Archives of Oral Biology 105 (2019) 5–12

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Salivary biological biomarkers for Alzheimer's disease

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ARTICLE INFO

Keywords:
Saliva
Alzheimer's disease
Biological biomarker

ABSTRACT

Alzheimer's disease (AD) is becoming a threat to aging population all over the world. The pathogenic process of AD is likely initiated many years before clinical onset, thus biomarkers for AD diagnosis are critical for the prevention and therapy for the disease at the early stage in order to reduce the global burden brought by the disease. Saliva is treated as a potential alternative and universal diagnostic fluid that can be collected non-invasively by participants with moderate training and without side effects. Several potential salivary biomarkers, which might prove to be significant diagnostic tools in AD, have been researched. We address here the present and the future of these salivary biological biomarkers for AD.

1. Introduction

Alzheimer's disease (AD) is the most common of the neurodegenerative diseases among the aging population, characterized by progressive memory impairment, significant cognitive deficits and irreversible changes in personality and behavior. Although the specific molecular and cellular mechanisms responsible for the etiology and pathogenesis of AD have not been defined, research has revealed that the main pathological characteristics of AD are amyloid-β (Aβ) aggregation and the hyperphosphorylation of tau protein, which eventually develop into senile plaque and neurofibrillary tangles. Together with associated processes, such as inflammation and oxidative stress, these pathological cascades contribute to progressive neurodegeneration (Blennow, de Leon, & Zetterberg, 2006).

The majority of AD cases are sporadic late onset types, which are believed to result from environmental factors to a great extent. Age is a significant risk factor for AD. Accompanying the increase in longevity, the prevalence of AD is expected to rise dramatically, causing a large economic and caring burden for health and social services, families, and individuals (Kamer et al., 2008). Thus, it is a great challenge to diagnose and monitor disease progression.

A definitive diagnosis of AD currently relies on clinical assessment and pathological evidence only available at postmortem. Current diagnostic options in the living is largely based on clinical assessments, which include a combination of clinical history, cognitive and mental state examination using clinical exclusion criteria such as DSM-V, ICD-10, NINCDS-ADRDA and NIA-AA criteria and the exclusion of other causes of cognitive impairment (Lopez, McDade, Riverol, & Becker, 2011). The assistance of structural imaging with computed tomography (CT), magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT) or positron emission tomography (PET) could also be useful to diagnose and differentiate AD from other types of dementia (Fletcher et al., 2013).

The pathogenic process of AD probably initiates decades before clinical onset of the disease. AD diagnosis is not possible until significant dementia has set in, which means diagnosis is often made in the late period of disease progression when it is too late to take measures to prevent or treat the disease successfully. Disease-modifying drugs will probably be more effective at the early stage of AD prior to the pathological changes spreading throughout the brain. Advances in preventive and therapeutic strategies for AD that lead to even small delays in onset and progression of the condition would significantly reduce the burden of the disease (Herukka et al., 2017; Semla, 2007). Thus, it is quite essential to provide early AD diagnosis for the patients. The symptoms of AD progress slowly. The term "mild cognitive impairment" (MCI) is often used to represent the prodromal phase of AD or other neurodegenerative disorders. However, there are many causes of MCI, so there is a need for more accurate diagnostic tests to identify MCI patients in whom AD may be the underlying cause (Gordon & Martin, 2013).

Extensive research has focused on potential biomarkers, which might prove to be significant early diagnostic tools in AD. According to

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https://doi.org/10.1016/j.archoralbio.2019.06.004
Received 7 April 2019; Received in revised form 31 May 2019; Accepted 10 June 2019

