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REVIEW ARTICLE

Mitochondrial Dysfunction in Alzheimer's Disease: Opportunities for Drug Development

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Abstract: Alzheimer's disease (AD) is one of the major reasons for 60-80% cases of senile dementia occurring as a result of the accumulation of plaques and tangles in the hippocampal and cortical neurons of the brain leading to neurodegeneration and cell death. The other pathological features of AD comprise abnormal microvasculature, network abnormalities, interneuronal dysfunction, increased β-amyloid production and reduced clearance, increased inflammatory response, elevated production of reactive oxygen species, impaired brain metabolism, hyperphosphorylation of tau, and disruption of acetylcholine signaling. Among all these pathologies, Mitochondrial Dysfunction (MD), regardless of it being an inciting insult or a consequence of the alterations, is related to all the associated AD pathologies. Observed altered mitochondrial morphology, distribution and movement, increased oxidative stress, dysregulation of enzymes involved in mitochondrial functioning, impaired brain metabolism, and impaired mitochondrial biogenesis in AD subjects suggest the involvement of mitochondrial malfunction in the progression of AD. Here, various pre-clinical and clinical evidence establishing MD as a key mediator in the progression of neurodegeneration in AD are reviewed and discussed with an aim to foster future MD based drug development research for the management of AD.

Keywords: Alzheimer's disease, apoptosis, β-amyloid plaques, mitochondrial dysfunction, oxidative stress, tau proteins.

1. INTRODUCTION

About 50 million people are suffering from dementia globally (who.int). Alzheimer's Disease (AD) being the sixth leading cause of death in the United States, affecting an estimated 5.8 million people aged 65 years or above is one of the major health care concerns round-the-globe. According to the estimates, the number of cases of Alzheimer's disease in the United States is expected to rise to 13.8 million by the middle of the century [1]. AD is a class of neurodegenerative disorders, that is associated with problems with thinking, learning, and behavior and accounts for about 60-80% cases of dementia. This is due to the degeneration of hippocampal and cortical neurons of the brain which are involved in memory, learning, and cognition [2]. Most of the AD cases are late-onset AD or sporadic AD, which occurs without any well-defined cause or reason while very few numbers of cases are with early-onset or familial AD, which occurs due to the inheritance of autosomal dominant traits (Table **1**).

Based on the classical theory of cholinergic deficit, acetylcholinesterase inhibitors (tacrine, donepezil, rivastigmine and galantamine) or NMDA-receptor antagonist (memantine) are the only FDA approved drugs that are being employed clinically for the management of AD. Although these medications are effective in providing symptomatic relief and slow down the disease progression, they fail to cure the disorder [2].

This review explains the relation of mitochondrial dysfunction or mitochondrial cascade hypothesis with classical AD biomarkers and *vice-versa*, forming a common link between all the associated AD pathologies and hypotheses. The preclinical and clinical trials of drugs targeting mitochondria that are currently under investigation and completed are also discussed alongwith the various challenges being encountered in the development of drug targeting mitochondrial dysfunction.

2. PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

The brain is made up of billions of neurons interconnected by synaptic spaces through which they communicate by generating nerve impulses *via* various neurotransmitters. The neurons in each part of the brain are specialized to perform definite functions and work in tandem to perform those functions. The primary hallmarks of AD include disruption in the neurotransmitter signaling especially of acetylcholine, along with the accumulation of a large number of plaques and tangles [3]. The network

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Table 1. Risk factors associated with the Alzheimer's disease.

Risk Factors	Reason	References
Age	Persons with age 65 or above are more likely to develop this condition	[2]
Family history	Persons with a family history of AD have increased chances to develop this disorder	[1]
Genetics	The genetic susceptibility of the individual is linked to the ApoE genotype. Though there are many forms of this gene present in individuals, but the ones that inherit ApoE4 are much more susceptible. Other genes majorly responsible are Presenilin 1, Presenilin 2, and APP gene.	[22, 25, 26]
External factors	Serious head-injury can increase the risk of developing AD later in life. Other environmental risk factors include hyperlipidemia, hypertension, homocysteinemia, diabetes mellitus and obesity.	[2, 149-151]

activities supporting cognition are altered even before the appearance of symptoms in AD patients [4-6]. These network alterations include activation and deactivation deficits, abnormal oscillatory rhythmic activity and network hypersynchrony [4, 7, 8]. In people carrying high risk factors for AD development, abnormal activation and deactivation of specific networks can be diagnosed decades before the onset of clinical disease [4, 9-11].

The plaques are formed by the accumulation of multiple fragments of β-amyloid protein in the neuronal synapse. The tangles are formed by the twisting of tau protein fibers inside the neurons. The plaque formation leads to the death of neurons by interfering with the transfer of signals between one neuron to another, whereas tau tangles interfere with the movement of nutrients or other important molecules inside the cell leading to neuronal degeneration. The course of AD starts with the build-up of plaques and tangles in the parts of the brain affecting learning and memory, which later progresses to various other parts of the brain leading to cell apoptosis or cell death. A large number of AD cases also exhibit abnormal accumulation of the presynaptic protein α-synuclein and an RNA-binding protein TDP-43 in the brain [12]. α-synuclein can also self-assemble into pathogenic oligomers and form larger aggregates known as lewy bodies. Both Tau and α-synuclein may also get released into extracellular space and may infect other cells rapidly [6,12]. Impaired or defective cholinergic transmission due to selective degeneration of cholinergic neurons in the hippocampus, frontal cortex, amygdala, and downregulation of muscarinic and nicotinic receptors is also the main identifying feature of AD. Since cholinergic and glutaminergic neurotransmission work in coordination, disruption of cholinergic neurotransmission leads to impairment in glutaminergic signaling [13] that causes neuronal excitotoxicity by hyper activating the NMDA and AMPA receptors, which help regulate the physiological calcium signaling [14]. Increasing Aβ load also leads to hyperactivation of NMDA, AMPA, and voltage-gated ion channels in the cytosol of the cell leading to increased calcium influx, causing cytosolic calcium overload [15-17]. To maintain calcium homeostasis mitochondria actively takes up calcium ion (calcium buffering) and this excessive influx causes membrane depolarisation which activates mitochondrial permeability transition pore (mPTP) and enhanced reactive oxygen species (ROS) production, metabolic dysfunction and cytochrome C release causing the initiation of neuronal apoptosis *via* caspases activation (Fig. **1**), [18-20]. There are three major signaling pathways (Fig. **2**) involved in the activation of caspases that in turn trigger the neuronal apoptosis. The first pathway involves the increased oligomerization of surface death receptors like FADD (Fas-associated death domain protein), TNFR, DR3, TRAIL-R4, and TRAIL-R5 triggering the activation of caspase-8 and -3 [21]. The second mechanism or the mitochondrial pathway of apoptosis involves the release of cytochrome C from the damaged mitochondria, which forms a complex with Apaf-1 (apoptosis protease activating factor-1) and procaspase-9 in the presence of ATP resulting in the increased activation of caspase 9A. This activated caspase-9A further cleaves and triggers caspase-3, -6, and -7, which initiate the release of death substrates like gelsolin and actin leading to DNA fragmentation and cell death. The third mechanism involved the impairment of Ca^{2+} metabolism due to the stress conditions prevalent in Endoplasmic Reticulum (ER) with excessive accumulation of misfolded proteins in ER lumen, which further activates the caspase-12 triggering apoptosis [22, 23].

