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Exosomes - The Innovative biomarkers in diagnosis and management of Alzheimer's disease

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ABSTRACT

Most eukaryotic cells produce exosomes (EXOs), which are tiny extravascular vesicles (30–150 nm in diameter) in the endosomal compartment. They are present in all eukaryotic fluids and are surrounded by phospholipid bilayers, which facilitate a number of crucial cellular processes. Early-stage identification of Alzheimer's disease (AD) is difficult since there are few reliable biomarkers and limited patient access to diagnostic procedures like imaging and cerebrospinal fluid (CSF) studies. According to recent studies, we can say that exosomes could be innovative diagnostic agents for AD because they contain specific chemicals that may be employed as prospective biomarker candidates for early disease detection and prediction before the development of symptoms. Particularly exosomal proteins and microRNAs (miRNAs), which make up the molecular components of exosomes, show considerable promise as new biomarkers for clinical diagnosis and treatment. Due to their presence in the majority of biological fluids, such as blood, urine, saliva, synovial fluid, amniotic fluid, vaginal fluid, breast milk, semen, etc., their extremely high disease-specific bio-molecular signature or profile, the ability of exosomes to carry and exchange various cargos between cells, and their capacity to cross the blood-brain barrier, there has been an increased interest in using exosomes for the diagnosis of neurodegenerative disorders in recent years. In this review, we will discuss the role of exosomes in the treatment and diagnosis of Alzheimer's disease.

Key words: Alzhiemer's disease, Exosomes, Neurodegenerative disease, cargo, miRNAs, treatment, diagnosis

INTRODUCTION

Alzheimer's disease (AD) is progressive neurodegenerative disease marked by memory loss, increasing cognitive decline and interference with daily tasks which ultimately results in death. The presence of senile plaques (SPs) and neurofibrillary tangles (NFTs) in AD brains have been described as the two main disease histopathological indicators [1].AD can be further categorized into early-onset (EOAD, 65 years) and late-onset (LOAD, >65 years) forms depending on when it first manifests. 10% of all instances of AD are EOAD, commonly known as familial AD.Contrarily, LOAD, often referred to as sporadic AD, represents 90% of all AD patients [2].Reactive oxygen species (ROS) generation and scavenging efficiency are out of balance, which is referred to as oxidative stress. Neuronal damage can result from the accumulation of ROS in neurons, which can cause mitochondrial malfunction and cell apoptosis [3].

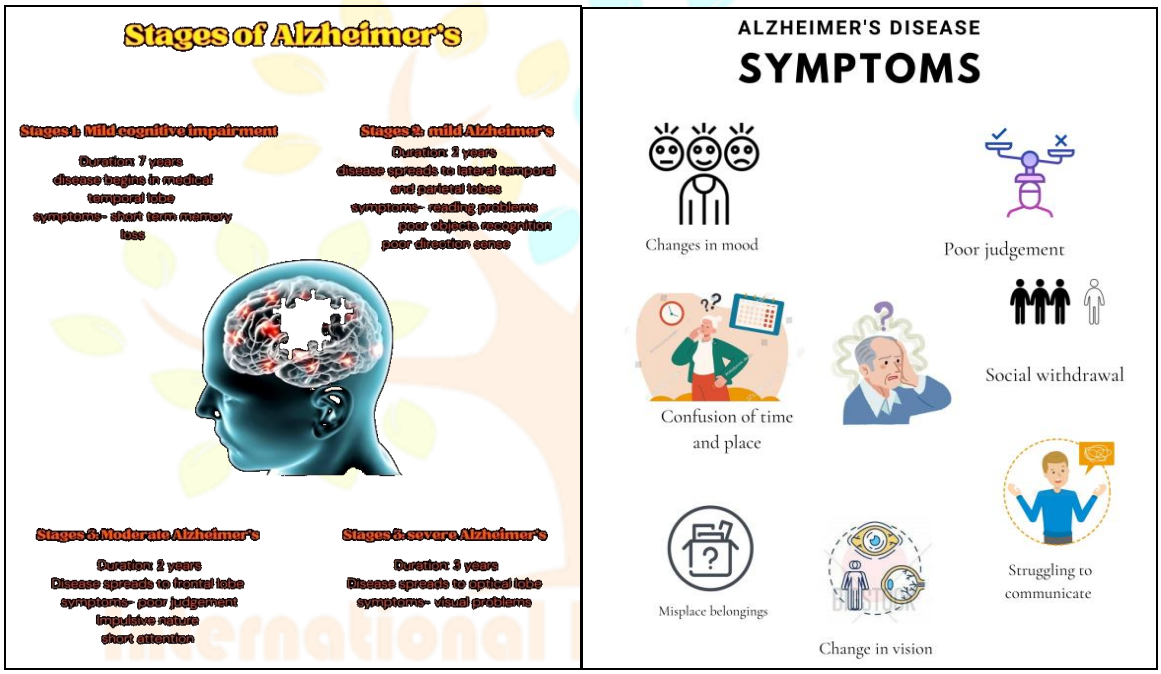


Figure:

