Progression of Cognitive Impairment to Alzheimer's Disease: Through the Lens of Salivary Extracellular Vesicles

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Simran Rastogi[1], Komal Rani[1] and Saroj Kumar[1,2] (iD)

[1]Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India. [2]Department of Health Science, Luleå University of Technology, Luleå, Sweden.

ABSTRACT: The elusiveness encircling around the domain of cognition, its impairment, and the poor prognosis of Alzheimer's disease has made early diagnosis a necessity. The noticeable symptoms in these conditions appear years later after the neuropathological changes occur in the brain. Exosomes, a small-sized extracellular vesicle facilitate intercellular communication of disease pathologies and their cargo can provide molecular information about its place of origin. The study titled "A novel approach to correlate the salivary exosomes and their protein cargo in the progression of cognitive impairment into Alzheimer's disease" was an attempt toward understanding the role of salivary small-sized extracellular vesicular (EV's) cargo in monitoring the progression. Outcomes of the study represent, that the salivary small-sized EV's (ssEV's) levels were higher in the cognitively impaired and Alzheimer's diseased as well the differential expression of the protein in the cargo correlates well with the disease severity staging. Thus, it can help in the development of an early non-invasive screening method.

KEYWORDS: Exosomes, neurodegenerative disease, Alzheimer's, extracellular vesicles

Background

Alzheimer's disease, a neurodegenerative disorder has its incidence ranging from 2% to 3% with age from 70 to 75 years to 20% to 25% among those with the age of 85 years.[1] According to the World Health Organization's report, approximately 55 million individuals are currently suffering from this disease, with 60% of the cohort belonging to countries with a low and middle-income group of people. The number of suffering individuals may rise to 78 million by the year 2020. AD is the most prevalent form of dementia. According to the Global Burden of Disease estimates for the 2003 World Health Report, dementia contributed 11.2% of years lived with disability in people aged 60 years and older.[2] It is known, after the age of 70, 16% of people have the condition of mild cognitive impairment (MCI), and 14% experience dementia. Two-thirds of individuals suffering from dementia are observed in population studies have Alzheimer's disease (AD). On the whole AD account for 70% of dementia cases followed by VD accounting for 20% to 25% of cases.[3,4]

Dementia, an umbrella term, which is commonly used for any impairment in the domains of cognition when accompanied by memory loss. The decline in cognitive abilities of an individual constitutes a multitude of changes in different domains of cognition that is memory, language, learning, and visuospatial skills. The domains of cognition are observed to follow a hierarchical order, keeping thinking and execution tasks on the top tier followed by perception, foresightedness, remembering ability at the lower-most tier.[5] The neuropsychological examinations devised for neurological and neuropsychiatric conditions are based on the various combinations of the sub-domains of these major domains. They are in a questionnaire format, where the suspected individual is needed to perform different assigned tasks. The degree of decline in abilities has categorized cognitive impairment into some sub-divisions. The condition of SCI: a person can perform well in objective tests, but lacks in subjective tests; MCI, a person has a loss in multiple domains of cognition and the impairment is severe enough to hamper the daily life activities. Majorly the behavioral symptoms in cognitive impairment occur later in a lifetime in comparison to the neuro-pathological changes, which conclusively lead to poor prognosis of the disease. The progression to the condition of AD has become a point of prime importance, as the majority of dementia cases are dominated by the pathology of Alzheimer's disease.

The prominence of early diagnosis

Alzheimer's disease has no cure. The facts and figures presented by the National Institute of Aging: the treatment of AD, neither regresses the rate nor stops the progression rather it helps in the management of only some symptoms of an individual. Currently, there are many molecular as well neuroimaging-based markers present,[6,7] however, they come in use only with the individuals showing any symptoms. The poor prognosis of these conditions is one of the major lacunae in the diagnosis and therapeutics of AD.

