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Could Vitamin B12 Have Beneficial Role on Alzheimer Disease Patients ?

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Abstract: Dementia is not a specific disease but is rather a general term for the impaired ability to remember, think, or make decisions. Alzheimer's disease (AD) is a devastating progressive neurodegenerative disease and the most common form of dementia for which no effective cure was found until today. Alzheimer's disease is characterized by memory loss, impaired spatial and temporal orientation, agnosia, language disturbance, mood disturbance, wandering, and other cognitive and neuropsychiatric functions that reduce the ability to carry out the activities of daily living. B vitamins, including vitamin B12 (cobalamin), are essential water-soluble micronutrients that have to be taken up in sufficient quantities from one's diet. They are crucial for maintaining neuronal health and hematopoiesis. Vitamin B12 is believed to be an antioxidant vitamin by different mechanisms, including direct scavenging of reactive oxygen species (ROS), particularly superoxide in the cytosol and mitochondria, and indirectly stimulating ROS scavenging by preservation of glutathione. Vitamin B12 supplementation exerts positive effects in respect to AD pathology not only in transgenic AD model mice but also in wildtype animals. Vitamin B12 supplementation in hyperhomocysteinemic rats could antagonize homocysteine-induced changes in APP processing and tau phosphorylation. Vitamin B12 protected scopolamine-injected rats and inhibited hippocampal inflammation and apoptosis and preserved pre- and post-synaptic proteins and possibly synaptic integrity in hippocampus by increasing synaptic plasticity

Keywords: *Vitamin B12, Alzheimer Disease*

Introduction

Dementia is not a specific disease but is rather a general term for the impaired ability to remember, think, or make decisions. Alzheimer's disease (AD) is a devastating progressive neurodegenerative disease and the most common form of dementia for which no effective cure was found until today**.** Currently more than 55 million people live with dementia worldwide, and there are nearly 10 million new cases every year **.** It stands for 60-70% of dementia cases **(1).**

Alzheimer's disease is characterized by memory loss, impaired spatial and temporal orientation, agnosia, language disturbance, mood disturbance, wandering, and other cognitive and neuropsychiatric functions that reduce the ability to carry out the activities of daily living**.** It can be present in two forms, the sporadic late-

onset form of the disease (the most prevalent type) and the familial early-onset of AD, that accounts for 1–5% of all cases **(2).**

There are two types of neuropathological changes in AD which provide evidence about disease progress and symptoms and include:

1- Positive lesions which are characterized by the accumulation of neurofibrillary tangles (NFTs), Beta amyloid plaques (Aβ), dystrophic neurites, neuropil threads, and other deposits found in the brains of AD patients **(3)**.

Amyloid is a general term for protein fragments that the body produces normally. Beta amyloid is a protein fragment snipped from an APP. In a healthy brain, these protein fragments are broken down and eliminated**.** The senile plaques are extracellular deposits of Aβ with different morphological forms, including neuritic, diffuse, dense-cored, or classic and compact type plaques that clump together between the nerve cells (neurons) in the brains of AD patients (**4).**

Proteolytic cleavage enzymes such as β-secretase and γ-secretase are responsible for the biosynthesis of Aβ deposits from the transmembrane APP These enzymes cleave APP into several amino acid fragments: 43, 45, 46, 48, 49, and 51 amino acids, which reach the final forms Aβ40 and Aβ42**.** There are several types of Aβ monomers, including large and insoluble amyloid fibrils which can accumulate to form amyloid plaques and soluble oligomers that can spread throughout the brain **(5).**

Aβ plays a major role in neurotoxicity and neural function, therefore, accumulation of denser plaques in the hippocampus, amygdala, and cerebral cortex can cause stimulation of astrocytes and microglia, damage to axons, dendrites, and loss of synapses, in addition to cognitive impairments **(6)**.

