STARRING ROLE OF MITOCHONDRIAL IMPAIRMENT IN ALZHEIMER’S DISEASE NEUROPATHOLOGY

Ayesha Yousaf¹, Farah Deeba¹*, Sidra Younis², Nadia Iqbal¹, Farheen Aslam¹

¹Department of Biochemistry and Biotechnology, Women University Multan, Pakistan.
²Department of Biosciences, National University of Medical Sciences, Rawalpindi

Corresponding Author Email: farah.9003@wum.edu.pk

Abstract

Alzheimer’s disease is the leading predominant demyelinating and degenerative ailment, described by the loss of cognitive function because of advanced neuronal loss in the brain. There are two pathological hallmarks in the brains of AD sufferers. One is the buildup of abnormal Tau proteins in neurons and the other is the formation of amyloid plaques. Although we still don’t know the comprehensive mechanism that is involved in AD pathophysiology; there are enormous studies that suggested that malfunctioning of mitochondria plays a substantial function in the pathology of AD. As we know that mitochondria are very dynamic organelles and the powerhouse of the cell (generate ATP). And it is said that a healthy pool of mitochondria is very essential because it provides energy to the neurons to perform the most important functions and it also protects the neurons by reducing the oxidative damage related to mitochondria. These organelles also play many important cellular functions such as regulation of intracellular calcium ions, bioenergetics processes, a scavenging system for free radicals, and stimulation of cell death that is mediated by caspases. But these functions can be adversely affected by amyloid beta-mediated mitochondrial dysfunction. In this article, I recapitulated the current advancement that highlights the starring role of mitochondrial impairment in AD pathology and summarize how different types of mechanisms are involved in mitochondrial impairment in the pathophysiology of AD.

Keywords: Alzheimer’s disease, Bioenergetics, mitochondrial dynamics, mitochondrial biogenesis.
INTRODUCTION

Alzheimer’s disease is the most predominant degenerative and demyelinating ailment globally (Feigin et al., 2016). It is described by forfeiture of retention and intellectual function. Two neurodegenerative processes involved in the neuropathology of AD first process is amyloidogenesis (formation of amyloid beta peptide) in this deposition of amyloid $\beta$-peptide ($A\beta$) occurs and the second process is the development of phosphorylated tangles of Tau protein intracellularly. Cell death and neuronal dysfunction occur due to the presence of these abnormal structures. Several pieces of evidence suggest that mitochondria show a vital role in neurodegenerative disorders, also in AD and the mitochondrial cascade hypothesis proposed that the main result in the pathology of AD is mitochondrial impairment (Swerdlow et al., 2010). Mitochondria are cellular organelles that play an important function in the bioenergetics processes and also play role in maintaining different cellular processes like calcium ion homeostasis, free radicals generation, and in the metabolism of amino acid, lipids, and steroids and apoptosis triggering. The brains require highest energy to perform their different cellular processes and in the synapses the number of mitochondria are elevated and mitochondrial damage might be severe concern intended for the endurance of neurons and it may lead to neuronal death which an alarming sign of AD (Tillement et al., 2011). Some evidences show the relationship between amyloid $\beta$-peptide ($A\beta$) accumulation in mitochondria and mitochondria mediated toxicity. Various observations shows that impairment in the energy metabolism is an early sign of clinical emergence of AD therefore mitochondrial impairment is considered as a predominant characteristic of AD (Swerdlow, 2018). And in this article we will review the different evidences that show the mitochondrial dysfunction and different mechanism underlying the mitochondrial impairment in the brain of AD subjects which help in offering novel therapeutic targets for the future and discuss in which manner the functionality of mitochondria is affected by the presence of amyloid beta protein.

Neuronal cell’s mitochondria

Human brains require great metabolic energy for the aforementioned opposite work. We know that neuronal cells have some degree of glycolytic ability therefore these cells are reliant on the energy produced by mitochondria. Neuronal cells have a specific structure and shape and extend their dendrites and axons from millimeters to meters. Although mitochondria are present along the entire length of neurons in some regions their presence is very high like in synapses, because in this region there is a high demand for energy consumption and ATP production. When synapsis releases the transmitter then ion channels in presynaptic membranes are opened permitting the inflow of ions and this propelling of ions requires great energy.
Mitochondrial ATP is also used up for axonal transport and for the action potential for restoring ion gradients. Different imaging studies were established, and these studies indicated that approximately in 1 second 4.7 billion ATP molecules were expended by a cortical neuron (Zhu et al., 2012). Mitochondrial trafficking of neuronal cells is very necessary for the survival of neurons because in neuronal regions correct distribution of mitochondria is very important for ATP and calcium ion homeostasis. Different imaging and biochemical studies were also performed which established that mitochondrial-based proteins dynein and kinesin and two other mitochondrial-based proteins such as Milton and miro is forms the adaptor/motor complex that helps in the mitochondrial translocation (Schwarz, 2013). Mitochondria is also very important for controlling the survival and death of cells by different apoptotic pathways and it also contributes to different cellular functions like maintaining calcium ion homeostasis, cell cycle regulation, maintaining the plasticity of synapsis, and maintaining cell redox potential besides of its role in energy metabolism. Mitochondria also play fundamental function in maintaining the polarity of neurons by dropping the calcium ion concentration near the presumptive axon base of neurons by promoting the differentiation and rapid growth of neurons and also by promoting the rapid polymerization of microtubules and all these processes are escorted by increasing the number of mitochondria/cell. These observations were achieved by different studies in which treatment of different chemical substances was done like chloramphenicol and oligomycin. Chloramphenicol treatment prevented the differentiation of the cells, chloramphenicol is the inhibitor of mitochondrial protein synthesis, while oligomycin did not prevent the differentiation of the cells, oligomycin is the inhibitor of mitochondrial ATP synthase. All these observations suggested that ATP production is not required for the differentiation of neuronal cells instead of it increased mitochondrial mass is mandatory for it (Vayssière et al., 1992).

