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Profiling of microglial-originated microvesicles to unearthing their lurking potential as potent foreseeable biomarkers for the diagnosis of Alzheimer's disease: A systematic review

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Abstract:

BACKGROUND: Alzheimer's Disease is a neurodegenerative disease characterized by accumulation of phosphorylated tau and amyloid deposits within the brain tissues in the elderly population. Numerous studies established that amassment of these toxic accretions within the brain tissues initiates neuronal demise and synaptic impairment which becomes the underlying basis for memory loss and cognitive abnormalities in these patients.

HYPOTHESIS: Hypoxia, oxidative stress, and inflammation are commonly encountered perils in the neuronal milieu that derail the neuron-synapse interactions and maneuver them to undergo apoptosis. A spinoff from neuronal desecration is microglial activation which forms a cardinal role in mounting innate immune defenses for warding off and reversing off toxic stimulus encountered.

RESULTS: A potential ramification of microglial activation in this context is assembly, processing and exuding of micro-vesicles into the extracellular space. These micro-vesicles will be packaged with amyloid and tau deposits which accumulate intracellularly within microglial cells secondary to their professional scavenging function. These microglial MVs are prone to seed tau and amyloid beta into the surrounding neuron-synapse framework, thus are implicated in spreading the disease pathology in AD.

CONCLUSIONS: Therefore, these MVs can be considered as an omen for disease initiation, progression, monitoring as well gauging the treatment response in the clinical AD cohorts. We speculate future research studies to unmask the dormant potential of these microglial MVs as reliable markers for diagnosis, evaluating the disease progression as well as treatment in AD. This will open the door for early diagnosis of AD so as to prioritize management and optimize clinical outcomes..

Keywords:

Activation, Alzheimer's disease, amyloid beta, biomarkers and neuronal microvesicles, microglia, microvesicles, neuronal death, tau, neurodegeneration

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Introduction

According to the Alzheimer's Association Report 2023, currently, there are approximately 6.7 million American population with confirmed Alzheimer's disease (AD) diagnosis, and these figures are projected to substantially multiply and eventually reach to around 13.8 million by 2060.[1] It is not surprising that AD is currently the fifth leading cause of death, and deaths secondary to AD have increased by 145% in the recent years.[1] The symptoms of AD can be neuropsychiatric (depression, apathy, agitation, hallucination, hypokinesia, paranoia, rigidity, tremors, and psychosis), sleep disturbances, and memory disturbances (loss of working memory and long-term declarative memory).[2-5]

Earlier and specific diagnosis of AD dementia is essential so that these patients so that these patients can be risk stratified for earlier referral, administration of clinical interventions and treatment monitoring. It is important to understand that dementia in the elderly populations can mirror dementia from other neuronal pathologies. Late-onset dementia can be classified into two categories based on the presence or absence of motor symptoms. Alzheimer's disease, frontotemporal dementia (FTD), Creutzfeldt–Jakob disease, and prion diseases are typically characterized by dementia without associated motor disturbances.[6] However, dementia with motor symptoms is commonly seen in diseases such as Parkinson's disease (PD), progressive supranuclear palsy, corticobasal ganglionic degeneration, hydrocephalus, Huntington's disease, and vascular dementia.[6]

If the clinicians suspect the diagnosis of AD based on the clinical profile of the patients, then a battery of non-invasive tests including blood [Aβ42/Aβ40 ratio, APP669-711/Aβ42, Tau, NFL & GFAP] and CSF [Aβ42, Aβ42/Aβ40, T-tau, P-tau, & Neurogranin] biomarkers are currently available to risk-stratify these patients.[7-9] Positron emission tomography (PET) scan has been helpful in the early detection of AD by picking up glucose hypometabolism in the parietotemporal association cortices, posterior cingulate cortex, and the precuneus initially, from where it slowly spreads to the frontal cortex.[10]

Previous studies indicate that accumulation of phosphorylated tau in CSF (p-tau217 and p-tau181) and brain amyloid by PET scans is associated with white matter pathology, vascular abnormalities, and cognitive impairment in AD.[11,12] PET scan is also extremely useful in gauging amyloid and tau burden in the brain tissues, a hallmark pathological feature that symbolizes and characterizes the severity of AD disease burden.[13,14] In most of the cases, clinicians rely on the combination of blood, CSF, and PET imaging tests to ratify the diagnosis of AD.[9]

Early diagnosis of AD is imperative, thanks to the current arsenal of therapeutic interventions that can modestly mitigate the symptom profile and cognitive disturbances, henceforth providing a ray of hope for these patients. The currently available drugs for AD can be classified mainly as three groups mitigators of behavioral and psychological symptoms of Dementia (BPSD) [brexipiprazole, & Suvorexant], mitigators of Cognitive decline [Donepzeil rivastigmine, galantamine & memantine] and disease modifying drugs [aducanumab and lecanemab].[15,16] These disease-modifying drugs were recently approved by the FDA, and these are monoclonal antibodies which exert their action by direct binding to the fibrillar forms of parenchymal amyloid plaques in the brain.[15,16] In the clinical trials employing FDA approved drugs, clinical researchers witnessed shrinking of amyloid/tau burden, along with concomitant regaining of cognitive functions, thus increasing the chances of patient independence in the treated AD cohorts.[17,18]

