# Salivary Biomarkers for Alzheimer’s Disease

Subjects: Clinical Neurology

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Alzheimer’s Disease (AD) is the most common neurodegenerative disease which manifests with progressive cognitive impairment, leading to dementia. Potential biomarkers include mainly proteins, metabolites and even miRNAs. Based on meta-analysis, in AD patients, salivary levels of beta-amyloid42 and p-tau levels were significantly increased, and t-tau and lactoferrin were decreased at borderline statistical significance.

Keywords: neurodegenerative diseases ; Alzheimer’s Disease ; saliva ; biomarkers

## 1. β-amyloid

β-amyloid (Aβ) is a protein produced mainly in neuronal endosomes via amyloid precursor protein (APP) hydrolysis with β- and γ-secretases. In normal conditions, Aβ release is regulated by synaptic activity, which is, in turn, influenced by Aβ. Interestingly, Aβ may play an immunoprotective role [1]. Nevertheless, the accumulation of aggregated Aβ fibrils leads to the creation of Aβ plaques, which is a pathological phenomenon characteristic of AD [2].

In 2010, Bermejo-Pareja et al. [3] measured levels of $A\beta_{40}$ and $A\beta_{42}$ in the saliva of AD patients. Apart from healthy controls, 70 patients were enrolled, which were divided into three groups: the mild, moderate, and severe stages of AD (29, 24, and 17 patients, respectively). The results show that salivary levels of $A\beta_{42}$ were significantly increased in patients in the mild AD stage. Moreover, a similar tendency was observed in moderate and severe stages although with a high standard deviation. Additionally, the authors observed a correlation between salivary $A\beta_{42}$ concentration and sex. On the other hand, no significant differences were found in $A\beta_{40}$ levels between AD patients and the control group.

Ten years later, another research focused on salivary $A\beta_{42}$ levels. In this case, 60 healthy subjects and 60 patients with a probable diagnosis of AD were recruited and selected by geriatricians. There was no distinction between disease stages. $A\beta_{42}$ levels in saliva were higher in AD patients but not significantly compared to healthy subjects [4].

In a study by Cui et al. [5], salivary $A\beta_{40}$ and $A\beta_{42}$ levels were assessed in a smaller sample (30 patients). Similarly, $A\beta_{40}$ levels did not differ significantly between controls and patients, and $A\beta_{42}$ levels were significantly increased. The performed ROC analysis revealed no significant predictive value for salivary $A\beta_{40}$ and $A\beta_{42}$ and their ratio.

On the other hand, Katsipis et al. [6] measured $A\beta_{42}$ levels in the saliva of 60 participants (20 AD patients). Again, the results indicated that salivary $A\beta_{42}$ levels significantly increased in AD patients compared to healthy individuals and MCI patients.

Consistent results were obtained by Boschi et al. [7] in a group of 100 participants (18 AD subjects, 18 controls, 64 patients with dementia other than AD). Salivary $A\beta_{42}$ levels were significantly elevated in patients affected by AD compared to non-demented controls. No considerable correlations between gender or MMSE score and salivary $A\beta_{42}$ level were observed. Interestingly, salivary $A\beta_{42}$ concentrations were significantly and negatively associated with CSF $A\beta_{42}$ levels in all diagnostic groups except for the AD group. Nevertheless, the ROC analysis revealed satisfactory performance of salivary $A\beta_{42}$ in AD diagnosis (AUC 0.806, specificity 68%, sensitivity 84%, with a cut-off value of 92.5 pg/mL).

Furthermore, Sabaei et al. [8] investigated salivary $A\beta_{1–42}$ levels in the study of 70 participants, including 24 patients with mild AD. Similarly, salivary $A\beta_{1–42}$ levels were significantly higher in AD patients in comparison with healthy controls with a slightly lower difference after age adjustment. In addition, the ROC analysis confirmed the satisfactory performance of this potential biomarker with both the cut-off point equal to 60.3 pg/mL (AUC 0.81, specificity 91%, sensitivity 62.5%) and 15.5 pg/mL (AUC 0.77, specificity 59.1%, sensitivity 91.7%).

