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Review Article

PERSPECTIVE REVIEW ON CLEARANCE OF AMYLOID BETA (AB) PEPTIDES IN ALZHEIMER'S DISEASE: FOCUSING ON ABC TRANSPORTERS

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ABSTRACT

Alzheimer's disease (AD) is an age-related disorder and the highest cause of death in elderly. Accumulating evidence describes that generation of Aβ peptides induces AD related synaptic dysfunction. Several mechanisms have been proposed for clearance of Aβ including enzyme mediated degradation and receptor mediated clearance across blood brain barrier (BBB). Now these mechanisms have become impaired in AD patients but still various mechanisms remain elusive. ABC transporters have been suggested in the regulation of cerebral Aβ proteostasis. These transporters harness the energy of ATP and escort the substrate across the plasma membrane. As per recent study it was concluded that ABCC1 has an important role in cerebral Aβ clearance and accumulation. Lack of ABCC1, a membranous protein substantially increased cerebral Aβ levels. Apart from these mechanisms low levels of serum 25-hydroxyvitamin D3 are related with increased risk of (AD) as well as vascular dysfunction. So, serum Vitamin D plays a role in clearance on Aβ from the brain. This review summarizes the recent findings on ABC transporters and vitamin D 3 mediated regulation of Aβ in AD.

KEY WORDS: Alzheimer's disease (AD), amyloid-beta peptides (Aβ), amyloid precursor protein (APP), presillin (PS1 and PS2), ABC transporters (ABCC1).

Abbreviations:

Alzheimer's disease (AD), amyloid precursor protein (APP), amyloid-beta (Aβ), blood brain barrier (BBB), neprilysin (NEP), insulin-degrading enzyme (IDE), ATP-binding cassette (ABC).

INTRODUCTION

Apoptosis is a beneficial process as it eliminates excess cells. An inappropriate initiation of apoptosis can cause various neurodegenerative diseases [1]. Recent researches observed that an age-related decline in proteostasis capacity representing the hallmark of various protein-aggregation diseases, including Alzheimer's disease and Parkinson's disease [9]. AD is an irreversible, fatal and progressive neurodegenerative disorder characterized by loss of neurons and synapses in selected areas of nervous system. Cerebral accumulation of amyloid beta peptide is a critical characteristic of AD [5]. About 25% of body cholesterol is present in the CNS and this is essential for both structure and function of CNS through synaptogenesis and optimal neurotransmitter release. Degradation of that cholesterol homeostasis is thought to be closely related to the development of neurodegenerative diseases, such as AD and multiple sclerosis [32]. However, it was suggested that increased level of amyloid-β causes AD may be due to reduced clearance of amyloid-β from the brain. There are various proposed mechanisms are available for clearance of amyloid-β and make it flow to

the bloodstream from the brain such as receptor mediated transcyotic transport through influx and efflux of amyloid-β by lipoprotein receptor related protein (LRP1) and receptor for advanced glycation end products (RAGE), degradation by enzymes [neprilysin (NEP) and insulin-degrading enzyme (IDE)] and by perivascular drainage of fluid. Apart from that another recent development for clearance of Aβ shows that, 3 members of the ATP-binding cassette transporter family (ABC Transporters) are prone to eliminate Aβ including ABCA1, ABCB1, and ABCG2 [36]. Yet the role of ABC transporters in contrast to Alzheimer's disease has not been investigated. But it has been observed that the transporter ABCC1 strongly affects transport and accumulation of amyloid-β peptides in vivo. Cerebral Aβ levels increases substantially due to deficiency of ABCC1. Thus, represents a novel target for regulating Aβ proteostasis in the brain [17].