The ongoing developments and continuous studies did aid in better understanding of the disease, nonetheless, there is no such medication, which can be perfectly referred to as disease-modifying therapy. Many FDA trials on amnestic MCI to mild and severe AD patients are carried in the hope of better AD therapeutics. The dearth of better therapeutics can be overcome by the constant efforts to ease the malaise around AD. Therefore, the search for a novel molecular marker or a methodology, that is targeted toward early detection of neuropathological changes, has gained prominence worldwide.

The principal character: Small sized extracellular vescicles

Recently, small-sized extracellular vesicles, with sizes ranging from 30 to 150 nm have gained high significance due to their precise representation of the conditions of their origin. Small-sized extracellular vesicles facilitate intercellular communication as well as have established their role in the spread of disease pathologies. The ssEV's cargo content is defined as the fingerprint of the molecular status of its origin, due to the heterogeneity of its content: nucleic acids, proteins, and lipids in its cargo. They are known to be abundantly present in all biofluids. In AD the role of these vesicles is well known. The ssEV's protein cargos containing amyloid precursor protein (APP), Aβ, and tau facilitating intercellular communication and further propagation of Aβ and tau pathologies is a vividly researched phenomenon.[8]

Description of the study

The study was designed to comprehend the role of neuronal salivary ssEV's cargo in monitoring the progression of cognitive impairment conditions to Alzheimer's disease.[9] Exosomal concentration in biofluids tends to increase during conditions of neurodegeneration, neurological disorders, cancer, and other disease pathologies.[9-12] Conditions like neurodegeneration have shown increased ssEV secretion in the peripheral system. The rationale of this study was the neuronal ssEV's concentration in saliva could play the role of a progression marker of cognitive impairment paving the path for early determination of AD. The study consisted of different categories of subjects, namely cognitively impaired, Alzheimer's disease, and vascular dementia. The disease severity staging assessment of the subjects performed using Addenbrooke's cognitive examination III (ACE III) & MMSE (Mini-Mental State Examination) scoring test which are the normal clinical assessment. ACE III, a subjective test, was instilled to detect cognitive impairment, as well the cognitive functioning in dementia cases. Majorly the variation in scores of different cognition domains, aids in differentiation between frontal-temporal dementia and Alzheimer's disease. MMSE, a generalized and more commonly used assessment to determine the cognitive functions in patients, although contains some neuropsychological issues in contrast to ACE III.[13]

The study used the clarified saliva samples of the subjects from which small-sized extracellular vesicle were isolated through polyethylene glycol-based chemical precipitation. Antibody-based validation of isolated small-sized extracellular vesicles via western blot profiling using the surface marker CD63, followed by CD171/L1-CAM marker to prove their neuronal origin.

Morphological characterized small sized extracellular vesicles through transmission electron microscopy were quantified quantification using the Nanoparticle Tracking Analysis. The salivary ssEV's concentrations (particle/ml) resulted to be higher in cognitively impaired and Alzheimer's diseased individuals in comparison to healthy age-matched controls. CD63 Alexa fluor 488 antibody-based NTA was also performed that corroborated with the western blot analysis of CD63. The investigations of the study targeted the contents of the ssEV's cargo and studied the differential expression of pathological hallmarks.

The amyloid-beta and phospho-tau (p-tau) play a central role in Alzheimer's pathology. A Capillary electrophoresis-based automated western blotting system was used for the determination of expression of AD hallmarks. Moreover, the findings of the study suggested, the expression of amyloid-beta oligomer was found to be higher in CI & AD in comparison to HC. The amyloid-beta oligomer/fibril ~180 KDa was seen to be higher in AD and oligomer ~60 KDa in CI. One of the striking observation was the increased levels of phosphorylated-tau in the cognitively impaired individuals when compared to healthy control and Alzheimer's disease patients, which could be primarily due to the formation of the neurofibrillary tangles and its pattern of spreading in the brain in accordance to Braak and Braak staging. The existing studies also show the higher expression of p-tau was almost equivalent in the small-sized extracellular vesicles of patients who are at higher risk of developing AD and those who suffered from MCI progressed to AD in 3 years.[14] The increased ssEV's tau in CI in comparison to AD can be associated with more small-sized extracellular vesicle secretion, thus more seeding of p-tau across the neurons. The temporal discord as it is stated in the case, whether amyloid-beta regulates or induces tauopathy or vice-versa is still a conflict. The amyloid hypothesis: prime causative agent of AD is amyloid-beta, however, the pattern of impairment in cognition relates better with the tau protein. In-vitro, in-vivo studies provide evidence for the oligomeric amyloid-beta induced neuro-fibrillary tangle formation. Although tau protein depletion has also disrupted the homeostasis of amyloid-beta in mouse models.[15]