Neurofibrillary tangles are abnormal filaments of the hyperphosphorylated tau protein that in some stages can be twisted around each other to form paired helical filament (PHF) and accumulate in neural perikaryal cytoplasm, axons, and dendrites, which cause a loss of cytoskeletal microtubules and tubulin-associated proteins. The hyperphosphorylated tau protein is the major constituent of NFTs in the brains of AD patients **(7).**

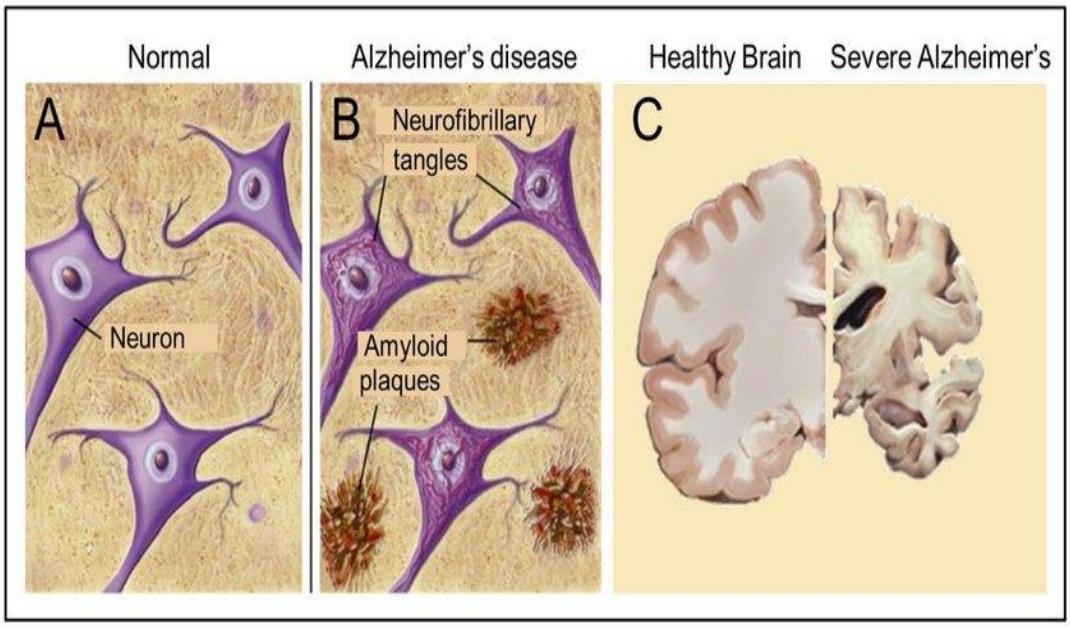


Figure 1. Major pathological hallmarks of AD are amyloid plaques and NFTs (B) that are absent in healthy brain (A). Major apoptosis occurs in late stages of AD in the human brain **(8).**

2- Negative lesions that are characterized by large atrophy due to a neural, neuropil, and synaptic loss. Besides, other factors can cause neurodegeneration such as neuroinflammation, oxidative stress, and injury of cholinergic neurons **(8).**

A synaptic damage in the neocortex and limbic system causes memory impairment and generally is observed at the early stages of AD. Synaptic loss mechanisms involve defects in axonal transport, mitochondrial damage, oxidative stress, and other processes that can contribute to small fractions, like the accumulation of Aβ and tau at the synaptic sites. These processes eventually lead to a loss of dendritic spines, pre-synaptic terminals, and axonal dystrophy **(9).**

Causes of Alzheimer's disease:

The underlying cause of the pathological changes in Alzheimer's disease is still unknown. Several hypotheses were proposed as a cause for AD but two of them are believed to be the main cause; the first one is an impairment in the cholinergic function, while the other is alteration in amyloid β-protein production and processing is the main initiating factor**.** However, at present, there is no accepted theory for explaining the AD pathogenesis **(10).**

I- Cholinergic Hypothesis:

In the 1970s, neocortical and presynaptic cholinergic deficits were reported to be related to the enzyme choline acetyltransferase (ChAT), which is responsible for the synthesis of acetylcholine (ACh). Due to the essential role of ACh in cognitive function, a cholinergic hypothesis of AD was proposed **(11).**

ACh is synthesized in the cytoplasm of cholinergic neurons from choline and acetyl-coenzyme A by the ChAT enzyme and transported to the synaptic vesicles by vesicular acetylcholine transporter (VAChT) **(11).**

In the brain, ACh is involved in several physiological processes such as memory, attention, sensory information, learning, and other critical functions. Degeneration of the cholinergic neurons was found to take place in AD and to cause alternation in cognitive function and memory loss. B-amyloid is believed to affect cholinergic neurotransmission and to cause a reduction in the choline uptake and a release of Ach**.** Studies demonstrated that cholinergic synaptic loss and amyloid fibril formation are related to Aβ oligomers' neurotoxicity and to interactions between AChE and Aβ peptide. **(6)**.