Pathogenesis of Alzheimer's disease:

The histopathological changes of AD is characterized by the extracellular deposition of amyloid- β peptides derived from amyloid precursor protein (APP) proteolysis often termed as neuritic or senile plaques and intracellularly as a phosphorylated form of microtubule-associated tau which forms neurofibrillary tangles [2]. Proteolysis of APP cause production of Aβ i.e. Aβ 40 and Aβ 42 which are progressively aggregated to form plaque and release cytokinine that activates microglial cells [2]. Another one characteristic involved in pathogenesis of AD is over activity of CDKs on cell cycle which causes increase in production and deposition of Aβ and neurofibrillary triangles [33]. Primarily amyloid plaques are composed of a peptide with 40-42 amino acids; sequential cleavage of APP forms Aβ by beta and gamma secretases [3,4]. Recently β secretase marked as the novel aspartic protease BACE1. So BACE1 may be an important target for future therapies to reduce the production of Aβ in AD [6]. In neuronal functions, peptide and its precursor (APP) plays a key role; thus, for slowing down the progression of AD we have to control the physiological Aβ levels instead of complete inhibition as a strategy to decrease the accumulation of senile plaques [8, 9, 4]. Local hypoxia and microhaemorrhages and even local infarcts induce by the deposition of Aβ to cerebral capillary walls [8]. Severity of AD can be measured by amount of amyloid plaques in brain [3]. AD is associated with several neuronal abnormalities like hypoxic stress. Signaling pathways are stimulated by hypoxic stress which disturbs the energy metabolism and Ca 2+ balance leading to activation of hypoxia-inducible factor-1a (HIF-1a) [8]. It has been demonstrated that HIF-1a was involved in activation of BACE1 and subsequently increased Aβ production [8]. Current clinical studies however suggest that there are three characteristics which particularly correlate with clinical dementia. They are:

(a) The number and distribution of neurofibrillary changes

(b) A raised level of soluble Aβ in the brain and

(c) The severity of CAA [12].

Misfolding of proteins:

Misfolding means alteration in conformation of normal proteins that tends to forms large insoluble nonfunctional aggregates. These aggregates also tend to stick to cell membranes as shown in fig 1. The alterations in conformation are favored by specific mutations of protein or by infection with prions. In nervous system these aggregates generally known as an amyloid deposit which is the main feature of AD. (41)