Younger patients without 'typical' hippocampus volume reduction or non-amnestic symptoms may not have AD dementia. In a group of young-onset AD patients with neuropathological confirmation, 53% of those with atypical presentations received the incorrect diagnosis, compared to 4% of those with normal symptoms. Non-amnestic traits can now be detected in vivo more effectively thanks to biomarkers. Recent biomarker studies offer knowledge about the pathophysiology of both typical and atypical AD, including geographical fragility and prospects for early detection, in addition to new findings from neuropathologically characterized AD subtypes [4]. Endogenous exosomes, which can be released by brain cells and predict the severity of brain damage, may be employed as biomarkers to indicate the onset and progression of disorders. They are anticipated becoming chemicals or drug carriers to treat neurodegenerative illnesses since they can be targeted to brain tissue through the BBB [5].

Extracellular vesicles havebeen found to perform a wide range of tasks, including understanding the molecular processes involved in inflammation, angiogenesis, programmed cell death, and the transportation of morphogens [6]. In addition, exosomes are an important source of quality biomarkers for diagnostics because

including blood, plasma, CSF, saliva or urine [7]. MicroRNAs are a class of tiny, noncoding RNAs which usually range in number from 22 to 32 nucleotides and constitute one of the most important classes of molecules included in exosomes. A single miRNA can repress translation or degrade more than 100 mRNAs, and several miRNAs are involved in regulation of a single mRNA [8].

The following characteristics suggest that exosomes can act as biomarkers:

1) Exosomes can be removed from a wide range of bodily fluid.
2) Most cells in the body, including neurons and other cells, release exosomes.
3) Exosomes pass through a bilayer lipid structure in the BBB.
4) Proteins, messenger RNAs, microRNAs and other components (collectively referred to as 'cargos') have been identified in exosomes and the membrane protects these substances from enzyme degradation [9].

Pathophysiology of AD

Alzheimer's disease is characterized pathologically by external amyloidal protein deposits and intracellular neurofibrillary tangles that result in senile plaques. Research into innovative pharmacological therapies that are more focused on the pathophysiological events of the disease has been stimulated by advancements in the study of pathogenesis during the past 20 years [10]. Plaques were a well-known side effect of senile dementia in the late 1800s, but Alzheimer is responsible for the first description of the neurofibrillary tangle (NFT) in 1907. It has been asserted that the NFT was also recognized before Alzheimer's description because of the extensive description of "neurofibrils" by Fuller a few months before Alzheimer's description of Auguste D. Some ultra-structural details of NFTs were subsequently added to literature, but it was not until 1986 that NFTs were purified and the microtubule-associated protein tau determined as the major protein component [11].

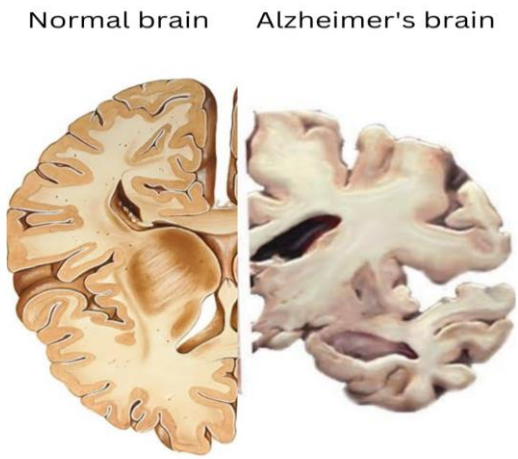


Figure:

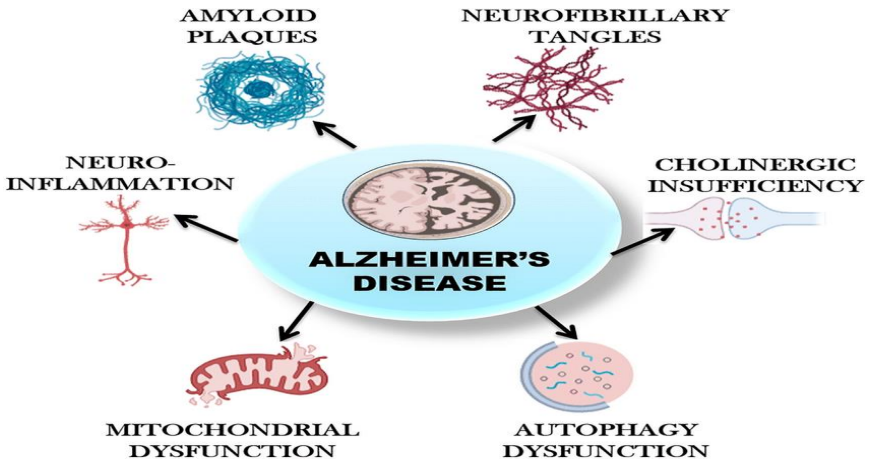
As a microtubule-associated protein that decreased the concentration at which tubulin assembles into microtubules in the brain, tau protein was initially identified.Significantly, tau polymers are also present in other neurodegenerative diseases known as tauopathies, which are characterized by the presence of phosphotau clumps.Thus, tau's phosphorylation and aggregation are related to the pathology of AD [12]. A40 and A42, two by-products of APP metabolism, are the main components of improperly folded amyloid plaques, which are extracellular accumulations. Due to its increased rate of fibrillization and insolubility, A42 is more common