Along with neurons, microglial cells also secrete microvesicles (MVs) into the CSF and blood of AD patients as the disease process unfolds and innate defenses are set in motion [Figure 1]. It has been speculated that these microglial-secreted MVs serve as paracellular messengers by transporting these toxic amyloid and tau aggregates from the microglial cells to the vicinity of neurons, thus aiding in neuronal death and progression of neurodegeneration in AD. Hence, performing research into these microglial-originated MVs in the body fluids of AD patients will become the Launchpad for unearthing novel biomarkers that can be useful for detecting preclinical and clinical AD. Apart from isolating these MVs, ascertaining cargo for pathological Aβ42 and pT181 and pT217 will provide crucial information whether these serve as mediators for abetting neurological death in AD.[19-27] Quantification of MVs and their cargo in the body fluids can be standardized as a screening assay for differentiating early preclinical and clinical AD cases from age-matched control population. Furthermore, they would also be utilized for monitoring disease progression and treatment monitoring in AD. Earlier diagnosis of preclinical and clinical AD is very much essential, so that patients can be risk stratified and enrolled in clinical trials for the administration of therapeutic interventions in a timely manner. This will lead to optimizing clinical outcomes as well as substantial reduction in mortality and morbidity in AD. This review addresses the important question of delving into MVs circulating in the body fluids such as CSF and blood to ascertain the appropriate and reliable marker that can adequately relay the subtle brain tissue alterations relevant to the onset and progression in AD

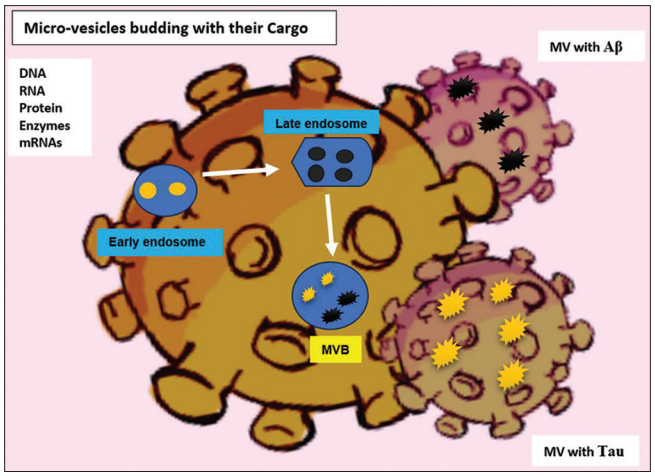

Figure 1: Microvesicles (MVs) assembly and release: MVs will be assembled within the cytoplasm of the microglial cells and packaged with necessary protein, DNA, RNA, enzymes, microRNAs, and other factors. Their lifecycle transitions from early endosome, late endosome to multivesicular bodies containing numerous cargos. These Mulvetisicular bodies (MVBs) fuse with the plasma membrane resulting in the release of MVs from the budding of the plasma membrane. Once released, these MVs carrying their cargo will traverse the extracellular space. Their functional significance mainly comes into play when interacting with neighboring cells including neurons and astrocytes through paracellular communication. Due to this, they might influence the physiological as well as pathological processes in neurodegenerative diseases such as Alzheimer's disease. mRNAs: MicroRNAs, MV: Microvesicle, MVB: Mulvetisicular bodies

pathogenesis. Our review is a small step to highlight the potential utility of microglial secreted MVs in forecasting the incipient disease changes indicative of MCI (Mild cognitive impairment) and AD. In a study by Joshi *et al*., microglial MVs floating in the CSF showed an upward trend in MCI and AD groups as compared to control population.[28] Similarly, frail MCI patients have higher circulating levels of microglial MVs as compared to control cohorts.[29] The main purpose of this review is to underscore the importance of these microglial MVs and to highlight their potential contribution in the neurodegeneration of AD. Furthermore, this review summarizes studies from the literature that makes to postulate that subtle brain tissue alterations induced by amassment of Aβ and tau are associated with significant alterations in these microglial-based MVs in the CSF and blood. This review highlights the value of the current literature by indisputably describing their toxic role in provoking neurodegeneration as well as spreading the toxic pathology (Aβ and tau) to the surrounding neurons, thereby abutting the neurodegenerative process in AD. By emphasizing their pathologicalsignificance transparently, we tend tounderscore the presumption that subtlemicroglial MV fluctuations in the body fluidswillbe directly proportional to the diseaseprogression in the preclinical and clinical AD disease models.

Methodology

We performed PubMed search of the relevant articles that enumerate the origin, circulation, cargo material and paracellular communication of these microglial secreted micro-vesicles (MVs) in the body fluids of AD and related neurodegenerative diseases. We particularly highlighted their pathological role in permeating the disease pathology and their relative importance in provoking neuronal demise in AD. We performed the literature review not using Mesh (Medical Subject Headings). We searched PubMed using words including MVs, exosomes, microglial activation, microglial exosomes, microglial cargo, MV lipids, paracellular communication of MVs, and biomarkers. We also searched PubMed for keywords including Alzheimer's disease, neuronal death, brain atrophy, amyloid beta, tau, neurodegeneration, and biomarkers. The inclusion criteria were MVs, exosomes, and microglial cells. The exclusion criteria were neuronal-derived MVs.