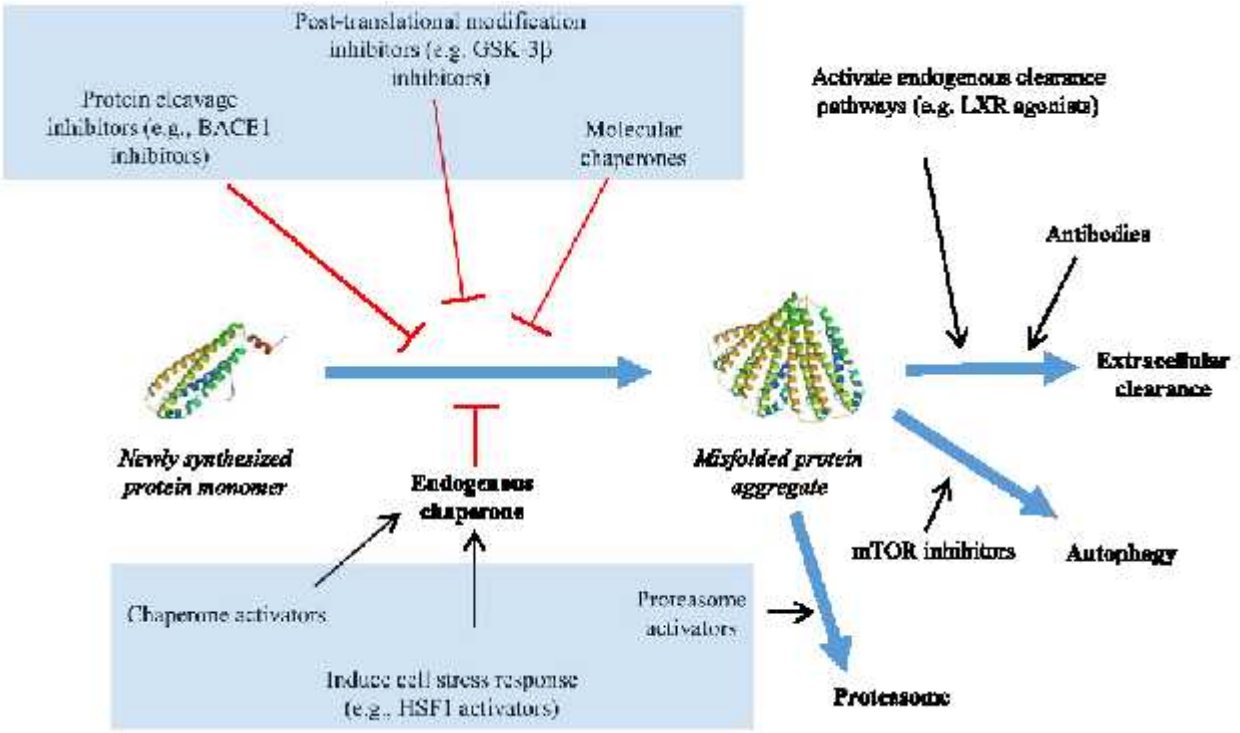

Fig.1. Protein misfolding: a process associated with neurodegenerative diseases [41].

Role of Proteostasis:

Recent researches observed that an age-related decline in proteostasis capacity allows the manifestation of various protein-aggregation diseases, including AD and Parkinson's disease (PD). AD is oftenly known as "protein misfolding" disease due to involvement of critical conformational changes of proteins and misregulation of protein maintenance [9]. Mostly proteins are folds into defined three-dimensional structures to gain functional activity. Proteostasis regulates the production, folding, binding, localization, and disposal of individual proteins making up the proteome oftenly through transcriptional and translational changes or by readapting the innate biology of the cell [1]. A patient with AD was more prone to disequilibrium of proteostasis which leads to the accumulation of fibrillar and oligomeric multimers of amyloid-β peptides in the brain. Approximately 30% clearance of Aβ across BBB is reduced in AD patients compared with healthy controls [4].

Formation and accumulation of Aβ in Alzheimer's brain:

Amyloid in the brains of Alzheimer's disease is mainly composed of amyloid peptides, the soluble Aβ peptide is converted into an intermediate insoluble prefibrillar form leads to 8–10 nm amyloid fibrils under the influence of Aβ concentration, pH and a variety of tissue factors with a highly ordered b-pleated sheet structure [12]. Primarily AD damage arises from small oligomeric amyloid forms of Aβ peptide. The principal component of plaques is amyloid β-peptide (Aβ; consists of 39–43 amino acids in length). Aβ is formed from protoelytic cleavage of APP that localizes to the plasma membrane, trans-Golgi network, endosomal, lysosomal and mitochondrial membranes. Single amyloid plaque formation represents a template-dependent process which critically involves the presence of phagocytosis-competent cells. Internalized Aβ peptide has found in multivesicular bodies where fibrils grow out. Cells undergo cell death on plaque formation and intracellular amyloid structures become released into the extracellular space. In Alzheimer's disease (AD), Aβ peptide forms amyloid fibrils. Cross-β structure has developed due to aggregation of fibrillar polypeptide or amyloid fibrils. These fibrils are structurally related to the infectious prions from Creutzfeldt-Jakob disease. The Aβ peptide is formed at cholesterol-rich regions of neuronal membranes and released into the