The genes like the ApoE4, amYloid-β Precursor Protein (APP) gene, presenilin-1 (PS-1), and presenilin-2 (PS-2) carry the dominant traits for the inheritance of early-onset Familial AD [24]. Studies elucidate that these epigenetic mechanisms modulate the AD risk [6, 25]. To identify the role of these epigenetic mechanisms in AD pathology, a study was conducted on human postmortem samples, peripheral leukocytes and transgenic mouse models, which showed the association of aging and AD with epigenetic dysregulation at multiple levels including abnormal DNA methylation and histone modifications [25]. AD-associated mutations in PS-1 and PS-2 localized in ER accelerate the cellular apoptosis by damaging the stress responses of ER [23]. The presenilins being the catalytic part of the γ-secretase complex, exhibit the cleavage of APP, leading to the formation of Aβ and maintaining the calcium homeostasis and their transmission across the synapses [26]. The G206D mutation of the PS1 gene decreases the interaction of presenilin enhancer-2 (Pen2) and elevates the Aβ42/Aβ40 ratio and aggravates the accumulation of Ca^{2+} in the ER [27]. PS1 may also disrupt autophagy of abnormal proteins by delaying lysosomal proteolysis [28]. Mutations in APP genes lead to increased production of Aβ42, which accumulates and forms senile plaque and cause AD progression [29]. ApoE4 increases the instances of tau and Aβ mediated

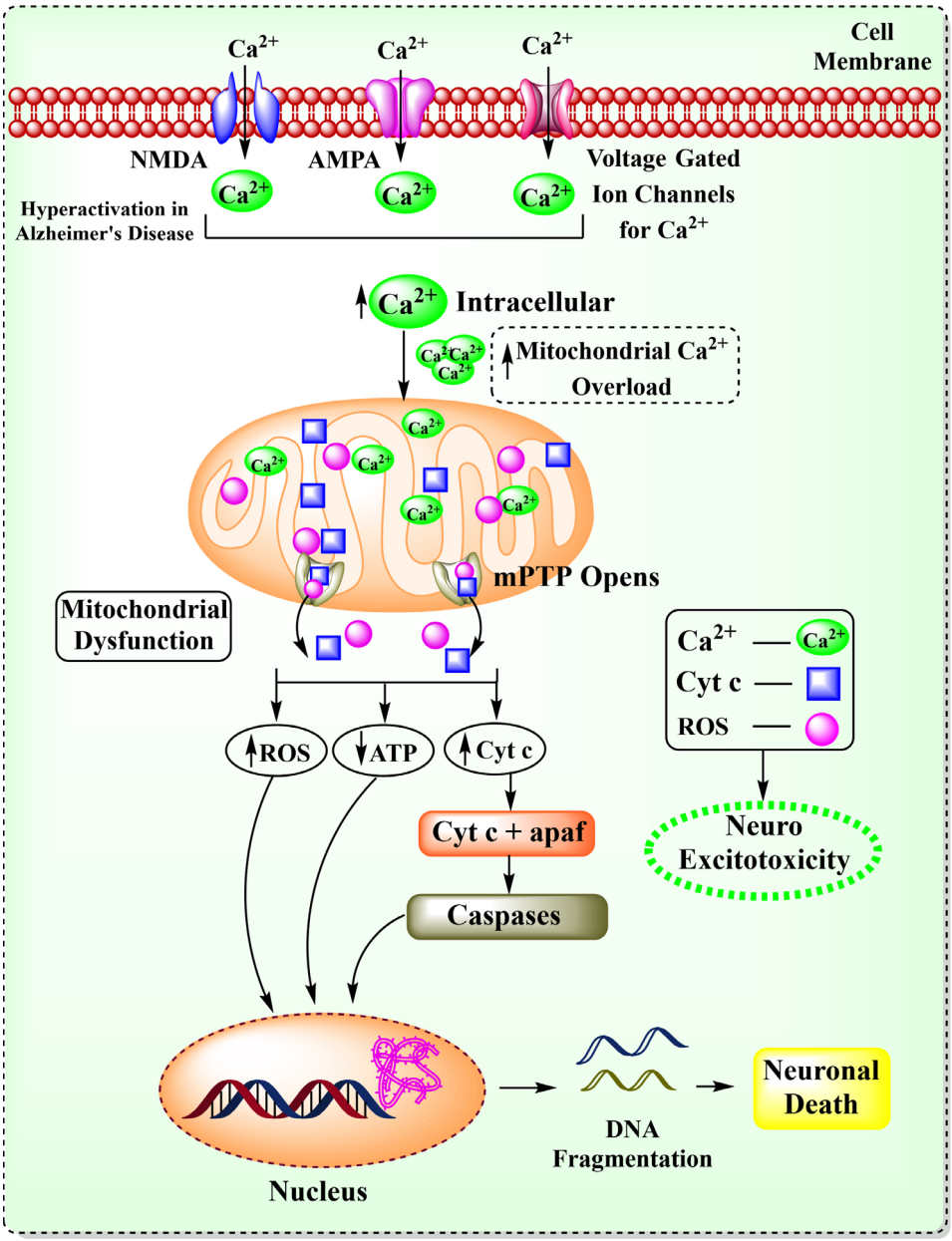

Fig. (1). Role of calcium signalling in mitochondrial dysfunction mediated neuronal apoptosis. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

neurodegeneration due to hyperexcitability of neurons of brains of patients carrying ApoE4 allele. This is carried further by reduced sensitivity of excited neurons to the GABAergic inhibitory signals [30, 31]. ApoE4 and its fragments cause age- and tau-dependent impairment of somatostatin positive GABAergic interneurons in the hilus of dentate gyrus, progressing to learning and memory deficits [32]. This was studied by preparing P301S tau transgenic mouse models on human ApoE knock-in or ApoE knockout background. The study reported that P301S/E4 mice had higher levels of tau protein in the brain than P301S/E2, P301S/E3 and P301S/EKO mice. P301S tau-expressing neurons co-cultured with E4-expressing glia results in markedly higher levels of Tumour-Necrosis Factor-α (TNF-α) secretion and reduced neuronal movements as compared to the other ones [33]. This emphasizes that ApoE4 is more potent among its 3 isoforms (ApoE2, ApoE3, and ApoE4) in stimulating Aβ production. The presence of different ApoE isoform differentially manipulate the brain metabolism. The ApoE4 brains exhibits decreased glucose utilization in areas affected by AD due to the noticeably decreased mRNA expression of GLUT3 transporters observed in them [34]. Although, evidences demonstrate crucial role of ApoE4 in the AD pathogenesis, literature review showed the significant variation in the ApoE4 carrier status [35]. The gene pravelance came out to be quite lower in subjects recruited from Asian and southern European/Mediterranean communities as compared to the subjects recruited from North America and Northern Europe [35]. The said gene was also not found to play pivotal role in AD in Nigerian population [36]. Thus, ApoE4 only could not be considered a determinant of the disease.

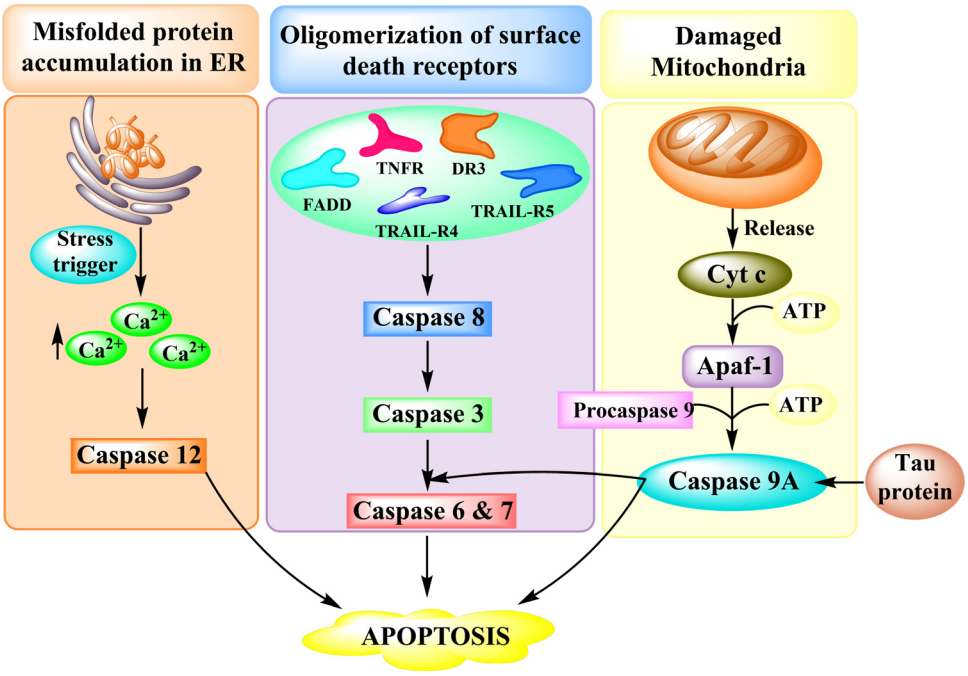

Fig. (2). Mechanisms involving caspase dependent neuronal apoptosis. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