the Consensus Report of the Working Group on Molecular and Biochemical Markers of AD, a biomarker should have both sensitivity for detecting AD and specificity for distinguishing other dementias of at least 80%. It should also be reliable, reproducible, non-invasive, simple to perform, and inexpensive (Thies, Truschke, Morrison-Bogorad, & Hodes, 1999). To date, biological, morphological, and functional brain imaging exams have been utilized to diagnose and monitor the progression and the outcome of treatment of AD at the early stage (Chintamaneni & Bhaskar, 2012). Compared with biological biomarkers, which reflect directly pathological changes of the brain, morphological and functional brain imaging exams such as medial temporal lobe atrophy assessed by MRI, the cerebral glucose metabolism assessed by fluorodeoxyglucose PET, and amyloid deposition in the brain assessed by amyloid-PET are not only the indirect reflection of the consequences of the disease, but also to some extent time consuming or expensive (Bobinski et al., 2000; Nordberg, 2004; Petrie et al., 2009). It is well known that brain biochemical modifications are reflected in the CSF. The majority of the studies of validated AD biomarkers to date have been carried out using samples of cerebrospinal fluid (CSF) obtained by lumbar puncture, which is an invasive procedure that requires highly trained professionals and may cause discomfort or side effects in some patients. It is also reported that the stress response of lumbar puncture leads to increased secretion of cortisol, which may be associated with any observed changes in biomarkers (Bohnen, Terwel, Twijnstra, Markerink, & Jolles, 2010). As a result, the screening of patients is often difficult and follow-up analysis of the same patient over several years is hard to achieve. Consequently, the use of a body fluid with minimal discomfort for the patient can be beneficial.

Recently, it has been shown that identification of blood biomarkers may allow the development of tests for AD. Several groups have investigated plasma or serum markers and the findings of those cross-sectional studies are conflicting (Hansson et al., 2010; Irizarry, 2004; Lambert et al., 2009). However, in a cohort with long-term follow-up, the plasma Aβ42/Aβ40 ratio was shown to be a useful biomarker for identifying cognitively normal elderly white subjects at risk for developing MCI or AD, which changed the outlook of plasma Aβ to an moderately promising AD biomarker (Graff-Radford et al., 2007). Notably, the developments of ultrasensitive immunoassays and novel mass spectrometry techniques give promise of plasma biomarkers such as neurofilament light proteins which need to be validated further (Zhao, Xin, Meng, He, & Hu, 2019).

Saliva testing is currently used in areas of toxicology, endocrinology, infectious diseases, and drug testing. Owing to the rapid progress made in salivary studies, researchers have proposed the concept of salivaomics, which include genomics, transcriptomics, proteomics, metabolomics and microRNA analysis (Zhang et al., 2016). It has been well recognized that salivary biomarkers can be exploited for the early diagnosis of some oral and systemic diseases. Besides being treated as potential alternative and universal diagnostic fluid, saliva can be collected noninvasively by participants with moderate training and without side effects. Several researches have focused on the associations between AD and AD induced impairment of a salivary gland (Ship & Puckett, 1994; Ship, DeCarli, Friedland, & Baum, 1990). AD-related proteins are expressed in salivary glands (Oh & Turner, 2006). Also, it is important to note that changes in the cerebrospinal fluid may perhaps be reflected in the saliva (Reuster, Rilke, & Oehler, 2002). Salivary biomarkers are promising in the diagnosis of AD.

In this paper, we will examine the current status of potential salivary biomarkers (Table 1), which might prove to be significant diagnostic tools in AD.

1.1. Aβ as biomarker for AD

Although the molecular mechanisms involved in the etiology and pathogenesis of AD have not been completely defined, the accumulation of β-amyloid (Aβ) and the hyperphosphorylation of tau in the brain are pathological hallmarks of AD. The amyloid peptide Aβ is normally produced from the processing of a transmembrane precursor protein called amyloid precursor protein (APP) by two enzymatic cleavages, β- and r-secretases in the brain, with the most important ones cleaved at amino acid position 40 (Aβ40) or 42 (Aβ42). Aβ peptides have a high tendency to aggregate, forming toxic oligomersthat are thought to contribute to the disruption of synaptic function and neurodegeneration (Benilova, Karran, & De Strooper, 2012).

