EVALUATION OF DIAGNOSIS OF PULMONARY TUBERCULOSIS USING THE ENZYME - LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF IgG ANTIBODY TO PURIFIED PROTEIN DERIVATIVE

Thesis

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Of The Master Degree in Basic Medical Sciences
(Bacteriology)

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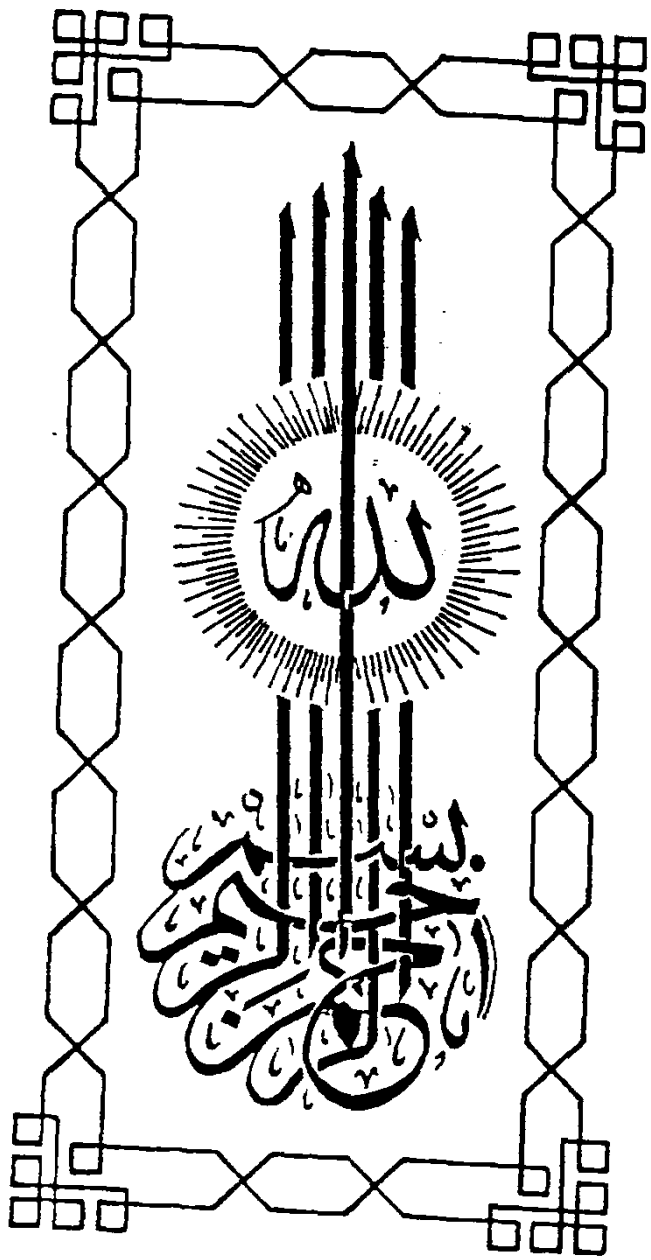