**APP metabolism (amyloid precursor protein) and Aβ production**

AD is a widespread disease in which cognitive impairment and dementia occur and it is instigated by the buildup of Aβ protein, which is due to the overproduction of amyloid beta or impairment in the clearance mechanisms. A hefty originator protein known as APP (amyloid precursor protein) is cleaved sequentially and generates Aβ. APP is an integral plasma membrane protein with only one membrane traversing helix and its amino-terminus is large extracellular and glycosylated while its carboxyl-terminus is shorter and it is in the cytoplasm. There are several different isoforms of APP but the most common isoform is (APP695) which is located in the brain and this isoform is produced primarily in neurons (Mattson, 1997).

There are two pathways of APP processing, one is the amyloidogenic pathway and another one is the non-amyloidogenic pathway (Figure 1). The non-amyloidogenic pathway is initiated by alpha secretases, alpha
secretases cut APP within the Aβ domain and in this pathway, there is no generation of amyloid beta because APP is cut inside the Aβ domain and alpha-secretase averts the discharge of amyloid beta. While in the amyloidogenic pathway beta-secretase (BACE1) is involved and initiates the amyloidogenic pathway and it helps in the generation of soluble beta-secreted APP amyloid beta (sAPPβ) and another membrane-bound fragment CTFβ is also generated. These two fragments act as a substrate for gamma-secretase, gamma-secretase is a multisubunit protease complex and it comprises two proteins PS1 (Presenilin 1) and PS2 (Presenilin). Then further processing of CTFβ is done and this processing release the amyloid beta fragments which are amyloidogenic. Because gamma-secretase cleaves the CTFβ exactly in the middle and this suggests the hypothesis that the generation of different amyloid-beta is directly related to the properties of the membrane. There are different forms of amyloid beta species such as Aβ38, Aβ40 and Aβ42. The lipophilic properties of each amyloid beta are different and their propensities to produce oligomers and masses are also very different. Especially, Aβ40/Aβ42 ratio is of medical importance in AD (Choy et al., 2012). In AD, amyloid beta monomers that are produced during the amyloidogenic pathway are aggregated to form oligomers and these oligomers are then self-aggregated into oligomers of varying sizes to form amyloid beta plaques. These oligomers and amyloid beta plaques are the strong toxins of synapsis and inhibit the proteasome function and mitochondrial activity, stimulate different inflammatory processes and change the levels of intracellular calcium ions (Walls et al., 2012).

**Figure 1:** Amyloidogenic and non-amyloidogenic pathways are initiated by dissimilar types of the dispensation of APP: sAPPα and CTF-α fragments are produced by the splicing of both α- and γ-secretase while the sAPPβ and Aβ fragments and the intracellular AICD fragment were produced by cleavage with β-secretase in different APP site. Fibrillar aggregates are formed through a misfolding step of Aβ.
Mitochondrial amyloid beta

Various studies have shown that amyloid beta and its extracellular established segmentation have been floated in diverse intracellular compartments like in the endoplasmic reticulum (ER), Golgi apparatus, or in the trans-Golgi network and in recycling lysosomes. Amyloid beta has also been found in the mitochondria and various studies have also proved that amyloid beta builds up in the mitochondria of both human and mouse models of AD. However, it remains unclear whether the amyloid beta is generated in situ or is imported from outside. Various observations from both human and mouse models of AD indicated that amyloid beta is not produced in situ, it is imported from outside. And the following postulation is held by the statement that the capability of gamma-secretase to splice the APP is unknown. Therefore, it is thought that amyloid beta is obtained from the extracellular or intracellular pool of amyloid beta, and for the internalization of amyloid beta into mitochondria a cellular trafficking process is involved. Some recent findings in which secluded mitochondria of rat is used have shown that for the intake or import of amyloid beta into mitochondria a special type of uptake mechanism is used in which translocase enzyme at the base of the outer membrane (TOM complex) is present that help in the import of amyloid beta into mitochondria. Another experiment was performed in which confocal microscopy was performed and the results show that the Aβ42 co-localizes with the second complex of the respiratory chain, outer mitochondrial membrane, and chaperon proteins Hsp60 of mitochondrial matrix (Gordon et al., 2018).

Relationship between impaired energy metabolism and mitochondrial impairment in AD

Impaired energy metabolism is closely associated with mitochondrial impairment because mitochondria is the main cell’s powerhouse. Compromised metabolism of energy is a unique initial and utmost remarkable feature of AD. The brain of humans encompasses 2% of the overall weight of the body and in a resting awake state human brain consumes about 25% of the overall glucose of the body and about 20% of the overall body oxygen. Because the brain is one of the highly energy-utilizing structures, even slight fluctuations in the metabolism of energy severely disturb nervous function. During normal circumstances, the human brain consumes glucose as the main energy substrate, and to evaluate the energy metabolism in the brain utilization of glucose is measured. A large number of results show that utilization of glucose is drastically reduced in AD and it is considered the initial characteristic of AD and this usually occurs a decade ago earlier than the beginning of the disease (Weise et al., 2018).

Use of FDG-PET for the measurement of energy metabolism in AD brain
FDG-PET (fluoro-2-deoxyglucose positron-emission tomography) is used for the evaluation of glucose utilization. When using FDG-PET it has been seen that glucose consumption was steadily decreased in the brain (cortex and hippocampus) of AD subjects as matched to the healthy people. In the initial stages of AD, the cingulate cortex is the most metabolically affected area among all of the other brain areas. Decreased glucose utilization or glucose hypo metabolism was also detected in the MCI subjects it is an introductory phase of AD and it is also proposed that it has also very imperative function in the initial stages of AD. A longitudinal study of 84 months was performed and this study demonstrated that glucose metabolism was decreased in ApoE4-associated brain region in perspective of MCI. The result of longitudinal study was also supported by the results of which experiment on young adults performed, the results showed that there were peculiarly low proportions of the utilization of glucose in susceptible brain areas of young individuals that have apoE4 allele during 20s, more than a few years ago earlier the probable emergence of disease. There is a correlation between symptom severity and extent and topography of glucose hypo metabolism and in AD it also imitated the local dissemination of condensed synaptic density and synaptic activity (Altmann et al., 2013).