AD brains also exhibit abnormal microvascular structure resulting in a reduced supply of blood as well as oxygen and glucose to certain regions of the brain leading to cerebral hypoperfusion, impaired brain metabolism, and cognitive failure [37-39]. Other changes in the brain that take place are atrophy of cortical and subcortical regions of the brain like the bilateral, medial-temporal region, and posterior brain regions [40]. The brain immune system comprises of microglia being the prime phagocytes acting as the first line of defense and astrocytes being neuroprotective and supportive in function regulate the neurotransmission through synapses, maintenance of synapses, and Long Term Potentiation (LTP). Both of these get activated during the initial stages of the AD and exert neuroprotective effects by clearing accumulated Aβ by secreting Aβ-degrading proteases like neprilysin (NEP), matrix metalloproteinases (MMP-2, MMP-9), Insulin-Degrading Enzymes (IDE) followed by phagocytosis. Microglia also gets activated in response to stimulation of the TREM2 receptor (triggering receptor expressed on myeloid cell 2) and engulf soluble and fibrillar Aβ followed by the release of ROS and proinflammatory mediators like interleukins (IL-1β, IL-6, IL-12, 1L-23), TNF-α and cyclo-oxygenase-2 (COX-2). In AD patients, the overexpression of the TREM2 receptor was found which down-regulates the microglial phagocytosis and up regulate the release of proinflammatory markers reducing Aβ clearance [41]. The prolonged duration of reactive gliosis is detrimental as it increases the release of ROS and other proinflammatory cytokines leading to excessive inflammation in surrounding areas, increased Aβ accumulation, and tau hyperphospho-rylation [42]. Hyperactive astrocytes exert toxic effects and thus synaptic functions and neurotransmission is impaired leading to cognitive deficits [42-44]. A research aimed at establishing the relationship between neuronal inflammation and β-amyloid plaques and tau protein loads using Positron Emission Tomography (PET) concluded that increasing β-amyloid load lead to increasing microglial activation and inflammation [45].

Various evidence-based studies conclude AD being a multifactorial disease arising out of tau phosphorylation, amyloid-β metabolism, impaired calcium homeostasis, increased inflammatory responses, insulin resistance, acetylcholine biosynthesis, mitochondrial dysfunction, oxidative stress, and neuronal apoptosis [46]. Out of all the causes, mitochondrial dysfunction or mitochondrial cascade hypothesis is an important part which needs to be addressed as it plays a central role (Fig. **3**) between all the associated AD pathologies [47-53].

3. MITOCHONDRIAL DYSFUNCTION: IMPLICATION IN THE PATHOGENESIS OF AD

Mitochondria are the most dynamic organelle responsible for fulfilling the energy requirements of the cell. They regulate cellular energy metabolism and cell apoptotic pathways [54]. This section discusses the various preclinical and clinical pieces of evidence available in the literature that reveals how mitochondrial dysfunction results in neuronal damage and plays a central role in the pathogenesis of AD.

3.1. Abnormal Mitochondrial Morphology, Distribution, and Mitophagy

Mitochondria maintain their morphology by undergoing fission and fusion reactions to maintain their shape and size [55]. The fission and fusion reactions are balanced in normal conditions whereas, in AD, this balance gets impaired due to abnormal expression of genes such as Dynamic-related protein-1 (Drp-1), mitochondrial fission-1 (Fis-1) and fusion proteins (Mfn1, Mfn2, and Opa1) that are required to maintain the normal functioning of mitochondria [56].

Immunoblot studies have revealed the decrease in the concentration of Drp-1, Mfn1, Mfn2 and Opa1 while increase in Fis-1 in the tissues of AD subjects. These alterations suggest that AD subjects exhibit greater extent of mitochondrial fission as compared to mitochondrial fusion [57]. Further, decreased concentration of Drp-1 is said to be responsible for abnormal mitochondrial distribution, morphology and misbalancing of mitochondrial division [58]. Possibly due to alterations in these crucial protein levels, the mitochondria procured from AD patients appear to have swollen surface, distorted cristae, and increased mitochondrial size [59]. In a study conducted on human fibroblast cells procured from normal as well as AD patients, the mitochondrial size and shape were found to be significantly increased. The elongated mitochondria are found to cluster together in perinuclear areas as compared to normal cases where there is a uniform cytoplasmic distribution of mitochondria [58].

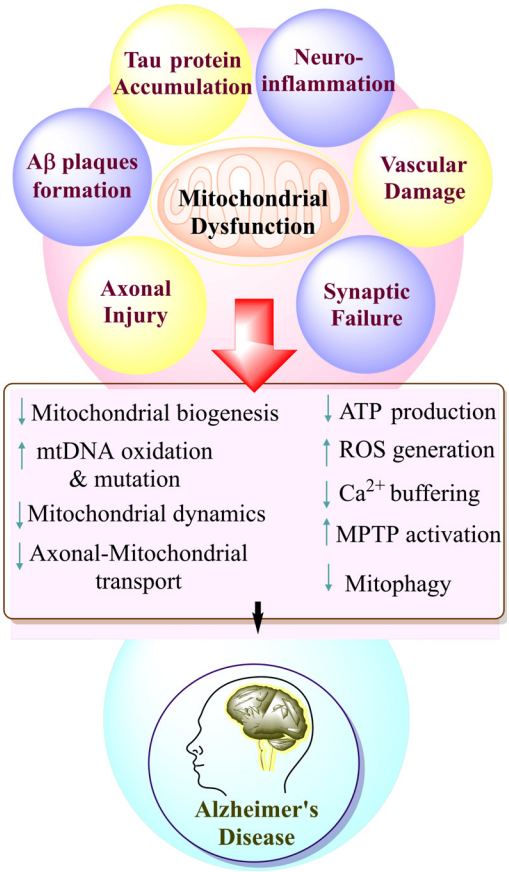

Fig. (3). Interplay between mitochondrial dysfunction and classical theories of Alzheimer's disease. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

Mitochondria frequently undergo changes in their shape and size to move through the mitochondrial traffic to distribute their functions in a dynamic and non-uniform manner. They move along the cell body to axons, dendrites, and synapses in an anterograde manner and return to the cell body *via* retrograde manner [60, 61]. In patients with AD, β-amyloid plaques tend to accumulate in mitochondria along with the neuronal synapses. The studies have found out that deposition of β-amyloid plaques impairs the normal mitochondrial anterograde and retrograde transport in the neurons of the hippocampal region leading to the degeneration of synapses in AD brains. A study found out that the exposure of mouse hippocampal neurons to β-amyloid decreased the number of motile mitochondria, altered mitochondrial distribution, and reduction in the number of anterograde moving mitochondria's accounting for the reduced ATP supply and synaptic degeneration associated with the disease [62].

For maintenance of mitochondrial homeostasis, mitochondria frequently undergo autophagy known as mitophagy. It is the process of engulfment of damaged mitochondria by the autophagic vessels and their fusion with lysosomes followed by subsequent degradation [63]. In AD patients, the mitophagy phenomenon tends to be highly compromised in the hippocampal region [64], whereby reducing the clearance of damaged mitochondria coupled with rising oxidative stress leads to continuous accumulation of dysfunctional neurons in AD [63].

