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Detection of IS6110 insertion sequence in serum extracellular vesicles of tuberculosis patients reported to Kandy Chest Clinic

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Background: Detection of sufficient bacterial RNA in latent tuberculosis biomarker identification is challenging. The discovery of extracellular vesicles (EVs) in biological fluids opened a new field of study. EVs carry both bacterial and host derived molecules. The content transported via EVs depends on the cell type and the pathological state of the cell. EV content is mostly derived from the host RNA and proteins (99%) and the pathogen content is comparatively less (1%). The insertion sequence IS6110 is an essential genetic sequence and a gold standard epidemiological marker for *Mycobacterium tuberculosis* complex (MTBC).

Objective: To identify the presence of serum EV-derived mycobacterial RNA in both active and latent tuberculosis patients using IS6110 insertion sequence.

Method: Total RNA was extracted from serum derived EVs (500 µl) using combined 8% polyethylene glycol (PEG)6000 precipitation and filtration (0.22 µm-Nylon filter) method. Complementary DNA (cDNA) was synthesized from 16 RNA samples collected from active tuberculosis (ATB, n=10), and latent tuberculosis (LTB, n=6) patients (age ≥ 18 years) from males (n=9) and females (n=7) attending the Kandy Chest Clinic. A conventional polymerase chain reaction (PCR) was carried out using IS6110 insertion sequence in a 25 µl PCR reaction mix containing 5 µl of template (6ng), 5x PCR buffer, 0.4 mM dNTPs, 2.0 mM $MgCl_2$, 1.5 units of Taq polymerase (Promega), 0.4 mM of Pt8 (Forward) and Pt9 (Reverse) primers. The amplified PCR products were run on 2.0% agarose gel along with 100bp ladder to visualize the band pattern.

Results: Four ATB samples (n=4/10) and one LTB sample (n=1/6) showed positive results with a total of five positive samples (n=5/16) among all tuberculosis patients.

Conclusion: Mycobacterial RNA could be detected in serum derived EVs using conventional PCR. The low detection rate of mycobacterial RNA may be due to the lack of optimal isolation protocol specific to the chosen biological sample.

Keywords: *Conventional PCR, Extracellular vesicles, IS6110, Serum, Tuberculosis*