In turn, Tvarijonaviciute et al. [9] concluded that salivary $A\beta_{42}$ levels are decreased in AD based on the sample of 69 patients. Analysis of the univariate logistic regression models revealed that individuals with decreased $A\beta_{42}$ levels in saliva were more likely to be in the AD group. Moreover, no significant association between disease stage and salivary $A\beta_{42}$ level was observed.

## 2. Tau

Tau belongs to the microtubule-associated protein group responsible for stabilising neuronal microtubules. In pathological conditions, tau may be hyperphosphorylated, which results in aggregation and neuronal toxicity [10]. Tau hyperphosphorylation and aggregation are connected with impaired both long- and short-term synaptic plasticity, which is a phenomenon observed in AD [11][12]. Tau protein has 85 phosphorylation sites, and in normal conditions, only 10 are phosphorylated, which is significantly less than the 55 in AD [13].

In 2011, Shi et al. [14] investigated both $A\beta_{42}$ (previously mentioned) and tau levels in saliva. In comparing AD patients and controls, a non-significant decrease in t-tau concentrations in patients was observed; however, no difference was found after standardisation by total salivary protein levels. On the other hand, both absolute and standardised p-tau levels tended to increase in AD patients, but this was also insignificant. Nevertheless, a significant increase in the p-tau/t-tau ratio was observed in patients affected by AD.

Similarly, four years later, another study did not succeed in measuring salivary $A\beta_{42}$ levels, but both p-tau and t-tau concentrations were detected. No significant differences between controls and patients with AD were found, although salivary p-tau tended to increase in the latter group. Moreover, none of these three biomarkers reflected the disease progression [15].

Interesting results were obtained by Ashton et al. [16] in a bigger sample of 53 AD patients using the Sioma platform. In contrast to the study mentioned above [14], salivary t-tau concentration tended to increase in the patients' group compared to healthy subjects, although not significantly. In addition, the authors noticed a non-significant tendency in elevated t-tau levels associated with poorer cognitive abilities.

In a previously mentioned research study by Cui et al. [5], salivary p-tau and t-tau levels were also analysed. The Spearman rank analysis of both proteins' salivary concentrations revealed no significant relationship. However, the p-tau/t-tau ratio increased significantly, which was consistent with a study by Shi et al. [14]. The ROC analysis showed no significant predictive value for t-tau and p-tau nor their ratio. Nevertheless, when p-tau, t-tau, $A\beta_{40}$, and $A\beta_{42}$ were combined, the ROC analysis revealed excellent diagnostic relevance (AUC 0.921).

On the other hand, Dos Santos et al. [4] noticed a statistically significant change in salivary t-tau levels in AD patients compared to healthy individuals. The median salivary t-tau of subjects without AD was significantly higher than that of AD patients. Conflicting results were obtained by Eldem et al. [17] in their proteomic study. In a group of 57 participants, 17 AD and 21 MCI patients were enrolled. T-tau levels were analysed using Western blot, and no significant differences between diagnostic groups were observed. Katsipis et al. [6] investigated p-tau levels in saliva.

Interestingly, although Marksteiner et al. [18] did not detect salivary $A\beta_{40}$ and $A\beta_{42}$ levels, the authors collected results about tau levels in saliva. T-tau levels significantly decreased in AD patients, especially in females. P-tau levels were significantly increased in MCI patients; slightly lower and not significant elevation in p-tau concentrations was observed in AD patients. Moreover, no statistically significant differences were found in the p-tau/t-tau ratio.

In 2019, Pekeles et al. [19] investigated the salivary p-tau/t-tau ratios among AD patients, MCI patients, and healthy controls, considering various phosphorylation sites. Interestingly, no significant differences were observed regarding one of the most extensively studied tau sites, T181. In contrast, analysis of both S396, S404, and a combination of S400, T403, and T404 sites showed significantly elevated levels of the p-tau/t-tau ratio in AD patients compared to the control group. S396 was most significantly increased and had better specificity than S404; however, it had worse sensitivity (S396 sensitivity 73%, specificity 50%, S404 sensitivity 83%, specificity 30%).

Sabaei et al. [8] also investigated salivary p-tau concentrations. Once again, significant elevations of p-tau levels were observed in the AD group compared to healthy subjects both regardless of the age-confounding variable and after adjusting the age variable. Moreover, the ROC curve analysis revealed satisfactory performance of this biomarker (AUC 0.78, specificity 63.6%, sensitivity 91.7%).

