Published in final edited form as:

J Alzheimers Dis. 2011 ; 27(2): 299–305. doi:10.3233/JAD-2011-110731.

Salivary tau species are potential biomarkers of Alzheimer disease

Min Shi, PhD¹, Yu-Ting Sui, MS¹, Elaine R. Peskind, MD^{2,3}, Ge Li, MD, PhD², HyeJin Hwang, PhD¹, Ivana Devic, BS¹, Carmen Gingham, MD¹, John Scott Edgar, PhD⁴, Catherine Pan, DDS¹, David R. Goodlett, PhD⁴, Amy R. Furay, PhD^{1,2}, Luis F. Gonzalez-Cuyar, MD¹, and Jing Zhang, MD, PhD^{1,*}

¹Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA

²Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine, Seattle, WA, USA

³Mental Illness Research, Education, and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA, USA

⁴Department of Medicinal Chemistry, University of Washington School of Pharmacy, Seattle, WA, USA

Abstract

Phosphorylation of tau protein is a critical event in the pathogenesis of Alzheimer disease (AD). Increased phosphorylated tau and total tau levels, combined with reduced concentrations of amyloid beta 1–42 (A β ₄₂) in cerebrospinal fluid (CSF), but not in plasma or serum, have been generally accepted as sensitive AD diagnostic markers. However, obtaining CSF is a relatively invasive procedure that requires participation of specially trained medical professionals, i.e., CSF is not an ideal sample source for screening or early diagnosis of AD, which is essential to current and future neuroprotective treatments for the disease. Here, we identified tau, but not A β species, with mass spectrometry in human saliva, a body fluid that is much more accessible compared to CSF or even blood. Quantitative assessment of salivary levels of total tau, phosphorylated tau, and A β ₄₂ using highly sensitive Luminex assays revealed that, while A β ₄₂ was not detectable, the phosphorylated tau/tau ratio significantly increased in patients with AD compared to healthy controls. These results suggest that salivary tau species could be ideal biomarkers for AD diagnosis, especially at early stages of the disease or even screening asymptomatic subjects, allowing for a much larger therapeutic window for AD patients.

Keywords

Alzheimer disease; Amyloid beta-Peptide; Biomarkers; Saliva; Tau protein

Introduction

Diagnosis of Alzheimer disease (AD) is largely based on clinical assessments, psychological testing, and more recently with the assistance of neuroimaging [1]. In general, however, AD diagnosis is not possible until significant dementia has set in, when no disease-modifying strategies are available [2, 3]. Therefore, inexpensive molecular biomarkers that are

* Address correspondence to: Jing Zhang, Department of Pathology, University of Washington School of Medicine, HMC Box 359635, 325 9th Ave, Seattle, WA 98104, USA. Phone: 1-206-897-5245, Fax: 1-206-897-5452, zhangj@uw.edu.

reproducible and reliable are needed to enhance diagnosis, particularly to help clinicians successfully identify patients and initiate disease-modifying treatment at the earliest possible stage, as well as to monitor disease progression or the outcome of treatment.

Increased total tau (t-tau) or phosphorylated tau (p-tau) protein, along with decreased amyloid beta peptide 1–42 ($A\beta_{42}$), in cerebrospinal fluid (CSF) have been shown to have high sensitivity and specificity in differentiating AD patients from controls [4–6]. Additionally, these markers performed well in predicting the conversion of patients with mild cognitive impairment (MCI), a prodromal state, to full AD dementia [7, 8]. Nonetheless, testing CSF biomarkers involves lumbar puncture that requires highly trained professionals and causes occasional discomfort/side effects in some patients. Several groups have investigated plasma/serum markers including $A\beta$ peptides (tau is largely undetectable in human plasma/serum) as potential AD biomarkers [9]. Nonetheless, to date, the findings of cross-sectional studies of plasma $A\beta$ levels are conflicting, with none of the studies demonstrating enough sensitivity or specificity to diagnose AD [9].

The submandibular gland, which produces most of the total resting and stimulated salivary volume, is significantly impaired in AD patients [10, 11]. Additionally, tau mRNA was reported to be highly expressed in salivary glands [12], and amyloid- β precursor protein ($A\beta$ PP), the precursor protein of $A\beta_{42}$, was found to be expressed in human salivary epithelial cells [13]. Thus, in the present study, we asked whether tau and $A\beta$ are present in human saliva – a body fluid that is easily accessible and collected in a less invasive manner compared to CSF or even blood – and if so, whether they could be used as biomarkers for AD diagnosis. Our pursuit was also motivated by the findings that key proteins unique to Parkinson disease (PD) have been recently identified in saliva [14]. Notably, during our investigation that started about one year ago, Bermejo-Pareja *et al* [15] reported that $A\beta_{42}$ and $A\beta_{40}$ were detectable in human saliva and salivary $A\beta_{42}$ levels increased in mild AD patients.