extracellular space. Aβ (1–40) represents the most abundant Aβ species in normal and AD brains, followed by the Aβ (1–42). Aβ (1–40) and Aβ (1–42) are able to get many differently shaped aggregates including amyloid fibrils as well as nonfibrillar aggregates that are sometimes termed as Aβ "oligomers". These extracellular Aβ oligomers can be formed in the presence of GM1 ganglioside on the cell membrane. This Aβ oligomer induces neuronal cell death mediated by nerve growth factor (NGF) receptors. A cellular prion protein (PrPC) acts as an Aβ oligomer receptor with nanomolar affinity, and mediates synaptic dysfunction. Aβ (1–42) distributes in brain and vessel walls whereas Aβ (1–40) present in the major form of amyloid in artery walls in CAA [12]. Addition of known amyloid inhibitor CR or by replacing Aβ with Aβ variant having a low intrinsic aggregation propensity, plaque formation can be reduced [7]. An imbalance between its production and its degradation can explain the accumulation of peptide [4, 6]. A complex hypothesis was then considered: that preventing amyloid formation reduces Aβ cytotoxicity because certain extracellular protein produces complex Aβ; formation of amyloid and the consequent toxicity occurs when this hypothesis fails. Accumulation of amyloid-β (Aβ) peptides is a critical yet poorly determined. For regulation of Aβ levels in brain ABC transporters have been suggested.

Clearance Amyloid-β (Aβ) from brain:

Recent findings accumulating that elevation of amyloid-β levels may be due to reduced clearance of amyloid-β from the brain. There are various mechanisms which have been proposed for Aβ clearance i.e. –

- Receptor-mediated transcytotic Aβ transport across the blood–brain barrier through LRP1 and RAGE on endothelial cells.
- Degradation [by enzymes (i.e. neprilysin (NEP) and insulin-degrading enzyme (IDE), autophagy and the proteasome complex] [14].
- Perivascular drainage of the extracellular fluid [16].
- Vitamin D3 mediated efflux of Aβ

For the reason of developing drugs for AD, these mechanisms have become potential targets. Recent development shows that, 3 members of the ATP-binding cassette transporter family (ABC Transporters) — have been shown to eliminate Aβ including ABCA1, ABCB1, and ABCG2 [17, 41]. Yet the role of ABC transporters in contrast to (AD) has not been investigated. To find out a solution for this question, the ability of specific ABC transporters (ABCB1, ABCG2, and ABCC1) was compared to reduce Aβ level in vitro in genetically modified mice. Then it has been observed that the transporter ABCC1 strongly affects transport and accumulation of amyloid beta peptides in vivo. Cerebral Aβ levels increases substantially due to deficiency of ABCC1 without making any change in the expression of most enzymes which favors the production of Aβ from the amyloid precursor protein. Thus represents a novel target for regulating Aβ proteostasis in the brain [17]. The module 1 represents production and removal of Aβ from Aβ monomer pool. This shows that abundance of insoluble aggregates effects the removal of monomeric Aβ through active ABC transporters. Whereas module 2 represents growth and nucleation of monomeric Aβ by addition of Aβ monomer. Equation of that model is restricted to 3 general mechanisms: (a) regulation of monomer abundance through production and clearance of Aβ; (b) nucleation; and (c) irreversible growth by addition of Aβ monomer [17].

ABC transporters:

The largest and arguably family of transmembrane (TM) proteins represents the ATP-binding cassette (ABC) genes. To access the transport of various molecules across all cell membranes, these proteins bind with ATP and use the energy [1, 3]. Based on the sequence and structure of their ATP-binding domain(s) which is also known as nucleotide-binding folds (NBFs), proteins are classified as ABC

