Sys Rev Pharm 2023; 14(6): 383-392
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453 / DOI: 10.31858/0975-8453.14.6.383-392

Review Article

An Overview on Biomarkers of Neurodegenerative Disease: Brain Aging

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Article History: *Submitted: 29.05.2023* *Accepted: 23.06.2023* *Published: 30.06.2023*

ABSTRACT

Brain aging is a neurodegenerative disorder, whose prevalence has increased worldwide. According to World Health Organization (WHO) guidelines, dementia due to neurodegenerative disorder is 22%, i.e., ≥ 80 million people among the world's population are affected by aging. According to a survey in 2010, 8% of the Indian population was affected by aging, which may reach 19% by 2050. Neurodegenerative damage contributes to persistent diseases such as Alzheimer's disease, Parkinson's disease and stroke in the aging brain. Functional impairment in cognition is mainly due to the formation of Amyloid beta (Aβ) plaques and neurofibrillary tangles in the brain. The main causes of brain aging are the generation and accumulation of free radicals, i.e., Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), gene regulation, mitochondrial dysfunction, apoptosis, and telomere shortening. A recent approach to overcome aging is to identify novel biomarkers, such as urinary and molecular biomarkers.

In the present review, we emphasize different theories and biomarkers of ageing that lead to neurodegeneration, which helps in identifying the severity and progression of brain aging in healthy and diseased people. From this review, we learn about the long-term goal of identifying new therapeutic targets in drug discovery that can reduce the prevalence rate of neurodegeneration.

Keywords: Amyloid beta (Aβ) plaque, Dementia, Theories of aging, Molecular biomarkers, Neurodegeneration

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ABBREVATIONS

AchE: Acetylcholinesterase Enzyme; AD: Alzheimer's Disease; APP: Amyloid Precursor Protein; CSF: Cerebrospinal Fluid; DNA: Deoxyribonucleic Acid; GABA: Gamma Amino Butyric Acid; HbA1C: Glycated Heamoglobin or Heamoglobin A1c; HPA: Hypothalamic Pituitary Adrenal Axis; IGF-1: Insulin like Growth Factor 1; NMDA: N-Methyl D-Aspartate; RNS: Reactive Nitrogen Species; ROS: Reactive Oxygen Species; TACE: Tumor necrosis factor Alpha Converting Enzyme; TNF: Tumor Necrosis Factor

INTRODUCTION

Brain aging is a neurodegenerative disorder that is associated with cognitive decline due to pathophysiological processes in the brain (Morel GR, *et al*., 2017). Alzheimer's disease is also a neurodegenerative disease. These two are the leading causes of dementia/cognitive impairment (Shiel Jr WC, 2016). Recent statistics state that almost 46.8 million people are affected by these diseases worldwide at the age of greater than or equal to ≥ 65 years (Gleerup HS, *et al*., 2019). This prevalence will increase to approximately 88 million people by 2050 (Alzheimer's Association, 2019). Aging is the major cause of Alzheimer's disease. Aging is mainly due to the generation of reactive oxygen species, which affect the organelles of mitochondria (Yankner BA, *et al*., 2008). Aging is mainly due to increased free radical generation, which affects mitochondria. These reactive oxygen species damage mitochondrial DNA, which causes a loss of mitochondrial function, and interferes with the production of Adenosine Triphosphate (ATP) and the metabolism of energy (Nikhra V, 2017). Another cause of neurodegeneration due to aging is the formation of amyloid-beta plaques (Gleerup HS, *et al*., 2019; Swerdlow RH, 2011) and tangles by tau phosphorylation (Gleerup HS, *et al*., 2019). Neuronal cell death leads to neurodegeneration and includes Alzheimer's disease, Parkinson's disease, and mild cognitive impairment (Nikhra V, 2017). Cognitive decline is mainly due to the degeneration of the temporal lobe, frontal lobe, parietal lobe, visual cortex and hippocampus (Nikhra V, 2017; Panizzutti R, *et al*., 2014).

Neuronal changes with aging

Not all brain regions are affected to the same extent (Panizzutti R, *et al*., 2014). The frontal and temporal lobes are affected more than the occipital and parietal lobes (Gleerup HS, *et al*., 2019; Lockhart S, *et al*., 2014). Shrinkage of gray matter and loss of white matter are expressed with age (Panizzutti R, *et al*., 2014; Lockhart S, *et al*., 2014). Recent studies state that synaptic dysfunction leads to aging (Azpurua J and Eaton BA, 2015). Strong evidence of aging and Alzheimer's Disease (AD) is due to the complete loss of synapses, which was found in recent studies (Azpurua J and Eaton BA, 2015).