**Energy metabolism in autosomal dominant and sporadic AD**

Different multimodal imaging studies were performed and different biomarkers were used in that study like FDG-PET and amyloid PET and the results of these studies showed that there was a direct correlation between glucose metabolism and amyloid beta plaque formation. Longitudinal amyloid beta plaque depositions in the autosomal dominant mutations of AD were increased in almost all cortical regions before 20-25 years of the expected age of the beginning of AD and glucose metabolism was reduced in the cortical region after 5-10 years (and this showed that amyloid beta plaques were the first biomarker of AD) and all these results suggested that reduced glucose utilization could be the second biomarker and amyloid beta depositions were the primary biomarkers in the pathophysiology of Alzheimer’s disease.

Though there is a direct relation between reductions in the regional glucose metabolism and overall amyloid neuropathology, there is no strong association between local Aβ pathology and regional hypometabolism of glucose while a comparison of both is done side-by-side. The destructive relationship between Aβ plaque deposition and glucose metabolism was shown by only one out of 404 regions of interest (Jagust et al., 2012). The following study showed that although glucose hypo metabolism was a subordinate incident in the cases of autosomal dominant AD it plays a very crucial role in the subsequent emergence of Alzheimer’s disease.
In contrast, energy hypo metabolism also plays a very crucial role as a prime incident in patients with sporadic AD. Another study was performed on a population of sporadic AD subjects. In this study majority of the individuals (subjects) approximately 60% cases of Aβ positive subjects followed the amyloid first biomarker profile pathway and 27 % cases followed the abnormal FDG-PET that suggested that the energy hypo metabolism was the first biomarker in some preclinical AD. Some FDG-PET studies have also identified that in carriers of apoE4 there was no amyloid plaque dismissal but there are chances that these carriers might develop amyloid pathology in upcoming years because they also followed the hypo metabolism first profile pathway (Szablewski, 2017). It is probable that in the above-discussed cases other etiologies like Tau protein or TDP43 and dysmetabolism of amyloid beta before the formation of amyloid plaques may underline energy hypo metabolism. Energy hypo metabolism first biomarker profile pathway in presymptomatic AD might have a primary function in the pathophysiology of subsets of patients of sporadic AD.

There are different steps convoluted in glucose metabolism like intracellular metabolism of glucose and transportation of glucose because it is a multi-step process. In the brain of AD victims, the abnormalities in these processes were identified like abnormalities in the transportation of glucose such as insulin resistance, abnormal blood flow or glucose transporter (Sang et al., 2018) and abnormalities in the intracellular glucose metabolism such as aberrations in the different cytosolic processes (for example pentose phosphate pathway and glycolysis), abnormalities in mitochondria-dependent processes also occur like abnormalities in TCA cycle and OXPHOS (oxidative phosphorylation) all these abnormalities lead to the glucose hypometabolism. It is not very difficult to identify which factor play more vital functions than other in triggering glucose hypometabolism in AD, however, potentially it’s very difficult to detect the pathfinder that has given intricate relations between these factors and there may be variances in key factor convoluted in different variants of AD.

However, glucose hypometabolism is generally taken as an impairment in energy metabolism via oxidative phosphorylation that strongly showed the relationship of impairment in mitochondria in causing AD during the early phase of the disease. There was a correlation between glucose hypo metabolism in parietal, frontal, and temporal cortices and reduced levels of different enzymes and coenzymes there was a reduction in the Thiamine diphosphate (TDP) levels it is a very important PDH complex (PDHC) coenzyme and alpha-ketoglutarate dehydrogenase complex (KGDHC) in TCA cycle and transketolase in HMP shunt (Pan et al., 2016). Declines in TDP and TDP-dependent enzyme activities are also common and remarkable features of AD victims like glucose hypo metabolism (Lajoie et al., 2017). PET (positron emission tomography) is most
commonly used to measure oxygen metabolism, an additional primary measure is also used for the measurement of energy metabolism it is the detection of Oxygen-15, and this measure directly provides a signal for mitochondrial function through ETC in the brain of AD subjects. There is an association between the extremity of impaired thinking and decrease in the cerebral metabolic oxygen rate, because cerebral metabolic oxygen rate was meaningfully reduced in the parietal, frontal, and temporal cortex in AD subjects (Mastroeni et al., 2017). An important correlation was also found by another type of study between diminished oxygen metabolism and EEG that show parietal-temporal occipital areas of the brains of AD subjects. All these types of studies demonstrated that there were defects in the bioenergetics machinery particularly in TCA and ETC, in mitochondria of AD patients’ brain. All these studies showed that mitochondrial impairment play very important function in the pathophysiology of AD and in the glucose hypometabolism and impairment in energy metabolism in AD.