Materials and Methods

1. Sample Collection and Preparation

For mass spectrometry identification of tau and $A\beta$, whole saliva was collected from several controls as previously described [14]. For preliminary quantitative comparison of biomarkers in AD versus controls, 21 patients with AD and 38 healthy controls were included in this study. All procedures were approved by the Human Subjects Committees of the University of Washington and the VA Puget Sound Health Care System and all subjects provided written informed consent. The clinical diagnosis of probable AD was made using NINDS-ADRDA criteria [16] applied at a clinical consensus conference in the Clinical Core of the University of Washington Alzheimer's Disease Research Center. All control subjects were community volunteers. Control subjects had Mini Mental State Scores ≥ 27 , paragraph recall scores > 6 , no history of neurological disease, and no history or evidence of cognitive or functional decline. The demographic and clinical data of all subjects are listed in Table 1. Saliva was collected by placing a dental cotton roll between the cheek and gum of the subject's mouth for at least one minute (without chewing or swallowing). Once moistened, the cotton roll was spun inside a Salivette (Sarstedt, Nümbrecht, Germany) to obtain saliva following the manufacturer's instructions. The saliva sample was then aliquoted and stored at -70°C until use. Total protein concentrations were measured using a Bicinchoninic Acid Protein Assay kit (Pierce, Rockford, IL, USA).

2. Immunoprecipitation and Mass Spectrometry Identification of tau and A β

Immunoprecipitation was performed as described previously [17] to enrich tau or A β from saliva. Briefly, a mouse monoclonal anti-tau antibody (Cat.# AHB0042, clone TAU-5; Invitrogen, Carlsbad, CA, USA) or a mouse anti-A β antibody (clone 10H3; Pierce/Thermo, Rockford, IL, USA) was covalently conjugated to tosylated Dynabeads (Invitrogen) and then incubated with 15 mL of saliva at 4°C for 48 hours. Enriched proteins were eluted from the beads and then digested with trypsin. The digest was analyzed using a 4800 matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF)-tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA) and an LTQ-Orbitrap hybrid mass spectrometer (Thermo Scientific Corp, San Jose, CA, USA) for peptide and protein identification as previously described [14, 18]. For identifications with MALDI-TOF, the mass spectrometry (MS)/MS spectra were extracted and searched against the International Protein Index (IPI) human protein database (version 3.73) with ProteinPilot™ software (version 3.0, Applied Biosystems) using the Paragon™ method with the Gel-based ID and phosphorylated emphasis pre-settings. For identifications with LTQ-Orbitrap, spectral information was searched using SEQUEST (version 27) against the IPI human protein database (version 3.68) with the following parameters: +15.99 Da for oxidized Met, +57.021 Da for carbamidomethylated Cys; +79.97 Da for phosphorylated Ser/Thr/Tyr; mass tolerance, 500 ppm. Protein identifications were then filtered using PeptideProphet/ProteinProphet as described previously [14].

3. Luminex Assays

Salivary t-tau, p-tau, and A β_{42} levels were measured using established Luminex assays as described previously [19] with minor modifications to minimize the matrix effect in saliva and obtain better assay accuracy. In brief, 50 μ l of saliva was treated with 0.24% Triton X-100 for 1 hour on ice and then diluted to 100 μ l with the diluent from the INNO-BIA AlzBio3 kit (Innogenetics, Gent, Belgium) before incubating with coated beads and the detection antibody (final Triton concentration: 0.1%). 25 μ l of the diluent was added to all background and standard wells to make the total volume (125 μ l) equivalent between samples. All samples were analyzed in a single plate using a LiquiChip Luminex 200™ Workstation (Qiagen, Valencia, CA, USA) in each of the two independent experiments. The limits of detection were determined using the MILLIPLEX Analyst software (version 3.4, Millipore, Billerica, MA, USA) as the following: t-tau, 1.60 pg/mL; p-tau, 1.65 pg/mL; A β_{42} , 21.2 pg/mL. The limits of quantification, determined using a sample/standard of concentration near the expected limit of detection, were 2.6–4.0 pg/mL for t-tau/p-tau. The assays' accuracies were measured by spiking different amounts of standards into a pooled saliva sample; the average recovery rate for the proteins was 50–70%.