Results

We searched the Pubmed with microglial microvesicles and it yielded 22 results. We included studies that mainly discussed microglial MVs associated with neurodegeneration in AD. Minimal brain tissue alterations induced by piling up of Aβ, and tau is associated with significant alterations in these microglial based MVs in the body fluids. These microglial based MVs are mainly incriminated in neurodegeneration as well as spreading the toxic pathology (Aβ and tau) to the surrounding neurons thereby propagating the neurodegenerative process globally in the brain regions in AD. Thus, we postulate them to be future biomarkers that can reliably predict the inception and progress of pathological process in AD.

Discussion

What are microvesicles: Definition, types, and significance?

MVs or exosomes are membrane-bound vesicles released from budding of the cell plasma membrane due to membrane remodeling and disruption of cytoskeleton.[30] They are usually released under conditions such as cellular toxicity, pro-inflammatory environment, hypoxia, and oxidative stress.[31,32] These MVs are secreted to meet the needs of intercellular communication as they accommodate and transport numerous cargo materials ranging from receptors, proteins, lipids, and carbohydrates to microRNAs (miRNAs).[33] MVs can be isolated from brain tissues of AD patients, and these are notable for substantial differences in their biology, contents, and functional characteristics (pro-inflammatory and neuropathological features) in proportion of degree of early disease in preclinical AD.[34] MVs isolated from the brain tissues of tau transgenic rTg4510 were revealed to incite seeding of tau into the FRET (Fluoroscence

Resonance Energy Transfer) Tau Biosensor cells in a threshold dependent manner, thus underscoring their mechanism for intercellular spread of tau pathology in the AD disease models.[35] In parallel to these findings, MVs isolated from cortical gray matter of AD patients were demonstrated to have higher levels of pS396 tau and Aβ1-42 as compared to their control cohorts.[36] MiRNAs which are the most important cargo present in the blood and CSF MVs were previously assessed to be a potential biomarker for the diagnosis of preclinical and clinical AD with modest success.[37-40]

MVs can be secreted by neurons or microglial cells. Neuronal derived MVs are implicated in sustaining the pro-inflammatory M1 phenotype as well as upregulating complement factors, a combination that paves the way for microglial mediated neuronal destruction.[41,42] On the contrary, few studies show that neuronal MVs offered protection and were associated with neurogenesis, brain repair, and abating microglial pro-inflammatory activity.[43,44] In fact, some studies even report that neurons secrete MVs utilizing sphingolipid metabolizing enzymes, and these are instrumental in transfiguring the toxic Aβ aggregates into nontoxic fibrils, so that they can be subsequently purged by neighboring microglia.[45]

Microglial-secreted MVs are shown to be involved with numerous functions such as proliferation, metabolism, neurogenesis, oxidative stress, apoptosis, autophagy, synaptic activity, and neuroinflammation [Figure 1].[46-48] Depending on the current circumstances surrounding the neuron–synapse framework, microglial cells can secrete MVs loaded with neurotrophic or neuroinflammatory factors, which can ultimately modify the neuronal milieu.[49,50] Under neuroinflammatory conditions, microglial cells will assemble and engineer MVs that harbor pro-inflammatory mediators (interleukin-1 [IL-1] β, Aβ, and caspases) and miRNA (miR-155 and miR-146-5p) that can potentially incite neuronal changes including revamping excitatory transmission, desecration of dendritic spines, and dissipation of Aβ toxicity.[51] In a recent study by Yang, Y. *et al*., LPS stimulation of BV2 microglial cells resulted in the massive production of MVs with pro-inflammatory cytokines (TNF-alpha [Tumor Necrosis Factor-α & IL-6 [Interleukin-6]) cargo and increased synthesis of specific proteins that primarily functional in translation and transcription of MVs.[52] Conceivably, blocking of inflammatory pathways utilizing TNF inhibitors resulted in substantial reduction in the MVs produced and their associated proteins.[52] Microglial MVs released during traumatic brain injury (TBI) and transient middle cerebral artery occlusion were neuroprotective by attenuating neuronal loss, reducing infarct volume, and curbing neuroinflammation.[53,54]

Significance of microglial-released microvesicles in perpetuating neurodegeneration in Alzheimer's disease

Microglial MVs are seemed to effectuate double-barreled functions in AD brain including production, propagation, and amplification of Aβ pernicious effects as well as destruction and purging of these toxic aggregates.[55] A previous study revealed that the administration of statins is beneficial as it favors the degradation of extracellular Aβ aggregates via stimulating microglial secretion of MVs enriched with insulin-degrading enzyme.[56,57] Furthermore, microglial micro-vesicles carrying TREM2 (Triggering receptors expressed on myeloid cells 2) receptors can bind, and engulf extracellular Aβ aggregates, a process that reconfigures the inflammatory milieu around the neurons by purging these toxic aggregates from their vicinity.[58] In a study by Gabrielli *et al*., microglial-released MVs were shown to revamp dendritic morphology and derail the synaptic plasticity, thereby greasing the wheels for inciting deficits in long-term potentiation in entorhinal cortex and dentate gyrus.[59]

