Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 11 (2019) 53-60

Alzheimer's & Dementia

Diagnostic Assessment & Prognosis

Development and validation of a salivary tau biomarker in Alzheimer's disease

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Abstract

Introduction: Total tau (t-tau) and phosphorylated tau (p-tau) are abnormally elevated in the brain and cerebrospinal fluid of individuals with Alzheimer's disease (AD). Tau is also present in the salivary gland tissue and saliva, and salivary measures might produce an accurate, accessible, and inexpensive biomarker.

Methods: Using unstimulated saliva and Western blot analysis, we quantified the p-tau/t-tau ratio at different phosphorylation sites.

Results: We found that for one phosphorylation site, S396, p-tau/t-tau ratio was significantly elevated in patients with AD compared with normal elderly control subjects. The elevation in saliva, however, did not correlate with cerebrospinal fluid tau or with brain measures such as hippocampal volume.

Discussion: There is significant elevation of p-tau/t-tau ratio for the S396 phosphorylation site. Large variation in the AD salivary tau levels, however, limits the utility of this test as a clinical biomarker.

Keywords: Alzheimer; Tau protein; Biomarkers; Phosphorylation; FTD

1. Introduction

An ideal biomarker for Alzheimer's disease (AD) would be inexpensive, easily obtained, accurate, safe, and repeatable [1]. In cerebrospinal fluid (CSF), amyloid β1−42 is reduced in AD, whereas total tau (t-tau) and phosphorylated tau (p-tau) are elevated [2,3]. P-tau181 is the most studied phosphorylation site and often used in clinical studies [4]. Together, CSF amyloid β1−42, t-tau, and p-tau181 can be used to identify AD with good accuracy; combined, these biomarkers have better diagnostic ability than each separately [4,5].

Saliva has considerable advantages as an easily obtained biofluid. Shi et al [6] used mass spectrometry and highly sensitive Luminex assays to assess the level of salivary t-tau, p-tau, and amyloid β42 in AD. The researchers found that the p-tau/t-tau ratio was significantly higher in patients with AD than that in controls [6]. However, mass spectrometry is expensive, and the collection method was suboptimal [7].

We hypothesized that salivary tau could be developed as a reliable and easily attainable clinical biomarker for AD. Our method of tau analysis, the Western blot, is less expensive than mass spectrometry and has the potential to be carried out in almost any laboratory [8].

In the present study, we wished to determine if p-tau/t-tau ratio was abnormally elevated in the saliva of AD subjects compared with normal elderly control (NEC) subjects. We wished, in addition, to determine the sensitivity and

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https://doi.org/10.1016/j.dadm.2018.03.003

specificity of salivary p-tau/t-tau as a biomarker for AD, and if salivary p-tau/t-tau was abnormally elevated in some subjects with mild cognitive impairment (MCI) [9,10]. Not all MCI subjects will go on to develop AD, but a significant portion of them will, with a conversion rate to AD of about 10%–12% per year [11]. Therefore, if salivary tau was a good early marker, we would have expected a significant portion of MCI subjects to have similar profiles to AD subjects. We also wanted to determine if salivary p-tau/t-tau was abnormally elevated in frontotemporal dementia (FTD) subjects, a heterogenous clinical diagnostic group that includes some individuals with abnormal accumulation of tau (tauopathies) [12].

We also investigated whether salivary tau correlated with indirect brain measures. Specifically, since it is known that in AD there is elevation in CSF p-tau/t-tau, we hypothesized that similar elevation in saliva would correlate with increases in CSF as well. Furthermore, it is known that the brain deposition of tau in AD occurs preferentially in the medial temporal region and correlates with hippocampal atrophy [13,14] and degree of episodic memory impairment [14–16]. In early AD, neurofibrillary tangles (NFTs) and tau pathology are primarily in the medial temporal lobe (hippocampus, amygdala, and parahippocampal gyrus), an area associated with episodic memory [37–40]. If salivary tau was a good reflection of brain tau pathology, we would anticipate a correlation between salivary tau and hippocampal volume and episodic memory scores (but not on tests of general cognitive function).

2. Materials and methods

2.1. Sample collection

All subjects gave informed consent. Collection was carried out in the morning whenever possible to control for possible diurnal variation of salivary tau. Unstimulated saliva was collected by having the subject spit one sample of 4 to 5 mL into a sterile 50 mL polypropylene tube.

2.2. Sample processing

The saliva was then transferred immediately to an identical tube with inhibitor cocktail already in it and kept on ice. The saliva was then put in Eppendorf Tubes and in a hot water bath (100°C) for 20 minutes, then centrifuged at 5000 rpm or 10,000 rpm for 10 minutes at 4°C. The supernatant of each tube was extracted, and all supernatants from one subject combined and vortexed in a 15 mL tube to create a homogenous sample. The sample was redistributed in 0.5 mL aliquots into 1.5 mL Eppendorf Tubes. The 0.5 mL aliquots were stored in −80°C freezer for later analysis. The tau-4 antibody was used to measure t-tau, and all t-tau measures reported in this article used this antibody. Phosphospecific antibodies targeting Thr^{181}, Ser^{396}, and Ser^{404} and a combined antibody for Ser^{400}/Thr^{403}/Ser^{404} were used for the analysis of phosphorylation levels at tau sites T181, S396, and S404 and a combination of sites S400, T403, and T404, respectively. The samples were analyzed for tau using Western blot, and we report in all cases p-tau/t-tau ratios at all sites. For brevity, we will refer to these measured ratio levels as T181, S396, and S404 and the combination S400, T403, and T404 site. For technical reasons, the analysis was carried out in two rounds, the first with 150 samples including AD, MCI, and NEC subjects and the second with 200 samples including AD, NEC, FTD, neurology, and young normal (YN) subjects.