3.2. Mitochondrial Dysfunction and Cell Bioenergetics Failure

Since mitochondria are the powerhouse of the cell, the primary evidence of mitochondrial malfunctioning being the reason behind the progression of AD comes from the abnormal energy hypometabolism observed in certain parts of the brain of AD patients [65]. Energy hypometabolism in the brain occurs as a result of altered microvasculature in some parts of the brains of AD patients leading to ischaemic conditions. Reduced blood and oxygen supply to neurons lead to a reduction in ATP formation resulting in oxidative stress, inducing dysfunction of the Na^+K^+ATPase pump and collapse of signal transduction, neurotransmitter dysfunction, and impaired cleavage of APP causing elevation of Beta-site Amyloid precursor protein Cleaving Enzyme-1 (BACE1) concentration and excessive formation of Aβ. Also, impaired energy metabolism causes the formation of abnormal protein molecules by aberrant misfolding, disassembling, and cleavage of protein molecules that further has deleterious effects on cellular structures. Tau hyperphosphorylation due to abnormal metabolism also leads to the damage of microtubules. The hippocampal and cortical brain regions are relatively more vulnerable to energy hypometabolism and associated biochemical changes resulting in cognitive deficits and memory impairments [39]. Another major reason behind brain hypometabolism in AD is the high energy demands of the hyperactivated microglia or the highly activated brain immune system in AD, which further limits the energy availability to neurons [31, 66-68]. The disrupted glucose metabolism in brain sacrifices the transmembrane ion transport, vesicle recycling and synaptic signalling [69, 70], which further leads to hyperexcitability, encitatory-inhibitory imbalance and functional impairment of cortical neurons, which further compromises brain's energy efficiency [31].

Evidence of impaired glucose metabolism in AD subjects comes from the cohort autopsy conducted within the Baltimore Longitudinal study of aging evaluating the ratios of concentrations of glycolytic amino acids, serine, glycine, and alanine to glucose and quantifying the protein levels of neuronal (GLUT3) and astrocytic (GLUT1) transporters. The study found that decreased glycolytic flux downregulated

GLUT3 levels and increased tissue glucose concentrations were linked to the severity of AD symptoms. The study established that impaired glucose metabolism due to decreased glycolytic flux was connected to the progression of AD [71]. Cross-sectional Positron Emission Tomography (PET) studies in ApoE4 gene allele carrying heterozygotes showed that e4 carrying heterozygotes had an abnormally low Cerebral Metabolic Rate for glucose (CMRgI) in the vicinity of temporal, posterior cingulated, prefrontal cortex, basal forebrain, parahippocampal gyrus, and thalamus. The declines observed were significantly higher than those in the e4 noncarriers [72]. Another MRI-guided 2-[^{18}F]fluoro-2-deoxy-D-glucose/Positron-Emission Tomography (FDG/PET) study examined the decline in glucose metabolism as a determining factor in the transition of patients suffering from mild cognitive impairment to AD [73]. The Croteau *et al.*, in 2018, compared the brain metabolism of glucose and ketone bodies using dual-tracer PET and MRI and reported reduced glucose metabolism (about 11%) in parietal, temporal lobes and in cingulate gyrus in AD patients compared to the control group. AD patient's MRI scans also revealed a decreased volume of grey matter and cortical thinning [74]. Another study considering increased lactate levels in cerebrospinal fluid (CSF) of AD as a consequence of energy hypometabolism in AD, explored the relationship between this upregulated lactate levels and cerebral glucose metabolism *via* 2-deoxy-2-[^{18}F] fluoro-D-glucose positron emission tomography in AD. The AD patients showed a remarkable increase of lactate in cerebrospinal fluid and brain glucose hypometabolism compared to the control group. A significant correlation was also established between upregulated CSF lactate levels and reduced glucose uptake in the left middle pre-frontal cortex, orbitofrontal cortex, and parahippocampal gyrus [75].

The mitochondrial biogenesis and metabolism is controlled and modulated by the expression of estrogenic receptors and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) [76,77], which in turn triggers various transcription factors like Nuclear respiratory factors 1 and 2 (NRF1, NRF2) that control the expression of mitochondrial transcription factor A (TFAM) regulating the biogenesis of new mitochondrial DNA and proteins from the existing ones [78-80]. A study evaluated the protein expression levels of cAMP protein kinase catalytic subunit-α (PKAC-α), cAMP response element-binding protein (CREB), PGC-1α, NRF1, NRF2, and TFAM in the early age of 3xTg-AD mice and the expression levels of all these were found to be remarkably reduced in the mice at a very early age with no notable Aβ deposition, implicating that PGC-1α mediated impaired mitochondrial biosynthesis precedes mitochondrial dysfunction and further progression of AD symptoms at later ages [81].

3.3. Mitochondrial Dysfunction and Oxidative Stress

Since mitochondria are the major producer of ROS [82], oxidative imbalance and significant rise in the number of ROS in AD subjects is another evidence indicating faulty mitochondria in Alzheimer's pathology. Plasma levels of various endogenous antioxidants like plasma bilirubin, uric acid, and albumin are reported to be lower in AD patients indicating the oxidative imbalance in the brains of AD patients [65, 83]. This imbalance causes a substantial increase in cerebral lipid peroxidation and protein and DNA/RNA oxidation that leads to neuronal death [84-86]. In fact, a significant increase in multiple oxidized bases was found in nuclear and mitochondrial DNA of frontal, parietal, and temporal lobes and cerebellum of 8 AD subjects in contrast to 8 age-matched control, which was consistent with higher levels of ROS in mitochondria. The level of 8-hydroxyguanine (a biomarker of DNA damage) was also found to be 10-fold higher than other oxidized adducts indicating that oxidative damage to mitochondrial DNA is a contributing factor in neurodegeneration associated with AD [87].

3.4. Abnormal Enzyme Concentrations

The impaired levels of enzymes involved in ETC and Kreb's cycle (TCA) is also another evidence suggesting abnormal mitochondrial activities and thus, energy hypometabolism comes from the significantly lower levels and activity of various enzymes that form a part of the Electron Transport Chain (ETC) [88]. Post-mortem studies conducted on AD patients revealed a decrease in the levels and activity of α-ketoglutarate dehydrogenase complex (a rate-limiting enzyme of ETC cycle) in the temporal cortex, parietal cortex, and hippocampus regions [89-91]. Cytochrome oxidase and pyruvate dehydrogenase enzymes were also found to be deficient in AD patients [92-94]. The β-amyloid protein dose-dependently reduced the effects on ETC complex 4 (cytochrome C oxidase) [95]. The interaction of Aβ with Aβ-binding alcohol dehydrogenase in the mitochondrial matrix aggravates Aβ-mediated mitochondrial and neuronal permeability, production of ROS and permeability transition pore (mPTP) failure and leads to synaptic degeneration and cognitive decline [96].

4. MITOCHONDRIAL DYSFUNCTION- A KEY MEDIATOR IN AD PATHOGENESIS

For years now, cholinergic, Aβ, and tau dysfunction have been the primary focus of all the AD therapies. In fact, most of the clinical trials and studies still focus on these classical targets to delay disease progression and improve the quality of life of AD patients [97, 98]. Various efforts to understand the multi-etiological AD have revealed the interactions between mitochondrial dysfunction and classical targets of AD (Aβ, tau, ApoE). Though there have been evidence listed on the interactions of protein species with mitochondrial dysfunction and how this triggers mitochondrial insult, there are also plenty of evidence to prove its attenuation. This section summarises and establishes the link between mitochondrial dysfunction and classical AD targets and demonstrates the relevance of targeting mitochondria and cell bioenergetics for AD treatment [99].

To establish a link between mitochondrial DNA (mtDNA) and AD, Ntera2D1 (NT2) teratocarcinoma cells were depleted of endogenous mtDNA and re-populated with platelet mtDNA from AD and non-AD patients (control), resulting in depression of cytochrome C oxidase activity and elevation in ROS production and free radical scavenging enzyme activities in the cytoplasmic hybrids (cybrids) from AD patients [48]. Furthermore, mtDNA deletions increase levels of proteins involved in cell cycle arrest (p21/p53) and

decrease in cytochrome C oxidase activity which involved pathways associated with ROS causing neuronal damage [100].

