

**Chronological primacy of oxidative-induced neuronal damage in the
pathogenesis of Alzheimer's disease**

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Abstract

The current study comprises the major oxidative modifications associated with the pathological lesions of neural damage in Alzheimer's disease including: repeated exposure to aluminum and heavy metals, mutations in a number of chromosomes and genes, diabetes, cardiovascular diseases, brain injury, amyloid beta and senile plaques.

We surmise that there is clearly a need for the identification and development of panels of biomarkers for accurate diagnosis and early detection of sporadic Alzheimer's disease. Meanwhile, a collection of the most globally manifested expeditious diagnostic tools for early detection of AD' disease is outlined.

Once AD symptoms are identified, here we disclose targeted therapeutics specifically tailored to AD patients including a survey of the vast clinically approved therapeutic modalities for restricting and even treating the symptoms of AD' disease. These arguments provide useful information in both understanding pathogenesis as well as accessing the novel treatment approaches for AD' disease.

Keywords:

Alzheimer's disease, pathogenesis, diagnosis and prognosis, therapeutic modalities.

Introduction

Alzheimer's disease was only named in the early twentieth century by German psychologist Alois Alzheimer, but the history of Alzheimer's disease may have started well before that. In Egypt, in the ninth century B.C., the Maxims of the Ptah Holy describe a form of Alzheimer's disease. A Roman physician, Claudius Galen, who lived from 130 to 200 A.D., recounts symptoms of age-related forgetfulness in his journals. And in fourteenth century in England, there was even a verbal test to check for forgetfulness. Today many countries have rising aging populations and are facing an increased prevalence of age related diseases, such as Alzheimer's disease (1). There is a study carried out in Assiut governorate in 1998, recorded the prevalence of Alzheimer's disease and other dementing disorders was a case per 100 population over the age of 60 and the age-specific prevalence tends to be doubled every 5 years (2).

Alzheimer's disease (AD) is a neurodegenerative disorder characterized clinically by progressive memory loss and subsequent dementia, and neuropathologically by senile plaques, neurofibrillary tangles and synapses loss (3). The abnormal accumulation of extracellular amyloid-beta peptide ($A\beta$) and the intracellular neurofibrillary tangles (NFTs) are believed to be responsible for the neuronal loss and the degeneration of the cholinergic system (4). Other essential abnormalities are gliosis, chronic inflammation, excitotoxicity and oxidative stress (5). The vast majority of patients diagnosed with AD also have cerebral amyloid angiopathy (CAA) (6).

Risk factors

The main risk factors for AD are age, age-related diseases such as cardiovascular disease, diabetes, obesity and cancer, low educational levels, head trauma and repeated exposure to aluminum and heavy metals such as copper, iron and zinc (7; 8). Aluminum is a prooxidant induced its neurotoxicity related to cell damage via free radical production and stimulation of A β oxidation. Moreover, aluminum has been shown to be colocalized with both the amyloid plaques and the neurofibrillary tangles in AD (9; 10).

Pathogenesis of Alzheimer's disease

Recent evidence indicates that oxidative stress occurs early in the progression of Alzheimer's disease, principally before the development of the hallmark pathologies, namely neurofibrillary tangles and senile plaques. Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles (11). Researchers from Mayo Clinic (USA) linked late onset of AD's disease to a locus on chromosome 10 that affects processing of the amyloid protein to form the amyloid plaques in the brain. Furthermore, Sigurdsson et al. (12) reported that genetic analyses of families with early-onset AD have revealed mutations in chromosome 21, within A β sequence, in addition to mutations within presenilin 1 and 2 genes. Most of these mutations lead to increased production of A β 1-42 and/or total A β .

99 Noteworthy, there is increasing consensus that the production and
100 accumulation of beta-amyloid ($A\beta$) peptide is central to the pathogenesis of
101 Alzheimer's disease **(13; 14)**

102 Amyloid-beta ($A\beta$) peptides derived from proteolytic cleavage of
103 amyloid precursor protein (APP) are thought to be a pivotal toxic species in
104 the pathogenesis of Alzheimer's disease (AD) **(15)**. Proteolytic processing of
105 APP occurs through two different pathways, a non-amyloidogenic and an
106 amyloidogenic pathway **(16)**