Role of brain neurotransmitters

Alterations of neurotransmitters and their receptors in different regions of the brain take place during the aging process (Nikhra V, 2017). Excitatory and inhibitory amino acids play a role at synapse glutamate, and Gamma-Aminobutyric Acid type A ($GABA_A$) shows excitatory and inhibitory action at the synapse (Rissman RA, *et al*., 2007).

Dopamine plays a role in cognitive control and the reward pathway. A decrease in dopamine levels takes place from adulthood. Therefore, decreased dopamine levels with age lead to cognitive and neurological decline (Nikhra V, 2017). N-methyl-D-aspartate (NMDA) receptors, which are excitatory in function, act on learning and memory. A recent review states that cognitive impairment in ageing is due to decreased NMDA receptors (Panizzutti R, *et al*., 2014). Acetylcholinesterase breaks down the acetylcholine into acetate and choline molecules. AchE enzyme levels are altered/raised during aging (Sirviö J and Riekkinen PJ, 1992).

LITERATURE REVIEW

Theories of aging

Many theories have explained the aging process (Davidovic M, *et al*., 2010). Theories of aging are broadly categorized into two

categories: Programmed and error theories (Sergiev PV, *et al*., 2015). Three subdivisions exist in programmed theory and they are a) programmed longevity, b) neuroendocrine theory and c) immune theory (Jin K, 2010). The error theory contains (a) wear and tear theory, b) theory of free radicals, c) rate of living theory, d) cross-linking theory, e) gene regulation, f) somatic mutation theory, g) apoptosis and h) cellular senescence/telomere theory (Jin K, 2010; Weinert BT and Timiras PS, 2003) (*Figure 1*).

Programmed theory

Program of longevity: This theory says that people who face moderate stress during the starting stage of life, have a long life. Moderate stress includes environmental variations and dietary habits (high-calorie diet) (Davidovic M, *et al*., 2010; Jin K, 2010; Kahn A and Olsen A, 2010). Therefore, consuming a low caloric diet delays the aging process.

Neuroendocrine theory: Central Nervous System (CNS) and ductless glands together called neuroendocrine systems. The endocrine system is a part of the cerebrum, i.e., the hypothalamus acts as a control centre, which regulates several functions by secreting some hormones. Hypothalamic-Pituitary-Adrenal (HPA) plays a major role in the response to stress (Jin K, 2010; Weinert BT and Timiras PS, 2003; van Heemst D, 2010). After several studies on primates, we gained information about the overactivity of the HPA axis (Aguilera G, 2011). HPA axis overactivity leads to neuronal degeneration associated with aging.

Immune theory: According to this theory, the immune system progressively increases in puberty and then gradually declines its function. Due to a decrease in immunity, there is decreased protection against infectious diseases. Therefore, the immune system plays a role in aging (Jin K, 2010; Weinert BT and Timiras PS, 2003; Fulop T, *et al*., 2014). Recently, researchers have been working in this view by enhancing immune action in older people, the aging process is delayed (Fulop T, *et al*., 2014; Park DC and Festini SB, 2017).

Error theory

Error theory includes free radicals, wear and tears, gene regulation, mitochondrial DNA damage, and the cellular senescence/telomere theory. This theory is also known as the damage theory, because it is due to progressive damage to tissues at various levels (Jin K, 2010; Weinert BT and Timiras PS, 2003).

Wear and tear theory: Essential parts of cells and tissues that wear out which leads to aging. The repeated use of body parts leads to wear out. This theory was first explained by Weismann A, a German biologist, in 1882 (Jin K, 2010; Weinert BT and Timiras PS, 2003).

Free radical theory: This is the best theory to explain brain aging. Free radical generation and accumulation cause oxidative damage to macromolecular components of the cell such as DNA, lipids and proteins (Jin K, 2010; Weinert BT and Timiras PS, 2003; Kumar H, *et al*., 2012). Free radicals consist of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). ROS include hydroxyl ions (OH^-), superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The generation of ROS and RNS is particularly the cause of neurodegenerative disorders (Kumar H, *et al*., 2012; Vina J, *et al*., 2013; Salminen LE and Paul RH, 2014). Free radical generation leads to mitochondrial dysfunction, which damages mitochondrial DNA, and causes cell death. This process leads to cognitive decline because white matter is affected by oxidative species (Vina J, *et al*., 2013; Salminen LE and Paul RH, 2014) (*Figure 2*).