Neuroinflammation is a potential trigger for secretion of microglial MVs with pro-inflammatory cytokine cargo, a risk factor for amplifying neuronal demise in AD.[52] Microglial MVs isolated from the cortex of TBI mice were capable to induce neuroinflammation in the control mice, thereby buttressing the postulation for their involvement in energizing the immune responses and augmenting the neuroinflammation.[60] According to Durur *et al*., microglial neuroinflammatory responses instigated by neuronal MVs containing Let. 7e miRNA can successfully perpetuate disease propagation and neurodegeneration in AD.[61] In a study by Sardar Sinha *et al*., MVs were shown to carry and shuttle Aβ oligomer cargo to the neighboring neurons, thereby abetting in their death and neurodegeneration in AD.[62] Microglial cells ingest these Aβ oligomers from the extracellular space and package them into MVs when their intrinsic degradation pathways are overwhelmed [Figure 2].[28,63] During this processing, insoluble Aβ will be transformed into soluble and neurotoxic Aβ due to the eccentric lipid profile of MV plasma membrane [Figure 2].[28] MVs budding from the microglial cell membrane traverse the extracellular space and offload their neurotoxic Aβ cargo upon reaching the neurons [Figure 3].[28] Eventually, toxic Aβ instigates neuronal death by various mechanisms including mitochondrial stress, oxidative stress, endoplasmic reticulum (ER) stress, reactive oxygen species, nitric oxide production, and c-Jun N-terminal pathway and microglial phagocytosis [Figure 3].[26,64-66] Furthermore, these microglial MVs carrying toxic Aβ pileups were unveiled to instigate neuronal desecration via mitochondrial clustering, elevation of fission protein drp1, linking to voltage-dependent anion

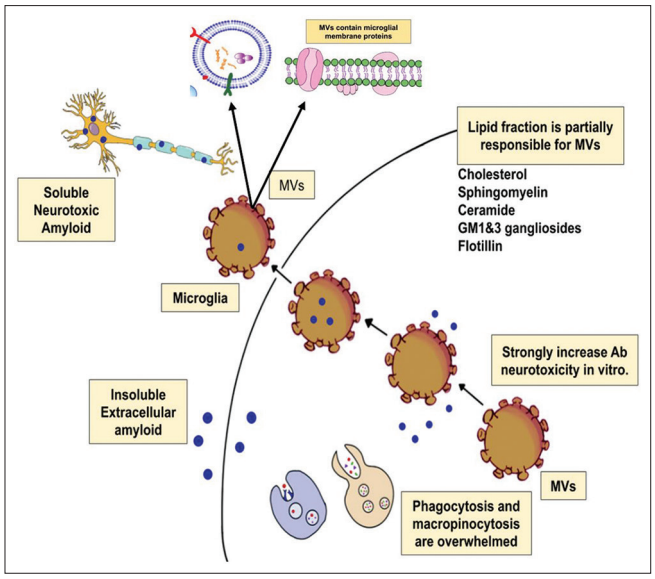

Figure 2: Microvesicles (MVs) modify the amyloid fibrils to be more neurotoxic in Alzheimer's disease. Insoluble extracellular amyloid will be ingested by wandering microglial cells through phagocytosis and macropinocytosis phenomenon. Once ingested, these amyloid aggregates will be processed through intracellular degradation pathways. As time progresses, these degradation mechanisms will be ultimately overwhelmed so that alternate mechanisms for processing take precedence. In such a context, the synthesis of microvesicles takes place and these nontoxic amyloid aggregates will be packaged within them. As a part of this processing, nontoxic amyloid will be converted into toxic amyloid due to the specific lipid profile of MVs. Ultimately, they get released by budding of microglial plasma membranes. Once the MVs are assembled intracellularly, the final step in their release is through budding from the plasma membrane. Due to this inherent step, MVs tend to harbor some of the key proteins imprinted in the microglial plasma membrane. This gives us the opportunity to identify the origin of these released MVs in the body fluids by profiling microglial transmembrane markers. Once these MVs are released, they traverse the extracellular space and reach the neurons and exude their toxic pileups onto the neurons. By this mechanism, these MVs originated from microglial cells and will be primarily implicated for spreading of disease pathology with the brain tissues. MVs: Microvesicles

