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Research Article

VACCINE TURNED TO DIAGNOSTIC TOOL

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ABSTRACT

The alarming increase in morbidity and mortality due to tuberculosis indicates the need to strengthen the clinical suspicion of the disease. Therefore this study is aimed at the use of antigens for the organism extracted from BCG (Tubervac) vaccine. *Mycobacterium tuberculosis* specific culture filtrate antigen is highly recognized by *M. tuberculosis* infected subjects which gave a sensitivity of 90 %. More importantly, the performance of the extracted antigens is suitable for the routine application for the laboratory diagnosis of TB and therefore best suited to laboratories in the developing nations.

Keywords: Tuberculosis, antigen extraction, BCG and Tubervac.

INTRODUCTION

The unceasing worldwide dominance of tuberculosis brings about morbidity and mortality[1, 2] which fuelled extensive research for faster and effective diagnosis. The serodiagnosis of tuberculosis has long been the theme of investigation[3, 4, 5]. Improvement and standardization of serodiagnosis is an imperative avenue for research in the diagnosis of TB. Serological assays provide an essential and needed tool for large volume testing for exposure to *M. tuberculosis*. They offer the key advantages of ease of use, assay speed and relatively low cost.

As the cultures may show no growth or take up to 6 weeks to become positive for *M. tuberculosis*, failure to diagnose the disease promptly in its early stages results in an increase in mortality[6]. Sensitive tests such as radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) for the detection of *Mycobacterium tuberculosis* antigen[7] or antibody[8] appear to be promising procedures for the early diagnosis of tuberculosis.

Proteins secreted into the extracellular environment by *Mycobacterium tuberculosis* constitute an important source of antigens that induce protective immunity and immune responses owing its diagnostic value which are usually targets of immune responses in the infected host[9, 10, 11].

The live attenuated vaccine strain, *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) is of dubious efficacy in providing immunity to tuberculosis[12, 13]. The development of more effective vaccines against tuberculosis has focused upon the identification and cloning of specific protective antigens from strain BCG[14].

In an attempt to design an alternative method that provide good discrimination between culture-positive pulmonary TB patients, culture-negative patients with other pulmonary disease and healthy individuals (non-TB patients) we have made use of the antigens extracted from BCG (Tubervac) vaccine for the diagnosis of the disease.

MATERIALS AND METHODS

Samples

Blood samples (30 samples) used for this study were collected from TB patients with active disease, subsided disease and also from patients with no history of TB were obtained from neighbouring diagnostic laboratory. Serum samples were stored at –20 °C after adding sodium azide (0.1%) for preservation.

Staining for AFB (acid-fast bacteria)

Preliminary and initial detection of occurrence of MTB was performed by Ziehl-Neelsen (ZN) method with the direct smear of the samples and graded as per RNTCP (Revised National Tuberculosis Control Programme, 1998) guidelines. Smear microscopy was considered positive when AFB was observed in at least two of the three specimens from each patient.

Culturing on Lowenstein-Jensen (LJ) medium

Readymade LJ medium slants (Himedia, Mumbai, India) were inoculated with 0.5 ml of each of the specimens and incubated at 37 °C for 8 weeks. The LJ slants were inspected weekly. Growth of the Mycobacterium colonies on the LJ slants i.e., rough, tough and buff-colored, was subjected to ZN staining to confirm the presence of acid-fast bacilli and exclude the contamination respectively[15]. Samples showing no growth in LJ after 8 weeks of incubation at 37 °C were considered as negative.

Culturing in modified Middlebrook 7H9 broth

Middlebrook 7H9 broth with indicator with PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) cocktail of antibiotics (Himedia, Mumbai, India) was inoculated with 0.5 ml of each of the specimens. The presence of an indicator makes it possible to visualize growth within 10–14 days. Antibiotic cocktail present in the system retards the growth of contaminating flora. All the isolated organisms were compared with the referral strain of *M. tuberculosis* ATCC 25177.

Strain

BCG strain, a commercially available form of BCG vaccine (Tubervac) supplied from Serum Institute of India, India was used.

BCG culture filtrate antigen (CFA)

BCG culture filtrate antigen was prepared as per by Collins[16] with some modifications. BCG was cultured in synthetic Sauton's medium (Hi-Media, Mumbai) enriched with 0.5% glucose, 0.5% sodium pyruvate and 0.05% tween 80 for 3 weeks (35 °C; 5 - 10% CO_2) and was centrifuged at 12,000 rpm for 30 min at 4 °C, and the supernatant fluid was sterilized by filtration through a 0.22 µm-pore-size membrane (Sartorius Stedim biotech). The proteins were precipitated with 80% ammonium sulfate in cold overnight, dissolved in sterile phosphate-buffered saline (PBS) and dialyzed at 4 °C and stored frozen (-20 °C) until use. The protein content was determined by the method of Lowry against a bovine serum albumin (BSA) standard[17] and adjusted to 5mg/mL[18]. The protease inhibitor phenylmethylsulfonyl fluoride (PMSF) was added to a concentration of 10 mM.