Finally, Tvarijonaviciute et al. [9] analysed salivary p-tau and t-tau in patients suffering from AD and non-demented individuals. No significant changes were observed. P-tau tended to decrease slightly in patients compared to controls. On the other hand, t-tau reached similar values in both groups.

## 3. Lactoferrin

Lactoferrin (LF) is a crucial protein that plays an important role in maintaining human health [20]. Antifungal, antibacterial, antiviral, anti-carcinogenic, anti-inflammatory, and iron-binding properties enhance its relevancy in biological processes [21]. LF may have neuroprotective effects in neurodegenerative diseases, such as AD. Several mechanisms in which LF likely alleviates cognitive impairment, Aβ accumulation, and neurodegeneration were reviewed in another paper [22].

In a study by Carro et al. [23], 116 AD patients were recruited. Also, patients affected by MCI, Parkinson's Disease (PD), and healthy controls were enrolled. Salivary LF levels were significantly lower in AD and MCI patients than in healthy controls (4.78 ± 1.11 vs. 10.24 ± 1.96 µg/mL). Moreover, a statistically significant negative correlation was found between AD and MCI severity and LF level in saliva. The analogical association was observed regarding the MMSE score. In addition, salivary LF was significantly correlated with CSF t-tau and $A\beta_{42}$. The performed ROC analysis, which included the MCI/AD group and healthy controls, reached 100% specificity and sensitivity with a cut-off value of 7.43 µg/mL.

Consistent results were obtained by González-Sánchez et al. [24] three years later. Significantly decreased salivary LF levels were observed in MCI patients with positive amyloid-PET scans and AD patients in comparison with cognitively normal individuals. No significant differences were observed between these two experimental groups. Similarly, such differences were not found between MCI patients with negative amyloid-PET scans and controls. Additionally, no significant correlation with disease stage was noticed. Nevertheless, salivary LF performance in differentiation between AD/MCI amyloid-PET positive patients and controls, visualised via the ROC curve analysis with a cut-off value of 5.63 µg/mL, showed satisfactory results (AUC 0.952, sensitivity 86.96%, specificity 91.67%).

In a study from 2021, Zalewska et al. [25] confirmed previously mentioned results. Indeed, in a smaller sample, LF levels, measured in stimulated whole saliva and analysed in µg/mg protein unit, significantly decreased in patients suffering from AD compared to non-demented controls. In this case, AUC was 0.6896. Again, no considerable relationships were observed between LF concentrations and disease stages. Opposite findings were presented in research by Gleerup et al. [26] from the same year. In a cohort of 222 participants, 71 AD patients were included. Surprisingly, no statistically significant differences between diagnostic groups were observed. Moreover, salivary LF tended to increase in AD patients compared to healthy controls. Standardisation by the total protein concentration in saliva did not reveal considerable results. The authors suggested that the inconsistency with previous studies may have appeared due to the inclusion of more heterogeneous and milder cases, which might have contributed to LF variations in their research.

## 4. Acetylcholinesterase, Pseudocholinesterase, Cholinesterase

Acetylcholinesterase (AChE) is an enzyme belonging to the serine hydrolases class, which is responsible for hydrolysing acetylcholine into choline and acetic acid and, therefore, finishing the action of this neurotransmitter [27]. AChE expression is performed in several forms, including homomeric and hetero-oligomeric states. This process can be observed in various tissues: peripheral and central nervous system neurons, skeletal muscles, and endocrine or exocrine glands [28]. AChE is considered a key target for the pharmacological treatment of AD, which is focused on the inhibitors of the hydrolysis of the neurotransmitter acetylcholine [29]. Additionally, higher AChE activity has been observed in several diseases, such as lung cancer, glaucoma, ALS, Hirschsprung's disease, pesticide poisoning, neurotoxicity, or essential hypertension [30][31][32].

Ahmadi-Motamayel et al. [33] investigated AChE activity in patients with AD and non-demented controls. Moreover, the authors measured the activity of pseudocholinesterase (PChE), which is a sister enzyme of AChE hydrolysing exogenous choline-based esters [33][34][35]. In a group of AD patients, salivary AChE and PChE activities were significantly elevated compared to healthy subjects. Furthermore, the increase in activity was higher in males than females, but this difference was insignificant.