**Discrepancies of mitochondria in AD**

**Disturbed mitochondrial bioenergetics**

Gene expression studies were performed and the results of these studies identified that there were defects in metabolic pathways that were directly related to mitochondria in AD, and the defects in these metabolic pathways confirm impaired mitochondrial bioenergetics machinery in the brain of AD. Genome-wide transcriptomic studies were performed in laser-capture micro-dissected neurons and the results showed that there was a larger proportion of down-regulated nuclear genes that encode the subunits of the electron transport chain of mitochondria in the posterior cingulate cortex than in the visual cortex, it is the section that is metabolically safe in control versus AD (Du et al., 2017). Impairment in ETC was disturbed among the two protein complexes of ETC such as in complex 1 and complex IV. Quantitative RT-PCR and microarray analysis studies were performed and the results of these studies showed that in AD victims there was downregulation in the genes of 15 members of the TCA cycle out of 51 members, glycolysis, OXPHOS, and other associated pathways. Further microarray data has shown that nuclear-encoded OXPHOS genes were downregulated in the brain of AD but there was no downregulation in the mitochondria-encoded OXPHOS genes (Sorrentino et al., 2017). In the early and final AD brain specimen, there was downregulation in complex I of oxidative phosphorylation while there was an increase in the expression of mRNA of complexes III and IV. OXPHOS pathway was considered the most significant pathway that was involved in AD and this was confirmed by bioinformatics analysis in which 4 datasets of hippocampus transcriptome of AD subjects were used. Mitochondrial import pathways disruption and downregulation of mitochondrial OXPHOS
pathways were two major hallmarks of AD according to the gene set enrichment analysis (Minjarez et al., 2016). Proteins of OXPHOS pathways were also downregulated in the cortex of AD subjects and it was proved by protein and proteome expression analysis (Adav et al., 2019). There was a difference in the altered mitochondrion in AD brain and age-related changes that suggested that dysregulation in the mitochondrial complexes were the major causes of AD pathology and these were identified by using different quantitative proteomics approaches (Woodling et al., 2016). Various complexes of OXPHOS were immune cytochemically stained and showed that there was decreased immune cytochemical staining in complex I and IV in numerous brain parts in AD. Posttranslational modifications were required for the regulation of the activities of some enzymes and it would be very important to determine the enzymatic changes in the brain of AD subjects. The activities of the enzymes of TCA cycle were changed in AD the activities of some enzymes become decreased while activities of some enzymes become decreased like activities of dehydrogenase/ decarboxylases were decreased [such as PDHC, ICDH, and KGDHC] while the activities of dehydrogenases [like SDH and MDH] were increased and all these changes were directly related with the clinical condition of AD. Different biochemical studies of enzyme activities showed that there was reduction in the activities of all the complexes of OXPHOS but reduction in the COX (cytochrome oxidase) activity was most significant of all reduction. Some of the studies reported the defect in the COX activity was more specific (Beck et al., 2016). COX activity was measured in the cingulate cortex that was metabolically more affected and in the metabolically least pretentious primary motor cortex and it was seen that Cox activity was reduced in the cingulate cortex than in the primary motor cortex and this COX activity was measured by histochemical quantification. Further studies indicated that the activity of mitochondrial ATP synthase was diminished in the AD victims because of the absence of oligomycin sensitive subunit of protein (Cha et al., 2015) or due to alteration in the O-GlcNAcylation of the alpha subunit of ATP synthase (Picone et al., 2013).

Impairment of mitochondria and increased oxidative stress

Mitochondria have a crucial function in cellular energy metabolism and it is also involved in the different metabolisms that are significant features of neurodegenerative processes like in the lipid, steroid, and amino acid metabolism, changes in intracellular calcium ion levels, regulation of apoptosis and free radical production. Although, a lot of research is going on to define the link between mitochondrial dysfunction and the pathophysiology of neurodegenerative diseases the exact mechanism is still not known. Nowadays, in neuroscience research, the most interesting topic is that the dysfunction of mitochondria plays a crucial role in neurodegenerative diseases. The direct cause of mitochondrial impairment is the rise in the production of
ROS that causes damage to DNA, RNA, protein, and lipids oxidatively. ROS are obvious byproducts in ETC and these are produced during aerobic respiration in mitochondria due to leakage of electrons at complexes I and III of ETC it is expected that more than 90% of ROS were produced by mitochondria. Mitochondria are important cellular organelles that consume oxygen and contain several redox enzymes that transfer only one electron to oxygen molecules and generate superoxide molecules. Moreover, mitochondria have an antioxidant defense system that helps in the detoxification of reactive oxygen species that are generated. Due to damage to mitochondria, the antioxidant defense system does not work properly and its defense mechanism decreases that further increasing the ROS production damage to mitochondria also increased causing the free radicals generation and a decrease or loss in the antioxidant capacity. ROS acts as a “redox messenger” and plays a crucial role in regulating intracellular signaling under normal physiological conditions. While cell death occurs, when there is an imbalance in the levels of ROS because an imbalance in ROS may lead to irreversible damage to the different components of the cell. Mitochondrial ROS can be originated from the different reactions of the Krebs cycle and ETC. In vitro studies showed that there was a link between elevated amyloid beta levels, oxidative stress, and mitochondrial dysfunction and all of these factors were convoluted in the etiology of AD. Furthermore, several studies also reported that amyloid beta is a very potent factor in oxidative stress, generation of free radicals plus mitochondrial dysfunction because it contributes to activating the cascade of events that lead to demyelination and degeneration in neuroblastoma LAN5 cells and seaurchin system (Dragicevic et al., 2010). When we were using use models of AD, then the results showed that the presence of amyloid beta in mitochondria adversely affects various functions such as it affects ROS production rates, and respiratory function of mitochondria as well it alters the membrane potential in different parts of the brain. The brain regions that have a major role in memory retention such as the cortex and hippocampus have been found that the high levels of amyloid beta and therefore mitochondrial dysfunction was also high. While lower levels of amyloid beta were found in the amygdala and striatum. Remarkable evidence was also found for the association between mitochondrial dysfunction and loss in cognitive function in AβPP and AβPP/PS1 mice and this was one of the first evidence for the link between mitochondrial and cognitive impairment in transgenic mice model of AD (Liou et al., 2003). Oxidative stress is also involved in activating the different signaling pathways that help in altering the APP or Tau processing. For instance, in the presence of oxidative stress the expression of beta-secretase increases by the stimulation of p-38 mitogen-activated protein kinase (MAPK) and c-Jun amino-terminal kinase, and phosphorylation of Tau protein increases by the stimulation of glycogen synthase kinase 3-β (GSK3-β). Some specific types of molecules were inactivated by the oxidant. For example, PINI (prolyl isomerase) was downregulated in patients with
AD because PINI is extremely sensitive to oxidative stress. Because of oxidative damage to PINI, isomerase activity was a loss that is responsible for the creation of neurofibrillary tangles. Further studies have shown that PINI also catalyzes the conformational changes in the protein that disturbs the Tau and APP processing. When PINI was knockout in transgenic mice then it increased the intracellular amyloid beta levels and amyloidogenic APP processing. It has also been seen that in PINI-knockout mice Tau hyperphosphorylation, neuronal degeneration, and behavioral and motor discrepancies occur (Swomley et al., 2015).