107 In the nonamyloidogenic pathway, the cleavage of APP is initiated by
108 α -secretase in the middle of $A\beta$ peptide thus inhibiting the generation of $A\beta$
109 **(17)**. During this cleavage a soluble ectodomain of APP ($sAPP\alpha$) is released
110 and a 10 kDa C- α terminal fragment (α -CTF) remains within the membrane
111 **(18)**. The soluble ectodomain of APP is known as a neurotrophic and
112 neuroprotective peptide **(19)**. Also, a further action of γ -secretase on C- α
113 terminal fragment (α -CTF) generates a nonamyloidogenic peptide p3 **(17)**.
114 At least 30% of APP is processed by this pathway **(20)**. However, in the
115 pathological amyloidogenic pathway $A\beta$ is produced by the cleavage of APP
116 via the action of two aspartyl proteases, β - and γ -secretases **(17)**. Beta-
117 secretase cleaves the APP, thereby generating the N-terminal $A\beta$ peptide
118 **(16)**. The remaining membrane bound C- β terminal fragments (β -CTF) of
119 APP are substrates for γ -secretase. Cleavage of β -CTF at different sites by γ -
120 secretase leads to the formation of $A\beta$ that varies in its length **(16)** and are
121 released into extra- or intracellular space **(4)**. In both pathways, APP
122 intracellular domain (AICD) is released into the cytosol where it participates
123 in gene transcription. The main variants are 40 and 42 amino acids long and

are called A β (1–40) and A β (1–42), respectively (16). Under normal conditions, about 90% of secreted A β peptides are A β 40, which is a soluble form of the peptide that only slowly converts to an insoluble β -sheet configuration and thus can't be eliminated from the brain. In contrast, about 10% of secreted A β peptides are A β 42 species that are highly fibrillogenic and deposited early in individuals with AD (20).

The apolipoprotein E ϵ 4 genotype, a major risk factor for Alzheimer's disease, leads to accelerated deposition of amyloid, and the generation of anti-amyloid antibodies in humans with Alzheimer's disease seems to ameliorate the disease process (21; 22; 23; 24; 25). Apolipoprotein E (APOE) genotype influences the average age at which AD pathology begins and thus may account for the risk of when dementia onset occurs later (26). Alternate hypotheses regarding the pathophysiology of Alzheimer's disease place great emphasis on the potential role of tau-protein abnormalities, heavy metals, vascular factors, or viral infections (27).

Dwyer et al. (28) reported that elevated brain homocysteine level is involved in iron dysregulation/oxidative stress cycle that has a central role in the pathogenesis of AD. Also, hydrogen sulphide (H₂S), a neuromodulator agent, is severely reduced in AD as a result of reduced activity of the enzyme cystathionine- β -synthase (C β S) which is the source of H₂S in the brain. Sulphydral compounds like homocysteine are electron donors in mixed function oxidation system, acting with the transition metals iron and copper, and thus by interfering with iron sequestration by ferritin *in vivo*, would explain the increase in redox-active iron in AD neurons with

concomitant oxidative stress with subsequent trigger accumulation of amyloid in the AD brain (28).

Smith et al. (29) found AD is often associated with abnormal localization of iron regulatory proteins (IRP's). Specifically, alteration of IRP-2 might be directly linked to impaired iron homeostasis, leading to neurofibrillary tangles, senile plaques and neuropil threads in AD patients.

Oxidative stress, the direct result of the imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and intracellular antioxidant defences, is invariably involved in the onset of diabetes and neurological pathologies such as Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis (30 ; 31; 32).

The major species responsible for oxidative stress is the overproduction of ROS and RNS, a major source of which is mitochondrial dysfunction (33). ROS which include superoxide anion radical and hydroxyl radicals, are involved in the damage of lipids, DNA, and protein modifications. Minor modifications of the nucleic acid bases are repaired through base excision repair involving DNA glycosylase and AP endonuclease, which are located in nuclei and mitochondria. The progression of AD is associated with the diminished expression of these DNA repair enzymes (34; 35). The accumulation of the oxidatively damaged nucleic acids and proteins likely exceeds the limit of cellular repair and detoxification mechanisms and leads to the onset or progression of diabetic and neurological pathologies. In general, accumulations of oxidatively damaged proteins, lipids, and nucleic acids correlate with the onset of age-related diseases, especially in diabetes

and AD, indicative of one and the same common pathological mechanisms (36).

