Microbiology Abstract
International Journal of Clinical And Diagnostic Research
Volume 2, Issue 5, Sep-Oct 2014.

"MYCOBACTERIAL SECRETORY PROTEOME WITH ES-31, ES-41, ES-43, ES-20, ES-38 AND ES-6 PROTEINS OF DIAGNOSTIC INTEREST AND SERINE PROTEASE AS CELL MARKER AND DRUG TARGET"

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Tuberculosis (TB) remains a major threat to public health worldwide and has been declared as global emergency by WHO in 1993. According to the World Health Organization (WHO, 2009), 9 million new TB cases including 4 million smear positive cases with 15% HIV-Positivity have been reported in 2007.

The secretory proteome of mycobacterium tuberculosis consists of large number of actively secreted proteins and enzymes of interest in diagnosis, immunoprophylaxis and as drug targets. In our laboratory proteins SEVA TB ES-31, ES-43, ES-41, ES-20 and ES-6 were isolated from short term M. tuberculosis H37Ra culture filtrate by FPLC, affinity chromatography and SDS-PAGE and explored in diagnosis. The seroreactivity of these purified antigens were assessed in pulmonary tuberculosis, extra pulmonary tuberculosis, different stages of pulmonary tuberculosis (fresh, relapse, chronic and latent) and in HIV-TB co-infection. Analysis of immune response to these purified antigens by indirect and sandwich ELISA using highly sensitive Penicillinase enzyme immuno assay, showed ES-31 antigen as having good diagnostic potential in pulmonary tuberculosis and in certain groups of extra pulmonary tuberculosis in particular tuberculous lymphadenopathy and tuberculous meningitis, whereas ES-41 was found to be more seroreactive in abdominal and bone & joint tuberculosis. ES-43 antigen was primarily recognized by serum antibodies in relapse cases. Further it has been shown that ES-6 and ES-20 have serodiagnostic potential in latent infection and lymphnode tuberculosis respectively. The later is observed at elevated level in weak immune patients. Immunomonitoring for presence of antigen in TB patients under ATT, showed that ES-31 antigen assay was useful in determining the effectiveness of therapy and patient's compliance. Further a cocktail of ES antigens SEVA TB ES-31, ES-43 and EST-6 (containing 38 kDa and 41 kDa protein antigens) and their affinity purified antibodies were explored and found useful in diagnosis of pulmonary and extra pulmonary tuberculosis sera of patients attending a tertiary care Kasturba Hospital. The antigen assay was found to be more sensitive than antibody based assay for detecting tuberculosis with HIV co-infection. Biochemical characterization showed that in vitro released ES-31, ES-43 and ES-20 antigens as glycoproteins while ES-41 and ES-38 antigens were found to be lipoprotein in nature.

User friendly Peroxidase ELISA has been developed for detection of circulating SEVA TB ES-31 antigen – a serine protease (Free antigen and Immune complexed antigen) with 70-75% sensitivity and 90% specificity and with a sensitivity of detection of antigen is 1 ng/20l (0.5 0g / ml serum). SEVA TB ELISA using cocktail antigen and cocktail antibody is being routinely done for screening of patients suspected of

tuberculosis attending Kasturba Hospital, a tertiary health care centre located in rural area.

Further study revealed that ES-31 antigen is a zinc containing serine protease with protease and lipase activities. Serine protease inhibitors such as pefabloc, 3, 4 dichloroisocoumarin, phenyl methyl sulphonyl fluoride (PMSF) and metalloprotease inhibitors such as ethylene diamine tetracetic acid (EDTA) and 1, 10 phenanthroline and orlistat (lipase inhibitor) inhibited 65 – 92 % serine protease activity in vitro. Isoniazid showed 95 % inhibition on mycobacterial ES-31 serine protease. These inhibitors also showed decreased bacterial growth in axenic culture and inhibition is further confirmed by decreased amount of ES-31 serine protease in culture filtrate. In human macrophage culture, highly inhibitory pefabloc, 1, 10 phenanthroline and isoniazid inhibited infectivity of virulent as well as avirulent M. tuberculosis bacilli to macrophages. The enhancement of mycobacterial cell growth in the presence of mycobacterial ES-31 serine protease while 90% inhibition (as observed by decreased CFU count) in the presence of anti ES-31 serine protease antibody was observed showing the importance of enzyme for entry and multiplication of bacilli and thus may be used as drug target. Orlistat may have therapeutic use in isoniazid resistant cases and in suspected cases of tuberculosis. Immunofluorescence study using FITC labelled anti ES-31 antibody conjugate showed the presence of ES-31 serine protease on mycobacterial cell surface and thus found to be a sensitive and specific biomarker to confirm M.tb in cell cultures and to differentiate from non tuberculous mycobacteriae (NTM). Further our findings support the concept that SEVA TB ES-31 as extracellular protease of M. tuberculosis which is readily accessible target of these impermeable organisms may be useful in rapid screening of potential antitubercular drugs.