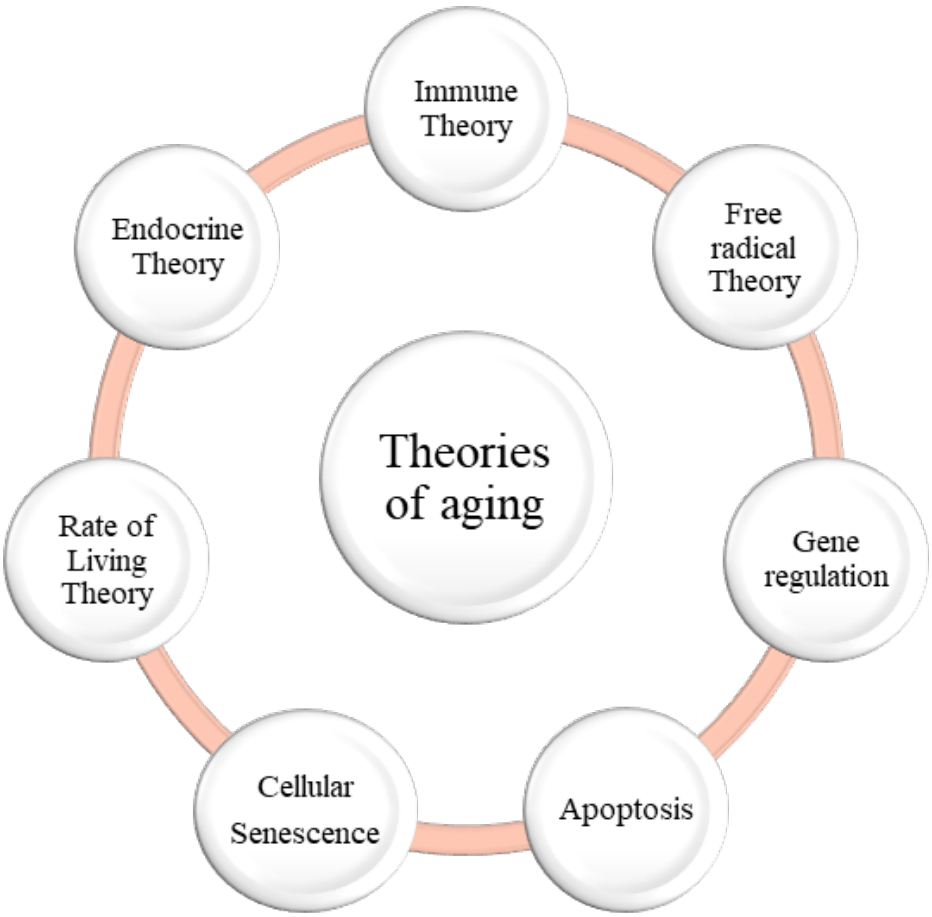

Figure 1: Different theories of aging

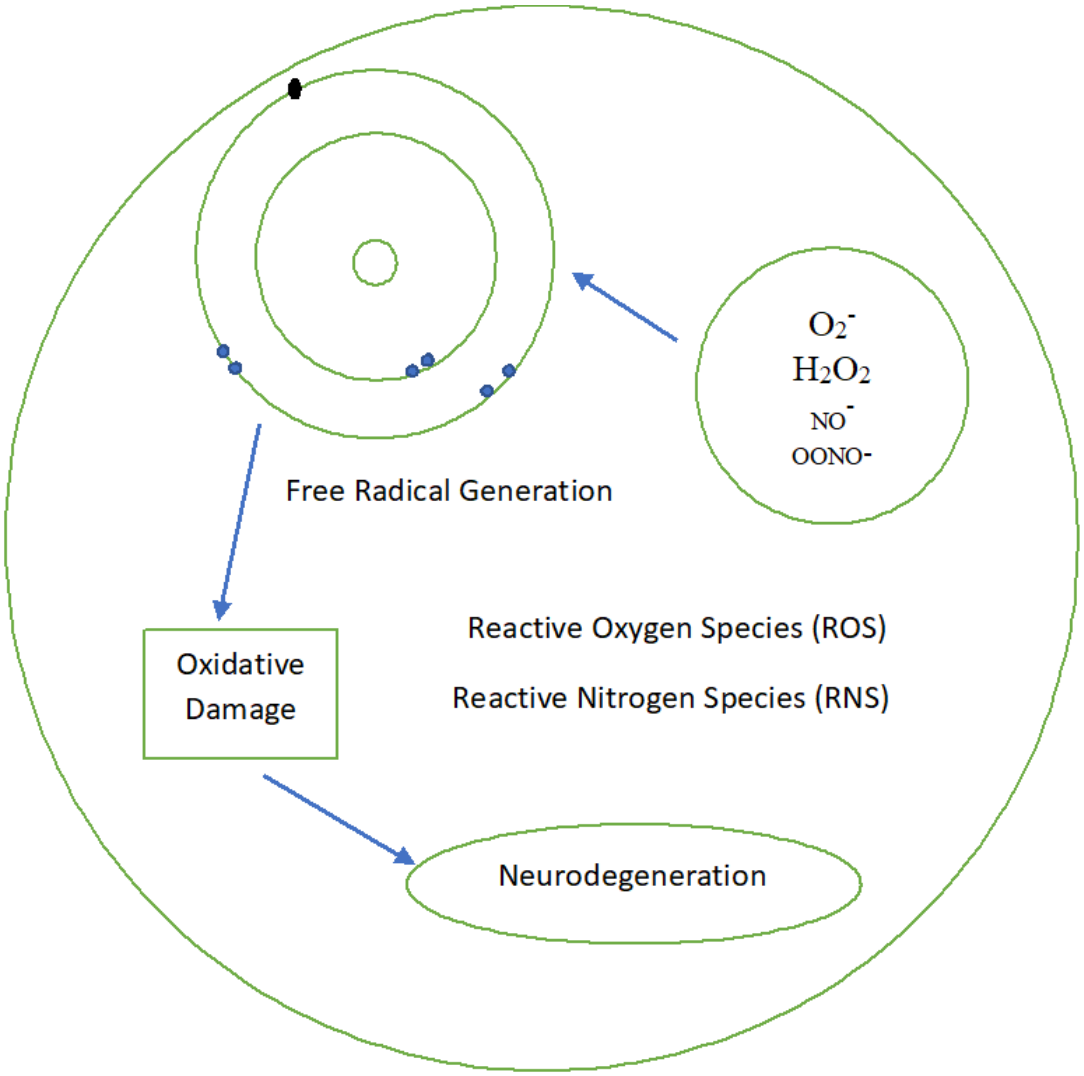

Figure 2: Brief representation of free radical theory of aging

Rate of living theory: This theory explains that, based on the metabolic potential of living organisms, assume the life span. Greater the metabolic potential, short term its life span. This theory cannot completely explain the reasons for the greater life span (Jin K, 2010; Weinert BT and Timiras PS, 2003; Vina J, *et al*., 2013; Brys K, *et al*., 2007; Hulbert AJ, *et al*., 2007).

Crosslinking theory: Aging is due to the assembly of cross-linked proteins that impair the functions of cells and tissues (Jin K, 2010; Bjorksten J and Tenhu H, 1990).

Gene regulation: Changes in gene expression lead to aging. Recent research in aging focuses on the Insulin-like Growth Factor-1 (IGF-1) pathway regulates the aging process in rodents (Weinert BT and Timiras PS, 2003; Tatar M, *et al*., 2003). IGF-1 has more than one action on the brain, such as neuroprotection and the production of neurons.

Somatic mutation theory: According to this theory, impairment of cellular function is due to mutations in somatic cells (Jin K, 2010; Weinert BT and Timiras PS, 2003). One of the causes of mutations in mitochondrial DNA (mtDNA) is increased production of reactive oxygen species, leading to neurodegeneration (Kennedy SR, *et al*., 2012; Schulz TJ, *et al*., 2007).