Amyloid beta protein (A β) in Alzheimer's disease induces oxidative stress through several mechanisms, including stimulation of nitric oxide synthase (NOS) activity (37). NO synthase synthesized nitric oxide (NO) through converting L-arginine to L-citrulline. NO caused brain lesion development and further progression of brain pathology and dementia (38). Peroxynitrite, a powerful oxidant produced as a result of diffusion limited reaction of superoxide anion O_2^- with nitric oxide in AD's disease. Peroxynitrite also causes the nitration of tyrosine residues so that nitrotyrosine immunoreactivity is increased in the neuronal cytoplasm of the cerebral cortex within regions of neurodegeneration in AD brains (39). Also, Alzheimer's disease induces oxidative stress by stimulating the hydrogen peroxide (H_2O_2) generation in isolated neocortex mitochondria, decreasing the activities of catalase and glutathione peroxidase in mitochondria (40). Hence, it is thought that oxidative stress may be an underlying mechanism in AD, and agents that prevent oxidative damage may be particularly efficacious in the treatment of AD (41; 42).

Cell dysfunction and cell death in nuclear groups of neurons, responsible for maintenance of specific transmitter systems, lead to deficits in acetylcholine, norepinephrine, and serotonin (43; 44). Alzheimer's disease is characterized by a dysfunction of central cholinergic systems. Acetylcholine (ACh) is the only classical neurotransmitter that, after release into the synaptic cleft, is inactivated by enzymatic hydrolysis. Acetylcholinesterase (AChE) was identified as the enzyme responsible for termination of

197 cholinergic transmission by cleavage of Ach to acetate and choline. AchE is
198 found in cholinergic synapses in the brain as well as in autonomic ganglia,
199 the neuromuscular junction, and the target tissues of the parasympathetic
200 system (45; 46).

201 Excitotoxicity is also considered to play an important role in the
202 pathogenesis of AD. It is triggered by excessive stimulation of glutamate
203 receptors (e.g. N-methyl-D-aspartate NMDA) due to either increased release
204 or decreased uptake of excitatory amino acids, mostly glutamate (47).
205 Alzheimer's disease brain is associated with glutamate receptor
206 overstimulation, since the moderate affinity NMDA receptor antagonist
207 memantine shows therapeutic efficacy in Alzheimer's disease. Many
208 investigations using cultured cortical neurons demonstrated that A β induced
209 cell damage by increasing glutamate release (48). As ion-channel glutamate
210 receptors are up-regulated following exposure to A β and their
211 overstimulation may lead to further oxidative events that account for
212 cumulative neural damage and death. The A β and excessive glutamatergic
213 tone may act synergistically in a reinforcing manner to induce oxidative
214 stress (49). Moreover, activation induced by glutamate results in massive
215 calcium entrance by NMDA-dependent channels with the consequent
216 triggering of apoptotic mechanisms (50).

217 It clearly indicates that neuronal dysfunction, not death, is the primary
218 event in Alzheimer's dementia. Alteration in the precisely regulated calcium
219 homeostasis is one of the known causes of neuronal malfunction. Proper
220 calcium signaling is of the most crucial importance for synaptogenesis and
221 dendritic spines plasticity. Cognitive decline and neurodegeneration of

synapses are the first symptoms of AD (51). In brain, calcium dysregulation is considered to be capable of eliciting increased A β formation and tau phosphorylation (5). Pierrot et al. (52) demonstrated that the increase in the cytosolic calcium concentration induces transient phosphorylation of APP and tau, leading to an increased production of intracellular A β . Amyloid- β oligomers have been shown to rapidly elevate intracellular calcium levels in human neuroblastoma cells and increase general membrane permeability and membrane conductance (53). It was indicated that A β triggers calcium release from endoplasmic reticulum (ER) stores by ryanodine receptor (54). Cytosolic calcium concentration can be additionally increased by the formation of cation selective ion channels by A β (55). Moreover, the mechanism by which A β disrupts intracellular calcium homeostasis is related to its ability to form reactive oxygen species that may induce membrane lipid peroxidation (56). This causes the alterations in membrane properties and affects the function of membrane transporters and ion channels leading to an elevation of intracellular calcium levels (57). These observations strongly suggest the existence of a positive feedback loop between A β generation and an elevated level of calcium ions in the cytosol (58). Calcium/calmodulin-dependent protein kinase II phosphorylates tau at the site of its interaction with microtubules (59). Moreover, it has been found that membrane depolarization of cultured neurons induces calcium-mediated transient phosphorylation of tau, followed by a dephosphorylation of this protein (52). This clearly demonstrates calcium-dependent regulation of tau phosphorylation status (60). Under pathological conditions, an increase in intracellular calcium results in overload of Ca²⁺ in mitochondria, causing mitochondrial dysfunction manifested by increased production of

ROS, deficiencies in key enzymes of energy metabolism and glucose utilization, most consistently α -ketoglutarate dehydrogenase and pyruvate dehydrogenase and also reduced glucose transport activity for the vessels in AD (61), release in cytochrome *c*, and eventually apoptosis (62).