than A40 inside plaques.Amyloid deposition does not always follow a stereotypical pattern of progression but broadly speaking develops in the isocortex, and only latterly affects subcortical structures. Unlike NFTs, amyloid plaques involve the entorhinal cortex and hippocampal formations to a lesser extent. Before spreading to the associative isocortex, neurofibrillary degeneration often starts in the medial temporal lobesallocortex (which includes the hippocampus and entorhinal cortex) [13].

The transentorhinal region (stage I) together with the entorhinal cortex proper typically sees the earliest NFTs, which are then followed by the CA1 region of the hippocampus (stage II). Then, NFTs grow and deposit in limbic regions such the amygdala, thalamus, and claustrum (stages III and IV), as well as the subiculum of the hippocampal formation. The associative areas were impacted earlier and more severely (stage V) than the major sensory, motor, and visual areas (stage VI), and NFTs eventually expanded to all isocortical areas (isocortical stage) [14]. Tragically, when microglia remain in this activated phase for an extended period of time, they emit cytokines and neurotoxic substances such nitric oxide (NO), reactive oxygen species (ROS), and IL-1, TNF, and IL-6 that can either directly or indirectly result in the death of neuronal cells. Additionally, retracted processes are a morphological trait of activated microglia that is associated with a decreased capacity to repair synapses. The decreased synaptic plasticity found in AD is a result of this impact [15].

Reactive astrocytes were found both in the brains of human AD patients and in a mouse model of AD. Pathological signals that cause astrogliosis in AD may involve damaged cells; β amyloid itself is a potent stimulator of astrocyte response. At the molecular level, the β amyloid induction of astroglioticremodelingis mediated by the release of Ca2+ from the endoplasmic reticulum; the latter inhibition suppresses the astrocyte response. In AD, astrocytes undergo relatively mild isomorphicglial proliferation and astrocyte domains do not overlap, which may indicate the defensive nature of the astrocyte response. Indeed, inhibitionof astrogliosisexacerbates β-amyloid accumulationand histopathology in AD mice. Ca2+ astrocyte over activity can promote the release of adverse factors, impair communication between neurons and glialcells, and alter conduction and synaptic plasticity [16].

Iron build-up in the same brain areas associated with Aβ deposition is a significant pathogenic finding of AD. First, iron inhibits furin transcription and lowers furin protein levels. Second, the production of iron-dependent reactive oxygen species (ROS) causes the aconitase to switch to the iron regulatory protein 1 (IRP1) form, which results in an erroneous signal of iron deficiency and increases cellular iron uptake. These circumstances would set off a domino effect that would gradually raise the intracellular labile iron pool (LIP), further suppress furin, and shift the secretase equilibrium in favour of the Aβformation [17]. Human AD tissue with toxic amounts of ferrous (Fe2+) iron can exacerbate inflammation by catalysing the Fenton reaction, which results in the formation of free radicals. In addition, ceruloplasmin levels are higher in AD, indicating a response upsurge to oxidative stress. Ceruloplasmin is a key plasma antioxidant that converts redox-active iron back to Fe3+ [18].



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Exosomes as diagnostic and therapeutic agents

Exosomes were formerly thought to serve simply as cellular trash cans. Exosomes are now known to be intercellular messengers and significant factors in both health and illness [19]. In healthy individuals and patients with various disorders, exosomes produced by cells into the bloodstream and body fluids show variable protein and RNA contents that can be evaluated as potential diagnostic markers. MiRNAs found in abundance in tumour-derived exosomes may act as tumour indicators. Exosomes have also been investigated as potential biological markers for a variety of non-cancer conditions affecting various organs, such as the CNS, kidney, lung, and arteries. Tau-induced neurodegeneration in the CNS is caused by extracellular build-up of tau protein that has been improperly digested [20]. By the time they are forty years old, every individual with Down syndrome (DS) has the neuropathology of Alzheimer's disease (AD) and the majority of them go on to develop AD dementia. It is frequently challenging to spot early signs of dementia in this population because patients with DS exhibit highly varying levels of baseline function. The word "biomarker" refers to accurate measurements of objective signs of an illness or disease. The Aβ peptides, Aβ1-40 and Aβ1-42, distinct species of P-Tau, pro-inflammatory cytokines including IL-6, TNF-α, and IL-1β, and pro-NGF are among the cerebrospinal fluid (CSF) indicators of AD that have been identified decades before dementia onset in both the general population and in individuals with DS [21].