Apoptosis: Generally, it is called cell suicide or cell death. This theory explains that due to extensive damage to DNA or genetic events, aging takes place (Weinert BT and Timiras PS, 2003).

Cellular senescence/telomere theory: This phenomenon was established by Flick H, 1965. Cell senescence is the process that decreases the number of cell divisions compared with normal cells. After a few divisions of cells, cell division stops due to changes in function (Weinert BT and Timiras PS, 2003; Schulz TJ, *et al*., 2007; Ogrodnik M, *et al*., 2019). At one particular time, cell division stops permanently. This process is known as replicative senescence (Schulz TJ, *et al*., 2007; Davalli P, *et al*., 2016).

Telomere theory explains that the replication of cells stops when the length of the telomere decreases. Due to these cells die. Finally, it causes the death of the organism. The shortening of telomeres is one of the causes of neurodegeneration (Schulz TJ, *et al*., 2007; Ogrodnik M, *et al*., 2019; Davalli P, *et al*., 2016; Ferrón SR, *et al*., 2009; Strimbu K and Tavel JA, 2010).

Biomarkers of aging

According to National Institute of Health (NIH), biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention (Crimmins E, *et al*., 2008; WHO, 2001; Butler RN, Sprott RL, 2004; de Gruttola VG, *et al*., 2001) (*Table 1*).