4. Statistical Analysis

Analyses were performed with Prism 4.0 (Graphpad, San Diego, CA, USA) and PASW Statistics 18.0 (SPSS/IBM, Chicago, IL, USA). Group differences were assessed using the Mann–Whitney *U* test. Values with $p < 0.05$ were regarded as significant.

Results

After trypsin digestion of the enriched tau proteins from saliva samples, five unique tau peptides were identified by mass spectrometry with an amino acid coverage of 22% (Fig. 1 and Table 2), indicating that tau protein is unequivocally present in human saliva. However, we were unable to identify A β with a similar approach.

Next, in a pilot investigation, using a 3-plex (t-tau, p-tau [181P], and A β_{42}) Luminex kit and a modified protocol to achieve minimal matrix effect and improved assay accuracy, we

quantified salivary t-tau and p-tau levels in 21 AD patients and 38 healthy controls ($A\beta_{42}$ was undetectable in this cohort; data not shown). As shown in Fig. 2A, t-tau levels tended to decrease in patients with AD compared to controls, but the difference diminished after normalizing to saliva total protein levels. There was a clear trend for increased p-tau levels in AD patients, whether normalized to saliva total protein levels or not (Fig. 2B). The p-tau/t-tau ratio (i.e., the proportion of the phosphorylated isoform of tau) increased significantly in patients with AD ($p<0.05$) (Fig. 2C), though the differences in t-tau and p-tau levels between patients and controls did not reach statistical significance.

Discussion

Tau is crucially involved in AD pathogenesis, and hyperphosphorylation of tau has been reported to be critical in the formation of neurofibrillary tangles, which constitute a pathological defining characteristic of AD [2, 3]. In fact, the load and distribution of neurofibrillary tangles are used currently to stage AD pathologically. Here, we explicitly demonstrated the presence of tau protein in human saliva with mass spectrometry. Furthermore, the p-tau/t-tau ratio was significantly different between AD patients and controls, suggesting that salivary tau may be shifted toward a phosphorylated form that is essential to disease development and progression in AD patients [2, 3]. This discovery could be quite significant because of the excellent accessibility of saliva, with essentially no sampling side effects. Once further evaluated and validated in larger studies, changes in salivary tau species could assist in identification of MCI or even preclinical AD patients. In addition, as the characteristics of tau species in saliva are better understood, they may not only serve as markers for disease diagnosis and/or progression but also provide feedback on patient response to pharmaceutical intervention. To this end, given that salivary glands are proximal to the central nervous system via the cranial nerves, and the function of the submandibular gland is significantly impaired with reduced salivary flow rate in patients with AD [10, 11], saliva could serve as another indicator to help with defining AD pathogenesis and progression in addition to providing an excellent source for AD biomarkers.

Several issues need to be addressed. First, in human CSF, both t-tau and p-tau substantially increase in AD over controls; but in saliva, t-tau appeared to remain unchanged or headed in the opposite direction. The second issue is that in the cohort analyzed, we observed much higher salivary p-tau levels than t-tau levels within the same subject (Fig. 2), which is quite different from what has been typically demonstrated in CSF samples. Because p-tau and t-tau were measured simultaneously in a competitive manner in the current assays, one interpretation of this phenomenon is that the majority of salivary tau was phosphorylated in a certain way, resulting in favorable capturing/detection of p-tau over t-tau. Another possibility is that the sample collection method affected the composition of tau species in the samples. Specifically, although the submandibular gland primarily contributes to saliva flow, it is possible that under stimulated conditions (see details in Methods for saliva collection for the current investigation), p-tau is preferentially secreted, possibly from other salivary glands. Comparison of saliva collecting methods and collecting glandular saliva may be necessary for further studies.