transporters. All ABC protein transporters consist of two membrane- spanning domains that persuade a translocation pathway for a specific substrate. Conformational changes occur as ABC cassettes bind and rearranged, that translocate the substrate from one side of the membrane to the other. Subfamily of ABCC hydrolyze ATP, which is transmitted to the membrane-spanning domains, and permit contains 12 full transporters with a broad functional spectrum which includes cell-surface receptor, ion transport and toxin secretion activities. Sulfonylurea binds to ABCC8 and ABCC9 proteins and regulates potassium channels which modulate insulin secretion. Other ABCC proteins of this subfamily are composed of nine MRP- related genes. In which ABCC1, ABCC2 and ABCC3 transport drug that shows conjugation to glutathione and other organic anions. The ABCC4, ABCC5, ABCC11, and ABCC12 lack an N- terminal domain and these proteins are smaller than the other MRP1-like gene products [13]. ABCC1 is also present at the choroid's plexus, where it may actively clear Aβ from the CSF. The ABCC4 and ABCC5 proteins include PMEA and purine analogs which confer resistance to nucleosides. Various human ABC transporters are medically relevant [14]. Therefore, transporter ABCC1 has played an important role in cerebral Aβ clearance and accumulation.

There are some ABC transporters whose functions have been defined by invitro assay and they may be useful in AD [42].

Gene symbol	Other names	Functions
ABCA1	ABC1	Convert cellular cholesterol and phospholipids to HDLs, defects in gene causes Tangier disease
ABCB1	PGY1, MDR1	PGY1=P-glycoprotein 1; MDR1=multidrug resistance protein 1, multidrug resistance P-glycoprotein, is an integral component of the blood-brain barrier, that can transfer a number of drugs from the brain into the bloodstream.
ABCC1	MRP1	MRP1=multidrug resistance associated protein 1, sphingosine-1-phosphate (S1P), enhances their migration and release from mast cells, uses glutathione as a co as a co-factor in mediating resistance to heavy metal oxyanions.
ABCC4	MRP4, MOATB	MRP4=multidrug resistance associated protein 4, MOATB=multispecific organic anion transporter B, mainly located in prostate, regulator of intracellular cyclic nucleotide levels, mediator of cAMP- dependent signal transduction to the nucleus.
ABCG1	ABC8, White1	Associated with transportation and efflux of intracellular cholesterol which is responsible for about 20% of cholesterol efflux to HDLs (reverse cholesterol transport)
ABCG4	White2	This gene is restricted to astrocytes and neurons; ABCG4 with ABCG1 helps in sterol transport and may increase lipidation of apoE in Alzheimer disease.

Role of ABC transporter:

According to the new research ABC transporters are involved in regulation of cerebral amyloid-β proteostasis. These transporters harness the energy of ATP and escort the substrate across the plasma membrane. From the seven subfamilies of ABC transporters (ABCA to ABCG) three contain

proteins to pump Aβ across the blood brain barrier [17]. BBB contains numerous membrane transporters on the endothelial cells causes influx and efflux of essential substrate such as electrolytes, nucleosides, peptides and glucose. ABC transporters in endothelia are sorted either to apical membrane, facing the luminal surface of vessels or to the basolateral membrane. This polarized arrangement of ABC transporters at the cell membrane enables guided transport through BBB [2]. Thus, ABC transporters fulfill an important function in elimination or clearance various compounds and amyloid peptides. In CNS, lipid accumulation in CNS leads to structural abnormalities of choroid plexus epithelial cells (CPE). Structural changes in CPE are associated with AD patients. CPE possesses Liver X receptor (LXR) that regulates the expression of various molecules associated with cholesterol homeostasis, including ATP-binding cassette transporter (ABC)A1 and ABCG1[35] and affect the development of AD pathology. Various studies demonstrated that ABCB1 and ABCG2 are critical to drug efflux at the BBB and whereas ABCC1 is essential for the blood-cerebral spinal fluid (CSF) barrier [29]. Apolipoprotein E present in brain work as a lipid carrier. Lipidation status of apolipoprotein E influences amyloid-β burden in Alzheimer's disease, caused by modulation of amyloid-β deposition or clearance rather than amyloid-β production. ATP transporters help in regulation of lipid homeostasis and apolipoprotein metabolism in brain [28]. The first ABC transporter that was detected in endothelial cells of the human BBB is P-gp [15]. It may serve as a general defense mechanism in BBB, protecting brain from toxicity of lipophilic compounds. It is present in luminal side of cerebral endothelial cells of various mammalian species including monkeys, rabbits, rats and mice [16] and plays a role in clearance as transports substrate directly into the bloodstream from brain. Multidrug resistance genes (MDR) encode P-gp. Mdr1a, Mdr1b and MDR2 are the potent gene products of P-gp which have been identified in rodents [17] whereas in humans two gene products MDR1 and MDR2 has been associated with AD [18]. Only MDR1 confers multi-drug resistance, since MDR2 is predominantly expressed by P-gp plays a neuroprotective and detoxifying role for CNS as its gene products (MDR1), MRP (MRP1, 2 and 5) and BCRP may function as an active efflux pumps that carries wide range of structurally diverse compounds from the CNS [30].

