serum IgG1vs IgG2 levels among TB positive and Control individuals

Based on the increase in the TB specific immunoglobulin (IgG) we further extended our study and estimated the levels of TB specific IgG subclasses (IgG1 and IgG2). We further observed the levels of TB Specific IgG subclasses such as IgG1 vs IgG2 during Treatment naïve, under therapy and healthy controls. Both the groups were comparable in terms of total Ig main class levels in the sera. Interestingly we noticed during active infection there was a preponderance of IgG1 (112 were Treatment naïve, 95 were under therapy and 61 were control groups) compared to IgG2 (98 were Treatment naïve, 82 were under therapy and 49 were control groups). This was more pronounced among the TB patients, where mean IgG1 were significantly higher in TB patients and compared to controls. Patients with disseminated forms of TB also had higher mean IgG1 ($P<0.01$) values. Based on the results shown in Figure 3 in IgG sub classes IgG1 were higher than in IgG2 in Treatment naïve and under therapy patients compared to healthy subjects. IgG1 and IgG2 levels of patients were significantly higher ($p<0.01$) than both TB patients. IgG1 was predominant isotype in antibodies against TB patients.

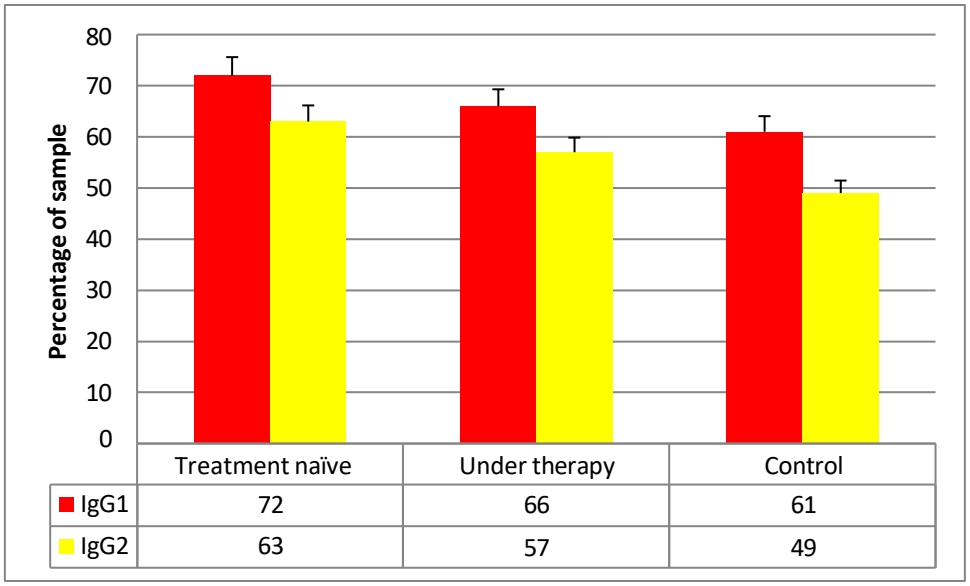


Figure 3: The antibody levels of percentage of positive sample with TB patients with Treatment naïve, under therapy and healthy controls.

Antibodies	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV (95% CI)	Accuracy (95% CI)
IgG	93.80% 88.14% to 98.93%	100.00 % 88.06% to 100.00%	100.00%	79.00 % 75.06% to 83.28%	95.33% 81.11% to 98.03%
IgM	95.04% 91.50% to 97.41%	100.00 % 93.84% to 100.00%	100.00%	82.86 % 73.57% to 89.35%	94.00% 93.12% to 97.92%
IgA	81.63% 76.21% to 86.28%	100.00 % 85.85% to 100.00%	100.00%	50.00 % 42.75% to 57.25%	81.67% 76.82% to 85.88%

Table 1: Comparative evaluation of three antibodies (IgG, IgM and IgA) for their sensitivity, specificity, positive predictive value (PPV), Negative predictive value (NPV), CI=Confidence Interval and accuracy.

The sensitivity, specificity, PPV, NPV and accuracy for IgG were 93.8%, 100%, 100%, 79%, and 95.3%, respectively. Totally, 300 (80.6%) TB patients and 76 (76%) controls had positive IgM results. Sensitivity, specificity, PPV, NPV and accuracy for IgM were 95.04%, 100%, 100%, 82.8%, and 94%, respectively. Out of 300 TB patients, 200 (66.6%) were IgA-positive. However, IgA antibodies, while highly specific at 100.00%, have a reduced sensitivity of 81.63%, which may indicate limitations in their capacity to detect the virus consistently. Nonetheless, all three antibody types have high positive predictive values (PPV) and negative predictive values (NPV). These findings highlight the importance of antibody testing as a helpful tool in comprehensive diagnostic techniques (Table 1).

Discussion

A comprehensive insight to immunoprofiling basics of antigen specific response plays a crucial role in not only understanding the disease pathogenesis, but also in developing diagnostic tests. In context of the humoral responses in this study, serum levels of three antibody isotypes, IgG, IgM and IgA, were evaluated in TB patients and control subjects. IgG antibody level was observed to be higher in the most advanced and extensive form of the disease. Patients with active TB usually exhibited a stronger IgG response but poor IgM and IgA responses (Lee *et al.,* 2020). Previous studies analyzing the IgG antibodies showed that the anti-MTB IgG antibodies increased in patients with the TB disease (Awoniyi *et al.,* 2017). In our results, the IgG antibody level was higher ($p<0.01$) in the TB patients than that in the healthy control, and the positive rate of IgG was highest among the three isotypes, indicating that the IgG antibody was the most prevalent antibody isotype. The authors of several studies have suggested that the IgM antibodies are produced mainly during the early phase of the primary TB infection (Jacobs *et al.,* 2016). Therefore, the IgM-positive patients usually indicate early stage of the infection process (Li *et al.,* 2020). Our results demonstrated that the positive rate of IgM was the second highest among the three isotypes. Several authors have observed in their study that IgM antibody production was not associated with any of the clinical phases and radiological factors (Demkow *et al.,* 2007). IgA secreted during exposure to MTB and/or its antigens with the mucosal surface stimulates the release of cytokines (Li *et al.,* 2012). In the present investigation, the positive rate of IgA was not significantly too high in TB.