Different redox proteomics studies were performed, these studies showed that in AD many enzymes that were convoluted in the antioxidant protection system were oxidized due to which the function of these enzymes become compromised and oxidative stress was increased. For instance, in the different brain parts of MCI and AD subjects the enzyme Glutathione-S-transferase Mu Peroxidin 6, GSH, and MDR protein 1 or 3 were seen to be nitrosylated or HNE-modified. This result showed that in the brains of MCI and AD subjects the proteins of different energy metabolic processes post-translational modification were found either in the form of lipid conjugation or by the reactions of these to reactive oxygen species. Such as the ATP synthase, the last enzyme that was involved in ATP production could be nitrated or HNE-modified in the hippocampus of AD and MCI patients. These modifications like carbonylation and HNE-modification were found in the creatine kinase in the ATP maintenance aconitase enzyme of the TCA cycle (Garone et al., 2018). All the above data showed that oxidative stress was a major contributor to disturbed metabolism of energy and mitochondrial impairment in AD.

**Disturbance in the mitochondrial genome homeostasis in AD**

Mitochondria have their specific genome, this DNA is well-known as mtDNA. This genome is multicopy means that 1-10 replicas of mtDNA existent in only 1 mitochondrion and around 13 mitochondrial proteins of the complexes of ETC and 22 tRNAs and 2 rRNA that are essential for the translation of proteins are encoded by mtDNA (Inczedy-Farkas et al., 2014). mtDNA is very important for the appropriate working of the mitochondria, and mtDNA is also susceptible to oxidative damage because it is near the site of the generation of ROS. It also gives rise to mutations because of the absence of protective histones and the lack of a proper repair mechanism. When mutations occur in mtDNA, which are either inherited or somatic variations these mutations are propagated by clonal expansion and deleterious effects occur when these mutations cross a certain threshold value and then affect the function of mitochondria and in result disease or cell death occurs.
Cognitive impairments that were similar to AD were seen in the patients who have only primary mutations in the mtDNA (Salvadó et al., 2019) which showed that mtDNA plays a critical role in good cognitive function. It is reported in various studies that motherly spread is more significant and common than the fatherly spread in the intimate history of dementia which is a more common cause of AD. It has also been seen that enlarged atrophy in specific brain regions that were susceptible to AD in the maternal family history of AD. In healthy people that have a normal cognitive function, a pattern of increased white matter hypersensitivity load in occipital and temporal hemispheres (Chen et al., 2016) and decreased glucose metabolism, pointed towards the family of origin effects. Maternal history of mtDNA showed that mtDNA plays a significant role in the inherited mtDNA variability of AD. No primary mutations in mtDNA play a direct role in causing AD. Additional studies have brought into being that germline modifications (i.e. haplogroups) and mtDNA SNPs have vital functions in AD (Table 1) such as haplogroup T being protective while the greater threat of AD was seen in haplogroup UK.

Susceptibility to AD may be changed by the interface between mtDNA congenital inconsistency and further type of factors like sex and apoE4 allele: for example, it has been seen that harmful effects of apoE4 allele are neutralized by the mtDNA haplogroups K and U; haplogroup U is concomitant with a greater possibility of developing the disease in males and the diminished possibility of developing the disease in females.

It has also been seen that somatic DNA mutations also have a role in AD, the most common somatic mtDNA mutation that was studied is 5 kb deletion (mtDNA Δ4977), and this mutation occurs between 8470-8482 and 13447-13459 positions and these mutations usually affects the different complexes of ETC especially complex I, III and V in Alzheimer’s disease. In the frontal cortex, the age-associated increase of this deletion was found in past qPCR studies. And in patients of AD that were aged less than 75 years a conspicuous 15 fold increase of this deletion was seen. In brains of AD, in addition to these deletion mutations, the most significant mtDNA rearrangement mutations were R-type and F-type rearrangements (Pienaar et al., 2017). Normally 63% rise in heteroplasmic mtDNA point mutations was seen in the control regions (CR) of AD brains, while definite AD brains protected the ailment-specific control regions point mutations up to 70-80% levels of heteroplasmy, all these events suppressed the transcription and translation of mtDNA and also altered the regulatory elements of mtDNA. But some conflicting results were also obtained because some groups don’t show any fluctuations in the accumulation of mtDNA point mutations between AD brains and control brains (Strobel et al., 2019), and this was probably due to the sample size is small or the use of different types of approaches. Variability may also be due to the absence of cell-specific mutations because recent studies by laser capture
microdissection and by in situ hybridization in single hippocampus glial cells or neurons by using multiplex qPCR method (Hoekstra et al., 2016) showed that neuronal occurrence of mtDNA Δ4977 was increased than glial occurrence in AD. Another study showed the increased ratio of mtDNA Δ4977 as compared to COX-negative neurons that were normal and selectively supplemented in AD. The particular phase of disease also contributed to mtDNA mutations. More recent studies were performed by using next generation sequencing method that eliminates the errors of PCR and other types of methods, showed remarkably increased levels of mtDNA point mutations in the hippocampus of initial phase AD victims (the individuals who have not intellectual dysfunction or dementia), but not in the confirmed patients of dementia (Stocco et al., 2017). This study showed not only the function of mtDNA mutations in early stage of AD but also showed that altered mtDNA mutations vanished as the disease advances because of the death of neurons.

Normally, it was thought that mtDNA mutations were increased due to an increase in oxidative stress that was seen in the AD subject's brains. The levels of oxidized bases were higher 10-fold in mtDNA as compared to the nuclear DNA and mtDNA undergoes 3 fold increase when oxidative stress occurs in the brains of patients of AD as matched to controls of the same age. However, in mtDNA, the high levels of oxidized nucleic acids were found to be notably increased in MCI and PCAD. Recent studies showed a decrease in base-excision repair (BER) and OGGI activity in both MCI, as well as AD subjects, suggesting that amplified mtDNA mutations were due to replication error transcription and function of mtDNA is also affected by other types of modifications. For instance, in the D-loop region of mtDNA elevated levels of 5-methylcytosine were found in the brain samples of AD, in contrast, decreased levels of methylation in the D-loop region were found in the LOAD patients (Corral-Debrinski et al., 1994).