Additional factors also contribute to the progression of AD, such as a reduction in nicotinic and muscarinic (M2) Ach receptors, located on presynaptic cholinergic terminals, and the deficit in excitatory amino acid (EAA) neurotransmission, where glutamate concentration and D-aspartate uptake are significantly reduced in many cortical areas in AD brains. This is in addition to the use of cholinergic receptor antagonists such as scopolamine, which was found to induce amnesia. This effect can be reversed by using compounds that activate acetylcholine formation **(12).**

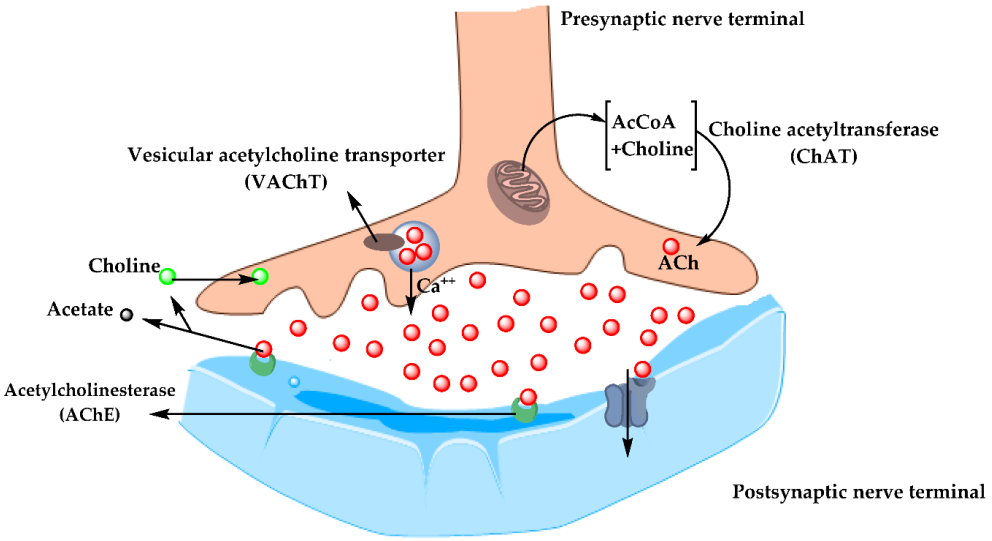


Figure 2. The pathway for the synthesis and transportation of acetylcholine between presynaptic and postsynaptic nerve terminals **(13)**

II- Metal ion hypothesis:

Metals are found in nature and biological systems and can be divided into bio-metals that have a physiological function in living organisms (e.g., copper, zinc, and iron), and toxicological metals which do not possess any biological function (e.g., aluminum and lead). Aluminum (Al) is used significantly in the industries such as processed foods, cosmetics, medical preparations, medicines, and others. In the body, Al is bound to plasma transferrin and to citrate molecules that can mediate the transfer of it to the brain. Studies demonstrated that Al accumulates in the cortex, hippocampus, and cerebellum areas, where it interacts with proteins and causes misfolding, aggregation, and phosphorylation of highly phosphorylated proteins like tau protein, characteristic of AD **(14).**

Lead competes with the binding site of bio-metals like calcium and can cross the blood–brain barrier (BBB) rapidly, where it can modify neural differentiation and synaptogenesis and cause severe damage. Studies revealed that an acute exposure to lead was associated with AD and caused an increase of β-secretase expression and Aβ accumulation. Cadmium is a carcinogenic water-soluble metal that can cross the BBB and cause neurological diseases like AD. Results have demonstrated that Cadmium ions are involved in the aggregation of Aβ plaques and the self-aggregation of tau in the AD brain **(15).**

III- Oxidative stress hypothesis:

The brain of patients suffering AD present a significant extent of oxidative damage associated with the abnormal marked accumulation of Aβ and the deposition of neurofibrillary tangles **.** Iron, zinc, copper play an important role in neurodegeneration and AB development**.** There are high affinity binding sites for copper and zinc on the N-terminal metal-binding domains of Aβ and its precursor APP while copper is a potent mediator of the highly reactive hydroxyl radical (OH•), and consequently contributes to the increase of oxidative stress characteristic of AD brain**.** In addition, high concentrations of zinc were associated with memory and cognitive regions of the brain, including the neocortex and amygdala, and hippocampus, which are mostly affected in AD pathology. This binding of zinc has a highly ordered conformational state of Aβ , leading to the production of toxic, fibrillary, Aβ aggregates **(16).**