To date, a moderate to marked decrease in CSF Aβ42 in AD has been found in most of the publications, which may be caused by reduced clearance of Aβ42 from the brain to the CSF or blood, as well as enhanced aggregation and plaque deposition in the brain; there is no change in CSF Aβ40 in AD (Benilova et al., 2012; Mehta et al., 2000). One of the most validated biomarkers for early detection in clinical use is lower level of Aβ42 together with elevated levels of tau and phosphorylated tau (p-tau) in the cerebrospinal fluid (CSF), which yields a combined sensitivity of > 95% and a specificity of > 85% (Benilova et al., 2012). Due to the drawbacks of CSF and blood, it is important to note salivary epithelial cells express APP and Aβ and changes in the cerebrospinal fluid may perhaps be reflected in the saliva (Formichi, Battisti, Radi, & Federico, 2006). Several researches on Aβ as a salivary biomarker for AD have been done. Pareja et al. compared a group of individuals with AD to a group of controls as well as individuals with Parkinson's disease and revealed that there was a statistically increase of Aβ42 in the saliva patients with mild AD while there was no noticeable change in the Aβ42 levels of either the severe AD or Parkinson's patients, which seemed to reflect a similar situation to brain Aβ generation(Bermejo-Pareja, Antequera, Vargas, Molina, & Carro, 2010). However, Kim et al. measured Aβ42 by an antibody-based magnetic nanoparticle immunoassay, which showed rather antithetical tendency to the former reports with higher Aβ42 levels in the severe AD stage than in the MCI stage that was also higher than in the normal stage (Kim, Choi, Song, & Song, 2014). They speculated that the salivary Aβ42 concentration could relatively increase as the severity of AD becomes higher due to the diminished amount of saliva caused by impairment of submandibular gland, this seemed to be unlikely because Pareja et al. validated that the protein concentration of the saliva samples obtained from the elderly control subjects was similar to those of the subjects either with AD or PD(Bermejo-Pareja et al., 2010). Both of the research revealed that Aβ40 levels remained unchanged for all AD cases. More recently, Lee et al. undertook a more detailed examination of salivary Aβ 42 in control and AD cases with adding thioflavin S as an anti-aggregation agent for Aβ 42 which was capable of detecting 5-fold of Aβ42 in the samples when compared to the method used by Pareja et al,finding out all AD cases secreted levels of Aβ 42 more than double those of controls (Bermejo-Pareja et al., 2010; Lee, Guo, Kennedy, Mcgeer, & Mcgeer, 2017). Similarly, Sabbagh et al. analysised the quantification of Aβ42 in the saliva of patients with mild to moderate AD compared to controls demonstrated that AD patients had a 2.45-fold increase in Aβ42 according to the protocol described by Lee et al (Sabbagh et al., 2018). The difference in results above may due to the different diagnosis criteria, the samples from patients in different stage of AD, saliva collection method, and the detection method of Aβ42. More studies are needed on the clinical validity and utility of salivary Aβ42 in predicting clinical progression in patients with MCI or AD. Thus, large and longitudinal studies with a greater number of samples will be necessary to determine conclusively whether there is a relationship between saliva Aβ42 levels and progression of AD. Since a marked decrease in the ratio of Aβ42/Aβ40 in CSF has been found in AD in several papers, which is more marked than the reduction in CSF Aβ42, the ratio of Aβ42/Aβ40 in saliva should be validated as well (Hansson et al., 2007).

1.2. Tau as biomarker for AD

Tau is found predominantly in axons, where it exists as a highly

Table 1
Summary of salivary biological biomarkers for Alzheimer's disease.