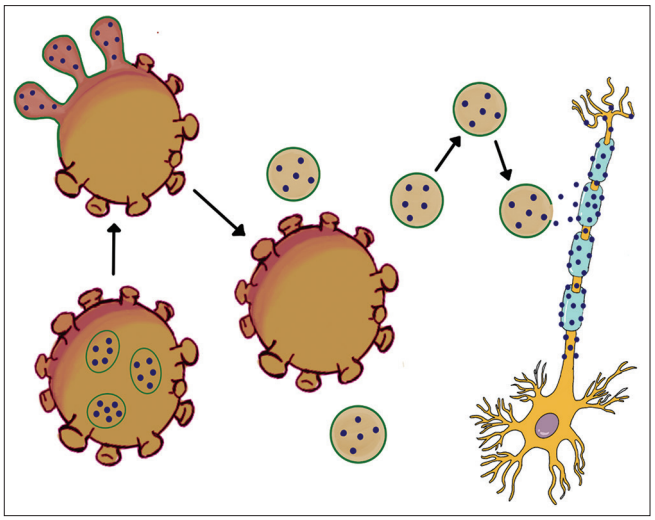

Figure 3: Microvesicles spread disease pathology and thus are noteworthy choices for biomarkers in Alzheimer's disease (AD). With their selective cargo such as amyloid fibrils and tau and paracellular communication, they spread the disease among the neighboring neurons and thus are responsible for aiding the neurodegenerative process in AD. Due to this functional capability, they might provide a harbinger for disease initiation and progression of AD. On top of that, they might also be harnessed for monitoring the disease prognosis as well as gauging the disease response to anti-amyloid medications in these high-risk preclinical and clinical AD cohorts. With few research studies performed so far to grasp their significance, their lurking potential as reliable biomarkers should be efficiently unearthed. This will be the starting point for including them in the armory of foreseeable biomarkers that can precisely forecast the nascent and subtle brain cellular changes symbolizing AD in the elderly cohorts

channel, and caspase activation.[67] Owing to the fact that these microglial micro-vesicles were implicated in disseminating these virulent Aβ within the neighboring neurons and by doing this triggering global disease pathology, therapeutic interventions designed to impede the formation, budding and secretion of these microglial based MVs tend to afford beneficial effects by halting the Aβ triggered neuronal demise.[62,68-70] In a recent clinical study by Agosta *et al*., CSF microglial MVs were shown to be substantially elevated in the MCI and AD cohorts as compared to controls. Presumptively, hippocampal microglial activation can be considered a repercussion of Aβ1-42 accumulation which subsequently results in the procreation of neurotoxic and myelinotoxic MVs.[71] These exuded pathological MVs are ultimately responsible for spawning white matter tract damage and progressive hippocampal atrophy in MCI and AD disease cohorts, respectively.[71] A report revealed that serotonin released from the serotonergic neurons acts on the $5HT_2$ and $5HT_4$ (serotonergic) receptors on the microglial cells, thereby sparking the release of exosomes into the extracellular space.[49] Furthermore, upon delving into the mechanisms involved in their release, energizing of cAMP-GEF1/2 (cyclic AMP-guanine exchange factor 1/2) signaling pathways and upregulation of cytosolic calcium levels causing fusion of MVs with plasma membrane were shown to be primarily responsible.[49]

Controversy associated with usage of neuronal microvesicles

Neuronal-derived exosomes are previously shown to be beneficial as biomarkers for diagnosis and monitoring disease progression in various neurological diseases including TBI, neuroinflammation, human immunodeficiency virus dementia, and AD.[72-77] The levels of Aβ42, T-tau, and P-T181-tau in neuronal-derived exosomes were revealed to be in higher concentration as compared to MCI and control cohorts.[12] Furthermore, this study highlighted the findings that the MVs isolated from CSF and blood harbor comparable levels of Aβ42, T-tau, and P-T181-tau in them, thus validating the potential utility of neuronal blood-based MVs as reliable biomarkers for the diagnosis of AD and MCI cohorts from control population.[12] That being said, by quantifying the levels of P-S396-tau, P-T181-tau, and Aβ1-42 in the blood neuronal-derived MV, it would be feasible to forecast the occurrence of AD-related disease changes 10 years before clinical onset.[78]

Nevertheless, recent reports indicate that these neuronal MVs are not ideal for the usage of biomarkers as their neuronal origin cannot be ruled out. The controversy surrounding the use of L1 cell adhesion molecule (L1CAM) (neuronal cell surface protein) for isolating neuronal MVs (neuron-derived extracellular vesicles [EVs]) has raised concerns about the reliability of studies utilizing these vesicles as biomarkers for brain damage, particularly in neurodegenerative diseases like Alzheimer's disease. Researchers led by David Walt at Brigham and Women Hospital, Boston, argue that L1CAM does not actually associate with these vesicles.[79] This challenges the validity of reported biomarker differences between healthy individuals and those with neurodegenerative conditions.

The study by Norman *et al*. suggests that L1CAM behaves as a soluble protein rather than associating with vesicles in CSF and plasma.[80] Thus, they recommend that L1CAM cannot be used in isolation protocols for identifying neuronal EVs from the body fluids.[80] This finding prompts questions about the accuracy of using L1CAM-based methods for studying changes in exosomes associated with conditions such as Alzheimer's, Parkinson's, and other neurodegenerative diseases.[73,81-85]

The search for alternative markers continues, with some proposing neural cell adhesion molecules but facing challenges in finding a suitable replacement. Studies using L1CAM to isolate EVs persist with the potential implications for the reliability of neurodegenerative disease biomarker research.