In recent years, a number of studies have investigated the potential role of various metal ions in the pathogenesis of Alzheimer's disease (AD) (63) and there is an Egyptian study carried out in Alexandria University Hospital in 1993 revealed a significant increase in the serum aluminum level in patients with Alzheimer's disease which supports the hypothesis that aluminum may be implicated in Alzheimer's disease in some form. Environmental exposure to Al was hypothesized to be related with the etiology and pathogenesis of AD (64). The potential association of aluminum and AD began with the observations of **Klatzo et al.** (65) who demonstrated neurofibrillary degeneration in rabbits following aluminum exposure. **Walton** (66) hypothesizes that Al could be implicated in the formation of neurofibrillary tangles in the brain and should therefore be considered as a causative factor in AD. This is because it induces misfolding and self-aggregation of highly phosphorylated cytoskeletal proteins such as neurofilaments or microtubule associated proteins which are implicated in AD (67).

Aluminum is found associated with amyloid-beta in the brains of AD patients (68). Since Al is a trivalent cation (Al^{3+}), it can interact with acidic groups of the peptides, and bind these peptides with one another (69). Also, Al causes the conformational change of A β peptides into the beta-sheet structure *in vivo* and *in vitro* (70).

AD involves a progressive mental deterioration manifested by cognitive impairments. The relationship between occupational Al exposure and the possible impairment of cognitive performance was also assessed (71; 72). It inhibits long-term potentiation, causes synaptic structural abnormalities, thereby resulting in profound memory loss (73). Some epidemiological studies indicate that occupational Al exposure can produce behavior impairments (71).

Aluminum is a potent cholinotoxin (74) and reduced cholinergic function (75). It causes apoptotic neuronal loss *in vivo* as well as *in vitro* (73) which is a characteristic symptom of neurodegeneration associated with AD (76).

Oxidative induced neuronal damage was shown to be one of the important mechanisms to indicate that aluminum has an association with the etiology of Alzheimer's disease (77). Aluminum is a nonredox active metal which is capable of increasing the cellular oxidative milieu by potentiating the prooxidant properties of transition metals such as iron and copper (78). An aspect of the biochemistry of this nonredox active metal is its prooxidant activity, which might be explained by the formation of an Al superoxide semi-reduced radical ion ($\text{AlO}_2^{\cdot 2+}$) (79). It has been shown that chronic aluminum exposure is involved in the impairment of mitochondrial electron transport chain (ETC) and increased production of ROS (80). Reactive oxygen species (ROS) interact with all biological macromolecules, including lipids, proteins, nucleic acids, and carbohydrates. The resulting stress increases neuronal death, which contributes to the neuropathology associated with several diseases (81). Also, Al induced depletion of glutathione (GSH)

298 and reduction of activity of glutathione peroxidase (GPx), glutathione S-
299 transferase (GST) and catalase (CAT) (82).

300 **Diagnosis of Alzheimer's disease:**

301 Even though there is a large literature demonstrating altered levels of a
302 range of biomarkers in patients with AD, attempts to identify a single
303 biomarker specific to AD have failed (83).

304 Biomarkers currently under investigation for the early diagnosis of AD
305 include: brain volume or activity measurements derived from neuroimaging
306 techniques, such as positron emission tomography (PET) or magnetic
307 resonance imaging (MRI) and chemical indices detected in various body
308 fluids (84). He states that decreased β -amyloid₁₋₄₂ and increased phospho-tau
309 protein levels in the cerebrospinal fluid (CSF), when measured together,
310 exhibit sensitivities and specificities in the range 80% -90%, and are
311 currently the most accurate chemical neurodiagnostics of sporadic AD.
312 Other candidate chemical biomarkers of the disease currently commercially
313 available are urine AD7C-neuronal thread protein, and CSF and urinary F₂ –
314 isoprostanes. Yet, CSF examination by Lumbar puncture is now invasive.
315 Carriers of the apolipoprotein E (APOE) ϵ 4 allele, a genetic modifier, are at
316 increased risk for the development of sporadic AD (84).