Salivary biomarker	Salivary biomarker	Analytical method	Consequence	Reference
A β	AD patients (n = 70, average age 77.20 years, 49 Females:21 Males), classification of mild, moderate and severe degrees of AD was performed; PD patients (n = 51, average age 72.96 years, 25 Females:26 Males); Healthy controls (n = 56, average age 74.35 years, 39 Females:17 Males).	ELISA	There was a small but statistically significant increase in saliva Aβ42 levels in mild AD patients compared to healthy controls.	Bermejo-Pareja et al. (2010)
	The AD group was classified into severe and MCI stages (n = 28); Healthy controls (n = 17).	Nanoparticle immunoassay	The salivary Aβ42 levels showed a statistically increase for AD patients compared with control group. Comparison within the AD group had a tendency toward an obvious increase in the Aβ42 level for the severe stage compared with the MCI stage.	Kim et al. (2014)
	AD patients (n = 7, aged from 57 to 86 years old, 4 Females:3 Males); PD patients (n = 1, aged 54 years old, 1 Male); pre-AD patients (n = 3, aged from 51 to 60 years old, 3 Females), Healthy controls (n = 26, aged from 16 to 92 years old, 9 Females:17 Males).	The saliva Aβ42 levels were firstly stabilized by adding thioflavin S as an anti-aggregation agent and sodium azide as an anti-bacterial agent and then quantitated with ELISA.	AD cases secreted levels of Aβ42 that were more than double those of non-AD cases.	Lee et al. (2017)
	AD patients (n = 15, average age 77.80 years, 8 Females:7 Males) ; Healthy controls (n = 7, average age 60.40 years, 5 Females:2 Males).	By the method of Lee et al. (2017)	the saliva levels of Aβ42 were significantly higher in AD patients than in controls.	Sabbagh et al. (2018)
Tau	AD patients (n = 21, average age 68.80 years,11 Females:10 Males); Healthy controls (n = 38, average age 69.0 years, 19 Females:19 Males).	Luminex assay	The p-tau/ t-tau ratio increased significantly in AD patients compared to controls.	Shi et al. (2011)
	AD patients (n = 53, average age 81.4 years, 30 Females:23 Males); MCI patients (n = 68, average age 79.8 years, 35 Females:33 Males); Healthy controls (n = 160, average age 78 years, 94 Females:66 Males).	Ultrasensitive single molecule array	No median difference in salivary t-tau concentration was found between AD and MCI or healthy elderly controls.	Ashton et al. (2018)
	Round one: AD patients (n = 46, average age 80 years, 22 Females:24 Males); MCI patients (n = 55, average age 78 years, 32 Females:23 Males); Healthy controls (n = 47, average age 73 years, 32 Females:15 Males). Round two: AD patients (n = 41, average age 80 years, 24 Females:17 Males); frontotemporal dementia patients (n = 16, average age 71.5 years, 5 Females:11 Males); Neurology patients (n = 12, average age 55 years, 7 Females:5 Males); Normal elderly controls (n = 44, average age 72 years, 30 Females:14 Males); Young normal (n = 76, average age 32 years, 45 Females:31 Males)	Western blot	There was significant elevation of p-tau/t-tau ratio for the S396 phosphorylation site in AD subjects compared to cognitively healthy elderly subjects.	Pekeles et al. (2019)
AChE	Patients with mild dementia who responded to AChE-I therapy (n = 22, average age 75 years, 15 Females:7 Males); patients with AD who did not respond to treatment (n = 14, average age 75 years, 10 Females:4 Males); Healthy controls (n = 11, average age 71 years, 5 Females:6 Males).	The enzyme catalytic activity was determined by a colorimetric assay system using acetylthiocholine as the enzymatic substrate. The expression of the AChE protein was determined by Western blot analysis.	There was a significant decrease in the average activity of the treatment nonresponders when compared with age-matched controls and those who responded to AChE inhibitor therapy.	Sayer et al. (2004)
	AD patients (n = 15, average age 83.5 years, 10 Females:5 Males) ; Vascular dementia patients (n = 13, average age 81.8 years, 4 Females:9 Males); Healthy controls (n = 13, average age 80.8 years, 6Females:7 Males).	By the method of Sayer et al. (2004)	There was no statistically significant overall difference between diagnostic groups.	Boston et al. (2008)
	AD patients that were under memantine therapy (n = 15); healthy controls (n = 15).	Ellman colorimetric method	There was no statistically differences in enzyme activity between AD patients and controls.	Bakhtiari et al. (2017)
	AD patients (n = 30, average age 70.57 years, 15 Females:15 Males); Healthy controls (n = 30, average age 66.50 years, 15 Females:15 Males).	Ellman colorimetric method	The activity of AChE significantly increased in the group with AD compared to the healthy subjects.	Ahmadi-Motamayel et al. (2019)
Lactoferrin	The cross-sectional study: AD patients (n = 80, average age 76.2 years, 49 Females:31 Males); MCI (n = 44, average age 75.16 years, 25 Females:19 Males); PD patients (n = 59, average age 69.5 years, 32 Females:27 Males); Healthy Controls (n = 91, average age 73.7 years, 59 Females:32	ELISA	Salivary lactoferrin levels were significantly reduced in MCI and AD patients compared with the healthy control group.	Carro et al. (2017)

(*continued on next page*)

Table 1 (*continued*)