<u>Stages of Alzheimer's disease:</u>

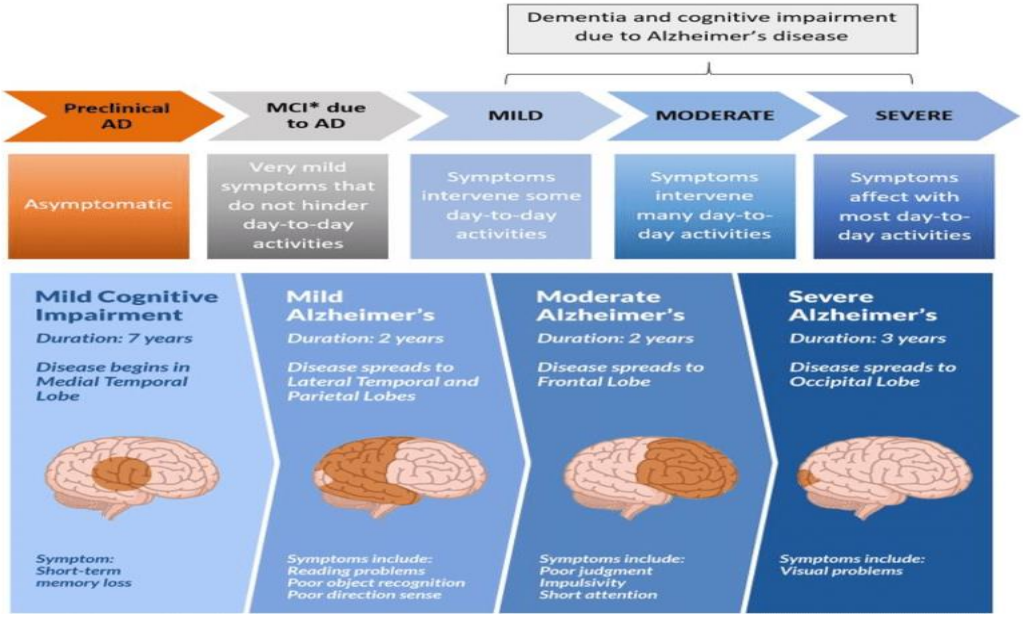


Figure 3. Progression of AD & brain changes **(17)**

The clinical phases of AD can be classified into:

1. pre-clinical or the pre-symptomatic stage, which can last for several years or more. This stage is characterized by mild memory loss and early pathological changes in cortex and hippocampus, with no functional impairment in the daily activities and absence of clinical signs and symptoms of AD **(18).**
2. The mild or early stage of AD, where several symptoms start to appear in patients, such as a trouble in the daily life of the patient with a loss of concentration and memory, disorientation of place and time, a change in the mood, and a development of depression **(18).**
3. Moderate AD stage, in which the disease spreads to cerebral cortex areas that results in an increased memory loss with trouble recognizing family and friends, a loss of impulse control, and difficulty in reading, writing, and speaking **(1).**
4. Severe AD or late-stage, which involves the spread of the disease to the entire cortex area with a severe accumulation of neuritic plaques and neurofibrillary tangles, resulting in a progressive functional and cognitive impairment where the patients cannot recognize their family at all and may become bedridden with difficulties in swallowing and urination, and eventually leading to the patient's death due to these complications **(19)**.

Vitamin B12

B vitamins, including vitamin B12 (cobalamin), are essential water-soluble micronutrients that have to be taken up in sufficient quantities from one's diet. They are crucial for maintaining neuronal health and hematopoiesis**.** Clinical vitamin B12 deficiency leading to myeloneuropathy or megaloblastic anemia is rare in developed countries, but subclinical vitamin B12 deficiency is common and can be found in 10 to 15% of individuals older than 60 years and in 25 to 35% of individuals aged over 80 years **(20).**

Subclinical vitamin B12 deficiency, defined as 119–200 pmol/L of serum vitamin B12, often remains asymptomatic over years. Based on the anti-oxidative property of vitamin B12, B12 deficiency might lead to oxidation of lipids, proteins and nucleic acids and might contribute to the development of age-related diseases, in which oxidative stress is believed to be a major factor, including AD, Parkinson disease and type 2 diabetes **(21).**