In general, all these studies showed that in the pathophysiology of AD mtDNA mutations, mtDNA variability and modifications in mtDNA play a crucial role. The mitochondrial cascade hypothesis proposed that inherited mitochondrial variants determine the susceptibility of the person and accretion of somatic mtDNA mutations in the brain that reflected how the environment and aging influenced the expression of phenotype. Additionally, it has also been seen that mtDNA mutations were not only specific to AD but mtDNA mutations were also found in other types of neurodegenerative disorders and further research is needed to describe how mtDNA specifically related to AD-type changes.
Table 1: Changes in mtDNA in Alzheimer’s disease

<table>
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Figure 2: Vital function of mitochondrial impairment in AD. Mitochondrial impairment performs a crucial function in AD alternatively as a main or minor event. In either event, augmented oxidative stress, the distressed genome of mitochondria, and diminished mitochondrial bioenergetics.
Different mechanisms involved in mitochondrial dysfunction in AD

There is extensive evidence along with the hypo metabolism-first biomarker profile pathway to preclinical AD that demonstrated that mitochondrial abnormalities could play a key role in the small subdivision of intermittent AD sufferers. In the autosome dominant AD mutations carriers, the hypo metabolism-first biomarker profile pathway could play the prime part in causing AD, whereas impaired energy metabolism could play a secondary or invariant role before the medical inception of the disease and these findings suggested that mitochondrial dysfunction. Irrespective of whether impairment in mitochondria plays a prime or inferior role in the pathophysiology of Alzheimer’s disease, there are some conspicuous manifestations of mitochondrial idiosyncrasies in AD such as increased oxidative stress, impaired mitochondrial bioenergetics plus distressed mitochondrial genome all of these idiosyncrasies cooperate to show the harmful effects of AD. However the comparative effects of these mitochondrial abnormalities may vary among different AD victims and it depends on the individual’s unique environmental, biological and genetic factors any one of these abnormalities further induces two or more abnormalities to complete a downward spiral in amplifying neurodegeneration and neuronal dysfunction (Figure 2). As we know that abnormalities in mitochondria play an important function in AD pathology, different mechanisms have been studied that was convoluted in the mitochondrial and neuronal impairment in AD and these mechanisms also generated new intuitions that offer new targets for future therapeutic development.

Impairment in the biogenesis of mitochondria in AD

For maintaining the good integrity of mitochondria in eukaryotes, biogenesis of new mitochondria is requires during its entire life cycle. When any damage to the mitochondrial proteins occurs then mitochondrial biogenesis could have the potential to rapidly react to these fluctuations due to impairment of mitochondria or due to environmental stimuli. Approximately, there are approximately greater than 1000 proteins that are present in the mitochondria of neurons, the mitochondrial genome encodes a total of thirteen proteins while the remaining proteins are encoded by the nuclear genome. For that reason, for the synchronized expression between the nuclear and mitochondrial genome, mitochondrial biogenesis is involved. The major controller of mitochondrial biogenesis is PGC-1 α and it also regulates respiration and energy metabolism with the help of a special type of transcription factors for example nuclear respiratory factor 1 (NRF 1) and nuclear respiratory factor 2 (NRF 2). The expression of many nuclear-encoded mitochondrial proteins was also controlled by these NRFs, it also
controls the expression of TFAM that is responsible for the replication and transcription of mtDNA. It has been also seen that expression levels of NRF 1 and NRF 2, PGC-1α and TFAM were reduced in both AD subjects and animal models like in the APPswe M17 cells and the above result suggested that mitochondrial biogenesis was drastically affected in the neurodegenerative disorders. Furthermore, it has been also thought that impairment in mitochondrial biogenesis might contribute to the impairment of mitochondria in AD (Sheng et al., 2012).

**Impairment in the mitochondrial dynamics in AD**

We know that mitochondria are active organelles because they can move all over the cell and constantly divide and fuse. Moreover, recent evidence showed that these mitochondrial dynamics become abnormal in neurodegenerative disorders, especially in AD. Impaired dynamics of mitochondria means that fission and fusion reactions become altered for example augmented fission and diminished fusion. These abnormal mitochondrial dynamics processes are mostly shown in the axons and dendrites as in all the cells and permit the interchange of constituents among the mitochondria. In non-neuronal cells when small contact occurs then it results in the exchange of proteins between different compartments of mitochondria. Further, it has been seen that impaired mitochondrial dynamics could result in a change in mitochondrial structure and these changes in the structure of mitochondria are rare. In AD abnormal fission and fusion reactions in mitochondria were reported. In the following study main aim of the authors was to conclude whether amyloid beta and APP could cause neuronal dysfunction or mitochondrial dysfunction over the inflection in the mitochondrial dynamics. Then confocal and electron microscopy analyses were performed and their results showed that if APP was overexpressed then it caused the fragmentation of mitochondria in neuronal cells. As we know, the shape of mitochondria is firmly organized by the equilibrium between fission and fusion between mitochondria and therefore it was theorized that mitochondrial fragmentation in neurons that were induced by APP could be the result of decreased fusion and increased fission. Moreover, recent studies that Drp1 which is also recognized as DLP1 plays a vital function in abnormal mitochondrial dynamics like in fission or fusion. Drp1 is a protein that has the most important role in maintaining and remodeling the mitochondria of mammals. Drp1 first of all interact with the amyloid beta and then phosphorylation of Tau protein occurs that resulting in the extreme fragmentation of mitochondria. The result of this is diminished axonal transport and trafficking of mitochondria and neurodegeneration and failure in cognitive function and behavioral...
deficits (Manczak and Reddy, 2012). Since we know that mitochondrial dynamics were changed in the neurons of AD subjects, major fluctuations in the expression of the protein that was involved in the fission and fusion reaction were also observed. And it has been also reported that the expression of all the fission proteins was increased like an expression of Fis1 while the expression of all the fusion proteins become decreased such as the expression of OPA1, Mfn1, and Mfn2 was reduced. These studies showed that abnormal mitochondrial fission might be used as a possible target for treating AD and more consideration was given to the specific dynamin-like protein (DLP1) because it gives more proof for the crucial function of abnormal mitochondrial dynamics during AD. In both the in vitro and in vivo models of AD it has been seen that mitochondrial fission could be inhibited chemically by the use of DLP1 specific inhibitor Mdivi-1 and by the decline of DLP-1 proteins genetically (Manczak et al., 2011).