Due to their microscopic size and some characteristics that make them similar to NPs, such as passive targeting ability and improved permeability and retention effects, exosomes are also referred to as nanoparticles (NPs). Additionally, they are the primary extracellular signalling mediators, can be secreted by all eukaryotic cells, including bacteria, and transport proteins, nucleic acids, lipids, and other active substances that can be found in bodily fluids like blood, urine, joint fluid, saliva, and breast milk. In order to diagnose diseases, exosomes can be employed as a liquid biopsy technique [22]. Exosomal miRNAs are "ideal" biomarkers for clinical diagnostic applications because they are shielded from RNase-dependent destruction and may be identified steadily in circulating plasma and serum. Exosomal proteins have been discovered to represent potential biomarkers for a growing number of illnesses. Exosomes include proteins and nucleic acids linked to cancer, as well as neurological, metabolic, viral, and other disorders, as various studies have shown over the past several years. Furthermore, exosomes are extremely desirable targets for diagnostic use since they can be separated from

readily available bio fluids like blood and urine [23].

Exosomal secretion is a fundamental process that affects both physiological and pathological processes and controls both the molecules on the exosomal surface and the composition of the exosomes. Exosomes can thus be used as biomarkers, vaccinations, and drug carriers and they can be rationally altered for therapeutic interventions. Accurate, effective, and targeted exosome identification, isolation, and quantification remain difficult tasks. Exosome theranostics is the use of exosomes in these applications, and more research on them will investigate their potential in translational medicine and open up new channels for the development of efficient clinical diagnostics and treatment approaches [24].Exosomes have been linked to the development and progression of a number of encephalopathies, offering biomarkers and therapeutic targets for the detection and treatment of disease. Exosome analysis in bodily fluids can detect small alterations in the pathophysiological process of neuropsychiatric illnesses, opening up new avenues for disease diagnosis, prognosis, and therapy options [25].

General composition of the exosomes includes:

a) Lipids
b) Proteins
c) Metabolites
d) Immune regulatory molecules
e) Nucleic acids

Characterization of exosomes [26]

Both physical and biochemical analysis are required for the characterization of exosomes.

Methods of Physical Analysis

1) ***Nanoparticle tracking analysis***: Taking into account the Brownian motion of the particles, NTA is a particle tracking technique that assesses the size (hydrodynamic diameter) and concentration of particles in a suspension.
2) ***TRPS or tunable resistive pulse sensing*:** The Coulter principle is the foundation of TRPS, which uses transitory variations in electric current produced by particles as they travel across a membrane with a pore that can be adjusted for size to determine the size of the particles.
3) ***Dynamic light dispersion*:** Although DLS is similar to NTA in that it is similarly based on Brownian motion and the Stokes-Einstein equation; it only offers information based on intensity-based distribution, hence the size of the particle is determined by the intensity's variation over time.
4) ***Electron microscopy (EM):*** Instead of using light, EM uses a concentrated beam of accelerated electrons that engages with the material to produce images with higher resolution than light microscopy. It includes two types- a) Scanning electron microscopy(SEM) and b) Transmission electron microscopy(TEM)
5) ***Atomic force microscopy (AFM):*** Based on Hooke's law, AFM is a sort of scanning probe microscopy that scans a sample's surface with a small mechanical cantilever to reveal the topography of the surface and the existence of substructures. AFM records two different types of data: amplitude-modulating and phase-modulating; the former provides information on surface topography and the latter records data on

substructures.

Methods of biochemical Analysis

1) ***Western blotting (WB) & ELISA:*** The most widely used methods to describe exosomes and show the presence of certain exosomal proteins are WB and enzyme-linked immunosorbent assay (ELISA), which are traditional methods based on the antigen-antibody interaction.
2) ***Flow cytometry (FCM):*** By measuring scattered light or fluorescence activation, FCM is a sensitive tool for characterizing tiny particles like exosomes. The samples are run through a laser of various wavelengths while being labelled with fluorescent tags or antibodies, which scatters light in forward scatter (FSC) and side scatter (SCC) patterns. While SCC is more sensitive than FSC and provides internal structure details, FSC indicates particle size. Lipophilic fluorescent tags or exosome-specific antibodies can be utilized for quantitative and qualitative investigation to characterize exosomes.