In the patient with stroke-related vascular dementia, where the major culprit of disease causation is not amyloid-beta, there was a significant decline in the expression levels of oligomeric forms of amyloid-beta and p-tau proteins from the isolated salivary small-sized extracellular vesicle from the patient. The findings of the study confirmed the neuronal origin of the salivary small-sized extracellular vesicles. The concentration of the isolated small-sized extracellular vesicles from all categories of subjects corroborated with the results of the western-blot analysis.
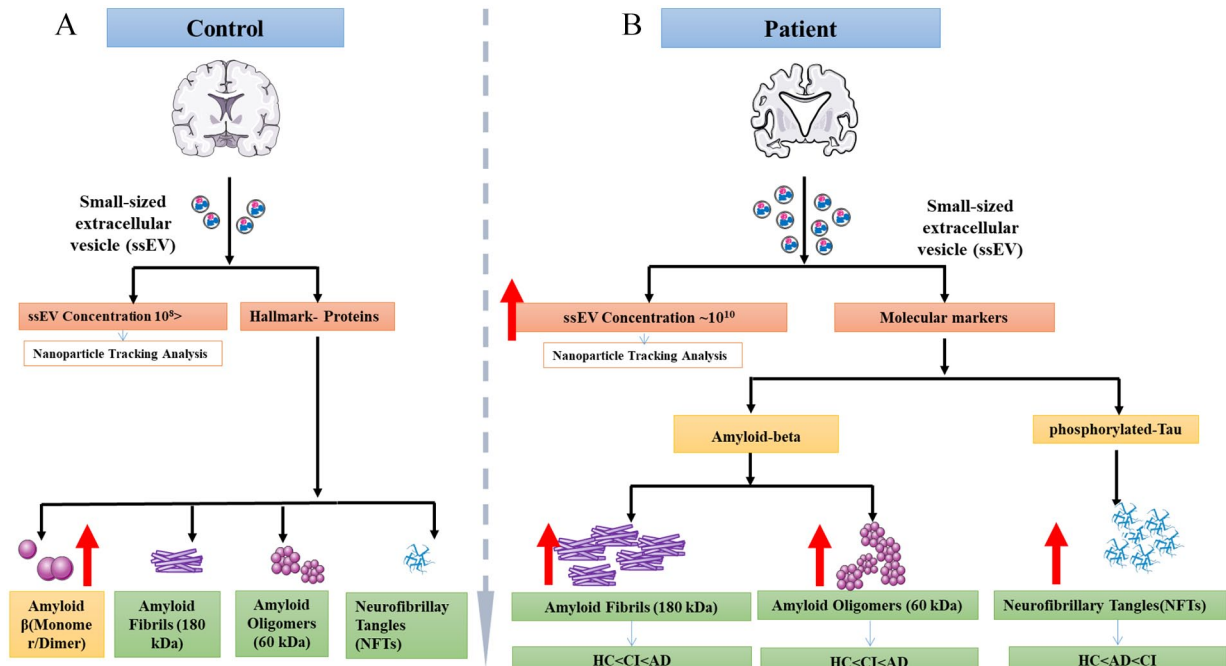
Pictorial representation of key-findings of the study. (A) The outcomes in the healthy controls. (B) The outcomes in patients.