Another research analysed AChE activity in the sample of 15 AD patients and 15 healthy controls. Surprisingly, AChE activity was lower in the AD group compared to controls; however, the difference was not significant. Neither age nor disease duration were clearly associated with AChE activity. Moreover, in contrast to the previous study, enzyme activity was generally lower in males than in females. It is noteworthy that all patients were on therapy with memantine, which is a neurological drug that does not inhibit AChE [36]. Discrepancies between these two studies [33][36] are difficult to explain;

however, unclear methods of diagnosis establishment, memantine therapy, and differences in the number of study participants might have influenced the results.

On the other hand, Tvarijonaviciute et al. [9] investigated salivary levels of cholinesterase. AD patients tended to have elevated levels of this enzyme compared to the control group; however, the results were not statistically significant.

## 5. Cortisol

Cortisol is the leading glucocorticoid hormone secreted by the adrenal cortex, fluctuating during the day [37][38]. Elevated cortisol level is associated with worse prognosis and the rapid progress of cognitive impairment in patients suffering from AD in the early stages or even the preclinical phase of the disease. Cortisol may contribute to the pathophysiology of AD by increasing both tau and Aβ pathologies as well as oxidative stress [39].

In 2008, De Souza-Talarico et al. [40] investigated salivary cortisol levels in mild AD patients (40 cases) and cognitively normal subjects (also 40 participants). Using a radioimmunoassay kit, AD patients presented significantly elevated salivary cortisol concentrations compared to controls. Slightly different times at sample collection between groups did not affect the results significantly. Interestingly, no significant correlation was observed between cortisol levels and working memory tests; however, AD patients with higher cortisol levels tended to have worse scores on one of the tests.

Different results were presented in another study published eleven years later. Peña-Bautista et al. [41] classified 97 participants into the AD group, consisting of both mild AD and MCI patients, who had positive neuroimaging and CSF biomarkers results. No significant association between AD and cortisol concentration in saliva was observed. Nevertheless, salivary cortisol levels in the AD group were increased compared to non-AD controls.

## 6. Biomarkers Related to Inflammation, Oxidative Stress or Redox Imbalance

Inflammation is clearly associated with AD pathology. Damage via various inflammatory mechanisms cumulates over years of disease progression and might considerably exacerbate pathogenic processes in this disorder [42]. Several factors participating in neuroinflammation concerning AD have been described, including cytokines, chemokines, caspases, complement system, and others [43].

Returning to research by Tvarijonaviciute et al. [9], several inflammation-related substances were also investigated. Salivary levels of haptoglobin, adenosine deaminase, and the ferric-reducing ability of plasma were decreased, whereas macrophage inflammatory protein-4, α1-antitrypsin, complement C4, and pigment epithelium-derived protein levels were increased in AD patients compared to controls. Nevertheless, only complement C4 alterations were considered significant.

On the other hand, Katsipis et al. [6] analysed salivary concentrations of glial fibrillary acidic protein (GFAP), interleukin-1β (IL-1β), IL-6, TNF-α, COX-2, and caspase-8. Interestingly, all these compounds presented significant changes between diagnostic groups. Levels of GFAP, COX-2, and caspase-8 were decreased, while IL-1β, IL-6, and TNF-α increased in patients affected by AD compared to MCI patients or healthy controls. The ROC analysis for distinguishing diagnostic groups revealed satisfactory results: between AD patients and healthy controls, AUC reached 0.998 or 1.000 (dot blot and ELISA methods, respectively), and between AD and MCI patients, AUC was 0.805 or 0.865 (dot blot and ELISA methods, respectively). Furthermore, a significant negative correlation between GFAP levels and COX-2, caspase-8, $A\beta_{42}$, and p-tau concentrations was found. Analogically, a significant positive association was noted in regard to TNF-α, IL-1β, and IL-6 levels as well as the MMSE score.