Table 1: An illustrated table showing key biomarkers of aging

S.No	Biomarker category	Subcategory	Biomarker	Mechanism of aging	Method of estimation	Reference
1	CSF biomarkers	β-Amyloid	Aβ-42, Aβ-40	Aβ senile plaques	ELISA	Blennow K, *et al.*, 2001
		Tau	Total tau	Increased tau levels leads to neuronal death	ELISA	Buée L, *et al.*, 2000; Chai X, *et al.*, 2012
2	Blood based biomarkers	Aβ and APP	Amyloid-β Precursor Protein	Increased APP increases A β which causes neurodegeneration	ELISA	Roher AE, *et al.*, 2018
		Diabetes marker	HbA1c	Increased HbA1c leads to diabetes. Diabetes is the cause for cognitive decline	High-Performance Liquid Chromatography (HPLC)	Raval DK, *et al.*, 2011
		Hormonal marker	NTproBNP	Higher NTproBNP leads to lowest systolic pressure causes cognitive decline	Chemiluminescent Immunoassay	Daniels LB, *et al.*, 2011
			IGF-1	IGF-1 production declines with age. Major role of IGF-1 in cell proliferation which regulates aging process	ELISA	Gubbi S, *et al.*, 2019
		Renal biomarker	Cystatin C	Low serum cystatin C is a biomarker of future risk of AD and cognitive decline	ELISA or Radio Immunoassay (RIA)	Mathews PM and Levy E, 2016
		Inflammatory marker	IL-6	Increased IL-6 reduced total brain volume	ELISA	Ridker PM, 2003; Gorelick PB, *et al.*, 2011
			CRP	Increased CRP reduced total brain volume	Nephlometry and immunoturbidometry method	
			TNF-α	Increased TNF-α leads to apoptosis which causes aging	ELISA and Highly sensitive enzyme amplified lanthanide luminescence immunoassay	
3	Salivary biomarkers	β-amyloid	Aβ-42, Aβ-40	From CSF these are secreted in to saliva	ELISA	Farah R, *et al.*, 2018
		Tau protein	P-tau, T-tau	P-tau, T-tau protein levels increased in neurodegenerative disorder	ELISA	Ashton NJ, *et al.*, 2018
		Enzyme	AchE	Decreased AchE enzyme, increases the Acetylcholine concentration which leads to Aβ plaque formation	Ellman's colorimetric method	Inestrosa NC, *et al.*, 1996
4	Urinary biomarkers	Hopeful biomarkers	Methionine, Desaminotyrosine, 5-hydroxy indole acetic acid, Taurine, N1-acetylspermidine	These proteins are elevated due to oxidative stress, which is the main cause of aging	Nuclear magnetic resonance (NMR) based metabolomics and Liquid Chromatography-Mass Spectrometry (LC-MS) based metabolomics	An M and Gao Y, 2015
		Prior to onset of cognitive decline	3-hydroxykynurenine Homogentisate Allantoin	These proteins are elevated due to oxidative stress, which is the main cause of aging	NMR based metabolomics	Lovestone S, 2010
5	Molecular biomarkers	DNA and chromosome	Leukocyte telomere length	Telomere length decreases with aging	Neuroimaging	Xia X, *et al.*, 2017
		DNA damage	DNA repair	DNA repair slow down by aging and unrepaired DNA causes aging	Gas Chromatography-Mass Spectrometry (GS-MS), RIA, ELISA and electrochemical methods	Maynard S, *et al.*, 2015
		RNA transcriptome	Transcriptome profiles	Heterogeneity of T-cells decreases or increases with aging	Dynamic Transcriptome Analysis (DTA) method.	Dillman AA, *et al.*, 2017
		Micro RNAs	mi-34a, miR21, miR-1263P, miR-151a-3P, miR-181a-5P and miR-1248	miRNAs function post-transcriptionally by inhibiting translation from specific target miRNAs. These small RNA molecules were thought to contribute to ageing or miRNA cause a general reduction of message-specific translational inhibition during ageing	HITS-CLIP (High Throughput Sequencing to Cross-linking Immunoprecipitation) and Northern blot	Grammatikakis I, *et al.*, 2014; Pincus Z, *et al.*, 2017

Importance of biomarkers

Neurodegeneration is the main cause of brain aging and it occurs due to the presence of senile plaques and by the formation of neurofibrillary tangles (de Gruttola VG, *et al*., 2001; Rao P, *et al*., 2013). The importance of biological markers is to predict, diagnose and monitor health problems in the human population. Biomarkers detect the disease, before the onset of symptoms. It is helpful for studying cross-sectional and longitudinal studies in humans (Xia X, *et al*., 2017; Wiltfang J, *et al*., 2002). Aging can be predicted by biomarkers. Different categories of biomarkers are discussed below.