Salivary biomarker	Salivary biomarker	Analytical method	Consequence	Reference
	Males). Sub-cohort study: AD patients (n = 59, average age 80.07 years, 44 Females:15 Males); Healthy controls (n = 68, average age 69.53 years, 43 Females:25 Males). 306 healthy participants from two 5 -year longitudinal cohorts were used for validation of the marker for conversion rate to MCI and AD.			
Metabolomics	MCI; healthy aging controls. The present classifications included 4 year follow-up independent assessments confirming stability of initial clinical status.	Liquid chromatography Fouriertransform ion cyclotron resonance mass spectrometry (LC − FTICR-MS)	Top-ranked 18 metabolites successfully distinguished the two groups, among which seven metabolites were putatively identified while one metabolite, taurine, was definitively identified.	Zheng et al. (2012)
	AD patients (n = 256, average age 78.6years, 132 Females:124 Males); Healthy controls (n = 218, average age 77.9 years, 116 Females:102 Males).	Faster ultra-performance liquid chromatog- raphy mass spectrometr y (FUPLC-MS)	Sphinganine-1-phosphate, ornithine, phenyllactic acid, inosine, 3-dehydrocarnitine, and hypoxanthine in the AD subjects were significantly different from the control subjects. The major contributor to the predictive model was sphinganine-1-phosphate, which was upregulated in AD, yielded satisfactory accuracy (AUC = 0.998), sensitivity (99.4%) and specificity (98.2%).	Liang et al. (2015)
	AD patients (n = 660, average age 78.6years, 332 Females:328 Males); MCI (n = 583, average age 78.9 years, 294 Females:289 Males).	Faster ultra-performance liquid chromatog- raphy mass spectrometr y (FUPLC-MS)	Ten metabolites in the saliva of AD subjects were significantly different from MCI subjects. Cytidine and sphinganine-1-phosphate were major candidate biomarkers for predicting conversion of MCI to AD.	Liang et al. (2016)
	AD patients (n = 9, average age 85 years, 6 Females:3 Males); MCI (n = 8, average age 83 years, 5 Females:3 Males); Healthy controls (n = 12, average age 82 years, 8 Females:4 Males).	^{1}H-NMR-Based Metabolomics	There were significant concentration changes in 22 metabolites in the saliva of MCI and AD patients compared to controls.	Yilmaz et al. (2017)
Inflammatory factors	AD patients (n = 15); Healthy age and gender matched controls (n = 10).	Luminex assay	Levels of salivary IL-1β, and TNF-alpha were significantly increased in AD patients in comparison to healthy controls.	Singhal and Anand (2013)
Trehalose	AD patients (n = 5); Healthy controls (n = 1).	Sensing salivary sugar using Drosophila cells expressing gustatory receptor (Gr5a) immobilized on an ion-sensitive field-effect transistor (ISFET) biosensor	A higher level of sugar was found in the saliva of AD than in that of the normal person.	Lau et al. (2014)
	AD patients (n = 20, average age 72.5 years, 12 Females:8 Males); PD patients (n = 20, average age 73 years, 11 Females:9 Males); Healthy controls (n = 20, average ag 66.1 years, 15 Females:5 Males).	Sensing salivary sugar using Drosophila cells expressing gustatory receptor (Gr5a) immobilized on an extended gate ion-sensitive field-effect transistor (EG-ISFET) biosensor	AD patients had higher sugar content than PD patients and the controls.	Hui Chong Lau et al. (2015)

soluble, phosphorylated protein that stabilizes and promotes the polymerization of microtubules mainly through the microtubule-binding domain (Hanger, Anderton, & Noble, 2009). Hyperphosphorylation and aggregation of tau protein are characteristic in AD. The abnormally phosphorylated tau (p-tau) loses its microtubule stabilization function and aggregates in neurons to form toxic neurofibrilary tangles. Recent evidence suggests that relatively early tau proteinphosphorylation is involved in the induction of neuronal death (Hanger et al., 2009).

The CSF biomarkers, total tau (T-tau) which probably reflects the intensity of the neuronal damage, and phosphorylated tau (p-tau), which reflects the phosphorylation state of tau, have been evaluated in numerous studies (Blennow & Hampel, 2003). As to the T-tau, a moderate to marked increase of CSF T-tau in AD or MCI that later progresses to AD with dementia has been found in numerous studies (Blennow & Hampel, 2003). However, high CSF T-tau levels will be found in a range of neurodegenerative disorders, such as Creutzfeldt- Jakob disease and some cases of frontotemporal dementia, proving that CSF T-tau is not specific for AD(Green, Harvey, Thompson, & Rossor, 1999; Otto et al., 1997). The specificity of CSF P-tau to differentiate AD from other dementias seems to be higher than T-tau; studies suggest that p-tau in CSF is not simply a marker for neuronal degeneration, but that it specifically reflects the phosphorylation state of tau and thus possibly the formation of tangles in brain of AD patients. At least 30 phosphorylation sites have been identified on the tau protein, the most prevalent being threonine 181, threonine231, serine 199, serine 396, and serine 404, etc. (Blennow & Hampel, 2003).