<u>The antioxidant properties of vitamin B12:</u>

Vitamin B12 is believed to be an antioxidant vitamin by different mechanisms, including direct scavenging of reactive oxygen species (ROS), particularly superoxide in the cytosol and mitochondria and indirectly stimulating ROS scavenging by preservation of glutathione**.** Furthermore, vitamin B12 might protect against inflammation-induced oxidative stress by modulating cytokine and growth factor production, including interleukin-6, tumour necrosis factor alpha (TNF-α) and epidermal growth factor. Notably, the involvement of neuroinflammation is reported to play a fundamental role in the progression of AD **(22).**

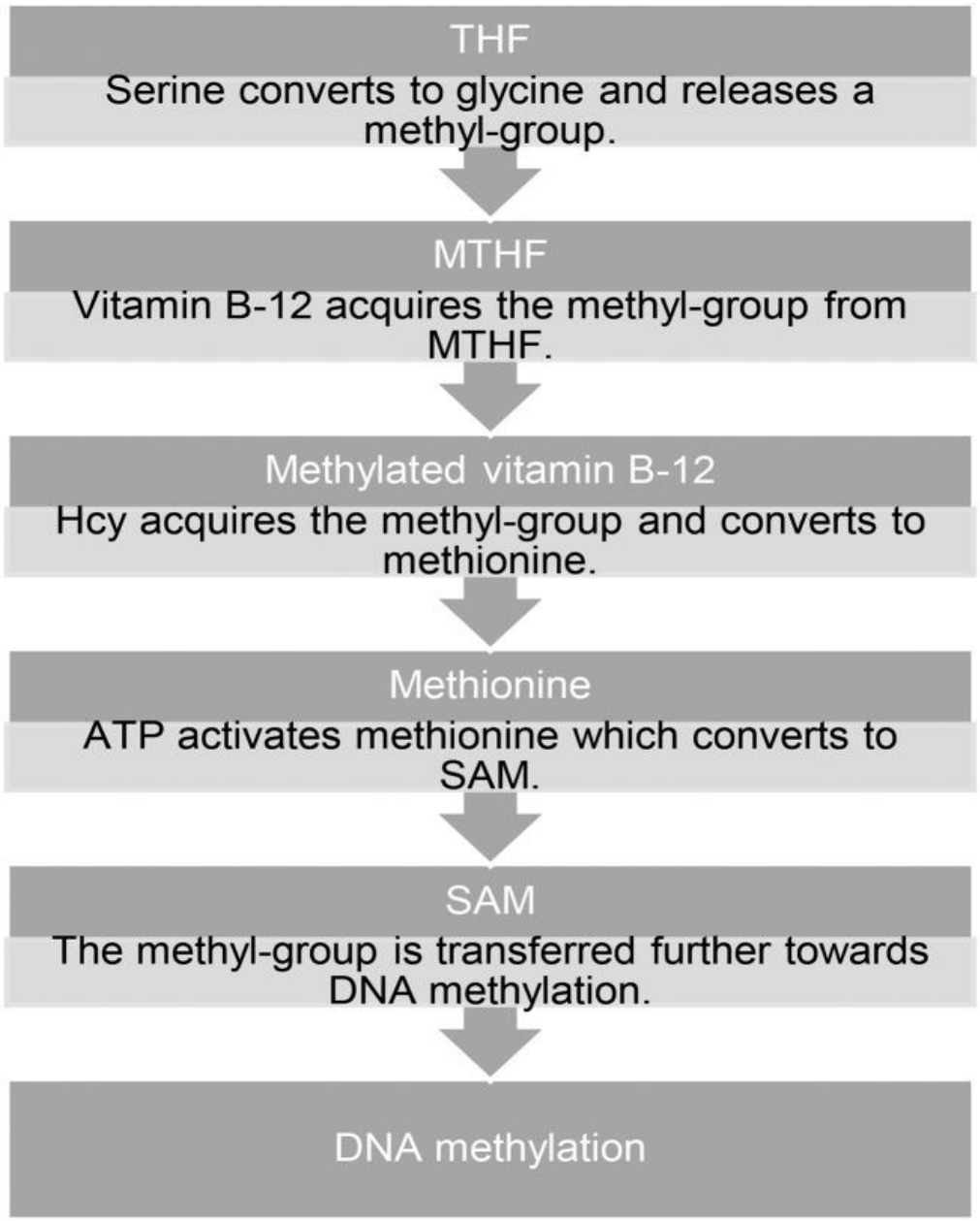
A reduced vitamin B12 status is associated with an increase in interleukin-6 production and TNF-α levels**,** and interleukin-6 has been shown to induce hyperphosphorylation of tau and TNF-α increases the Aβ burden by upregulation β-secretase production and increased γ-secretase activity **.** Another important antioxidative mode of action of vitamin B12 is closely linked to AD: a reduction in homocysteine-induced oxidative stress. Vitamin B12 is an important cofactor of methionine-synthase, converting homocysteine into methionine. Subclinical B12 deficiency reduces the conversion of homocysteine to methionine, leading to an elevated intracellular homocysteine level **(20).** Homocysteine has been reported as mediating ROS accumulation through multiple mechanisms, including autooxidation of homocysteine leading to H_2O_2 production, and by inhibition of cellular antioxidant enzymes, namely, glutathione peroxidase and superoxide dismutase (SOD) **(23).**