Vitamin D3 mediated Aβ clearance:

Often cerebral Aβ peptide clearance impaired due to cerebrovascular dysfunction. And low levels of serum 25-hydroxyvitamin D3 are related with increased risk of Alzheimer's disease, as well as vascular dysfunction. Vitamin D receptor (VDR) gene contains nucleotide polymorphisms which is responsible for cognitive function and developing AD. It was also found that low levels of serum 25 (OH) D3 are associated with vascular dysfunction which are indirectly responsible for increased risk of cardiovascular diseases, hypertension and diabetes mellitus [24] and further plays an important role in early progression of AD. Function of brain capillaries are affected by serum vitamin D, including the efflux transport of Aβ from brain to the blood. Physiologically active form of vitamin D, 1a, 25-dihydroxyvitamin D3 is responsible for eliminating human amyloid (1-40) across the BBB and also a decrease of human Aβ (1-40) binding to brain parenchymal cells or both [23].

Receptor-mediated transcytotic Aβ transport across the blood–brain barrier:

Through interstitial fluid (ISF) bulk flow into the bloodstream soluble Aβ can be eliminated slowly [1]. It was suggested that there are two receptors which are involved in clearance of Aβ in addition to large number of proteins across blood brain barrier. These are low density lipoprotein receptor and the receptor for advanced glycation end products (RAGE). LRP mediates the efflux of Aβ from the brain to the periphery whereas RAGE has been strongly implicated in Aβ influx back into the CNS. However, this mechanism can be successful for the clearance of only 10–15% of the total Aβ present in the brain [33].

LRP- mediated Aβ efflux:

LRP-a clearance receptor for Aβ. It consists of two chains-heavy chain have four ligand-binding domains, that bind an array of structurally unrelated ligands and light chain contains a transmembrane domain with phosphorylated cytoplasmic tail on serine which plays a role in intracellular signaling. Lipoprotein receptor-related protein (LRP)-mediates Aβ transcytosis across BBB initiates at abluminal side of endothelium and eliminates Aβ from brain to bloodstream. Aβ deposition in the mouse brain increased due to dysfunctioning of LRP, leads to reduced efflux of Aβ from the brain. In AD less presence of brain endothelial, LRP is often associated with positive Aβ staining of vessels [16].

RAGE-mediated Aβ influx:

RAGE, a multi-ligand and cell surface receptor in the immunoglobulin superfamily binds soluble Aβ with broad repertoire of ligands in the nanomolar range including products of nonenzymatic glycoxidation (AGE), and mediates transport of plasma Aβ across the BBB. RAGE is up- regulated by its ligands. RAGE-ligand interaction causes cellular dysfunction. RAGE mediates influx of free, unbound circulating Aβ across the BBB and downregulation of RAGE can suppress the influx of Aβ. RAGE expresses in affected cerebral vessels, neurons or microglia. RAGE expression is sustained at an increased level by excess amounts of Aβ in AD brain through a positive-feedback mechanism [16]. The transport equilibrium for Aβ across the BBB may be readjusted by upregulation of LRP and down regulation of RAGE by promoting its net efflux from brain into bloodstream. Activation of RAGE by Aβ could take place at an early stage of AD and leads to early neuronal dysfunction. The inhibition of RAGE–Aβ interaction at early stages of AD might be a useful strategy [16].