Table 1: Isolation techniques of exosomes [27, 28]

S.No	Techniques	Time required	Pros	Cons	Yield	Purity
1	Ultracentrifugation	4-10 hours	*Low cost *Used for large volume capacities	*Tedious process *Low efficiency	Low	Medium
2	Size exclusion chromatography (SEC)	0.3 hours	*High yield *Good purity and reliability	*Ultrafiltration may be needed before and after SEC	High	High
3	Density gradient centrifugation	17-90 hours	*High purity *No damage to exosomes	*Complicated and slow process *Less yield	Low	High
4	Immunoaffinity	5-20 hours	*Suitable for small scale samples *High purity and specificity	*Costly process *Based on Antibody specificity	Medium	High

5	Ultrafiltration	< 4 hours	*Easy to use *No special equipment's are required	*Cost effective *Exosomes with small diameter are lost	Mediu m	High
6	Polymer precipitation	0.4-12 hours	*Simple and easy process *Large-scale production	*Less purity *Cleaning is required before and after process	High	Low

Role of exosomes as biomarkers in AD diagnosis

Recent research has focused heavily on the combination of CSF p-tau and CSF A1-42 as a marker for diagnosis capable of separating AD from other dementias in the early stages. When assessing the level of CSF p-tau in patients with AD, dementia with Lewy bodies, and vascular dementia, it was higher in those with AD. In spite of the fact that CSF p-tau detection can help distinguish AD from other forms of dementia, comparing p-tau concentrations in exosomes is more useful for determining the degree and stage of AD by including symptoms based on a positive relationship between the amount of p-tau in CSF exosomes and the severity of AD. Additionally, since p-tau is present in CSF exosomes in the early stages of AD, it is useful for the early detection of the disease [29]. Exosomes from the CSF may be utilized as a biomarker to identify AD. However, at the moment, a tiny amount of CSF can also be used to identify p-tau. Application prospects are not improved by the identification of particular tau in CSF exosomes for the diagnosis of AD.

Even one to ten years before an AD diagnosis, the mean levels of exosomal total tau, P-T181-tau, P-S396-tau, and amyloid 1-42 (A1-42) in the AD group were considerably greater than in the healthy control group. These results suggested that exosomes can forecast the onset of AD. Yang et al.'s study also revealed that in the serum of AD patients, exosomal miR-135a, miR-384, and miR-193b were up-regulated while miR-193b was down-regulated. For early AD diagnosis, the combination of miR-135a, -193b, and -384 was found to be superior to one. MiR-342-3p expression was down-regulated in the plasma exosomes of AD patients. According to research, Alzheimer's patients' neurally generated plasma exosomes had downregulated levels of miR-212 and miR-132. According to studies, there may be one or more biomarkers for AD because of the significant differences in BACE1-AS-lncRNA levels in plasma and plasma-derived exosomes between the AD subgroup and the control group [30].

The surveillance of the A1-42/1-40, total Tau (T-Tau), and phosphorylated Tau (p-Tau) 181 triplet in the cerebrospinal fluid (CSF), which can help with AD-differential diagnosis, is the only molecular-based diagnostic that is presently universally accepted. Nevertheless, this method calls for a lumbar puncture, an invasive procedure that is challenging to use in medical environments such as those for basic health care. Since they would be less invasive, simple to use, and more affordable than other options, blood-based biomarkers could be an intriguing replacement for AD diagnosis [31]. The outcomes of cognitive and neuroimaging tests are correlated with exosomal miRNAs linked to Alzheimer's disease that have been found in serum. This

implies the possibility that alternative biomarkers, such as peripheral exosomal miRNAs, could be utilized to detect early-stage, preclinical Alzheimer's disease cases. Peripheral exosomes' diagnostic value, however, is currently unclear.To determine whether the tau protein and β-amyloid peptides are also present in exosomes from disease-related experimental models, more molecular studies are required [32].

Table 2: Potential exosomal miRNAs biomarkers for Alzheimer's disease clinical diagnosis [33]

Source	**Upregulated miRNA**	**Downregulated miRNA**	**Study Population**	**Exosomes Isolation Methods**	**miRNAs Detection Methods**
CSF	miR-132-5p miR-485-5p	miR-16-2 miR-29c miR-136-3p miR-331-5p	47 PD 28 AD 27 HC	Ultracentrifugation	microarray analysis
CSF		miR-9-5p miR-598	10 AD 10 HC	Commercial isolation kit	microRNA panel qRT-PCR
CSF	miR-125b-5p	miR-16-5p miR-451a miR-605-5p	LOAD 13 YOAD 17 HC 12	Commercial isolation kit	microarray analysis qRT PCR
CSF and Blood		miR-193b	51 DAT 43 MCI 84 HC	Commercial isolation kit	qRT-PCR
Plasma		miR-23b-3p miR-141-3p miR-185-5p miR-342-3p miR-342-5p miR-338-3p miR-3613-3p	46 AD 41 HC	Ultracentrifugation	NGS
Plasma		let-7i-5p miR-21-5p miR-23a-3p miR-126-3p miR-151a-3p miR- 451a	10 AD 18 DLB 15 HC	Size Exclusion Chromatography	NGS qRT-PCR