Beside the anti-oxidative function of vitamin B12, vitamin B12 exerts essential roles in the central and peripheral nervous system, maintaining the health of the nervous system including, e.g., the cellular energetic processes, myelin, and neurotransmitter synthesis**.**It's important for nerve metabolism (transmethylation processes), energy production, synaptogenesis and fatty acid and nucleic acid synthesis. It also has an impact

on the formation of myelin, by affecting the DNA synthesis of myelin-producing oligodendrocytes. Notably, recently it has been shown that myelin impairment may play an important role in AD pathology and that myelin pathology might even precede Aβ and tau pathologies of AD. The regeneration of nerves after injury has also been found to be supported by vitamin B12 **(24)**.

Vitamin B12 and DNA methylation:

B vitamins are substances well associated with the methionine cycle which includes the molecules homocysteine (Hcy), methionine, and S-adenosylmethionine and utilizes cofactors, such as vitamin B-12, B-9 (folate), and B-6. The methionine cycle and the reaction chain, beginning from folate and ending with DNA



methylation. The hypothesis is that nutritional deficits in B vitamins can lead to hyperhomocysteinemia and, consequently, to decreased S-adenosylmethionine concentration. The decrease in S -adenosylmethionine, which is a methyl donor, can induce demethylation of DNA, resulting in overexpression of genes involved in AD pathology **(25).**

Figure 4. DNA methylation. The reaction pathway by which the methyl group is transferred from tetrahydrofolate to DNA is shown. The figure also indicates the involvement of folate and vitamin B-12 in this process. Hcy, homocysteine; MTHF, methyltetrahydrofolate; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate **(26).**

Effect of vitamin B12 deficiency on the AB peptide level and AB deposition in Alzheimer's disease mice models:

Transgenic mice overexpressing the Swedish mutation of AD (Tg2576), leading to increased γ-secretase cleavage of APP and thus Aβ levels, fed with a diet deficient in folate, vitamin B6 and vitamin B12 for 7 months, revealed significantly elevated Aβ peptide levels in the hippocampus and cortex compared to Tg2576 fed with a control diet **(27).**

Vitamin B12 supplementation exerts positive effects in respect to AD pathology not only in transgenic AD model mice but also in wildtype animals. Vitamin B12 supplementation in hyperhomocysteinemic rats could antagonize homocysteine-induced changes in APP processing and tau phosphorylation**.** Vitamin B12 protected scopolamine-injected rats and inhibited hippocampal inflammation and apoptosis and preserved pre- and post-synaptic proteins and possibly synaptic integrity in hippocampus by increasing synaptic plasticity **(28).**

Effect of VB on aggregation kinetics of AB

Firstly the effect of VB on Aβ- 42 aggregation was monitored by ThT fluorescent assay. When ThT binds to the ordered cross beta sheet structure of amyloid fibrils, the fluorescence intensity of ThT increases significantly. Provided that binding is specific, ThT fluorescence gives a measure of amyloid population in the protein **(28).**

In summary, the combination of a wide range of biophysical, imaging and cell viability assays demonstrate that VB inhibits Aβ- 42 aggregation and protects against amyloid induced cytotoxicity. The ThT assay and TEM results demonstrate that VB inhibits the amyloid formation and effectiveness increases with increasing concentration of VB. The aggregates formed in the presence of VB contain less β-sheet structures, as depicted by the CD spectra. DLS data showed that size of aggregates got.

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