Another study by Zalewska et al. [25] focused on several biomarkers related to inflammation, oxidative stress, or redox imbalance. The ROC analysis indicated that salivary catalase, glutathione, glutathione peroxidase, the mean total antioxidant capacity/mean total oxidant status ratio (OSI), advanced glycation end products (AGEs), and IL-1β could be used to distinguish between AD patients and healthy controls clearly. The activity of salivary superoxide dismutase, glutathione peroxidase, and catalase as well as glutathione concentrations were significantly lower in the AD group compared to controls. In turn, NO, advanced oxidation protein products, AGEs, malondialdehyde, peroxynitrite, IL-1β, and nitrotyrosine concentrations, mean total oxidant status, and OSI were considerably increased in the same pattern. Moreover, a statistically significant association between the reduced activity of salivary peroxidase or superoxide dismutase and time elapsed from diagnosis of AD was observed.

On the other hand, McNicholas et al. [44] investigated the salivary levels of five inflammatory biomarkers (cystatin-C, IL-1 receptor antagonist, stratifin, haptoglobin, and matrix metalloproteinase 9) in a group of 16 AD, 15 MCI patients, and 29

non-demented controls. In general, cystatin-C, IL-1 receptor antagonist, and stratifin showed lower abundance in MCI and AD groups, whereas concentrations of haptoglobin and matrix metalloproteinase 9 were elevated. The results indicated that the levels of four of these biomarkers (without haptoglobin), adjusted for total salivary protein, were significantly altered in the AD group compared to healthy subjects, whereas only the absolute levels of haptoglobin and matrix metalloproteinase 9 were significantly changed in this comparison. Interestingly, in the MCI group, the absolute levels of all five biomarkers were significantly different compared to cognitively normal participants; however, after adjusting for total protein concentration, this significance dropped. Nevertheless, a panel consisting of the base model (only age, gender and APOEε4 allele status), cystatin-C, and IL-1 receptor antagonist (both adjusted for total protein concentration) showed excellent performance in distinguishing between AD patients and healthy controls (AUC 0.97). When matrix metalloproteinase 9 (adjusted for total protein concentration) and total protein concentration were added to this panel, it proved similar results in discriminating between either MCI or AD patients and non-demented individuals (AUC 0.97).

## 7. Amino Acids and Derivatives

Amino acids play an essential role in providing communication between neurons. These compounds can contribute to neurotransmission, acting as neurotransmitters, precursors, or neuromodulators [45]. Amino acids derivatives form an interesting group with a broad correlation spectrum, including obesity or neurological diseases [45][46][47][48]. Evidence shows that patients suffering from AD have impaired neurotransmission, which might be a result of a previously described accumulation of pathological compounds [49][50].

Interestingly, Peña-Bautista et al. [51] measured salivary levels of several amino acids and derivatives. Participants were divided into healthy controls (12 individuals) and the AD group, which consisted of patients with MCI due to AD and mild or moderate dementia due to AD (17 and 14 participants, respectively). Salivary acetylcholine levels were significantly higher in patients with mild AD than in controls, whereas creatine and myoinositol presented significantly lower concentrations in the AD group. Moreover, salivary levels of myoinositol, acetylcholine, glutamine, and creatine were significantly correlated with neuropsychological scales. In addition, myoinositol was considerably associated with CSF Aβ level. The performed ROC analysis revealed relatively satisfactory accuracy of glutamine and acetylcholine (AUC 0.777 and 0.660, respectively). Nevertheless, a multivariate analysis with combinations of previously mentioned biomarkers indicated that a set of all these compounds (myoinositol, glutamine, creatine, acetylcholine) showed the best performance and might be used to distinguish between AD patients and healthy subjects (AUC 0.806, sensitivity 61%, specificity 92%).

In more recent research by Marksteiner et al. [18], apart from previously described tau and Aβ, norepinephrine concentrations were also investigated. The performed HPLC-EC method analysis revealed a significant decrease in salivary norepinephrine levels in AD patients compared with healthy controls.

## 8. miRNAs and Sirtuins

MicroRNAs (miRNAs) form a group of small endogenous non-coding RNA that regulates target gene expression [52][53]. Ryu et al. [52] investigated miRNA-485-3p concentrations in salivary exosome-enriched extracellular vesicles (EE-EV) of 27 AD patients and 13 healthy controls. The results revealed that miRNA-485-3p concentrations in salivary EE-EV from AD patients were significantly elevated compared to the control group. The ROC analysis regarding differentiating between AD and healthy individuals showed good performance of this biomarker: AUC 0.895. Moreover, statistically significant associations were observed between miRNA-485-3p concentrations in salivary EE-EV and MMSE or Aβ PET results with a stronger association with the latter ones (AUC 0.754 and 0.922, respectively).