ELISA assay

Coating of ELISA plates was done as described in our previous study[19]. ELISA assay was performed as described by Chaturvedi[20] with some modifications. Briefly, 50 µL of the individual sample (40 µL of PBST milk + 10 µL of sample), were incubated at 37 °C for 1 hr 30 min. After washing with phosphate-buffered saline (PBST)

containing 0.05% Tween- 20, 50µL of IgG HRP conjugate (Sigma, USA) was added to the plate (1:3000 dilution) MTSE IgG and 50µL of IgM conjugate (Sigma, USA) was added to plate (1:1000 dilution) MTSE IgM and incubated for 1 h at 37 °C. The plates were washed again and added 75µL of the substrate (ortho phenylene diamine dihydrochloride in 0.1 M phosphate citrate buffer and 0.01% hydrogen peroxide), incubated in dark for 30 minutes. The colour change was noted, the reaction was stopped using the stop solution 1N sulphuric acid (50 µL) and absorbance was read at 492 nm. The cut off for the assay was determined using the mean optical density (OD) of sera from control group.

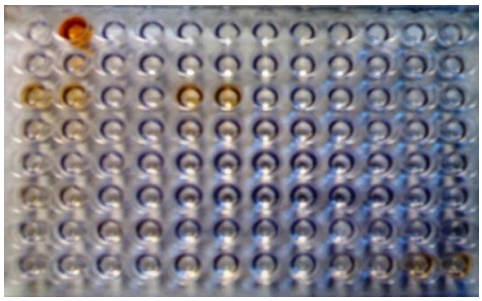
RESULTS AND DISCUSSION

The control of TB depends on early detection of cases and effective treatment. Various attempts have been made in the improvement of diagnosis of TB using clinical and routine laboratory methods[21, 22]. Laboratory diagnosis can be difficult: microscopic examination gives a low positive yield, whereas culture techniques require complex media and sometimes fail to detect the disease sufficiently early[23]. Research for detection of a suitable antigen for serodiagnosis of tuberculosis has come a long way, and yet there is lot more to be explored.

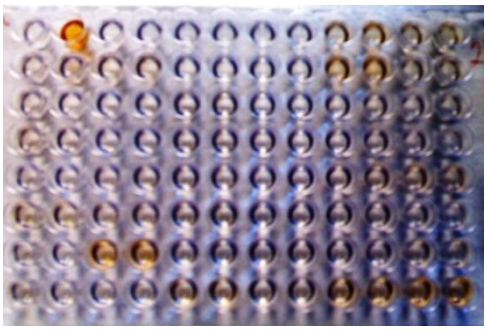
The development of the enzyme-linked immunosorbent assay[24] and of the dot-immunobinding assay[25] using mycobacterial antigens has stimulated interest in serological tests for the diagnosis of mycobacterial infections.

Thirty clinical samples were analyzed in this study out of which 25 appeared to be positive. Among the positive samples, 7 samples showed high IgG positivity; 9 samples showed high IgM positivity and remaining 9 samples showed both IgG and IgM positivity. IgG positivity showed chronic state of infection and IgM positivity showed acute state of infection. All samples were inoculated onto LJ slants for confirmation of infection. The sensitivity of the test appeared to be 90% (Fig 1). Sensitivity was calculated as follows:

$$\frac{\text{Total number of positive in ELISA}}{\text{Total number of positive cases in culture}}$$



(a)



(b)

Fig 1: Microtitre plates showing IgG (a) and IgM (b) response.

The majority of these results also agree with the results published formerly[8]. This study, although limited by a small number of samples, highlights the effectiveness of rapid and accurate diagnostic assay for the detection of TB, which may be particularly important in developing countries where TB is clinically intricate to diagnose.

The impact of antigen detection using ELISA for detection of tuberculosis remains obscure. In the present study, antigen detection was made the prime target to test the sensitivity of the above technique. Our observations reveal that the test standardised in our laboratory showed excellent sensitivity (90%). The BCG-CFA is rapid and easy, and is applicable for specimens, and does not require any special equipment. It can simplify the identification of *M. tuberculosis* complex strains, avoiding the technical complexity of PCR and similar identification techniques in clinical laboratories.

CONCLUSION

Our results suggest that BCG-CFA could be a valuable tool in the diagnosis of TB. It can endow simple and adequate results, predominantly in cases of tuberculosis, which are difficult to diagnose by the routine clinical and laboratory tests.

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