Cerebrospinal Fluid (CSF) biomarkers

CSF consists of biomarkers of neurodegenerative disease, in primates. Amyloid beta (Aβ) and Tau proteins that are present in CSF are biomarkers to estimate brain aging.

CSF Aβ: Aβ generally exists in many isoforms, i.e., Aβ1-42. Aβ1-40 is the most abundant isoform (Chen JA, *et al*., 2018; Masters CL, *et al*., 1985). Aβ1-42 is less abundant, but it is a major isoform that forms plaques in human brains (Haass C and Selkoe DJ, 1993). Amyloid Precursor Protein (APP) is a precursor and the proteolytic cleavage of APP generates Aβ (Buxbaum JD, *et al*., 1998). Aβ is cleaved from APP by the enzyme alpha-secretase. Researchers recently identified that ADAM (A Disintegrin and Metalloproteinase), ADAM 10 and Tumor necrosis factor-α-Converting Enzyme (TACE) also have alpha-secretase action (Lammich S, *et al*., 1999; Jarrett JT, *et al*., 1993). Aβ-42 aggregates more quickly than Aβ-40, which forms a senile plaque (Masters CL, *et al*., 1985; Blennow K, *et al*., 2001). Reduced levels of Aβ42 in CSF lead to aging. However, the decrease in AB-42 levels is due to the deposition of Aβ-42 as senile plaques (Motter N, *et al*., 1995; Tamaoka A, *et al*., 1997). It is little contemporary to find a strong correlation between levels of Aβ-42 and number of plaques. A recent study says that there is no change in CSF Aβ-40, but a marked decrease in Aβ-42 leads to aging, early AD and Mild Cognitive Impairment (MCI) (Fukuyama R, *et al*., 2000; Mehta PD, *et al*., 2000; Blennow K, *et al*., 1995). The most commonly used method to estimate CSF Aβ levels is Enzyme-Linked Immunosorbent Assay (ELISA) (Blennow K, 2004; Buée L, *et al*., 2000).

CSF tau: Tau protein is present in the axon of neurons. Six isoforms are based on size, i.e., 352 to 441 amino acids. Tau protein hyperphosphorylation leads to the formation of neurofibrillary tangles and senile plaques (Grundke-Iqbal I, *et al*., 1986; Franz G, *et al*., 2003).

An increase in CSF total Tau leads to neuronal death (Chai X, *et al*., 2012; Kohnken R, *et al*., 2000). It is also estimated by the ELISA method (Zetterberg H and Burnham SC, 2019).

Blood-based biomarkers

Biological markers in blood are present at very minute concentrations because the BBB (Blood Brain Barrier) prevents the entry of molecules between the central nervous system and blood compartments. However, some biomarkers related to neurodegenerative disorders are present in peripheral tissues, and are measured in the blood. Due to the pathological process of brain aging, other biological markers are estimated, i.e., inflammatory biomarkers, IGF-1, NT- and HbA1c (Justice JN, *et al*., 2018; Balducci C and Forloni G, 2014).

Plasma Aβ and Amyloid β Precursor Protein (APP): Recent studies have reported that the presence of APP (Amyloid β precursor protein) peripherally it increases Aβ. APP is a membrane protein that plays a major role in the growth and repair of neurons.

APP is increased, by the action of secretases. Aβ is capable of causing changes in the pathology of neurodegenerative diseases (Watts JC, *et al*., 2004; Mormino EC, *et al*., 2012; Roher AE, *et al*., 2017; Oh H, *et al*., 2014; Wagner KH, *et al*., 2016).

Inflammatory markers: Interleukin-6 (IL-6), CRP and Tumor Necrosis Factor- α (TNF-α) are collectively called inflammation markers (Wyss-Coray T and Rogers J, 2012). Inflammation also contributes to neurodegeneration (Tancredi V, *et al*., 2000). Neurodegeneration is due to neuronal apoptosis and synaptic plasticity and inhibits hippocampal neurogenesis (Balschun D, *et al*., 2004; Gu Y, *et al*., 2017; McCarty MF, 1999). According to Ridker PM and MC Carty, peripheral inflammatory markers cause cardiovascular diseases and these changes affect cerebrovascular pathology (Ridker PM, 2003; Gorelick PB, *et al*., 2011; Berelowitz M, *et al*., 1981).