Source	Upregulated miRNA	Downregulated miRNA	Study Population	Exosomes Isolation Methods	miRNAs Detection Methods
Serum	miR-15a-5p miR-18b-5p miR-20a-5p miR-30e-5p miR-93-5p miR-101-3p miR-106a-5p miR-106b-5p miR-143-3p miR-335-5p miR-361-5p miR-424-5p miR-582-5p miR-3065-5p	miR-15b-3p miR-342-3p miR-1306-5p	AD 23 MCI 3 HC 23	Commercial isolation kit	NGS qRT-PCR
Serum		miR-223	22 AD 10 VAD 16 HC	Commercial isolation kit	qRT-PCR
Serum	miR-135a miR-384	miR-193b	107 DAT 101 MCI 30 PD 20 VaD	Commercial isolation kit	qRT-PCR

CSF: Cerebrospinal fluid; **PD:** Parkinson's disease; **AD:** Alzheimer's disease; HC: healthy control; **LOAD**: late-onset Alzheimer's disease; **YOAD**: young-onset Alzheimer's disease; **DAT**: dementia of Alzheimer type; **MCI**: mild cognitive impairment; **VAD:** Vascular Dementia; **DLB**: dementia with Lewy bodies.

It is unclear whether alterations in synaptic proteins in the blood may be detected in the preclinical stages of AD, despite the fact that multiple synaptic proteins were shown to be altered in the CSF of individuals with MCI10–16. Exosomal synaptic proteins may be useful indicators to predict AD even in the preclinical stage, according to studies that show a lower level of many synaptic proteins in neuronal-derived exosomes in individuals with AD or preclinical AD. These investigations' data, which only included nine preclinical AD patients and were not verified by CSF analysis, were therefore limited in their ability to be applied clinically.The objectives of this study were to: (1) investigate the ability of exosomal GAP43, neurogranin, SNAP25, and synaptotagmin 1 to assist in making diagnoses of AD and amnestic mild cognitive impairment

(aMCI); (2) validate the outcomes of a preliminary discovery experiment with those from a subsequent validation stage with additional samples; and (3) examine the ability of exosomal biomarkers to identify preclinical A [34].

A unique technique was used to assess the concentration of all salivary exosomes using the NTA method, and then a western blot analyzer was used to confirm the presence of important exosomal cargo proteins. According to study findings, salivary exosome concentration based on nano-tracking technology has the potential to be employed as a low-cost screening strategy for spotting early signs of Alzheimer's disease [35]. Serous and mucous discharges are found in saliva, an easily accessible biofluid. In contrast to blood sample, salivary exosome isolation is a non-invasive, painless, and manageable process. Recently, the possibility of salivary exosomes as PD biomarkers has been investigated.

Absolute levels of α-synuclein oligomers and the proportion of α-synuclein oligomers/total α-synuclein are higher in PD patients compared to control participants in salivary exosomes obtained with the XYCQ EV enhancement kit. According to a different study, PD patients had higher quantities of neuronal exosomes in their isolated saliva from polyethylene glycol precipitation than healthy controls do [36]. Not just for kidney-related disease but also for non-kidney-related disorders, such as AD diagnosis, urine is an excellent source for finding new biomarkers. Furthermore, substantial amounts of urine can be collected non-invasively. According to a recent study, urinary exosomes make up around 3% of the total protein in urine. Because of their capacity to identify proteins of relatively low quantity and the simplified urinary proteome, urinary exosomes represent a promising source for the discovery of biomarkers. However, further research is necessary due to the importance and wealth of biological data present in the proteins found in urinary exosomes [37].

Current symptomatic approaches to Alzheimer's disease

Cholinesterase inhibitors

According to the cholinergic hypothesis of AD, cholinergic systems in the basal forebrain are harmed early on in the disease process, including loss of acetylcholine neurons and loss of enzyme function for acetylcholine synthesis and degradation, which causes memory loss and deterioration of other cognitive and noncognitive functions like neuropsychiatric symptoms. It has been suggested that utilising CIs to postpone acetylcholine breakdown between synaptic clefts will improve cholinergic transmission.

Antagonist of N-methyl-D-aspartate

Memantine is an additional therapy option for moderate-to-severe AD. An uncompetitive, moderate-affinity NMDA antagonist, this medication is thought to guard neurons from excitotoxicity. After 6 months of usage, persons with moderate to severe AD demonstrated improvement in cognition, ADL, and behaviours, according to a systemic evaluation of double-blind, parallel-group, RCT studies using memantine. Memantine may lessen the behavioural and psychological symptoms of dementia, according to another systemic review that included six RCT studies. Dizziness, headaches, and disorientation were the side effects of memantine studies that were most commonly reported.