Increased production of ROS and oxidative stress due to mitochondrial dysfunction increases the processing of APP to form Aβ. Thus, mitochondrial dysfunction also tends to increase the formation and accumulation of Aβ [101]. A study conducted on human AD subjects and the 3xTg-AD mouse model of AD showed the role of heat shock protein 60 (HSP60), a molecular chaperone localized to mitochondria and plasma membrane, in elevating abnormal APP levels and Aβ accumulation in mitochondria. The study also found increased mitochondrial Aβ, γ-secretase, and oxidative stress along with decreased ATP levels in brain mitochondria of AD subjects. The *in-vivo* and *in-vitro* models of AD exhibit increased interaction between HSP60 and APP [102].

Tau protein being another classical target of AD is also associated with mitochondrial dysfunction. A study examined cortical tissues from AD patients, control subjects, APP, APP/PS1, and 3xTg.AD mice, the interaction of VDAC1 (voltage-dependent anion channel-1) protein with Aβ and tau protein was observed. VDAC1 protein level was found to be significantly elevated in cerebral cortices of 6-, 12-, and 24-month old APP transgenic mice relative to the age-matched control non-transgenic wild-type mice. Mitochondrial dysfunction was found to be aggravated in these models leading to the conclusion that interaction of VDAC1 with Aβ, APP, and phosphorylated tau blocks mitochondrial pores contributing to mitochondrial dysfunction [103]. Tau oligomers were identified to produce mitochondrial dysfunction by lowering levels of ETC Complex I (NADH-ubiquinone oxidoreductase) and it also activated caspase-9, which is related to the apoptotic mitochondrial pathway [104]. Tau expression was studied in stably transfected CHO (Chinese Hamster Ovary) cells and differentiated neuroblastoma N2a cells and concluded that tau causes a change in cell shape, slowed cell growth, and altered distribution of various organelles. Mitochondrial transportation was also impaired as it tends to cluster near the microtubule-organizing center due to inhibition of kinesin-like motor protein which mediates transportation [105]. Tau overexpression leads to abnormal mitochondrial distribution and impaired mitochondrial trafficking as was determined in AD mouse models and rTg4510 mice models. There is a great decrease in cytoplasmic area fraction of mitochondria in cells with Alz50+ tau accumulations [106].

While these examples and researches encapsulate the role of tau protein in causing mitochondrial dysfunction, there are also studies to illustrate and establish mitochondrial dysfunction as the causative agent in tau hyperphosphorylation. Chronic oxidative stress leads to hyperphosphorylation of Tau proteins [107] which tends to play a pivotal role in neurofibrillary pathology is responsible for neurodegeneration [108].

Since ApoE4 is a major risk factor responsible for aggravating AD pathologies, its effects were examined on mitochondrial dysfunction in AD. ApoE4 expression was identified to decrease NAD^+/NADH ratio and aggravated the concentration of ROS and mitochondrial calcium. ApoE4 cells also demonstrated decreased levels of respiratory complexes like Complex V (ATP synthase) and impaired levels of mitochondrial endoplasmic reticulum-associated membranes, mitochondrial fission/fusion, mitochondrial translocation proteases and mitochondrial ribosomal proteins [109]. Up-regulation of ApoE4 (1-272) fragment causes significant dysregulation in activities of Complex III and IV [110]. The concentration of mitochondrial respiratory complexes was assessed in neurons cultured from brain cortices of neuron-specific enolase promoter-driven ApoE3 (NSE-ApoE3) or ApoE4 (NSE-ApoE4) transgenic mouse and it was found to be critically lower in NSE-ApoE4 neurons as compared to NSE-ApoE3. ApoE4 expression in neuroblastoma Neuro-2A (N2A) cells reduced the levels of mitochondrial respiratory complexes I, IV, and V which leads to reduced mitochondrial respiration. This research also concluded the importance of targeting ApoE4 to treat AD subjects by eliciting treatment with a small molecule disrupting the interaction with ApoE4 to restore mitochondrial complex IV levels [111]. The *in-vivo* PET imaging of the brain of young adult subjects carrying ApoE4 have shown a decline in glucose metabolism in brain areas most vulnerable to AD-like posterior cingulated cortex, decades before the onset of possible symptoms. ApoE4 carrying individuals also demonstrated decreased mitochondrial cytochrome oxidase activity [112].

5. BIOENERGETICS MEDICINE AS A NEW THERAPEUTIC PARADIGM

To date, the majority of therapies for Alzheimer's disease targeting the amyloid cascade hypothesis, but by far these only target cognitive symptoms rather than decreasing the rate of disease progression. Treatments targeting Aβ have repeatedly failed due to multiple reasons which include inadequacy of clinical trials or faulty choosing of the substrate [113-115]. Either they have been disappointing in altering the course of the disease or they have been observed to produce toxic side effects leading to their failure. Moreover, it is till date a serious challenge to develop drug that penetrates blood brain barrier and selectively inhibits the cleavage of APP without affecting cleavage of alternative substrates like Notch or voltage-gated sodium channel substrates [116,117]. After the failure of Aβ-targeted therapies, attention is shifted to tau-targeting interventions, but by far these are also not as advanced as the Aβ ones and there are speculations that these might also face difficulties like the earlier ones due to difficulty in testing these in a population already suffering from degeneration [118]. Though, tau therapies are effective in reducing intracellular levels of tau and improving cognitive functions [119].

After repeated failure of therapies related to the amyloid cascade hypothesis and the potential difficulties encountered during their clinical trials, the focus has now shifted towards multifactorial approaches. Due to the presence of neuroimaging tools and biomarkers measurement, we can assess the onset and progression of AD in a more refined way and this can also help to device a better therapeutic aid for the condition. A multifactorial model for AD was established depicting alterations in brain amyloid-β burden, glucose metabolism, vascular flow, resting-state functional activity, structural properties, and cognitive integrity, and how the disorder is a result of interactions of all these factors. The model establishes vascular dysregulation as the most initial pathological

event leading to Late-onset Alzheimer's Disease. The result also demonstrated the advantages of targeting multifactorial over single-target treatments [120,121].

Since, mitochondrial dysfunction is the main connecting link between all the proposed pathologies of AD, targeting mitochondrial pathologies could be a useful approach for mitigating neurodegenerative changes in AD. Drug regimen targeting dysregulated mitochondrial processes seem to compensate the bioenergetics failure, oxidative stress, and other enzyme abnormalities associated with the disease leading to corrections in the working of various metabolic pathways including Kreb's cycle, fatty acid β-oxidation, oxidative phosphorylation, heme biosynthesis, and glycolysis involved in energy production which were found to dysregulated due to the mitochondrial dysfunction and the genetic risk factors involved in the disease.

6. DRUGS TARGETING MITOCHONDRIAL DYSFUNCTION: PRECLINICAL AND CLINICAL EVIDENCES.

Classical AD treatments have only focused on amyloid cascade hypothesis for delaying the course of progression of the disease. The present review focuses on the clinical trials and interventions focussing on mitochondrial cascade hypothesis and its related pathologies. For accessing the information, various databases mentioned in Table **2** were searched (accessed July 19-24, 2020), using a systematic search method described further.

Table 2. Databases searched for reviewing various clinical trials on AD.