This brings the issue of where tau and/or p-tau may come from. Although the source remains unknown, we believe that there are at least several possibilities. First, given that the salivary glands are proximal to the CNS, and tau species can be secreted by neuronal cells to the extracellular space [20, 21], it is possible that salivary tau/p-tau are released from the nerves that innervate salivary glands. Additionally, there are also reports suggesting that CSF may communicate with peripheral compartments by transporting along cranial nerves [22, 23]. Based on our preliminary observations, while salivary t-tau levels remained largely

unchanged, the p-tau levels increased in AD patients compared to controls (Fig. 2); the latter is in line with the pathological changes in the AD brain and CSF. The second possibility is that the tau species are expressed and secreted by the acinar epithelial cells of the salivary glands, and salivary tau levels directly or indirectly reflect the pathological changes in AD salivary glands and brains. This is supported by the observation that tau mRNA was identified in salivary glands [12], although it is unknown whether the mRNA was present in nerves, epithelial cells, or some other type(s) of cells. It is also unknown whether the mRNA is translated into tau protein or not. Further investigations are necessary to examine the precise source(s) of salivary tau/p-tau. However, it is unlikely that salivary tau and p-tau are derived from blood through passive diffusion because they are largely undetectable in blood and their sizes are relatively large.

Another related issue is that high molecular weight tau (>100 kDa) or big tau, with an additional 254 amino acid insert located in the amino-terminal half, was reported to be expressed predominantly in the peripheral nervous system and other tissues [24–26]. Although the first two identified peptides listed in Table 2 are regular/small tau isoform specific, it is possible that both small tau and big tau proteins are present in human saliva, confounding the salivary tau levels to be detected. In our immunoblotting examination of tau proteins from saliva samples using the detection antibodies from the INNO-BIA AlzBio3 kit, however, only tau bands at 40–80 kDa were observed (Supplementary Fig. 1), suggesting that big tau, even if present in human saliva, is unlikely a dominant species to be detected by Luminex. Further confirmation of this and examination of the effects of other potential confounding factors such as disease state, age, sex, and circadian rhythms of salivary production as well as drugs used to treat AD patients are important for controlling variables when salivary tau and p-tau are used for AD biomarkers.

We were unable to identify A β in saliva using mass spectrometry, and the A β ₄₂ fragment was not detectable with the highly sensitive Luminex assay. As discussed earlier, Bermejo-Pareja *et al* reported detectable A β ₄₂ and A β ₄₀ signals in saliva from healthy controls and AD or PD patients using an ELISA kit from Invitrogen [15]. While A β ₄₂ was undetectable in our cohort using the Innogenetics Luminex kit, during our assay development with an Invitrogen Luminex kit (designed for CSF samples), we observed exceedingly high A β ₄₂ signal. However, the observation was accompanied by poor assay accuracy and specificity in pooled control saliva samples (data not shown). The difference between the Innogenetics and Invitrogen Luminex kits might be due to the antibody pairs used in the assays; however, it is unknown whether the antibody pairs included in the Invitrogen Luminex and ELISA kits are the same or not (proprietary information). The fact that A β could not be detected by mass spectrometry suggests that A β , if present in saliva, is low in abundance or heavily modified. Further investigation is necessary to confirm whether A β is indeed present in human saliva, and if so, whether it can be used as an AD biomarker. This is important since A β PP was reported to be produced in a human salivary epithelial cell line [13].

In summary, we successfully identified tau, an important protein related to AD pathogenesis, in human saliva. Furthermore, given the preliminary discovery that p-tau/t-tau levels are increased in AD, salivary p-tau and/or the p-tau/t-tau ratio could be excellent AD diagnostic markers. Our results warrant the necessity to conduct larger studies on the potential utility of salivary tau and p-tau as AD biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institutes of Health grants (AG033398, ES004696-5897, ES007033-6364, ES016873, NS057567, NS062684-6221, and AG005136), NINDS Institutional Center Core Grant-Neuroproteomics P30 (NS055088) as well as grants from the Cheng-Mei Shaw Endowment, Friends of Alzheimer's Research, Alzheimer's Association of Western and Central Washington and the Department of Veterans Affairs. We thank Clete Barrick for his assistance in preliminary experiments, and also deeply appreciate those who have donated their saliva for our study.