Microglial-secreted microvesicles are notable by their specific membrane signature proteins

Microglial-originated MVs will be released by budding of their plasma membrane. Due to this, these extravasated MVs tend to harbor some of the key proteins on them. These markers can be used to trace their origin and thus can be exploited for devising biomarkers for diagnosis and treatment monitoring in AD. The microglial plasma membrane proteins can be broadly classified into general microglial markers and activated state markers.[86]

In a recent study, a most commonly expressed microglial plasma membrane protein TMEM119 was utilized for isolating exosome fraction originated from microglial cells.[29] The most specific proteins that are strictly expressed in microglial cells include integrin αm (CD11b), TREM119, and purinergic G-inhibitory protein receptor, which can be which were exploited for segregating MVs emerging from microglial cells.[86-88]

Importance to patient care

Previous research studies suggest that quantifying exosomes in the body fluids was a viable option as their changes were proportional to the degree of neurological damage, brain tissue changes, and cognitive dysfunction in neurodegenerative diseases. In a study performed by Winston *et al*., quantification of neuron-derived MVs (NDMVs) and their cargo material (Aβ42 and tau) was successful in forecasting the conversion from MCI to full-blown AD dementia.[83] Intracerebral injection of plasma NDMVs isolated from MCI and AD patients in female mice resulted in tau aggregation pathology in the pyramidal neurons of the hippocampus due to inter-neuronal spread of these pathological aggregates.[83] Analysis of neuronal MV cargo load by enzyme-linked immunosorbent assay (ELISA) revealed that Aβ42, NRGN, synaptophysin, synaptotagmin, and synaptopodin were successful in differentiating MCI group from cognitive normal controls.[89,90]

In a case–control study by Deng *et al*., overexpression of exosome miR-146-5p in the serum was directly proportional to the cortical thinning by magnetic resonance imaging in the major depressive disorder.[91] Similarly in a study by Joshi *et al*., microglial MVs secreted into the CSF showed a significant increase in MCI and AD groups as compared to control cohorts [Figure 1].[28] This increase in MVs in MCI and AD groups suggests that they increase in proportionate to the disease propagation in AD. A recent study demonstrated that the rise in these MVs was related to early synaptic impairment in AD.[59] An important point to infer from this study is that the free-floating Aβ might not be able to deploy their neurosynaptic toxicity in a full-fledged manner besides their inability to propagate the disease pathology widely to the contiguous brain regions from their original point of origin.[59] These encumbrances would be surmounted by their efficient packaging in the MVs which would mutate them to be more virulent at lower concentrations and provide a medium to propagate the disease process widely.[59,92] Not to mention, the eccentric lipid composition of these MVs can transfigure them into more neurotoxic and synpatotoxic by solubilizing them and thereby increasing their virulent properties.[59,93,94] In an animal study by Martins, I.C. *et al*, lipid exposure to mature Aβ fibrils has triggered few morphological alterations so that they are configured to be more soluble and neurotoxic. As a consequence of this transmutation, they incited more neuronal death, excessive tau phosphorylation and severe cognitive deficits in AD disease models.[93]

In line with these findings, in an *in vitro* study by Johansson *et al*. which delved into the effects of polyunsaturated fatty acids on Aβ aggregation by size exclusion chromatography, it was unmasked

that docosahexaenoic acid had proficiently braced the solubilized form of Aβ wild-type profibrils, thereupon amplifying their neurotoxicity on PC12 neuronal cell lines.[94]

In this study, upon infusion of the microglial-induced MVs laden with Aβ into the mouse entorhinal cortex, they evidenced deterioration of long-term potentiation initially in the entorhinal cortex immediately, later on, the next day this disablement upsprang in the dentate gyrus.[59] That being the case, this dissipation of long-term potentiation deficits from one place to another can be fathomed due to the end-target effects of these MVs emanated from the microglial cells. As these MVs march from the one of part of the brain to other region, transmuted Aβ released from MVs can provoke morphological abnormalities in the dendritic spines as well as derail the synaptic plasticity at the entorhinal cortex-dentate gyrus circuitry, henceforth forming the underlying basis for propagation of memory abnormalities in these mice.[59] These findings provide credence to the notion that the turnover of microglial production of toxic MVs can be directly proportional to the breadth of neuronal mutilation, synaptic impairment, and early cognitive changes witnessed in AD.[28,59] In a study where they assayed the CSF of 106 AD patients, 51 MCI patients, and 29 healthy controls for microglial MVs, it was uncovered that significantly increased levels were discovered in the AD and MCI groups as compared to controls.[71] They hypothesize that massive production and release of these MVs into the CSF emanate in the presence of Aβ1-42 by the virtue of microglial activation in the hippocampus.[71] Once these toxic MV gets released into the CSF, they invoke numerous neurotoxic and myelinotoxic culminations ranging from widespread seeding of disease pathology, global white matter damage, sustained microglial activation and hippocampal atrophy.[71] On the grounds of these findings, it would be reasonable to assume that curtailing the production and release of these MVs into the CSF might have a fruitful outcome with regard to the disease pathogenesis in AD disease models. To test this hypothesis, 3-month-old P301S mice were treated with P2RX7-specific inhibitor GSK1482160 for 30 days, and this resulted in substantial downregulation in shedding of exosomes containing tsg101 and CD81 markers from the microglial cells.[95] This translated into reduced accumulation of misfolded tau in the hippocampal region, repression of pathological complex formation (Alz50+ and tsg101) in the hippocampal neurons, along with improved working and contextual memory in the treated mice as compared to controls.[95] The dissipation of tau from the entorhinal cortex to the hippocampal region in the early stages of AD is contingent upon the presence of the microglial cells by the reason of that these microglial cells abet in rampant dispersing of tau across brain regions through secretion of cargo-laden MVs.[96] In a study by Ruan *et al*. where they inoculated MVs with 300 pg of tau in the dentate gyrus of the 18-month-old C57BL/6 mice, they evidenced a spreading of MVs with inception of abnormally phosphorylated tau in the hippocampal regions within a span of 4.5 months.[92] Another interesting finding in this study is that these MV provoked endogenous misfolding of tau (oligomeric and sarkosyl-insoluble forms) in the hippocampus, thereupon fueling the conjecture that these MVs boost and consolidate the neurotoxicity of these tau forms by posttranslational modifications.[92] Keeping that in mind, depleting microglial cells with or without switching of MVs synthesis and their exudation into CSF was conducive to shrinking tau propagation in *in vitro* and *in vivo* disease models.[96]