Sirtuins (SIRT) belong to the histone deacetylases group and regulate processes like cell metabolism or gene expression via epigenetic mechanisms. Moreover, these enzymes might have neuroprotective effects [54][55]. Pukhalskaia et al. [54] enrolled 58 healthy participants and 64 AD patients in the initial or moderate stage of the disease. The results showed that SIRT1, SIRT3, and SIRT6 levels were significantly lower in the AD group compared to controls, while SIRT5 did not differ significantly. Among these biomarkers, SIRT1 and SIRT6 changed most considerably between diagnostic groups. Except for SIRT5, the rest of the mentioned SIRT significantly decreased along with patients' age, while only SIRT1 and SIRT6 were significantly lower in older healthy subjects.

## 9. Trehalose

Trehalose is a natural disaccharide which exhibits neuroprotective effects via several potential ways, including an induction of autophagy or modulation of inflammatory responses [56]. Lau et al. [15] used an improved extended gate ion-sensitive field-effect transistor (EG-ISFET) to measure salivary trehalose levels in patients suffering from AD or PD and

healthy controls. The findings showed that salivary trehalose levels were higher in AD patients compared to other diagnostic groups. Furthermore, the authors stated that using the EG-ISFET method, salivary trehalose levels of the AD group could be clearly distinguished from other diagnostic groups.

## 10. Metabolomics and Proteomics Panel Studies

Metabolomics, which analyses and profiles metabolites in biofluids, aids in the understanding of interactions between molecules and provides insights into mechanisms underlying diseases [57][58]. Similarly, proteomics evaluates both the structures and functions of proteins to better understand their characteristics in the organism [59]. In recent years, omics research has rapidly evolved and is predicted to develop even further [60].

In 2018, Huan et al. [61] developed a salivary diagnostic model of AD based on a metabolomic approach. A total sample of 109 participants (35 cognitively healthy, 25 MCI, and 22 AD patients) was divided into two phases: discovery (to determine the most significant metabolites that differentiate paired groups) and validation (to provisionally validate selected significant metabolites detected in the discovery phase). Using top-ranked but putatively identified biomarkers, a three-element panel to distinguish between AD and healthy controls was designed and consisted of methylguanosine, choline-cytidine, and histidinyl-phenylalanine. A similar panel for discriminating between AD and MCI groups included amino-dihydroxybenzene, glucosylgalactosyl hydroxylysine—$H_2O$, and aminobutyric acid + $H_2$. The performed ROC analysis revealed excellent results (overall AUC 0.997, sensitivity 98.52%, specificity 96.55%, and AUC 0.993, sensitivity 100%, specificity 97.70%, respectively). Analogically, using positively identified biomarkers, the designed panels included the following: phenylalanyl-proline, phenylalanylphenylalanine, urocanic acid (AD versus controls), and alanyl-phenylalanine with phenylalanyl-proline (AD versus MCI) (AUC 0.831, sensitivity 82.22%, specificity 73.56%, and AUC 0.843, sensitivity 81.90%, specificity 72.41%, respectively).

One year later, Marksteiner et al. [62] used targeted metabolomics to study salivary metabolomic changes in AD, MCI patients, and cognitively normal individuals; each group consisted of 25 participants. The results showed decreased salivary acyl-alkyl-phosphatidyl cholines (PCae) concentrations in AD and MCI groups compared to the control group. However, only alterations in PCae C34:1-2, PCae C36:1-2-3, PCaeCC38:1–3, and PCae C40:2-3 reached significant differences when comparing AD patients and healthy subjects. It is noteworthy that the significance was especially high when all these compounds were combined. Moreover, decreased salivary levels of PCae C36:1-2-3 significantly distinguish MCI patients from controls.

Another study investigated the metabolomic and proteomic parameters of saliva collected from 80 participants (20 AD, 20 MCI patients, and 40 cognitively normal controls). Statistical analysis revealed that 79 metabolites and 346 proteins were significantly altered in a comparison between AD and control groups. Interestingly, in the MCI group, 374 proteins and only six metabolites were considered significant compared to controls. All metabolites whose levels differed significantly between the MCI/AD and control groups (L-fucose, L-tyrosine, L-ornithine, L-aspartate, rhamnose, and serotonin) were upregulated (fold change > 2.0) [63].