Abnormal interaction between endoplasmic reticulum and mitochondria in AD

Mitochondria and endoplasmic reticulum are both constant tube-like, hollow connections of membranes that are present in the cytosol. Both of these organelles perform the synergistic function because of the presence of connection points between mitochondria and ER (Csordás et al., 2018). ER-mitochondria contact sites have very critical physiological functions such as the exchange of calcium between mitochondria and ER, control of phospholipid biosynthesis and its metabolism, tuning of dynamics and autophagy of mitochondria, apoptosis, and inflammasome activation all of these are important for the proper functioning of mitochondria. Recent studies revealed that the ER and mitochondria connection points have a crucial role in the function of a neuron and its survival. And MAM signaling was seen disturbed in neurodegenerative disorders including AD. Likewise, alteration in the mitochondria-associated ER membrane (MAM) role might also be the reason for mitochondrial impairment. MAM is a sub-compartment of the endoplasmic reticulum and it has a structure similar to a lipid raft that is convoluted in the phospholipid metabolism, cholesterol metabolism, mitochondrial function and dynamics, bioenergetics, cell signaling, and calcium ion homeostasis (van Vliet et al., 2014). MAM is biochemically, tangibly, and reversibly connected to the mitochondria. This connection point help provides a platform for intracellular signaling determining the life and death of a cell. Under stress conditions such as in the case of redox stress, MAM alters its functions and also alters the set of regulatory proteins. In the sub-compartment of ER presenilins and gamma-secretase enzymes are present in very high concentrations and it has been postulated that
changes in these factors either genetic or any biochemical changes that affects the MAM function should be responsible for the progression of AD and increased processing of APP. However, controversy is present between the presenilins effects on the MAM role and assembly, ER-tethering was impaired because tissues of AD victims contain PS1 E280A mutation this result was additionally proved by ex vivo studies (Sepulveda-Falla et al., 2014). The functionality of MAM is measured in terms of cholesterol and phosphatidylserine production was increased in the both model system and in the patients of both sporadic and familial forms of AD. Likewise, increased expression of MAM was also seen in the AD subject’s brains during postmortem.

**Impaired mitophagy in AD**

As we know that mitochondria are very crucial organelles that are active metabolically and in this organelle, approximately 90% of ROS are formed therefore mitochondria also cultivate quality control systems to manage the unescapable harm of ROS to the components of mitochondria or the whole organelle. In normal conditions, mitochondria that are impaired are tarnished by mitophagy. Damaged mitochondria activate the special type of mitophagy pathways that involves a special type of activation and stabilization of PINK1, present in the outer membrane of mitochondria and stimulated by the impaired potential of the mitochondrial membrane that is the major characteristic of impaired or damaged mitochondria (Nguyen et al., 2016). In this mitophagy pathway, ubiquitin is phosphorylated by PINK1 to nourish the Parkin-facilitated ubiquitination of the proteins of the outer mitochondrial membrane and tags the impaired mitochondria for breakdown by mitophagy pathway in addition to the phosphorylation of the E3-ubiquitin ligase and Parkin to mitochondria. It is of great importance that PARKIN and PINK1 are mutated in the early emergence of familial Parkinson’s disease, which is the 2nd widespread demyelinating and degenerative disease after AD (Pickrell and Youle, 2015). That suggested that PINK1 and PARKIN pathways have an important function in CNS. In AD brains mitochondria are the key targets of degradation by the mitophagy pathway and strong evidence showed that lysosome/autophagy are impaired in AD. In both the transgenic models of animals and in human AD individuals, damaged mitochondria were shown accumulated and this was shown by evidence of swollen appearance due to the presence of distorted cristae, and these results were shown by electron microscopy (Martín-Maestro et al., 2016). Activated but stalled mitophagy process was shown in the hippocampus of AD subjects, a transgenic model of APP or cell model that were expressing mutated PS1 or APP and this stalled mitophagy process was due to amplified PINK1 and
Parkin or due augmented mitochondrial membrane proteins ubiquitination that was present in the amassed mitochondria in pyramidal neurons (Fang et al., 2019) and in the result of impaired mitophagy capacity, the capacity of eliminating the great amount of impaired mitochondria loss in the other steps of mitophagy also occurs like in lysosomal degradation (Martín-Maestro et al., 2017), the number of damaged mitochondria was increased and mitochondrial homeostasis also become disturbed. A detailed mechanism of mitophagy in AD is still not known and remains to be worked out. When PINK1 was overexpressed in the transgenic mice model then it bring back the mitochondrial function, abridged the amyloid beta pathology and Aβ production, and also improved the synaptic function and also the intellectual and developmental functions (Du et al., 2017). When treatment of different compounds like actinonin and urolithin A that increase the mitophagy was done in C. elegans model that was overexpressing amyloid beta-42 or tau in PINK1 dependent manner then it has shown that memory function was restored (Fang et al., 2019). Likewise, these compounds also enhanced mitophagy in APP/PS1 mice model, which lead to the improvement of amyloid beta pathology and improvement of developmental and cognitive functions. Remarkably, the Aβ tangles become cleared because of the amplified efficacy of phagocytic cells of microglia who have also diminished mitophagy in APP/PS1 model mice and was restored by treatment with actinonin and urolithin A (Sorrentino et al., 2017).