Combining therapies

RCT studies on parallel groups of patients with moderate to severe AD revealed a significant benefit from the combination use of memantine and donepezil over the placebo group (memantine and placebo) in terms of cognitive function, language, ADL, behaviours, and overall state. Patients with mild to moderate AD, however, did not see this advantage [38].

Exosomes for Therapeutic Use

Exosomes can function as efficient natural carriers for the therapeutic delivery of chemicals with the potential to treat disease because they have the capacity to transmit bioactive compounds across cells across the BBB. MSCs are desirable prospective exosome makers because to their abundance in a range of tissues, their simplicity in isolation, and their significant capacity for in vitro proliferation. These MSCs are genetically adaptable, which further enables them to create exosomes that are abundant in the necessary therapeutic components. These cells are crucial as allogeneic and autogenous natural delivery systems because proteomic studies of MSC-derived exosomes revealed similar immunotolerance features as MSCs. Additionally, it has been hypothesised that MSCs' therapeutic effect on diseased regions is mediated via the exosomes they produce.

The unique migratory and homing properties of MSC-derived exosomes may open the door to their application as versatile theranostic agents in AD. Exosomes from MSCs may deliver their therapeutic components, particularly miRNAs, to recipient cells, changing gene expression and triggering a therapeutic response, according to a number of studies.

The innovative potential of exosomes as therapeutic delivery systems and research tactics to design and alter both the surface and content of these priceless biological entities are also present. Exosomes from curcumin-treated (primed) cells, known as Exo-cur, have been shown in a recent study to reduce the symptoms of AD by preventing the phosphorylation of Tau protein and to aid both in vitro and in vivo avoid neuronal death. Exosomes may hold significant potential to improve targeted drug delivery and neuronal functional recovery in AD therapy [39].

Aβ degradation

A potential treatment possibility for AD is the enhancement of Aβ degradation following delivery of neuronal exosomes or stimulation of exosome secretion. Using exosome-like synthetic liposomes with glycosphingolipids (GSLs), necessary for collecting Aβ and presenilin (PS), and their glial internalisation, in place of natural exosomes would offer various benefits, including consistency and being contamination-free.

The exosomes produced by microglia and mesenchymal stem cells originating from adipose tissue contain significant amounts of Aβ-degrading enzymes, insulin-degrading enzymes, and neprilysin. Exosomes have also been investigated as a delivery system for chemicals or short interfering RNAs. Exosomes with functional properties, liposomes that resemble exosomes, or even fusion vesicles of both types can be used as effective nanotechnological treatments for AD.

Microglia are capable of picking up neuronal exosomes that contain Aβ, helping to remove the peptide from the extracellular space and, in turn, lessening the disease associated with Aβ.Exosome surface proteins, such as the

cellular prion protein, can sequester Aβ peptides by exosome infusions as well. These infusions eliminated the disruption of synaptic plasticity that is generally brought on by Aβ. Neuronal exosomes or exosomes derived from hypoxic mesenchymal stromal cells were also shown to reduce Aβ accumulation and amyloid plaques when infused intracerebrally into APP transgenic mice. In APP/PS1 transgenic mice, the treatment of these exosomes improved memory and learning abilities while reducing inflammatory cytokine levels [40].

Protection form oxidative stress

Exosomes protect neurons from oxidative stress. As previously stated, exogenous exosomes can aid in the breakdown of A1-42 in AD and other neurodegenerative illnesses. Co-cultured injured cortical neurons with human adipose-derived mesenchymal stem cells (ADSCs) using a semi-porous membrane, and the results demonstrated that AMSCs-conditioned medium, enriched with exosomes, mediates direct neuroprotection by inhibiting neuronal cell apoptosis, promoting nerve regeneration and repair, and restoring bioenergy following energy depletion caused by glutamate excitotoxicit.

Cystatin C

These findings imply that exosomes derived from ADSCs and other foreign stem cells could be employed to treat nervous system illnesses.These findings imply that exosomes derived from ADSCs and other foreign stem cells could be employed to treat nervous system illnesses. The concentration of Neprilysin (NEP) in exosomes isolated from ADSCs was much higher than that of nerve cells, and this neutral endopeptidase was linked to the breakdown of Aβ. Cystatin C, which is likewise involved in the breakdown of Aβ, was found in exosomes isolated from rat primary neurons. The physiologically high content of cystatin C in CSF and its proliferative effect on neural rat stem cells suggested that it could have a trophic function in the brain. As a result, exogenous exosomes are regarded as promising medicines for the treatment of Alzheimer's disease [41].

Exosomal miRNA

Exosomes include miRNAs, which are necessary constitutive components that facilitate intercellular communication. They serve a regulatory role in the control of CNS function and are captured by nearby or distant target cells. Noncoding RNAs in the class of miRNAs have a length of about 22nt. They are said to control the activity of their target genes by preventing protein production or directly cleaving mRNA. Exosomal miRNAs have a significant role in cell development and division.