IGF-1: Insulin-like Growth Factor-1 hormone is produced from both endocrine and autocrine cells. IGF-1 hormone production is high at early years of age and at the puberty stage. IGF-1 production declines with age (Yamamoto H, *et al*., 1991; Tarantini S, *et al*., 2016). IGF-1 plays a major role in cell proliferation, which regulates the aging process (Gubbi S, *et al*., 2018; Deak F and Sonntag WE, 2012; Dar B, *et al*., 2015).

Haemoglobin A1c (HbA1c): The glycated haemoglobin HbA1c test is used to monitor the blood sugar levels in diabetes. A recent study revealed that HbA1c also helps to identify age-accelerating glycation. Increased HbA1c leads to an increase risk of diabetes. Dar B reported that diabetes is one of the causes of cognitive decline and neurodegeneration (Wu L, *et al*., 2017; Raval DK, *et al*., 2011; van Vliet P, *et al*., 2014), which leads to aging.

N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP): It is an inactive form of pro-brain natriuretic peptide hormone. NT-proBNP is a marker of congestive heart failure and MI. However increased NT-proBNP levels are not specific due to CHF in normal people (Daniels LB, *et al*., 2011).

Daniels LB, *et al*., 2011; Feinkohl I, *et al*., 2012 and Marksteiner J, *et al*., 2014 stated that higher NT-proBNP levels lead to cognitive decline when compared to healthy subjects. Recent studies stated that both higher NT-proBNP and lowest systolic blood pressure lead to cognitive decline when compared with other subjects (Daniels LB, *et al*., 2011). NT-proBNP reflects cardiac, neurovascular and neurodegenerative etiologies (Filler G, *et al*., 2005).

Cystatin: Higher serum cystatin C levels indicate Chronic Kidney Disease (CKD), cancer, hypertension, rheumatoid arthritis, cardiovascular disease and neurodegeneration (Sundelöf J, *et al*., 2008). A recent study by Sundelof J reported that lower serum Cys C leads to an increased risk of AD, which is not dependent on age. This study indicates that low serum Cys C is a biomarker of future risk of AD and cognitive decline (Mathews PM and Levy E, 2016; Mandel ID, 1987).

Salivary biomarkers

Saliva is a physiological fluid that is secreted by salivary glands. It plays a role in the digestion of carbohydrates, antibacterial action, and lubrication.

Saliva collection for the estimation of biomarkers is easy, inexpensive and painless when compared with blood and CSF. CSF proteins such as Aβ-42, Aβ-40, and tau are excreted into saliva (Farah R, *et al*., 2018). The following proteins are estimated in the saliva:

β-Amyloid: Aβ-42 and Aβ-40 are estimated in saliva. A recent study by Farah R reported that Aβ-42 predicts familial genotype neurodegeneration (Lee M, *et al*., 2017). Aβ-40 did not show any variation between two-state control subjects (Ashton NJ, *et al*., 2018). Aβ-42 and Aβ-40 levels were estimated by ELISA.

Tau protein: Phosphorylated tau (p-tau) and total tau (t-tau) were detected in saliva. P-tau and t-tau levels are increased in Alzheimer's disease. However, there is no significant difference, due to the undefined source that secretes the biomarkers into the saliva (Lau HC, *et al*., 2015; Whitehouse PJ, *et al*., 1981).

Acetylcholinesterase (AchE) activity: AchE is an enzyme that degrades

the acetylcholine neurotransmitter at the synapse. Recent research reported that the AchE enzyme decreases with age. Decreased AchE leads to an increased acetylcholine concentration, which damages neurons by enhancing Aβ-plaque formation (Inestrosa NC, *et al*., 1996; Rees T, *et al*., 2003; Boston PF, et al., 2008). Decreased AchE *i*s a biomarker of brain aging, which was estimated by Ellman's colorimetric method (van der Strate BW, *et al*., 2001).

Other biomarkers

Lactoferrin (Huan T, *et al*., 2018), spinganine-1-phosphate, ornithine and phenyllactic acid (Liang Q, *et al*., 2015), and inosine-3-dehydrocarnithine and hypoxanthine (Mucke L, 2009) are other biomarkers in saliva.

Urinary biomarkers: Neurodegenerative disorders were diagnosed by the estimation of biomarkers in CSF that take place at the late-stage of the disorder. Therefore, early diagnosis of neurodegenerative disorders can be diagnosed by the estimation of markers in urine.

Urinary biomarkers can be estimated in transgenic animal models (Fukuhara K, *et al*., 2013; Lovestone S, 2010). Biomarkers before the onset of cognitive decline are hydroxy-kynurenine, homogentisate, and tyrosine (An M and Gao Y, 2015). Other markers in urine are 1-methyl nicotinamide, dimethylamine, trigonelline, dimethylamine, citrate, urea, and 2-oxoglutarate, which are identified at the late stage of the neurodegenerative disorder (An M and Gao Y, 2015; Bratic A and Larsson NG, 2013).