A few studies have focused on the salivary tau. Though the precise source of salivary tau remains unknown, there are several possible pathways. Given that salivary glands are proximal to the central nervous system via the cranial nerves, it is possible that salivary tau proteins are released from the nerves that innervate salivary glands (Shi et al., 2011). Besides, tau is expressed and secreted by the acinar epithelial cells of the salivary glands; tau mRNA was identified in salivary glands (Conrad, Vianna, Freeman, & Davies, 2002). Shi et al. found the p-tau/t-tau ratio was significantly different between AD patients and controls by Luminex assays, which could be an excellent AD diagnostic marker (Shi et al., 2011). However, t-tau appeared to remain unchanged, which is different from that both t-tau and p-tau substantially increase in AD over controls in human CSF. Ashton et al found the similar results that No median difference in salivary t-tau concentration was found between AD and mild cognitive impairment or healthy elderly controls (Ashton et al., 2018). It should be noted study focused on only one phosphorylation site, threonine 181 and may not have detected other phosphorylated forms of p-tau. Pekeles et al. quantified the p-tau/t-tau ratio at different phosphorylation sites among an AD group, MCI group and control group by western blot analysis, finding that a significant difference in AD patients compared to cognitively healthy elderly subjects at phosphorylation sites, Ser396 (Pekeles et al., 2019). However, the large variation in levels of AD subjects suggests this test may not be useful as a diagnostic biomarker.

To date, the application value of salivary tau still need to be further studied. Future work should explore other phosphorylation sites and should further investigate the mechanism of tau in saliva. It is also important to use standard saliva collecting and protein analysis methods, for which can greatly affect the protein amount (Pekeles et al., 2019). Once further evaluated and validated in larger studies, changes in salivary tau species could assist in identification of MCI or AD patients.

1.3. AChE as biomarker for AD

The acetylcholinesterase enzyme (AChE) catalyses the acetylcholine (ACh) neurotransmitter to its constituent components of acetic acid and choline.Cholinergic neurons are critical to memory and learning, which are destroyed in the early stages of AD leading to a significant decrease in ACh levels (Nunes-Tavares et al., 2012). Therefore, one of the possible therapeutic approaches to treat AD is to inhibit AChE in order to maximise the potential of the released neurotransmitter. Most of the studies indicate that the drugs exhibit efficacy primarily in the early mild-to-moderate stages of AD disease when the cholinergic neurons keep at least partial activity (Rosler et al., 1999). To target the drugs to patients most likely to respond to therapy, a preclinical marker of brain cholinergic activity, which may provide an indication of the disease progression stage, would be of particular use. AChE has been proposed as a biomarker of cholinergic potential because of its biosynthesis exclusively by cholinergic neurons.

There are conflicting reports of changes in AChE activity associated with AD in peripheral body fluids. Some studies have indicated a reduction in activity in serum or CSF while other studies have reported no changes (Sayer, Law, Connelly, & Breen, 2004). This likely reflects differences in the sampling methods and potential nonlinear changes in enzyme activity associated with disease progression. Moreover, the enzyme derived from peripheral tissues may unlikely reflect the exact changes that occur in AD brains.

Saliva is produced from salivary glands and mucous membranes that are under cholinergic innervation and reproducible levels of salivary AChE catalytic activity can be detected in elderly subjects, therefore there is a broad correlation between enzyme activity and potential brain cholinergic function. Sayer et al. revealed that the AChE activity in AD patients who did not respond to AChE inhibitor therapy was significantly lower than the age-matched controls (Sayer et al., 2004). In addition, there was significant difference in activity between those who responded to AChE inhibitor therapy and those who did not, providing an indication of the stage of disease progression. Also, there was a significant age-associated decrease in enzyme activity in the control group, suggesting that changes in the salivary AChE activity appear to parallel the AD-associated decrease in brain cholinergic activity. However, Boston et al. found there was no significant differences in salivary AChE activity between people with AD and age-matched controls (Boston, Gopalkaje, Manning, Middleton, & Loxley, 2008). It is also not clear how far salivary AChE, as a peripheral marker, reflects changes in central cholinergic function. Bakhtiari et al. also found although the average of salivary AChE activity in people with AD was lower compared to the control group, there was no statistically significant differences, proving that salivary AChE activity was not significantly associated with AD (Bakhtiari et al., 2017). However, Ahmadi-Motamayel et al. found that AChE levels were increased in saliva samples of patients with AD in the early stage compared to the control group (Ahmadi-Motamayel, Goodarzi, Tarazi, & Vahabian, 2019). The difference between the results of these studies could be due to differences in the study design, for which treatment with an inhibitor may cause long term adaptive changes in the production of AChE.

