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RNA yield in serum extracellular vesicles of tuberculosis patients: using combined polymeric precipitation and filtration method

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Background: Extracellular vesicles (EVs) are an emerging platform for future biomarker discovery. EVs play a significant role in cell-to-cell communication and transportation of diagnostically significant molecules such as proteins, lipids, and nucleic acids. The most common method of EV isolation is differential centrifugation. However, more simple, fast, and cost-effective methods are demanding for resource-limited settings.

Objective: To identify a simple, fast, and cost-effective EV isolation method to obtain sufficient RNA yield for further downstream analysis.

Method: A total of 20 clinical samples were collected from active (ATB, n=14) and latent tuberculosis (LTB, n=6) patients (age ≥ 18 years); male (n=11) and female (n=9) attending the Kandy Chest Clinic. Serum was isolated from 4 ml of blood collected into separator tubes by centrifugation at 3,200 rpm for 15 mins at 4°C. For EV isolation, 500 µl of serum was mixed with an equal volume of 16% polyethylene glycol (PEG) 6000, 1.0 M NaCl, and incubated at 4 °C for 2h. Then the sample was centrifuged at 5,200 rpm for 20 mins and the subsequent pellet was washed three times with 50 µl of PBS. Finally, the pellet was resuspended in 1 ml of PBS and filtered through a 0.22 µm-Nylon filter. RNA isolation was performed using guanidinium thiocyanate–phenol–chloroform extraction and quantified using QuantiFluor® RNA System (Promega).

Results: The mean serum EV-derived RNA (EvRNA) concentration of ATB and LTB were 5.19 ng/µl±2.76SD and 7.84 ng/µl±4.45SD, and the total EvRNA yield was 196.38 ng±97.01SD and 277.66 ng±164.63SD, respectively. There was no significant difference between the total EvRNA yield of the two groups ($t(18)= 1.39$, $p>0.05$). However, the obtained total RNA yield was 20 times higher compared to the minimum necessary concentration for downstream analysis (10 ng).

Conclusion: Accordingly, the combined polymeric precipitation and filtration method gives sufficient serum EvRNA for further downstream analysis.

Keywords: *Extracellular vesicles, PEG, RNA, Serum, Tuberculosis*