References

1. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Meguro K, O'Brien J, Pasquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, Scheltens P. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol.* 2007; 6:734–746. [PubMed: 17616482]
2. Cummings JL, Cole G. Alzheimer disease. *JAMA.* 2002; 287:2335–2338. [PubMed: 11988038]
3. Semla TP. Alzheimer's disease: will advances made in the past turn the tide? *Ann Pharmacother.* 2007; 41:1494–1496. [PubMed: 17666576]
4. Hulstaert F, Blennow K, Ivanoiu A, Schoonderwaldt HC, Riemenschneider M, De Deyn PP, Bancher C, Cras P, Wiltfang J, Mehta PD, Iqbal K, Pottel H, Vanmechelen E, Vanderstichele H. Improved discrimination of AD patients using beta-amyloid(1–42) and tau levels in CSF. *Neurology.* 1999; 52:1555–1562. [PubMed: 10331678]
5. Tang BL, Kumar R. Biomarkers of mild cognitive impairment and Alzheimer's disease. *Ann Acad Med Singapore.* 2008; 37:406–410. [PubMed: 18536828]
6. de Jong D, Kremer BP, Olde Rikkert MG, Verbeek MM. Current state and future directions of neurochemical biomarkers for Alzheimer's disease. *Clin Chem Lab Med.* 2007; 45:1421–1434. [PubMed: 17970699]
7. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol.* 2006; 5:228–234. [PubMed: 16488378]
8. Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, Herukka SK, van der Flier WM, Blankenstein MA, Ewers M, Rich K, Kaiser E, Verbeek M, Tsolaki M, Mulugeta E, Rosen E, Aarsland D, Visser PJ, Schroder J, Marcusson J, de Leon M, Hampel H, Scheltens P, Pirttila T, Wallin A, Jonhagen ME, Minthon L, Winblad B, Blennow K. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA.* 2009; 302:385–393. [PubMed: 19622817]
9. Song F, Poljak A, Smythe GA, Sachdev P. Plasma biomarkers for mild cognitive impairment and Alzheimer's disease. *Brain Res Rev.* 2009; 61:69–80. [PubMed: 19464319]
10. Ship JA, DeCarli C, Friedland RP, Baum BJ. Diminished submandibular salivary flow in dementia of the Alzheimer type. *J Gerontol.* 1990; 45:M61–M66. [PubMed: 2313044]
11. Ship JA, Puckett SA. Longitudinal study on oral health in subjects with Alzheimer's disease. *J Am Geriatr Soc.* 1994; 42:57–63. [PubMed: 8277117]
12. Conrad C, Vianna C, Freeman M, Davies P. A polymorphic gene nested within an intron of the tau gene: implications for Alzheimer's disease. *Proc Natl Acad Sci U S A.* 2002; 99:7751–7756. [PubMed: 12032355]
13. Oh YS, Turner RJ. Effect of gamma-secretase inhibitors on muscarinic receptor-mediated calcium signaling in human salivary epithelial cells. *Am J Physiol Cell Physiol.* 2006; 291:C76–C82. [PubMed: 16467403]
14. Devic I, Hwang H, Edgar JS, Izutsu K, Presland R, Pan C, Goodlett DR, Wang Y, Armaly J, Tumas V, Zabetian CP, Leverenz JB, Shi M, Zhang J. Salivary alpha-synuclein and DJ-1: potential biomarkers for Parkinson's disease. *Brain.* 2011 published online February 24, 2011.
15. Bermejo-Pareja F, Antequera D, Vargas T, Molina JA, Carro E. Saliva levels of Abeta1–42 as potential biomarker of Alzheimer's disease: a pilot study. *BMC Neurol.* 2010; 10:108. [PubMed: 21047401]

16. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984; 34:939–944. [PubMed: 6610841]
17. Liu J, Zhang JP, Shi M, Quinn T, Bradner J, Beyer R, Chen S, Zhang J. Rab11a and HSP90 regulate recycling of extracellular alpha-synuclein. *J Neurosci*. 2009; 29:1480–1485. [PubMed: 19193894]
18. Hong Z, Shi M, Chung KA, Quinn JF, Peskind ER, Galasko D, Jankovic J, Zabetian CP, Leverenz JB, Baird G, Montine TJ, Hancock AM, Hwang H, Pan C, Bradner J, Kang UJ, Jensen PH, Zhang J. DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. *Brain*. 2010; 133:713–726. [PubMed: 20157014]
19. Shi M, Bradner J, Hancock AM, Chung KA, Quinn JF, Peskind ER, Galasko D, Jankovic J, Zabetian CP, Kim HM, Leverenz JB, Montine TJ, Ginchina C, Kang UJ, Cain KC, Wang Y, Aasly J, Goldstein DS, Zhang J. Cerebrospinal Fluid Biomarkers for Parkinson Disease Diagnosis and Progression. *Ann Neurol*. 2011; 69:570–580. published online October 528, 2010. [PubMed: 21400565]
20. Kim W, Lee S, Hall GF. Secretion of human tau fragments resembling CSF-tau in Alzheimer's disease is modulated by the presence of the exon 2 insert. *FEBS Lett*. 2010; 584:3085–3088. [PubMed: 20553717]
21. Kim W, Lee S, Jung C, Ahmed A, Lee G, Hall GF. Interneuronal transfer of human tau between Lamprey central neurons in situ. *J Alzheimers Dis*. 2010; 19:647–664. [PubMed: 20110609]
22. Mollanji R, Bozanovic-Sosic R, Zakharov A, Makarian L, Johnston MG. Blocking cerebrospinal fluid absorption through the cribriform plate increases resting intracranial pressure. *Am J Physiol Regul Integr Comp Physiol*. 2002; 282:R1593–R1599. [PubMed: 12010739]
23. Johnston M, Zakharov A, Papaiconomou C, Salmasi G, Armstrong D. Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in humans, non-human primates and other mammalian species. *Cerebrospinal Fluid Res*. 2004; 1:2. [PubMed: 15679948]
24. Couchie D, Mavilia C, Georgieff IS, Liem RK, Shelanski ML, Nunez J. Primary structure of high molecular weight tau present in the peripheral nervous system. *Proc Natl Acad Sci U S A*. 1992; 89:4378–4381. [PubMed: 1374898]
25. Goedert M, Spillantini MG, Crowther RA. Cloning of a big tau microtubule-associated protein characteristic of the peripheral nervous system. *Proc Natl Acad Sci U S A*. 1992; 89:1983–1987. [PubMed: 1542696]
26. Gu Y, Oyama F, Ihara Y. Tau is widely expressed in rat tissues. *J Neurochem*. 1996; 67:1235–1244. [PubMed: 8752131]

Figure 1. Identification of tau protein in human saliva with mass spectrometry

To determine the presence of tau in saliva, immunoprecipitation was performed to enrich tau from pooled saliva samples collected from several controls. Enriched proteins were analyzed with mass spectrometry (MS) and five unique fragments, corresponding to a total of 90 residues (of the 412 amino acids of tau isoform E as an example), were identified (see also Table 2). Shown are the fragmentation spectra of MS/MS for the sequences SGYSSPGSPGTPGSR (**A**) and TPSLPTPPTR (**B**), with y- and b-series ions interpreted.

Figure 2. Salivary t-tau and p-tau levels in healthy controls and patients with Alzheimer disease Total tau (t-tau) and phosphorylated tau (p-tau) protein levels in saliva were measured by Luminex assays in healthy controls (CTRL) and patients with Alzheimer disease (AD). The t-tau levels (A) tended to decrease in AD compared with the control group, but the difference diminished after normalizing to the saliva total protein levels. The p-tau levels (B) tended to increase in patients with AD compared to controls, whether normalized to the saliva total protein level or not. The p-tau/t-tau ratio (C) increased significantly in AD patients (*, $p < 0.05$ compared to controls). Means with SEM are also shown.

Table 1

Summary of the demographic and clinical data of the participants

	Control (n=38)	AD (n=21)
Mean age (range)	69.0 (40–88)	68.8 (52–85)
Sex (male/female)	19/19	10/11
Mean MMSE (range)	29.4 (27–30)	19.2 (4–29)
Mean CDRS (range)	0.05 (0–0.5)	1.05 (0.5–2)
Mean duration of disease, year (range)	-	4.4 (2–10)

AD = Alzheimer disease; CDRS = Clinical Dementia Rating Scale; MMSE = Mini Mental State Examination.

Table 2

Identification of tau protein in human saliva by mass spectrometry

IPI / Protein name	Coverage (%)	Peptide score*	Identified peptide sequence	Ions
IPI00220175 Tau	21.84	0.99	AEEAGIGDTPSLEDEAAGHVTQAR	13/46
		0.99	STPTAEAEAGIGDTPSLEDEAAGHVTQAR	15/58
		0.98	CGSLGNIHHKPGGGQVEVK	10/36
		1.00	SGYSSPGSPGTPGSR	17/28
		1.00	TPSLTPPTR	14/18
		0.86	IGSLDNITHVPGGGNK	21/60

* These are either the peptide probability scores (shown are *nsp adj prob* - the initial PeptideProphet probability [*init prob*] adjusted for the number of sibling peptides) generated from SEQUEST and PeptideProphet/ProteinProphet, or the Peptide Confidence scores (converted from a 0–100 scale to a 0–1 scale to be consistent with the peptide probability scores) from ProteinPilot scoring algorithm. IPI = International Protein Index.