While these studies demonstrate the possibility of salivary AChE as a useful biomarker, there have yet to be conclusive results verifying the diagnostic value of AChE levels and whether salivary markers truly reflect changes in central cholinergic function. There would be benefit in an experiment with large scale and longitudinal sampling by standard methods.

1.4. Lactoferrin as biomarker for AD

Evidence suggests that AD pathology may be initiated or exacerbated by infection with bacteria or viruses in the brain (Honjo, van Reekum, & Verhoeff, 2009). Antimicrobial peptides have been proposed in AD pathology as pathogen targeting agents and markers of brain infections that are involved in the pathogenic process of AD (Welling, Nabuurs, & van der Weerd, 2015).Saliva is one of the body's first lines of defense due to its composition of antimicrobial proteins. Lactoferrin, an Aβ-binding glycoprotein, one of the major antimicrobial peptides in saliva, plays an important role in modulating immune response and inflammation process, representing an important defensive element by inducing a broad spectrum of antimicrobial effects (Orsi, 2004).

Carro et al. first carried out an AD diagnostic cross-sectional study and found that decreased salivary lactoferrin perfectly classified all amnestic MCI and AD patients and all cognitively healthy subjects (Carro et al., 2017). Then they validated the saliva lactoferrin as an AD biomarker in two new blinded and independent longitudinal cohorts, finding that apparently healthy individuals with low levels of saliva lactoferrin were at a high risk of more than 77% of converting to amnestic MCI and AD dementia. Moreover, the accuracy for AD diagnosis shown by salivary lactoferrin was greater than that obtained from CSF biomarkers, including total tau and CSF Aβ42.

To our knowledge, this is the only research of antimicrobial peptides as a salivary biomarker and may provide an idea for future research on antimicrobial peptides. The evidence suggests that lactoferrin is greatly up-regulated in AD brain and may be involved in Aβ deposition, however, lactoferrin decreased in the saliva of AD patients (Carro et al., 2017). The relationship between salivary lactoferrin and the AD pathogenesis remains unclear, and needs further research. Longitudinal cohort analyses are also needed to address how the salivary lactoferrin marker may help to differentiate between AD and other neurodegenerative diseases.

1.5. Metabolomics as biomarker for AD

The pathophysiological changes associated with AD begin decades before the emergence of clinical symptoms. Understanding the early mechanisms associated with AD pathology is, therefore, especially important for identifying disease-modifying therapeutic targets. Metabolomics is a powerful tool that detects perturbations in the metabolome, a pool of metabolites that reflects changes downstream of genomic, transcriptomic and proteomic fluctuations, and represents an accurate biochemical profile of the organism in disease. Any unusual disturbances to activity in the metabolic network could be useful to better understanding the mechanisms of the disease. The application of metabolomics could help to identify biomarkers for early AD diagnosis, to discover novel therapeutic targets, and to monitor therapeutic response and disease progression (Carro et al., 2017).

Metabolomics research involves the identification and quantification of hundreds to thousands of small-molecular-mass metabolites in cells, tissues, or biological fluids. To increase the probability of finding specific biomarkers of diseases using salivary metabolomics, there is a clear need to develop more sensitive analytical tools to profile a large number of metabolites. Currently the ability to simultaneously measure dynamic changes in many molecules of biological samples become available through the utilization of such advanced analytical technologies as high resolution nuclear magnetic resonance (NMR) and mass spectroscopy (MS) coupled with either high or ultrahigh resolution liquid (LC), gas (GC) chromatography or capillary electrophoresis(CE), and the development of sophisticated methods of data analysis (Zheng, Dixon, & Li, 2012).