317 **O'Bryant et al. (83)** reported that the serum protein-based algorithm
318 biomarkers can be combined with clinical information to accurately classify
319 AD patients. These markers consistently distinguished AD cases from
320 controls in significant analysis of microarray, logistic regression and
321 Wilcoxon analyses, suggesting the existence of an inflammatory-related

endophenotype of AD that may provide targeted therapeutic opportunities for this subset of patients. **O'Bryant et al. (85)** reported that the serum BDNF levels are not significantly different between AD patients and controls and suggested that serum BDNF is not a useful marker of AD disease status.

Munzar et al. (86) reported that AD7C-NTP (neural thread protein 41-kD) is a brain protein that is selectively elevated in AD disease and is associated with the pathologic changes of AD, and overexpression of AD7C-NTP is associated with the cell death similar to that found in the AD brain. Urinary AD7C-NTP is validated as a sensitive biomarker for AD and has significant clinical usefulness **(87)**.

Georganopoulou et al. (88) demonstrated that the ultra high-sensitivity nanoparticle-based bio-barcode assay was used to measure the concentration of amyloid- β -derived diffusible ligands (ADDLs), a potential soluble pathogenic AD's disease marker in the CSF of AD patients. This method can be used to measure the concentration of the pathogenic ADDL in CSF at clinically relevant concentrations and proved that elevated levels of ADDLs correlate with the presence of AD disease and it points toward a potential reliable detection method for diagnosing AD that is faster, higher throughput, and less expensive than current imaging techniques.

Nagele et al. (89) demonstrated that AD can be linked to characteristic alterations in serum autoantibody expression profiles, and by using only 10 autoantibody diagnostic biomarkers, AD serum samples were readily distinguished from non-demented control (NDC) sera with a sensitivity of 96% and a specificity of 92.5%. This approach proved to be useful for AD

diagnosis throughout the full course of the disease, and may also be useful for early detection, perhaps including patients with mild cognitive impairment (MCI) and pre-symptomatic disease. They concluded that the relative non-invasiveness, low cost, and dynamism of protein microarrays make a diagnostic of this kind well-suited for incorporation into routine health care.

Recent research has shown that when the exosomes, present in the cerebral spinal column, contain high levels of tau and other proteins, that gives an indicator of advanced stages of Alzheimer's disease and may become revolutionary in diagnosing AD's disease in its early onset.

Therapeutic modalities for Alzheimer's disease:

Sigurdsson et al. (12) reported that to date there is no cure or effective therapy for reducing a patient's amyloid burden or preventing amyloid deposition in Alzheimer's.

Positron Emission Tomography (PET) scans have shown that patients with AD treated with genetically engineered tissue expressing nerve growth factor protein (NGF), inserted directly into their brains, realized decrease in the rate of cognitive decline and increased metabolic activity in the brain. Also, the PET scan showed an increase in the brain's uptake of glucose, a sign of increased brain activity. Evaluation of the Mini-Mental Status Examination (MMSE) suggested improvement in the rate of cognitive decline and amyloid toxicity **(90)**.

Hook et al. (91) reported that the cysteine protease inhibitors CA074Me and E64d were selected to inhibit β -secretase activity in regulated secretory vesicles that produced β -amyloid represented by cathepsin β . Treatment of APP mice model, expressing the wild type WT β -secretase site, with these inhibitors resulted in marked improvement in memory deficit accompanied by reduced amyloid plaque load, decreased A β -40 and A β -42, and reduced C-terminal β -secretase fragment derived from APP by β -secretase. The notable efficacy of these inhibitors provides support for CA074Me and E64d as potential therapeutic agents for AD patients expressing the WT β -secretase APP site (91).

Wang et al. (92) found that when a naturally derived grape seed polyphenolic extract was administered to Tg2576 mice, expressing high molecular weight (HMW) A β oligomers, this polyphenolic preparation significantly attenuated AD- type cognitive deterioration coincidently with reduced HMW soluble oligomeric A β in the brain, and they concluded that grape seed-derived polyphenolics may be useful agents to prevent or attenuate AD's disease in humans.

Alvarez-Erviti et al. (93) investigated the therapeutic potential of exosome-mediated siRNA delivery demonstrated by the strong mRNA (60%) and protein (62%) knockdown of BACE1, a therapeutic target in Alzheimer's disease in wild-type mice.

Lim et al. (94) used the APP^{sw}(Tg2576) transgenic mouse model to evaluate the impact of dietary omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) on amyloid precursor protein (APP) processing and amyloid burden and showed that DHA-enriched diets

reduced A β -42 levels and markedly reduced total A β by 70%. It is also possible that eicosapentaenoic acid, the other prominent omega-3 fatty acid in fish oil induced transthyretin TTR mRNA levels in aged rats. They suggested dietary DHA could be protective against β -amyloid production, accumulation, and potential downstream toxicity (94).