Exosomes can be employed as carriers to transfer nucleic acid fragments such as miRNA and siRNA for the treatment of Alzheimer's disease because to their RNA transport capacity, stable presence in bodily fluids, and ability to cross the BBB. Finally, the finding that exosomal miRNAs can exhibit dysregulation in AD patients makes them promising candidates for disease surveillance. To support their usage as disease biomarkers in clinical practise, more research is required. A possible technique for the future development of miRNA-based therapies for neurodegenerative illnesses is the correction of miRNA expression using miRNA agonists or antagonist oligonucleotides, regardless of the complexity of these methods.

Exosomal SiRNA

The double strand of small interfering RNA (siRNA) measures 7.5 nm in length and contains 21–23 pairs of nucleotides. The RNA-induced silencing complex (RISC) is created in the cytoplasm, where it degrades the

corresponding sequence of the mRNA to silence the gene. Preventing the translation of the mRNA into protein could be used to cure the disorders, which are caused by the overexpression of a gene [43].

A study published in 2011 claimed the first successful therapy of Alzheimer's disease in mice utilising exosomes with siRNA. Electroporation was used to load exogenous siRNA into purified RVG-targeted exosomes. Protein expression and Aβ deposition in the AD mouse brain were shown to be significantly lower. This demonstrated the efficacy of exosome treatment for neurodegenerative disorders via siRNA delivery. Another study found that delivering miR-124a via exosomes increased the expression of excitatory amino acid transporter 2 (GLT1) on the surface of astrocytes, which is important for controlling synaptic activity and increasing glutamate absorption. This approach is intended to reduce neuronal apoptosis in Alzheimer's disease patients.

The authors inserted siRNA against beta-secretase 1 (BACE1) into the exosomes before giving them to animals to further investigate the therapeutic potential of exosomes. An essential stage in the development of Alzheimer's disease is marked by the cleavage of the amyloid precursor peptide by BACE1.Both the BACE1 protein and mRNA expression showed a significant knock-down effect when cortical tissues from the animals were examined. Beta amyloid, which makes up the majority of Alzheimer's plaques, was also dramatically reduced, indicating a therapeutic impact of the exosomes loaded with siRNA. Exosomes are known to contribute to the accumulation of toxic peptides when the clearance pathway is overburdened as well as the breakdown of toxic Aβ according to recent research. Exosome miRNAs in the serum may indicate a research area for studying AD prevalence and treatment [44].

Challenges and Limitations

Although there is a growing body of literature relating to the rapid evelopment f exosomes derived from MSCs, the therapeutic use of these exosomes remains in its infancy. Many issues need to be resolved before MSC-derived exosomes can be used for clinical treatments. Significant research is still required in order to guarantee the long-term biological safety of these exosomes, and confirm the potential adverse effects and efficacy of exosome administration in patients with AD. We also need to know more about the time of administration, the most effective route of administration, including dose-response experiments, before considering MSC-derived exosomes for clinical application. Since these exosomes contain a huge number of chemicals that have not yet been characterised, the exact composition of exosomes formed from MSCs is still mostly unclear. The exosome contents have been identified using proteomics and microarray methods. Exosomes, however, may differ in content depending on the origin of the MSCs or the conditions of their cultivation. Although exosomes have previously been given the go-ahead to be used in a number of clinical trials, our understanding of exosome-related medicines is fast developing [46].

Conclusions

Exosomes have been shown to be a key player in the development of AD pathogenesis. Establishing AD models, early stage diagnosis, and gene therapy will all benefit from understanding the fundamental mechanisms at play. Additionally, it might shed light on potential treatments for other neurodegenerative conditions.

As small genetic fragments and toxic proteins could be carried by exosomes and transported between cells and extracellular fluids, which might also be the mechanism of the slow progress of neurodegenerative diseases, it can be assumed that in the co-culture system of nerve cells and isolated exosomes from plasma or CSF of patients with AD, nerve cells may be induced into AD-like injury cells. Furthermore, applying specific amounts of exosomes from AD to the CSF may induce AD symptoms in animal models.

Given that exosomes are more likely to transfer p-tau and A1-42 to extracellular fluids in the early stages of AD, their combination detection can significantly improve diagnostic sensitivity and specificity. The benefits of CSF exosomes are counterbalanced by the advancement of clinical laboratory technology, which makes it possible to identify harmful proteins in a tiny amount of CSF. It will be easier to diagnose AD early if hazardous proteins and certain microRNAs are found in plasma exosomes.

Finally, due to their safety, selectivity to target cells, and capability for small molecule drug administration, exosomes will emerge as a new hot point in the molecular treatment of AD. Furthermore, exosome dispersion is not constrained by biological barriers, giving exosome administration choices.

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