Enzyme-mediated Aβ degradation:

Aβ is generally degraded by various peptidases, mainly two zinc metallo endopeptidases are used which is referred to as neprilysin and insulin-degrading enzyme (IDE).

(1) Neprilysin:

Neprilysin is a rate-limiting enzyme present in brain, degrades Aβ. Generally, deposited amyloid in AD mouse brain remarkably decrease by intracerebral human neprilysin gene transfer and inhibition of neprilysin protein or disruption of the neprilysin gene leads to defect in Aβ degradation [36]. In cortex and hippocampus region of AD brain, there is marked decrease in the level and activity of neprilysin but not in other brain areas or peripheral organs. This correlation between neprilysin and Aβ peptide levels has been found in the vasculature of AD patients [37]. Amyloid precursor protein intracellular domain (AICD) transactivated the neprilysin gene promoters produced from gamma-secretase cleavage of APP-like proteins [38]. These presenilin dependent regulations of neprilysin modulate Aβ levels with varying levels of gamma-secretase activity [16].

(2)- IDE:

It is another major enzyme responsible for Aβ degradation in the brain. Level of IDE in the brain reduces as age increases. In AD brain it possesses distinct distribution with lower levels and IDE is being more oxidized in the region of cortex and hippocampus than in the cerebellum. Animal models show that, IDE leads to the impairment of Aβ degradation, whereas overexpression of IDE decreases Aβ levels or completely prevents amyloid plaque formation in the brain [39]. Other enzymes such as endothelin-converting enzyme (ECE) and angiotensin- converting enzyme (ACE) , also degrade Aβ through hydrolysis of Aβ in the brain. Various studies suggest that a decrease in activities of these enzymes because of genetic mutations and alterations in gene expression due to age or disease might increase the risk for AD. Prolongation of these degradation enzymes either through gene therapy or transcriptional activation represents a novel therapeutic strategy [16].

Perivascular drainage of Aβ peptide:

Failure of elimination of protein along perivascular pathways is also responsible for drainage of Aβ that occurs inside aging brain and further responsible for Alzheimer's disease. This is also known as "protein-elimination failure arteriopathy" (PEFA), commonly associated with the central and peripheral nervous systems. When LRP mechanism is blocked or fails and if neprilysin levels in the brain are reduced, perivascular drainage appears to compensate. In CAA, Aβ is deposited in perivascular drainage pathways. As soluble tracers which are draining from the brain, Aβ has almost same distribution along the basement membranes in the capillaries and arteries walls. Further Aβ is deposited in the basement membrane of capillaries and forms compact nodules.[13]

CONCLUSION

Accumulation of Aβ peptides in AD is critical yet poorly understood process. Equilibrium state of Aβ is maintained by its production and clearance. Recent evidence has suggested that enzyme mediated degradation of Aβ and receptor mediated BBB transport have become impaired in AD. Apart from that ABC transporters have play a major role in clearance and accumulation of Aβ. They regulate cerebral Aβ proteostasis by harnessing energy of ATP and escort the substrate from plasma membrane. Cerebral Aβ levels increases substantially due to deficiency of ABCC1 without making any change in the expression of most enzymes which favors the production of Aβ from the amyloid precursor protein. Thus, represents a novel target for regulating Aβ proteostasis in the brain. It was also found that low levels of serum 25 (OH) D3 are associated with vascular dysfunction which are indirectly responsible for increased risk of cardiovascular diseases, hypertension and diabetes mellitus and further plays an important role in early progression of AD. In that condition serum vitamin D plays a major role in eliminating Aβ. Therefore, these two mechanisms are the major therapeutic approaches for regulating clearance and accumulation of Aβ in AD.

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