Mohamd et al. (95) reported that estradiol administration to AlCl₃ – intoxicated rat model produced marked improvement in the most studied biomarkers and that the histological examination of the brain showed remarkable improvement in the feature of the neurons in addition to disappearance of amyloid plaques.

Pedrini et al. (96) found that both atorvastatin and semavastatin may be associated with a decreased risk for AD disease. Statin action is related to the drug's ability to activate α -secretase-cleaved soluble Alzheimer amyloid precursor protein ectodomain (sAPP _{α}). Statins also inhibit the isoprenoid pathway thus modulating the activities of the Rho family of small GTPase-RhoA,B and C. Rho proteins, in turn, exert many of their effects *via* Rho-associated protein kinases ROCKs. They suggest that the Rho/ROCK1 protein phosphorylation pathway might be involved.

Ahmed et al. (72) treated AlCl₃ –intoxicated animal model with either one of coenzyme Q₁₀, vitamin B complex or lethicin or a combination of all of them for three months and observed marked improvement in most of the biochemical markers and regression in neurological damage as indicated through histopathological examination of the brain tissue. Noteworthy, the combined nutraceuticals therapy revealed more pronounced role in delaying the progression of neurodegenerative disorders.

Carman et al. (97) reported that adenosine receptor (AR) signaling modulated the blood brain barrier (BBB) permeability *in vivo*. AR activation facilitated the entry of intravenously administered macromolecules including large dextrans and antibodies to β -amyloid. Treatment with an FDA-approved drug “Lexiscan” also increased BBB permeability in murine models. Treatment with a broad-spectrum AR agonist, intravenously administered anti- β -amyloid was observed to enter the CNS and bind β -amyloid plaques in a transgenic mouse model of AD’s disease. These results will aid in drug delivery and treatment options for neurological diseases such as AD.

Epidemiologic studies revealed that long-term use of a select of nonsteroidal anti-inflammatory drugs (NSAID) such as dapsone, meclofenamic acid and enantiomers of flurbiprofen reduced risk for the development of AD and lowered $A\beta$ -42 levels to greatest extent by targeting γ -secretase and reduced side effects related to inhibition of cyclooxygenase (COX). These drugs are excellent candidates for clinical testing to lower $A\beta$ -42 levels in Alzheimer’s patients **(98)**.

Shalby et al. (99) reported that treatment of AD that is induced in female rats with either one of the nutraceuticals: vitamin E, acetyl-L-carnitine (ALC) or α -lipoic acid (LA) restored the biomarkers: total homocysteine (tHcy), insulin, insulin-like growth factor-1 (IGF-1), interleukin 1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) to near normal levels as compared to those achieved by using the drug “donepezil”. These results provide evidence for the importance of dietary supplements in delaying the progression of age-related neurodegenerative diseases as AD.

Metwally et al. (100) investigated the effect of nattokinase (Natto) on growth factors in the brain of AD's disease induced in animal model for three months. They observed remarkable improvement in the biochemical parameters reflected by decreased brain transforming factor- β (TGF- β) level with significant increase in brain derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) levels. Histological examination of the brain tissue showed improvement in the brain morphological structure and disappearance of most of the amyloid plaques.

Researchers from the University of Sheffield used lasers to fabricate intricate scaffolds from a commonly used polymer, polylactic acid (PLA). These synthetic biocompatible materials degrade in the human body to form lactic acid that can easily be removed leaving the regenerated tissue behind in the required size, shape and structure and demonstrated their ability to effectively harness the growth of neuronal cells. The fabrication of these scaffolds is a vital step in the process of tissue engineering and must be fine-tuned if treatments for Alzheimer's and Parkinson's diseases are to be realized (101).

The protein produced by the KCC2 gene, a member of the cation-chloride cotransporter gene family, acts to prevent damage to neurons caused by neurodegenerative diseases such as AD. Artificial gain of KCC2 induced by overexpression of KCC2 gene function in preventing excitotoxicity and protect neurons from death (102).

The extracts of both blackcurrants and boysenberries containing anthocyanins and polyphenolics act as antioxidants and can protect against Alzheimer's by influencing the early gene expression in learning and

memory, which influences cell signaling pathways that help neuronal cells communicate with each other (103).

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