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Part I: Abstracts

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Biophysical characterization of PEG-based extracellular vesicles isolated from tuberculosis patients using Dynamic Light Scattering technique

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Dynamic light scattering (DLS) is a simple and efficient method for nano-particle size and shape measurements. Recent studies suggested the differences seen in the shape and size distribution of extracellular vesicles (EVs) in different biological fluids may have the potential to discriminate different pathological conditions and provide useful information for clinical diagnosis. In Sri Lanka, this is the first attempt to biophysically characterize EV subpopulations isolated using a low-cost PEG-based EV isolation method from tuberculosis patient sera which is applicable for TB diagnosis in low-middle income countries. A total of 60 serum samples were collected and grouped into clinical cohorts of active tuberculosis (ATB, n=15), latent tuberculosis (LTB, n=15), household contacts (CT, n=15), and healthy individuals (HC, n=15) attending to the Kandy Chest Clinic. The EVs were isolated using the combined 8% polyethylene glycol (PEG)6000 precipitation and filtration (0.22 µm-polyethersulfone) method. The isolated EVs were morphologically characterized using the Renishaw inVia Raman spectrometer (Renishaw, UK) and the Olympus CKX41 inverted phase-contrast microscope and were biophysically characterized using the Nanoparticle analyzer, nanoPartica SZ-100V2 Series (HORIBA) based on two parameters; intensity-weighted particle size (Z-average) and the polydispersity index (PDI). For each clinical cohort, 45 measurements were taken and the results were statistically analyzed using One-way ANOVA (CI<0.05%), SPSS software v.28.0.1.1. Firstly, EVs were morphologically identified as sphere-shaped vesicles with an intact membrane which is consistent with previous studies. Based on the Z-average (nm) measurements (HC=120.336±2.996, CT=168.054±14.773, LTB=157.394±9.142 and ATB=302.158±38.672) and PDI values (HC=0.493±0.013, CT=0.627±0.011, LTB=0.490±0.019 and ATB=0.559±0.033) following clinical groups were statistically significant; Z-average: ATB and LTB, ATB and CT, and, ATB and HC and PDI: HC and CT, CT and LTB, thus could be differentiated using the DLS technique. Furthermore, increased PDI values (0.1-0.7) indicate the samples were highly polydisperse. Differences among PDI values of different clinical cohorts may be due to the alterations of the lipid bilayer composition as a result of the pathological condition. In conclusion, DLS can be used to differentiate tuberculosis patients from healthy individuals mainly based on the Z-average. However, a major limitation of this technique is, that several freeze-thawing could affect the particle size measurements.

Keywords: Extracellular vesicles, dynamic light scattering (DLS), polydispersity index (PDI), serum, tuberculosis, Z-average

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