Interestingly, another proteomic study, described earlier in the tau section, identified transthyretin as a potential biomarker of AD. Proteomic analysis showed a significant decrease in salivary transthyretin in AD patients, which was additionally confirmed by Western blot. The results revealed a 0.5-fold reduction in both MCI and AD groups compared to the cognitively normal subjects [17]. Transthyretin is considered a highly amyloidogenic protein that is responsible for creating amyloid deposits in the nerves, heart, arterioles, or ligaments [64]. In contrast, this protein is also believed to be a neuroprotective factor in AD due to its interaction with Aβ and decrease in Aβ aggregation [65].

On the other hand, Liang et al. [66] performed metabolomic screening on the sample of 256 AD patients and 218 cognitively normal controls to determine salivary biomarkers of early AD. The results indicated that six biomarkers significantly differed between diagnostic groups: inosine and 3-dehydrocarnitine were downregulated, while sphinganine-1-phosphate, ornithine, hypoxanthine, and phenyllactic acid were upregulated in the AD group compared with controls. Furthermore, the ROC analysis revealed that sphinganine-1-phosphate, ornithine, and phenyllactic acid seem most promising as salivary biomarkers of AD (AUC 0.998, sensitivity 99.4%, specificity 98.2%; AUC, 0.927 sensitivity 81.9%, specificity 90.7%; AUC 0.831, sensitivity 79.5%, specificity 84.3%, respectively); whereas inosine, 3-dehydrocarnitine, and hypoxanthine proved worse performance (AUC 0.740, sensitivity 66.8%, specificity 77.0%; AUC 0.669, sensitivity 57.4%, specificity 84.2%; AUC 0.674, sensitivity 53.7%, specificity 73.9%, respectively).

In a recent study, Contini et al. [67] enrolled 36 patients affected by PD, 36 healthy controls, and 35 AD patients (13 in moderate and 22 in mild disease stage). Using a proteomic approach, significant differences between various compounds

in diagnostic groups were observed. AD patients had significantly higher abundances of thymosin β4, α-defensins—1, 2, 3, and sum of α-defensins, histatin 1 mono- and non-phosphorylated, statherin 2P, des 1-9 and des 1-13, P-C peptide, cystatin A, B-SSG, total cystatin B monomer, cystatin B S-S dimer, total cystatin B, S100A8-SNO, sum of S100A8-A8SNO, S100A9s, sum of S100A9s, and total S100A9 (s + l) compared to controls, and there were analogically significantly lower abundances of PRP1 0P. Moreover, 24 biomarkers were determined to distinguish between patients suffering from AD and PD—respectively, higher expression of α-defensins—1, 2, and sum, Hst1, Hst1 0P, Hst5, Hst6, statherin 2P, 1P, desD1, des1-9, des1-10 and des1-13, PRP1 1P, PRP3 2P, PRP3 1P, P-C peptide, cystatin SN and S100A9sox, and lower expression of SLPI, PB des1-5 and des1-12, SV1 and cystatin SA.

## 11. PD-Related Biomarkers in AD

One of the primary hallmarks of PD is α-synuclein [68]. Interestingly, in a previously mentioned study, Sabaei et al. [8] observed significantly decreased salivary total α-synuclein levels in AD patients compared to healthy controls either without age-confounding variables or after adjusting for age. Nevertheless, the ROC analysis with a cut-off point equal to 9.4 pg/mL did not prove the high reliability of this biomarker in AD diagnosis (AUC 0.71, sensitivity 66.7%, specificity 68.2%).

On the other hand, heme oxygenase-1 (HO-1) is associated with both AD and PD, since HO-1 dysregulation is linked with neuroinflammation presented in both disorders [69]. In a study by Galindez et al. [70], patients suffering from both diseases were included along with patients affected by other neurological disorders and healthy controls. Importantly, AD patients were combined together with MCI patients in one group. The results indicated that this group had significantly higher salivary HO-1 levels than healthy controls. After combining AD, MCI, and PD patients in one group (neurodegenerative) and non-neurodegenerative individuals in another, the ROC analysis revealed satisfactory results in distinguishing between neurodegenerative and non-neurodegenerative subjects (AUC 0.86, sensitivity 79%, specificity 80%).

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