MVs isolated from CSF of relapsing multiple sclerosis cohorts were shown to have a potential role in carrying and disseminating inflammatory signals to the neighboring neurons.[97] In glioma tumors, microglial-derived MVs were incriminated for creating an immunosuppressive environment and shielding the tumor cells against immune defenses, thus facilitating their survival and spread.[98] According to a recent study, microglial-derived MVs isolated from plasma were significantly higher in the frail MCI patients as compared to controls ($5.89 \times 10^9 \pm 3.98 \times 10^9$ vs. $3.16 \times 10^9 \pm 3.04 \times 10^9$ particles/ml, $P < 0.05$).[29] In addition, these microglial-derived MVs were more neurotoxic to neurons, thereby underscoring the presumption that these MVs hold and disseminate toxic cargo material to the neighboring neurons.[29] In parallel to these findings, proteomic analysis of the microglial-secreted MVs (CD11b+) isolated from the parietal cortex of BRAAK late-stage (V–VI) (staging defined by German anatomist Braak and Braak, 1991[99]) patients showed that there is an increase in the disease-associated microglial markers in Cd11b-positive microglial MVs (ferritin heavy chain 1 and TREM2) as compared to controls.

There are very few clinical trials that utilized these MVs are biomarkers for assessing the treatment response in AD disease models. In a placebo-controlled clinical study by Maja *et al*., MCI and AD cohorts were treated with 20 IU insulin or placebo and followed for 4 months. Close follow-up of these cohorts revealed that EV-based insulin resistance biomarkers (pS312-IRS-1, pY-IRS-1) were directly proportional to cognitive dysfunction in ApoE ε4 noncarriers.[100] In another study (NCT01811381), blood EV-based biomarkers were being currently used for measuring treatment outcomes in a clinical trial where AD cohorts were subjected to curcumin/yoga therapy or placebo.[100,101] In a randomized, double-blind, placebo-controlled trial by Winston *et al*., MCI cohorts and age-matched controls were treated with a 20-week

trial of GHRH administration or a placebo and neuronally derived exosomes were evaluated by ELISA for alteration of synaptic protein.[89,101] Upon completion of GnRH treatment, it was revealed that only exosome synaptic markers synaptophysin and synaptotagmin were increased, with no changes in other markers (β_{1-42}, NRGN, synaptopodin, ptau-S396, and GAP43).[89,101]

Technical difficulties with Simoa assay platforms

Simoa assay high definition (HD) platform might be a feasible approach for bigger pharmaceutical companies to assess the serial changes in tau and amyloid beta levels in plasma and CSF.[102] Despite this, its development and assay improvements will not be possible in the university research laboratories due to various factors including high costs involved, lack of throughput capacity, and single supply of assay reagents.[102] The currently used SiMoA Aβ40 and Aβ42 assays were designed to capture and quantify the levels of monomeric forms of Aβ40 and Aβ42 floating around in the body fluids.[103] Despite having a moderately high sensitivity (52%–78%) and specificity (75%–78%) in detecting brain amyloid burden by SiMoA Aβ40 and Aβ42 assays, it cannot be exclusively conclusive of AD pathology due to the fact other conditions such as hypertension, diabetes, cerebral microbleeds, and ischemic heart disease can even enkindle the rise in Aβ40 and Aβ42 levels in the blood; thence, future researchers should excise caution in interpreting these assays.[103,104] It is important to note that preanalytical procedures play a significant role in influencing final Aβ and tau concentration in the body fluids. Freeze-thaw cycles, type of tubes, delayed plasma centrifugation, delayed storage, and centrifugation temperature are some of the most common factors that can potentially affect the levels of Aβ and tau captured in the body fluids.[105-107] Tubes used to collect the sample should be kept the same because different tube type ethylene diamine tetraacetic acid (EDTA, serum, LiHep, and citrate) can engender different Aβ and tau concentrations, thus obscuring the final concentrations and delivering conflicting results.[107] Moreover, free-thaw cycles should be limited to <3 as a higher number of cycles can comprise the stability of protein in the sample, thence confounding the final concentrations.[107] In addition, reports suggest that Aβ42, Aβ40, and t-tau should not be measured in serum as these samples can increase the propensity to degradation to freeze-thaw cycles and foster different protein levels depending on the type of tube used to collect sample.[107]