1.	Clinicaltrials.gov
2.	Ctri.nic.in
3.	Clinicaltrialsregister.eu
4.	apps.who.int/trialsearch/
5.	Canada.ca/en/health-canada/services/drugs-health-products/drug-products/health-canada-clinical-trials-database.html
6.	Isrctn.com
7.	Chictr.org.cn
8.	Anzctr.org.au/trialsearch.aspx
9.	www.clinicaltrials.in.th
10.	Rctportal.niph.go.jp

The first database we searched was clinicaltrials.gov, we found 2357 studies for "Alzheimer disease", out of which 1950 were interventional studies. Making use of different keywords like "Mitochondria", "Oxidative stress", "Energy hypometabolism", "Hypometabolism", "Dynamic-like Protein", "Mitochondrial Fission", "Heme", "Pyruvate Dehydrogenase" and "Cytochrome Oxidase", we found 55 studies. The inclusion criteria followed for evaluating the study included interventional studies involving the investigation of a drug or a dietary supplement targeting mitochondrial dysfunction occurring in AD or any studies indicating even a slightest modulation in the mitochondrial pathway were included and the interventional studies in all the phases of the clinical trials were included. The studies were excluded on the grounds of being the investigation of a device or a behavioural intervention. Duplicate studies were also excluded. Other exclusion criterion for studies was if the particular study was terminated at an early stage due to lack of participation. All the interventional studies irrespective of their phases were included and the number of studies reduced to 46. Out of these 46 studies, each of the mentioned study was reviewed individually for its relevance. After critical appraisal of the obtained studies following the criterias described above, 14 studies that targeted mitochondrial cascade hypothesis or had antioxidant effect or caused immunomodulation resulting in improvement of mitochondrial function were selected and listed in Table **3**. Some of the studies targeted certain enzyme pathways associated with mitochondrial function.

The second database on our search list was ctri.nic.in. After using the keyword "Alzheimer Disease", we included studies in all phases and from all states of India and all recruitment statuses. We limited our search to interventional studies only and thus, came across 4 studies which didn't had any connection to our main focus area that is 'mitochondrial cascade'. Then after using the keyword 'mitochondria' along with AD, 1 study was obtained, which was excluded by us under exclusion criteria of it being a behavioural intervention. . Using other keywords such as "Oxidative stress", "Energy hypometabolism", "Hypometabolism", "Dynamic-like Protein", "Mitochondrial Fission", "Heme", "Pyruvate Dehydrogenase" and "Cytochrome Oxidase" along with AD, didn't yield any result.

After that, another database clinicaltrials.eu was searched. The database showed 355 results on using keyword "Alzheimer Disease". To refine our search results, we used other keywords along with "Alzheimer disease" that were mentioned earlier. Three studies were obtained using keyword "Alzheimer disease AND oxidative stress" and "Alzheimer disease AND hypometabolism". Out of these 6, only 1 study was found relevant and listed in Table **3**, the other 5 studies were rejected on the basis of our exclusion criteria. The study investigated the use of HF0220 drug in patients suffering from mild-to-moderate dementia (EudraCT Number: 2005-005791-32). The drug targeted the oxidative stress associated with mitochondrial dysfunction in AD.

Other database searched was apps.who.int/trialsearch/ which is the International Clinical Trials Registry Platform of World Health Organisation. The database yielded 0 studies on searching "Alzheimer disease AND mitochondria". After that, 7 results appeared on searching "Alzheimer disease AND oxidative stress". Out of these 7, only 1 study was found to be relevant to our search, which is to target the mechanisms associated with mitochondrial dysfunction and included in Table **3**. Of the excluded 6 studies, 2 were same as found while searching the clinicaltrials.gov database and were excluded under the exclusion criteria. The rest 4 were rejected as they didn't satisfy our inclusion criteria. Next, we searched for studies relating to "Alzheimer disease AND energy metabolism", which yielded 2 studies, again out of these 2, one was already listed study of 'Nicotinamide riboside' (Clinicaltrials.gov ID: NCT04430517) and the other one was excluded as it was the clinical study involving investigation of

Table 3. Clinical trials involving drugs targeting different phases of mitochondrial dysregulation in AD.

Name of the Intervention	Class of Drug/Established Mechanism of Action	Clinical Trial Phase	Participants	Study Design	Study Outcome	Completion Date	Source
MitoQ	Antioxidant	Not applicable	12 participants; All genders; no healthy volunteers; Age: 50 - 85 years	Randomized cross-over design	Results awaited	Estimated: May 2021	Clinicaltrials.gov ID: NCT03514875
S-Equol	Estrogen receptor-β stimulation, Generation of new mitochondria	Phase 1	15 participants; only females; Age: 60 - 90 years; no healthy volunteers	Single-group assignment	The pilot study established its efficacy for its safe use in conducting further full fledged Phase1 trials only after taking appropriate measures.	April, 2016	Clinicaltrials.gov ID: NCT02142777 [152]
S-Equol	Estrogen-like compound, stimulates estrogen receptor-β; Increase in mitochondrial activity	Phase 1 Phase 2	40 participants; All genders; Age: 50 - 90 years; no healthy volunteers	Randomized, cross-over design; Masking: double	Results awaited	Estimated: September, 2020	Clinicaltrials.gov ID: NCT03101085
Epigallocatechin gallate (EGCg)	Antioxidant, modulation of several signal transduction pathways, influence on expression of genes regulating cell survival responsible for programmed cell death, induction of alpha-secretase and endothelin-converting-enzyme, prevents β-amyloid aggregation to toxic oligomers	Phase 2 Phase 3	21 participants; All genders; Age: 60-100 years; no healthy volunteer	Randomized; Parallel assignment; Masking: Quadruple	No results available	February 2015	Clinicaltrials.gov ID: NCT00951834
Resveratrol with glucose, malate	Resveratrol: antioxidant; Glucose: precursor of oxidative metabolism; Malate: primer of Kreb's cycle	Phase 3	27 participants; all genders; no healthy volunteers; Age: 50 - 90 years	Randomized, double-blind, placebo-controlled trial, parallel assignment Masking: Quadruple	It is safe and well-tolerated. Scores of ADAS-Cog, MMSE, ADCS-ADL, NPI were found to change at 12 months and showed less deterioration as compared to control group. However, none of them reached statistical significance.	June, 2011	Clinicaltrials.gov ID: NCT00678431 [126]
Nicotinamide riboside	Boosts NAD^+ levels, Enhances mitochondrial function, slows or reverses age-related abnormalities	Early phase 1	50 participants; all genders; no healthy volunteer; Age: 55-89 years	Single group assignment; Masking: none	Results awaited	Estimated: May 2025	Clinicaltrials.gov ID: NCT04430517
Dimebon	Neuroprotective, antiapoptotic and inhibits mPTP opening, increases mitochondrial potential, improves ATP synthesis	Phase 3	598 participants with mild-to-moderate AD; genders; no healthy volunteers	Randomized; parallel assignment; Masking: Quadruple	Initial trials showed its beneficial effects on cognitive parameters. Phase 3 trials were Terminated early due to lack of efficacy.	December, 2009	Clinicaltrials.gov ID: NCT00675623 [153]

(Table 3) contd….