In our study, it was found that IgG and IgM was the most sensitive test (93.3% and 95% respectively) and for IgA, it was 81.6%. The most specific test was found to be IgG and IgM. In Pouthier *et al*., study, 36.5% of TB patients who had co-infection with HIV and 69.5% of HIV negative TB-infected patients had positive IgG against A-60 antigen. The combination of the IgA and IgG responses can serve to increase the accuracy of the serodiagnostic tests for active TB disease in the TB endemic settings (Baumann *et al.*, 2013). In this study, the sensitivities of the IgG test were very high when compared to those of the IgM test and this was in agreement with the previously reported. Their studies also revealed a decreased sensitivity in the case of IgA (from 16% to 94%) which was found to be in agreement with our study but in the case of IgM test, the sensitivity obtained by us was significantly higher ($p<0.01$).

In a study conducted in Saudi Arabia, the sensitivity and specificity of a modified Anda–TB ELISA test for TB detection was found to be 87% and 95%, respectively (Al-Hajjaj *et al.*, 1999). In our study IgG TB was found to be positive in 271 patients (142(91%) were treatment naïve and 129(90%) were under therapy), IgM TB was present in 242(124(79%)) were treatment naïve and 118(82%) were under therapy) and IgA antibodies alone were present in 200 patients (98(63%) were treatment naïve and 112(78%) patients were under therapy) of the patients. Based on this study results, it can be concluded that the variation in IgM & IgG antibody levels serves as an important index for determining the stage of tuberculosis as a result raised IgG & low level of IgM, can serve as a feature in the evaluation of the secondary disease (Steingart *et al.*, 2007). In the present study we evaluated that IgA TB had 81.63% Sensitivity, 100% specificity, 100% positive predictive value (PPV), 50% negative predictive value (NPV) and 81.6 % accuracy. Out of 300 TB patients, 271 (90.3%) were IgG positive; however, IgG was positive in 85 controls (85%). The sensitivity, specificity, PPV, NPV and accuracy for IgG were 93.8%, 100%, 100%, 79%, and 95.3%, respectively. Totally, 300 (80.6%) TB patients and 76 (76%) controls had positive IgM results. Sensitivity, specificity, PPV, NPV and accuracy for IgM were observed to be 95.04%, 100%, 100%, 82.8%, and 94%, respectively (Table 1). Bhatia *et al.*, 2003 reported in their study that they obtained a IgG sensitivity of 94% and IgM sensitivity of only 33% in the case of extra-PTB, whereas in the study by Maheshwari *et al.*, 2000 they observed IgG sensitivity of 75% and IgM sensitivity of 37.5% in tuberculoma cases. In a meta analysis, the results for all the available commercial kits for serological diagnosis of tuberculosis, varied significantly with the sensitivity ranging from 15.7% to 89.2% and

specificity in the range of 50% to 100% (Cho, 2007). When IgG, IgM, IgA and IgG subclass activities were subjected to evaluation before and after treatment in the TB patients, it was found that two of these, IgG and IgM, were affected by the anti-TB treatment. Our study also supports the statement due to the elevated levels of IgG1reponse shown by our Active TB patients. The Serodiagnosis methods employing ELISA can serve as great frontiers developing countries as they are relatively inexpensive and also the higher prevalence can increase its positive predictive value. Thus higher positive predictive & negative predictive values of the ELISA techniques based on IgG and IgM TB make it a remarkable tool for diagnosis by providing confirmation to the clinical suspicion and thereby could be potentially used for smear negative TB and those subgroups of patients from whom sample collection is complicated as in the case of extra pulmonary tuberculosis and childhood tuberculosis. Thus the evaluation of these assays showed that IgG ELISA had advantages over the other commercially available ELISA test kit in terms of the cost effectiveness and in providing higher sensitivity, specificity, positive & negative predictive value and thus can be of better clinical tool. Hence, the multicentric trials on the diagnostic utility of the test using a larger population of cases of tuberculosis are suggested. From this study the levels of IgG subclasses, in particular IgG1 and IgG2, are elevated in tuberculosis, and may have an important role in the disease process and can be further considered for TB diagnosis.

Conclusion

This study sheds light on the potential of serum antibody levels, specifically IgG, IgM, and IgA, as diagnostic markers for TB. IgG antibodies, particularly prevalent in advanced TB stages, and IgM antibodies, prominent in early infection, showed promising diagnostic sensitivity and specificity. Combining IgG and IgM tests improved diagnostic accuracy, especially in challenging TB cases. Evaluation of IgG subclasses, IgG1 and IgG2, suggested their role as additional TB markers. ELISA-based serodiagnostic methods, particularly IgG and IgM assays, emerged as cost-effective and reliable tools, outperforming other commercial kits. Multicentric trials with larger populations are warranted to validate these findings and explore the diagnostic potential of IgG subclasses further.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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