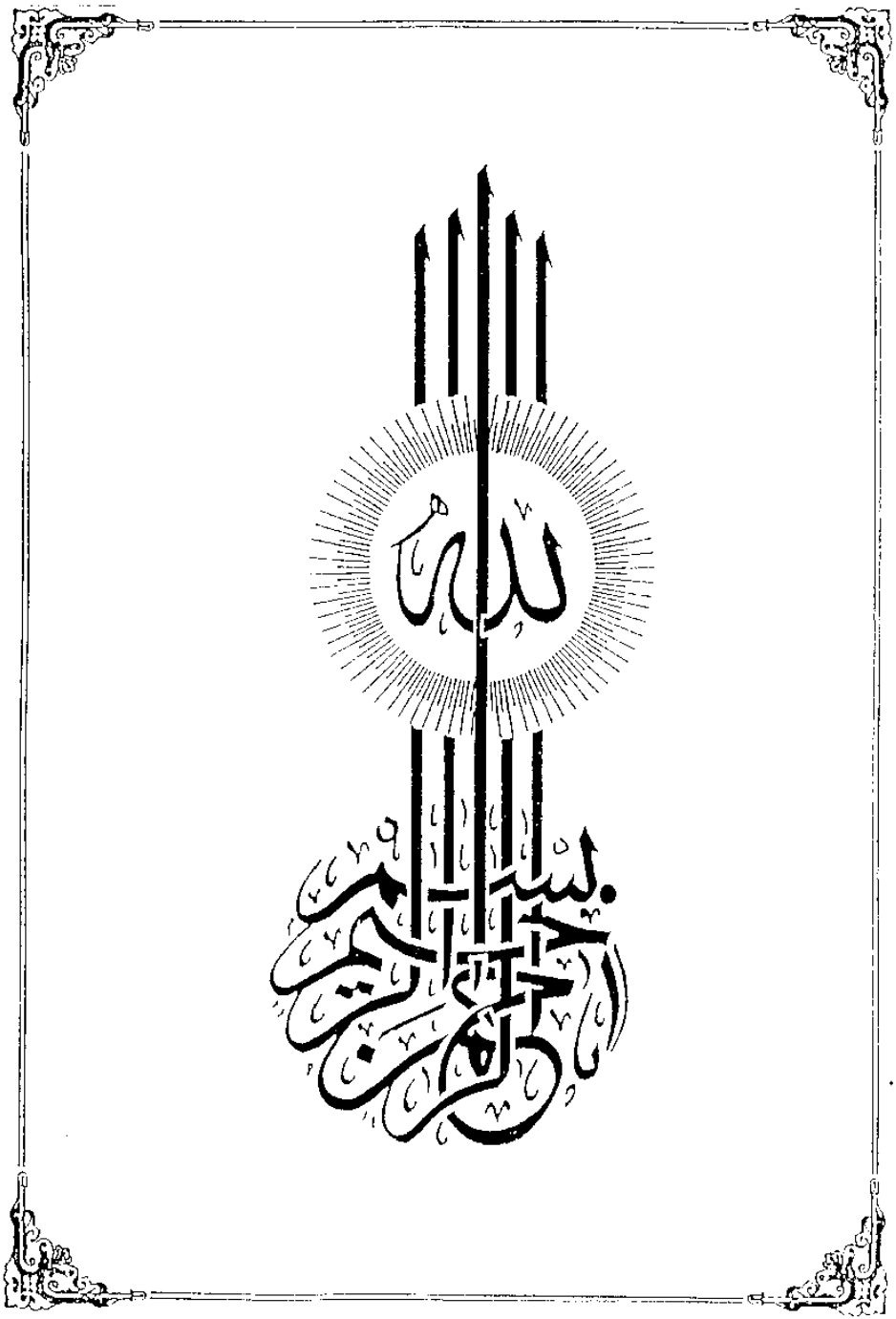

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Introduction

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Aim Of Work

INTRODUCTION AND AIM OF WORK.

Tuberculosis is prevalent in many developing countries including Egypt. Its laboratory diagnosis is routinely based on sputum smear examination for acid - fast bacilli and a positive result for tubercle bacilli. However false negative and false positive results may occur in sputum smears while a positive culture to confirm the diagnosis required weeks or months to develop. A rapid serologic test for active pulmonary tuberculosis would be of great clinical value and has been an erea of active research.

Antibodies to mycobacterial antigens have been identified in sera of tuberculous patients using a variety of techniques. Earlier techniques include the use of latex particles coated with culture filtrates of virulent Mycobacterium tuberculosis strain H37Ra (Duboczy & white 1969). Precipitin antibodies were detected by double diffusion in agar , and by immunoelectrophoresis using untreated culture filtrates (Forman et al. 1968 and Janicki et al 1971).

Haemagglutination technique has been used to study antibody to a mycopolysaccharide antigen of M. tuberculosis (Mitchison et al. 1977). Unfortunately , all of these assay lack the sensitivity and specificity required for use as a rapid diagnostic test in the indentification of infection with Mycobacterium tuberculosis (Kalish et al. 1983).

For this reason alternative methods , more sensitive and specific were needed. Nassau et al. 1976 used Enzyme - linked Immunosorbent Assay (ELISA) for detection of antibodies to mycobacterial culture filtrate in patients with tuberculosis and control subjects , however there was considerable overlap between patients and control values.

Zeiss et al. (1982) measured IgG antibody directed against Mycobacterium tuberculosis PPD by both radioimmunoassay and ELISA in patients with active tuberculosis and healthy controls with known tuberculin skin test reactions. There was a sharp delineation between patients and skin test positive and negative controls and no overlap in end point values.