Name of the Intervention	Class of Drug/Established Mechanism of Action	Clinical Trial Phase	Participants	Study Design	Study Outcome	Completion Date	Source
R+pramipexole	Antioxidant	Phase 2	20 participants; all genders; no healthy volunteers; Age: 55 years and older	Single group assignment; Masking: none (open-label)	Results unavailable	April, 2014	Clinicaltrials.gov ID: NCT01388478
Mito-food plan	Improve cellular health, decrease inflammation, improves cognition	Phase 1 Phase 2	5 participants; all genders; healthy volunteers taken; Age: 55-90 years	Single group assignment, Masking: none (open label)	Results not yet published	July, 2018	Clinicaltrials.gov ID: NCT03630419
Etanercept Dietary supplement: Curcum, Luteol.Theaflav. Lip.Acid, Fish oil, Quercet.,Resveratr. Other Names: Super Biocircumin Super omega-3 Optimized Quercetin Optimized Resveratrol	Immunomodulation, cognitive improvement	Phase 1	12 participants; with diagnosed AD; all genders; No healthy volunteer; Age: 60-85 years	Randomized; crossover assignment; Masking: None	In phase 2, it was well tolerated and showed some trends towards cognitive, functional and behavioural benefits	May 2016	Clinicaltrials.gov ID: NCT01716637 [154]
Fish oil, Alpha lipoic acid	Antioxidant, anti-inflammatory, slow AD progression	Phase 1 Phase 2	39 participants; diagnosed with probable AD or MCI; MMSE score between 18-26; all genders; no healthy volunteers; Age: 55 years and older	Randomized; parallel assignment; Masking: Triple	The combination of omega-3 fatty acids and alpha lipoic acid is safe and tends to slow down cognitive and function decline in mild-to-moderate AD patients over 12 months	August 2007	Clinicaltrials.gov ID: NCT00090402
Sulforaphane	Influences histone deacetylase mechanism and inflammatory biomarkers	Not applicable	160 participants; meeting the AD criteria; all genders; no healthy volunteers; Age: 50-75 years	Randomized; parallel assignment; Masking: quadruple	Results awaited	Estimated: December, 2022	Clinicaltrials.gov ID: NCT04213391
Photobiomodulation and ketogenic diet	Red and near infrared light *via* LED augments cellular energy metabolism, enhancing mitochondrial function, increasing cytochrome C oxidase activity, stimulates antioxidant protective pathways and promotes cell survival. Ketogenic diet shows metabolic and neuromodulatory benefits within CNS	Not applicable	30 participants; all genders; healthy volunteers accepted; Age: 18-80 years	Randomized, parallel assignment; Masking: none (open label)	Results awaited	Estimated: September, 2020	Clinicaltrials.gov ID: NCT03859245
Dapagliflozin	Sodium glucose co-transporter-2 inhibitor used in type-2 diabetes to improve glycemic control, lowers blood sugar	Phase 1 Phase 2	48 participants; all genders; no healthy volunteers; Age: 50-85 years	Randomized; Parallel assignment; Masking: Quadruple	Results awaited	Estimated: October, 2020	Clinicaltrials.gov ID: NCT03801642
HF0220 (7-beta-hydroxyepiandrosterone)	Antioxidant and reduces disease progression	Phase 2	40 participants with mild-to-moderate dementia; all genders; Age: above 18; no healthy volunteers	Randomized, controlled, double blind trial, parallel assignment	The drug was found to be safe and well-tolerated at all doses	August, 2008	Clinicaltrialsregister.eu EudraCT Number: 2005-005791-32

(Table 3) contd….

Name of the Intervention	Class of Drug/Established Mechanism of Action	Clinical Trial Phase	Participants	Study Design	Study Outcome	Completion Date	Source
Dietary *Centella asiatica* extract	Modifies oxidative stress markers	Not applica-ble	40 participants with MMSE score≤23; all genders; Age: above 65 years; no healthy volunteers.	Randomized controlled trial; parallel; Double blind.	Results not yet posted.	September, 2017	Clinicaltrials.in.th Study ID: TCTR20180228001

Abbrevations: AD: Alzheimer's Disease; MCI: Mild Cognitive Impairment; ADAS-Cog: Alzheimer's Disease Assessment Scale-cognitive subscale; ADCS-ADL: Alzheimer's Disease Cooperative Study-Activities of Daily Living; MoCA: Montreal Cognitive Assessment; NPI: Neuropsychiatric Inventory; MMSE: Mini-Mental State Examination; mPTP: mitochondrial permeability transition pore

a device. After that, we searched for other terms like "Alzheimer disease AND hypometabolism", "Alzheimer disease AND cytochrome oxidase", "Alzheimer disease AND heme", "Alzheimer disease AND pyruvate dehydrogenase" and all the other search keywords associated with the disease, but none of them yielded any result. So in all, only one relevant study was obtained out of this database which examined the effect of dietary Centella asiatica extract on the oxidative stress markers associated with the mitochondrial dysfunction in AD (Study ID: TCTR20180228001).

Other databases searched were canada.ca, which did not yield any result with any of the key search terms. Next, we moved on to our other database, which is isrctn.com; the database yielded 15 studies on AD alone, so we reviewed each of them to check for its relevancy to our subject, but no study was found targeting mitochondrial dysfunction or any of its associated pathologies. The Chinese clinical trials database didn't yield any study on Alzheimer's disease itself. The clinical trials database of Australia and New Zealand-Anzctr.org.au/trialsearch.aspx was searched, and filters for searching only interventional studies were applied. Putting "Alzheimer disease" in the health condition/problem studied column and choosing 'neurological disease' in condition category, 8 recorded studies were found. But none of these studies were focused on mitochondrial dysfunction.

Next database searched was Clinical trials database of Thailand- www.clinicaltrials.in.th, it yielded 1 result with the key search term "Alzheimer disease AND oxidative stress", which was the one we already listed from the WHO database itself, the study involving *Centella asiatica* crude extract (Study ID: TCTR20180228001). Rest 0 results were obtained for all other search terms.

The last database searched was the clinical trials database of Japan, rctportal.niph.go.jp. The database showed 328 studies for AD alone. But 0 studies were obtained for all the other key terms – "Alzheimer disease + Mitochondria", "Alzheimer Disease + Oxidative stress", "Alzheimer disease + alpha-ketglutarate dehydrogenase", "Alzheimer disease + cytochrome oxidase", "Alzheimer Disease + energy hypometabolism", "Alzheimer Disease + hypometabolism" and "Alzheimer Disease + heme".

All the studies were obtained after critical appraisal of the clinical trials after applying our inclusion and exclusion criteria and the studies relevant to our subject are all listed in Table **3**.

Of all the clinical trials, interventions like MitoQ, epigallocatechin-gallate, R+pramipexole, Resveratrol, HF0220, dietary *Centella asiatica* extract and combination of fish oil and alpha-lipoic acid exert antioxidant activities and improved cognitive functions. MitoQ or mitoquinone mesylate is an orally administered mitochondria-targeting antioxidant that inhibits the production of reactive oxygen species and reduces the extent of neurotoxicity caused due to Aβ. The drug was shown to intercept cognitive decline, oxidative stress, synaptic loss and activation of caspases in 3xTg AD mice model [122]. Another study found MitoQ prolongs lifespan and improves the depletion of mitochondrial lipid cardiolipin, delays Aβ-induced paralysis and protects ETC complexes Ⅰand Ⅳ in transgenic *Caenorhabditis elegans* model of AD [123]. Another research evaluated the effect of mitochondrial-targeting antioxidants like MitoQ, SS31 and resveratrol on neurons from Tg2576 AD mouse models and mouse neuroblastoma cells which demonstrated that the cells treated with MitoQ, SS31 and resveratrol showed impaired peroxiredoxins expression and mitochondrial functions were found to return to normal [124]. MitoQ drug is under a randomized clinical trial (NCT03514875) to check its effect on endothelial NO production, cerebrovascular oxygenation and carotid artery blood flow which is estimated to be completed in May 2021. Another anti-ageing and antioxidant molecule Resveratrol, which is a constituent of red wine, was given as a dietary supplement to improve AD markers. It decreased cognitive impairment *via* reducing amyloid burden and inhibiting tau hyperphosphorylation. Resveratrol prolongs life span of SAMP8 AD mice model [125]. Resveratrol has undergone Phase 3 clinical trial (NCT00678431) for its effect on cognitive impairment in AD patients showing some variations in assessed outcome measures with no adverse effects obtained [126].

S-Equol, another drug under clinical trials for its action on mitochondrial cytochrome oxidase enzyme activity (NCT02142777) and (NCT03101085) works by stimulating estrogen receptor-β and increase mitochondrial activity by stimulating the generation of new mitochondria. In a study aimed at comparing the efficacy of R/S-Equol phytoSERM treatment with S-Equol phytoSERM treatment on mitochondrial markers in neurons of mouse hippocampus. The results established both treatments to be having positive effects with S-equol having greater enhancing effect on mitochondrial function. They modulated genes that were involved in β-amyloid production and clearance, bioenergetic mechanisms, lipid metabolism and redox homeostasis [127].