Microvesicles' pathological relevance in other neurodegenerative diseases

MVs are incriminated in the carrying, processing, and releasing of aggregated α-synuclein, thus fostering inter-neuronal spread of disease pathology in PD.[108] Aggregated α-synuclein was notorious to instigate synaptic dysfunction, mitochondrial stress, ER stress, proteasome blockade, and axonal impairment that forms the foundational basis for provoking neurotoxicity and neuronal death.[108] Packaging, synthesis, and release of α-synuclein-enriched MVs entail lysosomal dysfunction, ubiquitination, and sumoylation.[109-111]

In the blood of PD patients, α-synuclein enriched MVs were among the most notable markers isolated from the neuronal L1CAM-positive EVs as compared to healthy controls.[112] In the FTD patients with carriers of N297K tau mutation, there appear to be fallacies in intracellular trafficking, and diminished lysosomes in the iPSC-derived neural stem cells, a synergistic combination of cellular derangements, that eventually engenders pileup of exosomes and endosomes.[113] Consequently, there is increased synthesis of intracellular vesicles and exosome formation in the frontal and temporal cortex of FTD patients.[113] CSF isolated from the FTD patients harbors exosomes with amplified expression of transactive response DNA-binding protein of 43 kDa protein with prion-like properties, thence providing a pivotal medium for enkindling intercellular spread of disease pathology.[114] Resultantly, U251 cells exposed to CSF of FTD patients developed tunneling nanotubes such as structures and exosomes of varying stages along with sprung of autophagy and apoptosis, henceforth underscoring the prion-like disease spreading traits of exosomes within them.[114] Efforts expended in limiting the synthesis, release, and propagation of exosomes in the CSF of FTD patients might be fruitful in derailing the disease progression in these patients.[114] In a study by Ngolab *et al*., inoculation of exosomes isolated from the brain of patients with Lewy body dementia into the brains of wild-type mice sparked off α-synuclein aggregation. Furthermore, in the *in-vitro* models, incubation of these exosomes with MAP2+, $Rab5^+$ neurons precipitated α-synuclein aggregation secondary to their intake via endocytosis. Taken together, research studies from various neurodegenerative diseases underscore the fact that MVs with their toxic cargo propagate overarchingly and seed disease pathology, hence taking part in the pathophysiological process of neurodegeneration. Gauging these MVs in the body fluids (CSF& blood) which mirror image the disease initiation and progression could be beneficial as their subtle alterations can signify unfolding neurodegeneration in AD. Since they are harbingers of progressive neuronal damage in AD, they can be exploited for their practical utility as biomarkers for diagnosis, treatment monitoring and prognosis in the neurodegenerative diseases. Any efforts directed in this regard would form a steppingstone for unraveling their untapped potential, thus giving rise to the inception of novel biomarkers for investigating disease progression in AD.

Limitations

Although microglial micro-vesicles are implicated in the neurodegeneration in AD, lot of research is needed to confirm their role in the neuronal demise in AD. Moreover, isolation, quantification and standardization of micro-vesicles can be quite challenging. This requires basic science and clinical research studies to ascertain their role and launch them as biomarkers by bringing them from bench side to clinic. These microglial micro-vesicles might not reveal the true picture of neuronal milieu and might possible serve as ancillary biomarkers and supplement neuronal imaging studies like CT scan/ MRI as well as plasma/CSF tau/Amyloid beta.

Conclusions

Taken together, these findings underscore the presumption that microglial-derived MVs serve as messengers to deliver neurotoxic cargo to the adjoining neurons which form the foundation for onset of neuronal demise, cognitive dysfunction, and dementia in AD patients. Thus, we provide the appropriate groundwork to buttress the hypothesis that these MVs originated from MVs are primarily involved in furthering the disease pathology in AD. Specifically, they are involved in solubilizing the cargo materials, namely Aβ and tau, thence making them more potently neurotoxic and synpatotoxic. On over and above this, Aβ and tau circulating in these MVs have an increased proficiency to spread rampantly throughout the brain, thus giving rise to overarching and global disease pathology in the AD models. Any efforts to muzzle this pathological modus operandi (synthesis and secretion of these microglial MVs) at an early stage might be interlinked with favorable disease outcomes in AD disease models.

Developing a reliable technique to quantify these MVs in the CSF and blood can provide indirect evidence for the status quo of the various stages of neurodegeneration in AD.[28,29,70,115] Therefore, we hypothesize that identification and quantification of these MVs in the CSF and blood would provide a mirror image for the neuronal injury, subtle brain changes, disease status, and progression in the AD disease continuum. Performing quantitative and qualitative analysis of CSF/blood MVs would yield an initial framework upon which reliable assays can be crafted for the risk stratification in the AD patients. Successful validation of these techniques in a larger patient sample would yield a reliable and sensitive assay that can be subsequently commercialized and used in the general population as a screening tool.

Author contributions

Conceptualization, S.H.K.; Writing– Original Draft Preparation, S.H.K & P.J.S.; Writing– Review & Editing, S.H.K.& P.J.S.; Figure Graphics: P.J.S. Visualization, S.H.K.; Supervision, S.H.K.

Ethical committee approval

Not Applicable.

Declaration of Helsinki

Not Applicable.

Patient consent

Not applicable.

Data availability statement

Data sharing not applicable to this article as no datasets were generated and/or analyzed during the current study.

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Conflicts of interest

There are no conflicts of interest.

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