Conclusion

The rationale of this study was to understand the role of salivary small-sized extracellular vesicle in understanding the progression of cognitive impairment to Alzheimer's disease. The results of the transmission electron microscopy, which is used for morphological characterization of small-sized extracellular vesicle revealed the lipid-bilayer of the neuronal vesicles as well confirmed the purity of isolated small-sized extracellular vesicles from the saliva samples of healthy control, cognitively impaired, and Alzheimer's disease patients. The quantification of small-sized extracellular vesicles by the scatter-mode-based NTA and the antibody-based NTA show the increased concentration of salivary small-sized extracellular vesicle in cognitively impaired and Alzheimer's disease in comparison to healthy controls, which corroborated with the pre-existing studies stating a change in concentration of vesicles. The expression level of the hallmark protein (amyloid-beta and p-tau) was determined using the automated western blotting system. The investigations carried out revealed that the concentration of the salivary small-sized extracellular vesicles of all the subject categories obtained by nanoparticle tracking analysis and the expression level of hallmark proteins in western blot analysis correlated well with the disease severity staging. Thus, the novel methodology based on the nanoparticle tracking analysis of salivary ssEV's cargo shows eminent potential to be developed as an early diagnostic tool for the detection of neuropathology in early-stage patients of CI and AD.

Author Contributions

Idea was conceptualize by SK. SR and KR were involved in drafting the manuscript.

ORCID iD

Saroj Kumar [iD] https://orcid.org/0000-0001-7029-1163

REFERENCES

1. Ferri CP, Prince M, Brayne C, et al. Global prevalence of dementia: a Delphi consensus study. *Lancet*. 2005;366:2112-2117.
2. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet*. 2011;377:1019-1031.
3. Morley JE, Morris JC, Berg-Weger M, et al. Brain health: the importance of recognizing cognitive impairment: an IAGG consensus conference. *J Am Med Dir Assoc*. 2015;16:731-739.
4. van der Flier WM, Scheltens P. Epidemiology and risk factors of dementia. *J Neurol Neurosurg Psychiatry*. 2005;76:v2-v7.
5. Harvey PD. Domains of cognition and their assessment. *Dialogues Clin Neurosci*. 2019;21:227-237.
6. Lee JC, Kim SJ, Hong S, Kim Y. Diagnosis of Alzheimer's disease utilizing amyloid and tau as fluid biomarkers. *Exp Mol Med*. 2019;51:1-10.
7. Márquez F, Yassa MA. Neuroimaging biomarkers for Alzheimer's disease. *Mol Neurodegener*. 2019;14:21.
8. D'Anca M, Fenoglio C, Serpente M, et al. Exosome determinants of physiological aging and age-related neurodegenerative diseases. *Front Aging Neurosci*. 2019;11:232.
9. Rani K, Rastogi S, Vishwakarma P, et al. A novel approach to correlate the salivary exosomes and their protein cargo in the progression of cognitive impairment into Alzheimer's disease. *J Neurosci Methods*. 2021;347:108980.
10. Rani K, Mukherjee R, Singh E, et al. Neuronal exosomes in saliva of Parkinson's disease patients: a pilot study. *Parkinsonism Relat Disord*. 2019;67:21-23.
11. Rastogi S, Sharma V, Bharti PS, et al. The evolving landscape of exosomes in neurodegenerative diseases: exosomes characteristics and a promising role in early diagnosis. *Int J Mol Sci*. 2021;22:440.
12. Dai J, Su Y, Zhong S, et al. Exosomes: key players in cancer and potential therapeutic strategy. *Signal Transduct Target Ther*. 2020;5:145.
13. Calderón C, Beyle C, Véliz-García O, Bekios-Calfa J. Psychometric properties of Addenbrooke's cognitive examination III (ACE-III): an item response theory approach. *PLoS One*. 2021;16:e0251137.
14. Saman S, Kim W, Raya M, et al. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J Biol Chem*. 2012;287:3842-3849.
15. Musiek ES, Holtzman DM. Three dimensions of the amyloid hypothesis: time, space and 'wingmen'. *Nat Neurosci*. 2015;18:800-806.