**Impairment in the mitochondrial proteostasis in AD**

A process known as mitochondrial proteostasis at the protein level of quality control mechanism monitors the damaged protein of mitochondria with the help of a connected network that consists of protease and chaperons in each section of mitochondria: proteases that are dependent on ATP for energy are liable for removing misfolded or damaged mitochondrial proteins while chaperons are responsible for translocation and folding reactions of mitochondria (Martín-Maestro et al., 2016). When these proteins become damaged then the capability of mitochondria to oversee, remove and repair the defective proteins become impaired that end in the accretion of defective proteins in mitochondria and also causes mitochondrial impairment (Deepa et al., 2016).

Other evidence showed that at sub organelle level, impaired mitochondrial substances such as mitochondrial DNA and proteins were accumulated in the AD, as well as mitochondrial proteostasis is also impaired. However, there is a balance between the development of introduced mitochondrial proteins and damaged protein breakdown. Upregulation of mitochondrial proteases in AD was also
seen in one study (Cha et al., 2015), it may be an inadequate protective response. These mitochondrial proteases were upregulated in the patients of MCI and 3XTgAD mice model leads Tau and amyloid beta pathology that suggested that it is an early event during AD (Minjarez et al., 2016). Moreover, it has been also seen that the increased mitochondrial proteostasis reduced the proteotoxicity of the Alzheimer’s disease transgenic model. Current studies concentrated on the two different types of proteases present in the mitochondria that are responsible for APP or amyloid beta processing or metabolism. One of the mitochondrial proteases is peptidosome, PreP and it is positioned in the matrix of mitochondria and it is responsible for the cleavage or maturation of presequence of mitochondrial matrix import proteins (Cha et al., 2015). Falkevall et al. were, first of all, acknowledged the role of PreP in degrading amyloid beta monomers 40 and 42 in the laboratory that showed similar amyloid-beta degradation mechanisms in the mitochondria of AD victims. Depending upon this study, Alikhani et al. also stated that the diminished Prep activity was shown in the transgenic mice model of AD because these hybrid mice have augmented oxidative stress that causes the diminished activity of Prep.

The second most common mitochondrial protease is HtrA2 or Omi in AD, it is a serine protease that is located in the IMS of mitochondria. In AD subjects, particular HtrA2 or Omi protease activity was seen considerably augmented while in the Swedish case-control studies feeble association was found in the HtrA2 A141S and AD. Then yeast two-hybrid assay was performed which showed the interaction of HtrA2 or Omi with amyloid beta with the help of PDZ domain at the carboxyl terminus and this was additionally proved by co-immunoprecipitation assay in HEK392 cells. It was seen that amyloid beta was present in the mitochondrial matrix but there is no unanimity on its localization in different sections of mitochondria. It is probable that amyloid beta also has admittance to the intermembrane space HtrA2 but it is still unknown how the interaction influences the HtrA2 activity. Another study also showed that HtrA2/Omi also performed the function of chaperons and remarkably deferrals the accumulation of Aβ1–42 peptide in vitro independent of the PDZ domain. We know that APP was localized in the mitochondria or also colocalized with the HtrA2. Remarkably, APP that was present in the mitochondria was cleaved by HtrA2 in both in vivo and ex-vivo AD models and released the C161 fragment into the cytosol. It is expected that HtrA2-mediated splicing of APP might relieve the mitochondrial malfunction that was induced by APP accumulation. Yet, it is still unclear what the C161 destiny-released fragment is associated with the amyloidogenic or non-amyloidogenic pathway. There was another most interesting function of HtrA2, HtrA2 cooperates with the presenilin in active gamma-secretase complexes that were positioned in the mitochondria and control the APP splicing.
This type of interaction also has a great impact on the HtrA2 protease activity because we know that PS1 C-terminus is a dynamic ligand aimed at HtrA2 PDZ domain and induced the cell death that was dependent on HtrA2 (Gupta et al., 2004).

In general, however, there was plenty of evidence that exhibited accretion of damaged mitochondrial proteins however only a few studies were present on changes in the mitochondrial proteostasis in AD. And it is still not clear how different mitochondrial chaperones and proteases are involved in different compartments of mitochondria. Moreover, current studies established “mitochondria as guardian of cytosol” where mitochondrial proteases when they were imported prevent the accumulation of cytosolic proteins that can become aggregated (Liu et al., 2018). That suggested that mitochondrial proteostasis could play a vital role in neuronal integrity and homeostasis of cytosolic proteins. Yet, further study is required for this important aspect of AD.

**CONCLUSION**

Mitochondrial impairment might play an essential role in Alzheimer’s disease either as a prime or inferior incident. Aβ is introduced in the mitochondria either intracellularly or extracellularly through TOM machinery. When these amyloid beta monomers get accumulated they result in abnormal mitochondrial functions and lead to neuronal damage and a decline in cognitive function. However, it is still not clear how amyloid beta monomer causes miotoxicity but there is various mechanism that describes the Aβ pathology. Mitochondrial dysfunction that is brought by Aβ contributes to the loss in energy metabolism, generation or buildup of mitochondrial reactive oxygen species (ROS), defects in the respiratory activity or enzyme that are involved in the respiratory activity, impaired mitochondrial biogenesis and impaired mitochondrial dynamics. Different types of mechanisms are convoluted in the mitochondrial dysfunction in Alzheimer’s disease. Moreover, further studies are needed to improve stratagems contrary to mitochondrial impairment. For the protection of neuronal function against amyloid beta toxicity, a specific type of mitochondrial specific therapeutic strategy should be discovered. Antioxidant-targeted therapy is the most basic therapeutic strategy against ROS in AD. One therapeutic strategy that specifically targets the mitochondria concentrates on controlling the different mitochondrial activities like bioenergetics. Remarkably, current studies showed that when complex I was mildly inhibited by CP2 compounds (CP2 is a tricyclic pyrone and it has a role in the prevention of amyloid beta-associated cell death) at FMN subunit, then it increased the respiratory activity and decreased the proton leaks. CP2 is developed to treat AD and it has also been seen that it
improved cognitive function and different pathological discrepancies in different animal models of AD. It also suggested that bioenergetics modulators are potentially used for the treatment of AD.

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