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ISOLATION OF EXOSOMES, COMPARISON OF EXOSOMAL AND SERUM PROTEINS FROM HEALTHY AND TB PATEINTS

Anuska Singh[1], Ranjan Nanda[2] and Ausaf Ahmad[3]

[1,3]Amity University, Lucknow Campus, Lucknow - 226 010, India.
[2]International Center for Genetic Engineering and Biotechnology, New Delhi - 110 067, India.
email: aahmad@lko.amity.edu

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ABSTRACT : Exosomes from peripheral serum of tuberculosis (TB) patients contain mycobacterial proteins, although the precise makeup of other exosomes is still unknown. In the current work, serum exosomes from individuals with active TB (ATB) underwent a thorough proteomics analysis. SDS PAGE was used to examine exosomes from ATB patients. This work identified several possible biomarkers linked to TB infection and gave a thorough description of the exosome proteome in the serum of ATB patients.

Key words : Exosomes, TB patients, serum proteins.

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INTRODUCTION

The pathogen of tuberculosis, *Mycobacterium tuberculosis* (*M. tuberculosis*), continues to pose a serious threat to global public health (Aguzzi *et al*, 2009). The World Health Organisation (WHO) reported in 2021 that there were 5.8 million new cases of TB worldwide and that TB-related illnesses claimed the lives of approximately 1.5 million people. A new generation of techniques, including genome sequencing and proteomics, have been created in the last ten years to help us better understand how viruses cause disease in host cells after being eliminated by the immune system (Alipoor *et al*, 2016). Direct microscopy demonstration, culture, and polymerase chain reaction (PCR) are the basic methods for diagnosing active TB. The lengthy and intricate nature of these conventional diagnostic techniques prevents tuberculosis from being diagnosed quickly. Finding diagnostic markers for quick tuberculosis detection is thus extremely important (Azmi *et al*, 2013).

Exosomes are external membrane vesicles that range in size from 30 to 150 nm and because they are produced by both haematological and non-hematopoietic cells and are engaged in intercellular communication, they provide a potential study target for the diagnosis and treatment of tuberculosis infection. Exosomes have crucial roles in immunomodulation and signal transduction (Bellingham *et al*, 2012) and the movement of materials including lipids, proteins, nucleic acids, and other biological components (Cappello *et al*, 2017), additionally, by transferring different chemicals from donor cells to destination cells, they serve as cellular "trash bags" to remove surplus intracellular compounds and facilitate intercellular communication (Chen *et al*, 2021).

Exosomes from M. tuberculosis-infected host cells, like macrophages and natural killer cells, cause a variety of immune reactions, including an inflammatory response and the presentation of antigens. These immune responses highlight the crucial role of exosomes in the immune response against *M. tuberculosis* (Cheng *et al*, 2014). Exosomes from *M. avium*-infected macrophages caused naive macrophages to produce proinflammatory substances including tumor necrosis factor-alpha (TNF-alpha) and inducible nitric oxide synthase (iNOS), which in turn controlled the activation of T cells (Cheng *et al*, 2014). Chu *et al* (2005) demonstrated that Bacterial pathogenic glycopeptidolipids are present in exosomes produced by macrophages infected with M. avium. Exosomes may carry a variety of *M. tuberculosis* antigens, according to multiple exosome proteomics studies. For example, Das *et al* (2018) revealed 41 M. tuberculosis proteins within exosomes from macrophages infected by *M. tuberculosis* or *M. tuberculosis* culture

the ultracentrifuge sucrose density gradient technique. In contrast to TB patients, whose protein bands were very thin and indicated lower concentrations, the healthy persons' protein exosomes were more abundant in both concentration and number. It can be ascribed to the physiological condition's altered metabolism.

DISCUSSION

Exosomes are essentially tiny EVs that function in several cells and are crucial for intercellular communication. Upon inhalation of toxic compounds, a range of lung cell types, including epithelial cells, alveolar macrophages, endothelial cells, circulating blood cells, and monocytes, alter the creation and composition of tiny vesicles and exosomes, which may have an impact on one's health.

Exosomes from different cell types are released and absorbed in response to physiological variables, including pH. It has been demonstrated that low pH, a trait of malignant tumors, plays a crucial role in improperly controlling Exosome mobility within the tumor aggregate.

The metabolic, transcriptome, and proteome components of several biofluids, including sputum, serum, and urine, of TB patients who are infected with drug-susceptible or drug-resistant Mtb strains show significant disruption (Bissig *et al*, 2013; Cho *et al*, 2020; Colombo *et al*, 2014). The biofluids of TB patients with dysregulated molecular patterns provide fresh perspectives on the disease's pathophysiology (Corrado *et al*, 2013; Das *et al*, 2018; D'Asti *et al*, 2012). Exosomes, also known as extracellular small and large micro vesicles, are hypothesised to serve as molecular information transporters, moving molecules including proteins, lipids, mRNAs, and miRNA between cells and organs that perform essential biological processes (Dickens *et al*, 2017; El Andaloussi *et al*, 2013; Frohlich *et al*, 2014; Gebremicael *et al*, 2017; Giri *et al*, 2010). Exosomes are good and potent sources of biomarkers for a range of diseases because they include cell-specific characteristics.

CONCLUSION AND FUTURE PERSPECTIVE

Two innate immunological checkpoints that regulate MTB immunity are found in exosomes from immune cells that have acquired MTB infection. Although, earlier research has mostly focused on exosomes produced by innate immunity cells, adaptive immune cells' exosomes are more important for battling MTB infection. As a result, investigating potential effects of innate immune cell exosomes on MTB infection will support the development of novel vaccines and therapies. Even today, there are still problems with identifying, preventing and treating TB, especially drug-resistant TB. Different RNA molecules were found in exosomes following MTB infection. Exosomal RNAs may be used as novel TB biomarkers for the creation of the following generation of TB diagnostic techniques, and relevant research has already begun in light of this new information. A more potent TB vaccine may be possible given that exosomes have shown substantial promise in the delivery of vaccine components (proteins, peptides and RNA) in a number of infectious diseases. Despite the lack of understanding regarding the functions of exosomes in anti-MTB medication delivery, macrophage-targeted drug delivery is made possible by their great capacity to internalize the nanosized system. Exosomes do play unfavorable roles in immunological activity, hence caution must be used when using them as medication delivery platforms. The targeting effects against particular cell types would be enhanced by further surface functionalization of exosomes with particular ligands, which serves as a reminder that some ligands with macrophage targeting properties may be advantageous for anti-MTB treatment by exosome functionalization and drug administration. Most importantly, creating cell systems that can generate useful anti-MTB exosomes will significantly increase the use of exosomes in creating vaccines or drug delivery systems.

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