Methionine, desaminotyrosine, taurine and N1-acetylspermidine are promising biomarkers (An M and Gao Y, 2015).

Molecular biomarkers: Alterations at the molecular level lead to aging. Molecular biomarkers predict and monitor the aging process (Kirkwood TB, 2005). Molecular mechanisms contributing to aging include, DNA damage, oxidative stress, changes in RNA expression and telomere shortening (Sedelnikova OA, *et al*., 2004; Dollé ME, *et al*., 1997).

Free radical theory is a common cause of DNA damage, mitochondrial dysfunction, and telomere shortening (Hayflick L, 2007).

DNA damage: DNA damage occurs due to free radical generation and accumulation. DNA repair slows down with aging. Unrepaired DNA damage causes genomic instability and aging. Damaged DNA causes mutations that affect neurons (Maynard S, *et al*., 2015).

Telomere shortening: Telomeres are located at the end of chromosomes, and after each replication, they become shorter. The length of the leukocyte telomere indicates aging. Leukocyte telomere length decreases with age (Nakamura KI, *et al*., 2007). Recent studies by Nakamura KI, stated that changes in telomere length reported a positive correlation with age in 40 older individuals (Lukens JN, *et al*., 2009). Longer telomere length is greater than 60 years, and increases life span (Cawthon RM, *et al*., 2003). Shorter telomere length reflected mortality in humans for less than 60 years (Leal SL and Yassa MA, 2015).

Ribonucleic Acid (RNA) and transcriptome: RNA quality, i.e., the DNA sequence, is one of the markers of brain aging (Dillman AA, *et al*., 2017). The RNA sequence contains a relatively large number of detected genes. Dillman AA reported that changes in RNA-sequence affect neurotransmitters at synapses, i.e., both excitatory and inhibitory neurotransmitters (Grolleau-Julius A, *et al*., 2010). Gene expression in the brain was affected by heterogeneous cellularity. RNA sequencing provides good insight into brain aging.

microRNAs (miRNAs): It is a noncoding RNA. Recent studies on miRNAs state that, they can be present in peripheral tissues, which can be used to identify changes in the origin of cells (Hooten NN, *et al*., 2013). miRNA is considered a significant biomarker of brain aging (Grammatikakis I, *et al*., 2014).

Two sources of RNA exist peripherally extracellular RNA and Peripheral Mononuclear Blood Cells (PBMCs). Blood, plasma and CSF help to develop miRNA markers for neurodegenerative disorders such as AD, brain aging and other neurological diseases (Pincus Z, *et al*., 2011).

Different miRNA markers are identified as miRNA-34a, miR-21, miR-126-3p, miR-151a-3p, miR-181a-5p and miR-1248 (Li X, *et al*., 2011). Recent work on miRNA-34a was reported by Li X, miRNA-34a is a tumor suppressor in the brain, and its absence leads to the development of tumors in the brain (Wagner KH, *et al*., 2015). However, a sharp increase in miRNA-34a is a noninvasive marker of neurodegeneration and age-dependent brain decline. Therefore, miRNA-34a is considered an early biomarker for changes in the brain (Pincus Z, *et al*., 2011; Wagner KH, *et al*., 2015).

Novel biomarkers

Recently, some biomarkers have been identified. Bilirubin is a novel marker for aging (Simm A, *et al*., 2015). Advanced Glycation End products (AGEs) a marker for age-related diseases, including neurodegeneration (Sharma S, *et al*., 2013). Metallothioneins act as free radical scavengers. It plays a role as a neuroprotector of the aging brain (Cenini G, *et al*., 2019).

DISCUSSION AND CONCLUSION

Multiple theories of aging have been proposed in the present review, including biomarkers of aging. Current evidence suggests that the free radical theory of aging may be associated with neurodegenerative diseases. Apoptosis and mitochondrial dysfunction due to the generation and accumulation of free radicals mainly increase oxidative phosphorylation in the body, which affects the normal aging process. Kumar H reported that as age increases, oxidative stress increases, which is one of the causes of many people prone to neurodegenerative disorders. Cenini G also stated that free radical accumulation leads to early neurodegeneration at normal age, which causes cognitive decline. Novel biomarkers at the molecular level are mentioned in the form of a concise table, which provides information about different markers that help in predicting human aging. From this review, the long term goal is to identify new therapeutic targets in drug discovery, that can reduce the prevalence rate of neurodegeneration.

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