Kalish et al. (1983) found that the mean ELISA values for IgG antibody to PPD were significantly greater in patients with active pulmonary tuberculosis than in patients with other pulmonary diseases or with inactive tuberculosis and that other classes of immunoglobulin antibody to PPD were less discriminating than IgG.

The encouraging results obtained with ELISA using PPD antigen for detection of mycobacterial antibodies stimulated our interest to study the diagnostic utility of ELISA in patients with pulmonary tuberculosis.

The aim of this study is to evaluate diagnosis of pulmonary tuberculosis using ELISA by detection of IgG antibody to purified protein derivative antigen (PPD) in the sera of patients in comparison with direct sputum smear examination by Ziehl - Neelsen stain as the results obtained from both methods would be available within 24 hours.

Another purpose of this study is to assess whether this ELISA test would be a useful method for follow up of patients under treatment and to

Review Of Literature

HISTORY

Tuberculosis is a disease of great antiquity and has almost certainly caused more suffering and death than any other bacterial infection. Even in 1980 , despite the availability of highly effective chemotherapy , many millions of human beings became infected. In the past tuberculosis has been referred to as the 'great white scourge' and by John Bunyan , as the 'captain of all of these men of death'.

In 1868 , Villemin produced a disease resembling human tuberculosis in rabbits by injecting material from human tuberculous lesions , both pulmonary and non pulmonary . The cause of these lesions could not be recognized , however until Koch discovered the human tubercle bacillus in 1882. He succeeded in culturing it on inspissated serum. Koch transmitted the disease to many animals of different species. Koch's classical study established without doubt that the bacillus he isolated was the cause of tuberculosis . In addition to culturing the causative organism , Koch succeeded in staining it by treatment with an alkaline solution of methylene blue for 24 hours. Subsequently Ehrlich (1882) improved the technique by using a hot solution of the arylmethane

dye fuchsin and it is this technique , slightly modified by Ziehl (1882) and Neelsen (1883) whose names it bears , that is still used today.

After Koch's discovery , acid-fast bacilli were isolated from cases of tuberculosis - like disease in various animals and were named after the host from which they were isolated. Five main types of tubercle bacilli were recognized - human , bovine , vole, avian and cold blooded. The 'cold - blooded' tubercle bacilli comprise two species of rapidly growing mycobacteria , namely Mycobacterium fortuitum (synonym M. ranae , the frog tubercle bacillus) and Mycobacterium chelonei (the turtle tubercle bacillus). Other acid - fast bacilli have been isolated from various environmental sources.

DEFINITION

Mycobacterium is the only genus in the family Mycobacteriaceae. They are straight or slightly curved rods , but coccobacillary , filamentous and branched forms also occur. Cells are gram positive , acid - fast , non - motile and non - sporing. Some strains are encapsulated by peptidoglycolipids (mycosides). Some strains produce a yellow pigment either in the dark or after exposure to light. Aerobic or microaerophilic. Acid is produced from sugars oxidatively. Nutritional requirements and temperature range of growth vary considerably. Two major subdivisions are recognized : rapid growers and slow growers. Cells contain large quantities of lipids. The qenus is distinguished by characteristic antigenic patterns and mycolic acid structures. G + C content of DNA : 66 - 72 moles per cent.

The generic name Mycobacterium (Lehmann and Neumann 1896) was given to a group of bacteria which grew as mould - like pellicles on liquid media. The genus contains over 30 species , most of which are well defined , including the causative agents of tuberculosis , leprosy and Johne's disease. Members of

other species , though occasionally the cause of disease in man and animals , usually lead a saprophytic existence in the natural environment. These species have been termed 'atypical' , 'anonymous' , 'paratubercle' , 'tuberculoid' and 'MOTT' (mycobacteria other than typical tubercle) bacilli (Francis and Abrahams 1982).

An important character of the mycobacteria is their ability to resist decolorization by acid after being stained by on arylmethane dye (acid fastness) but the genus may be more accurately defined by the chemical structure of its mycolic acids (Etemadi 1967) and its antigenic structure (Stanford 1973). The cultivable members of the genus are divisible into two major groups , the slow growers and rapid growers which differ in biochemical properties (Tuskamura 1967) , antigenic structures (Stanford and Grange 1974) and in DNA relatedness (Gross and Wayne 1970).

With very few exceptions , mycobacteria synthesize lipid - soluble iron - binding compounds , termed mycobactins , which differ in their chemical structure from functionally similar compounds in other genera (Patel and Ratledge 1973).