Nicotinamide Riboside enhances mitochondrial function by boosting NAD+ levels and thus, retards disease

progression (NCT04430517). It was found to decrease the accumulation of Aβ and enhance the cognitive function and the oxidative phosphorylation in transgenic AD mouse model (APP/PS1) [128]. It was found to improve cognition by enhancing the expression of PGC-1α in brain and BACE1 degradation and decrease in generation of Aβ in Tg2576 mice models [129].

Other drug interventions targeting mitochondrial aspect for providing relief include Anti-apoptotic and antihistaminic drug **Dimebon** (NCT00675623) also has neuroprotective effect and it is found to decrease the concentration of APP metabolites and Aβ by trigger mammalian target of Rapamycin (MTOR)- and AT65-dependent autophagy. Behavioural deficits were also found to be improved alongwith the prevention of autophagic failure in brains of TgCRND8 mice models [130].

Other clinical interventions for disease improvement include **Mito-food plan** (NCT03630419) which decreased inflammation and improved cognitive deficits associated with the disease. Another drug **Sulforaphane** which is currently under clinical trials works by influencing inflammatory biomarkers and histone deacetylase mechanism (NCT04213391). The study is estimated to be completed by December 2022.

Out of all the clinical interventions enlisted in Table **3**, some are under clinical evaluation and are estimated to be completed shortly. The compiled studies have shown efficacy in improving cognitive parameters assessed, though in some cases, noted changes are insignificant but still any occurrence of severe adverse event is not noted in any of these implicating there potential in being an effective future prospective.

Other therapeutic drugs that acted on mitochondria and alleviated mitochondrial dysfunction when tested preclinically are inhibition of mitochondrial complex I using a small molecule **CP2** in Familial AD mouse models decreased the concentration of amyloid beta and tau proteins and prevented the cognitive decline in 3 animal models of familial AD. It was also found to significantly improve mitochondrial bioenergetics [131]. Another mitochondria-targetting hydrogen-sulphide donor, **AP39** was found to increase the number of ATPs in brain, prevented mtDNA damage, reduced the generation of ROS and also prevented atrophy of brain in APP/PS1 transgenic mice [132]. Another treatment includes mitochondrial division inhibitor, **Mdivi-1** which is a derivative of quinazoline. It was found to prevent fragmentation of mitochondria, inhibit loss of mitochondrial membrane potential, generatiom of reactive oxygen species and also delayed synaptic depression in neurons treated with Aβ. It was also identified to enhanced memory and learning [133].

7. CHALLENGES TO DEVELOP DRUG TARGETING MITOCHONDRIAL DYSFUNCTION IN AD

Besides AD and other neurodegenerative disorders, mitochondrial dysfunction is implicated in aging with a variety of disorders, including cardiovascular diseases, migraine, infertility, cancer, kidney and liver diseases [134]. However, there are a whole lot of challenges faced during the development of formulations targeting mitochondria specifically, one of these is the troubles in diagnosing the extent of mitochondrial functioning and dysfunctioning and the dose of the drug required to produce the desired modulation in mitochondrial functioning or to ascertain if the already available therapeutic regimens have an effect on mitochondria or elsewhere. Hence, the need of the hour is the development of validated, sensitive and specific biomarkers for this diagnosis to enable designing and formulation of desired therapeutic dose and monitoring of patient's response to the treatment [135-137]. As of now, currently available AD diagnosis includes cognitive testing, imaging of Aβ and tau pathology in various parts of brain and assays of cerebrospinal fluid. But so far, these techniques have disadvantages including limited availability, high cost alongwith invasive procedures employed with their results and integrity under question [138] putting in a nutshell the inability to diagnose the exact disease status of the individual. But the advancement in technology has led to researchers speculating novel biomarkers involved in the pathogenesis of the disorder, which include specific measurements of Aβ oligomer or monomer forms, concentration of tau proteins in the peripheral plasma and CSF, apoptosis, neuroinflammation, alteration in activity of neurons, dysfunctioning of blood brain barrier, abnormal lipid metabolism, oxidative stress [139] and various other metabolites for early detection and diagnosis of disease progression. Most of these biomarkers are based on the earlier tau and Aβ hypothesis with mitochondrial based biomarkers still being in infancy. Development of mitochondrial biomarkers could be an excellent approach as mitochondrial functions are common to various cell types and are present in both sporadic and familial AD. The potential blood biomarker could be a protein that is known to cause or resultant of mitochondrial abnormalities. Measurement of complex II and complex IV of ETC and NAD^+/NADH ratio can be speculated as a possible biomarker as the decrease in functions of these markers is reported in both CNS and peripheral cells in AD. Since there are disturbed mitochondrial ETC system in AD, measuring the functions of ETC using metabolomic approach could be another useful approach to track AD pathogenesis [137]. Another way of improving the accuracy of diagonosis and identifying the time point and potential patient that could develop AD is to add mitochondrial assessment to currently employed battery of AD biomarkers and ATN (amyloid tau and neuronal injury) system [140]. However, before developing the mitochondrial funtion as a clinically useful biomarker, identification of metabolic deficits that are common in majority of AD patients is certainly required.

Another significant challenge encountered in designing the formulations that exert their action on mitochondria is to attain tissue selectivity in order for drug to reach mitochondria by penetrating the blood brain barrier [136]. Another associated challenge is to trigger the action of drugs only inside mitochondria to minimize other side effects [141] and some proposed ways to attain this can be selective activation of pro-drug by enzymes, combined delivery of more than one active compound targeting mitochondria that react with each other after reaching mitochondria or radiotherapeutic approaches [136, 142, 143].

CONCLUSION

Comprehensive evaluation of mitochondrial cascade hypothesis and clinical interventions targeting changes linked with mitochondrial dysfunction, establishes the superiority of this hypothesis in serving as a target for attenuation of neurodegeneration and improvement of cognitive functions as compared to other classical AD targets. But, besides the improvement in understanding the pathophysiology of AD and availability of abundant evidence implicating the role of oxidative stress and mitochondrial damage in AD and several attempts being made to slow down the progression of disease using putative mitochondrial protectors, no effective treatment has emerged so far. Looking through the preclinical models that mimic most of the aspects of human disease and enable reliable determinations of efficacy of drug in clinical studies, the number of such models tends to be very scanty [6]. Also the relevance of results from animal models to human disease is somewhat uncertain. None of the AD models till date has been proven complete faithful reproduction of human disease, indicating a lack of good AD model involving application of best principles of designing of clinical trials [144,145]. In the clinical trials, drugs targeting mitochondrial dysfunction based pathological changes, no severe side effects and adverse events are reported. Also, they do not worsen the existing conditions of patients, which validates their use in further clinical trials. As of now, most of the listed trials are either pilot studies that only establish the safety of the drug or have a limited number of participants that make it hard to evaluate the true potential of drug candidates in managing AD. Moreover, till date, most of the underway trials include patients that already exhibit clinical syptoms, which can turn the preventive measures ineffective in many cases. The future trials can incorporate individuals with a high risk of onset of disease so that the carefully planned early treatment can prevent the onset on the first hand more effectively. The heterogeneity of patient populations and modifier genes arising due to interaction of genetic, epigenetic, environmental factors and varied pathways have the potential to modulate the results of trials [6]. Therefore, mitochondrial cascade hypothesis being a potential therapeutic target is not researched and explored to its fullest. Further research into the mitochondrial pathways are needed for more clarity for designing effective drugs. Though several recent interventions have begun to uncover novel biomarkers of the disease, and the emergence of concepts of personalized medicine, genetic engineering, pluripotent stem cells culture has the potential to transform the trials [146-148]. As of now, the development of treatments targeting mitochondria are still in infancy and a major breakthrough has remained elusive, but the coming future carries hopes for discovery of various unresolved areas. Also, some clinical interventions are still under investigation, hopefully, they will have a positive impact in reducing the pace of advancement of this deadly disease in the near future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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