Metabolomic studies have identified candidate biomarkers in serum and plasma while there have yet to be any conclusive biomarkers. As to the salivary metabolomics studies, Zheng et al. validated the effect of MCI on metabolome changes compared to age and gender matched controls with LC—MS, revealing that taurine was definitively identified with lower concentrations in MCI patients (Zheng et al., 2012). Taurine is essential for the central nervous system, which is involved in several physiological actions in the brain, such as osmoregulation, neurotransmission, and membrane stabilization (Olive, 2002). Liang et al. used the sme LC−MS method to find the metabolic changes of the salivary metabolome from AD patients compared to age-matched controls, revealing that sphinganine-1-phosphate, an intermediate in the metabolism of glycosphingolipids and sphingolipids, was up-regulated in AD yielding satisfactory sensitivity (99.4%) and specificity (98.2%) (Liang et al., 2015; Tomlinson, Rafii, Ball, & Pencharz, 2011).They also examined metabolic differences in saliva samples from MCI subjects and age-matched AD subjects, revealing the major contributors were cytidine and sphinganine-1- phosphate (Liang, Liu, Li, & Zhang, 2016). Yilmaz et al firstly presented the ^{1}H-NMR based metabolomics study discriminating MCI sufferers, AD patients, and healthy controls from each other, demonstrating that there were significant differences in the concentrations of 22 salivary metabolites in AD and MCI versus controls (Yilmaz et al., 2017). Differences were also found when the AD and the MCI groups were compared. The results demonstrated that saliva metabolite profiling may contribute to understanding the pathogenic mechanism of AD and MCI.

The results of the research mentioned above were conflicting because they focused on the different condition of AD even with the same analysis method. However, this form of exhaustive biochemical analysis could offer a unique perspective on the pathologic pathways of AD that could further ascertain useful diagnostic markers.

1.6. Other biomarkers for AD

Recently, inflammation within the brain is thought to play a pivotal role in the etiology and pathogenesis of AD. Studies suggest that peripheral infection or inflammation might affect the inflammatory state of the central nervous system (Watts, Crimmins, & Gatz, 2008). Inflammatory factors related to inflammatory pathways are currently used as diagnostic tools to confirm AD such asIL-1β, and TNF-α (Singhal & Anand, 2013). However, an inflammatory response is associated with a variety of disorders, which should be used in conjunction with other biomarkers of AD to make sure of the specificity.

High salivary sugar levels were previously reported thought to be associated with the occurrence of diabetes mellitus (Satish et al., 2014). Several reports have proposed an association between diabetes mellitus and the progression of AD; AD patients were also observed to have a high occurrence of diabetes mellitus (Sims-Robinson, Kim, Rosko, & Feldman, 2010). Therefore, salivary sugars may serve as potential biomarkers for AD. Lau et al. utilized two kinds of cell-based biosensors to significantly distinguish salivary sugar trehalose of the AD group from the control groups (Lau et al., 2014; Lau et al., 2015). Although the source of salivary trehalose remains unknown, salivary sugars could be associated with disease development and serve as a possible diagnostic tool for AD.

2. Conclusion

So far, there are not many studies on the salivary biological biomarkers of AD, most of which are mainly involved in the pathological process of AD. Though salivary biomarkers are supposed to be a great value for the early diagnosis of AD, there still need further verification in the future. Although aforementioned salivary biomarkers have been identified, there still lacks longitudinal or large-scale studies for specificity and sensitivity tests, especially the precise distinction of AD and MCI patients in whom AD may be the underlying cause and other neurodegenerative disorders. Combined biomarkers should be validated to improve the specificity and sensitivity of the saliva diagnosis. Most of the current salivary biomarkers are preliminary verified because they are associated with the pathogenesis of AD, but need further research on the relevant underlying mechanisms. Besides, with the development of more advanced detection methods, the validation of salivary biomarkers will be more accurate and effective. The secretion of saliva is usually affected by the systemic illness or medication. Consequently, there may exist differences in biomarker concentrations between patients and healthy controls due to different secreted concentrations of total salivary proteins. Hence, it is recommended to normalize measurements of salivary biomarkers against total salivary proteins. As to the methods to quantify the salivary biomarkers, we should establish standard operating criteria, such as the classification of subjects with different disease progression, protocols for saliva sample collection and detective methods of target proteins. Notably, there may exist diurnal variation or circadian effects on salivary biomarkers.

Circadian rhythms for salivary cortisol secretion and chromogranin A levels and diurnal variations in salivary redox homeostasis have previously been described in human saliva (Su et al., 2008). Hence future researches would be needed to consider these variability of salivary biomarkers in an individual and standardize the timing of specimen procurement.

Author contributions

D.L. drafted the original paper, and H.L. conceived of the overall study and reviewed the manuscript. All authors have read and approved the final manuscript.

Conflicts of interest

The authors state no conflicts of interest.

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