2.3. Subjects

AD, MCI, and FTD patients were recruited from the Memory Clinic and Neurology clinics of the Jewish General Hospital in Montreal, a McGill University teaching hospital. Diagnoses were established by neurologists or geriatricians highly trained in the diagnosis of neurodegenerative diseases. Characteristics of probable AD include meeting the McKhann et al. [17] criteria for dementia, including significant cognitive impairment and symptoms sufficient to interfere with work or daily activities, a gradual onset of symptoms, with either deficits in learning and recall or language, visuospatial, or executive problems, and absence of other neurological diseases causing the symptoms [17]. Evidence of pathophysiological processes, such as NFTs or amyloid plaques, increases certainty of diagnosis [17]. A clinical diagnosis of MCI was made if the subject displayed subjective memory complaints, had normal activities of daily living and general cognitive function, demonstrated objective evidence of memory impairment on testing, and was not demented [10,11,17]. A diagnosis of FTD was made according to accepted current criteria [18]. FTD consists of a set of neurodegenerative diseases, which involve predominant degeneration of the frontal and temporal cortices [16]. Subjects with "mixed" pathology or a mixed AD/Parkinson's diagnosis were excluded. FTD diagnosis was supported by imaging results on magnetic resonance imaging and fluorodeoxyglucose positron emission tomography, and all subtypes (behavioral variant, aphasic variant, and mixed) were accepted. The NEC subjects, aged ≥60 years, were recruited by advertisement. They were screened with the Montreal Cognitive Assessment (MoCA) [19]. Subjects had to score 25 or higher to be included as a control [20]. As well, saliva was collected from healthy YN controls, aged 18–60 years, to determine any age-related difference in salivary tau levels. These volunteer subjects were recruited from clinics of the Jewish General Hospital. To better determine specificity, saliva was also collected from neurology patients with brain diseases not associated with abnormal tau, such as chronic stroke, epilepsy, and multiple sclerosis. These patients were recruited in the Jewish General Hospital Neurology clinics. The neurology patients were also administered the MoCA, or their score was obtained from their treating neurologist and only recruited if their MoCA score was greater than 25/30 to indicate normal cognitive function.

Although in stroke there is a transient increase in CSF tau, there is no chronic change in p-tau [22]. We recruited only subjects with chronic stroke and not acute (i.e., at least 3 months since the stroke).

2.4. Subject clinical and neuropsychological testing

Episodic memory tests and other neuropsychology tests [21] were carried out. The Logical Memory 2 score from the Wechsler Memory Scale (i.e., delayed memory on a paragraph recall test, maximum score = 25) was obtained as a measure of episodic memory [23–25], and the clock drawing test (maximum score = 10) was obtained as a measure of general cognitive function [26,27]. MoCA scores (maximum score = 30) were also obtained.

CSF p-tau and t-tau levels were obtained from 12 individuals from among our study cohort undergoing a lumbar puncture for diagnostic purposes. The cerebrospinal (CSF) tau levels were evaluated by Athena Diagnostics, which uses enzyme-linked immunosorbent assay for analysis and the phosphorylation levels at T181 site as a measure of p-tau.

Hippocampal volumes were obtained from 12 subjects who had a magnetic resonance imaging carried out for another research project and consented to have their scans used for this study. Scans were analyzed through FreeSurfer software [28] to determine the volume of the left and right hippocampus, while accounting for intracranial capacity.

2.5. Statistical analysis

Kruskal-Wallis tests were used to determine differences in NEC, MCI, and AD of data from round one and in AD, NEC, neurology, and FTD, for round two data. Mann-Whitney U tests were used for pairwise contrasts with a Bonferroni-Holmes correction to correct for multiple comparisons. Mann-Whitney U tests were used to determine differences in p-tau/t-tau in YN compared with NEC subjects. Spearman correlations were used for salivary tau correlations with CSF tau, hippocampal volume, and neuropsychology scores. Statistical analysis was carried out using SPSS, version 20, and Prism. Values with $P < .05$ were considered significant, except for the Shapiro-Wilk test, where $P < .05$ indicates a nonnormal distribution.

3. Results

3.1. Round one results

There were two "batches" of samples analyzed, termed round one and round two. Individuals were included in only one batch. One hundred fifty subjects with AD, MCI, or NEC were assessed in the first round of analyses, but only 148 were analyzed. One subject was excluded due to a final diagnosis of "mixed pathology," and one NEC was excluded for not having a MoCA score in the acceptable range. Demographic information for round one data is listed in Table 1. For this round, several of the Western blot analyses were rejected for technical problems, resulting in a reduced sample size for pS404.

The Shapiro-Wilk tests for normality suggested that the data did not conform to a normally distributed population, $P < .05$ for the data of each phosphorylation site. Data for each phosphorylation site (T181, S396, and S404 and the combination S400, T403, and T404 site) were therefore analyzed using nonparametric tests. We will always be presenting data in terms of ptau/t-tau ratios in all cases.

At all the phosphorylation sites tested, results followed a similar trend, with NEC having the lowest and AD having the highest median p-tau/t-tau levels (Fig. 1). We excluded extreme outliers (three outliers at S396, two outliers at the combination S400, T403, S404, one outlier at T181, and two outliers at S404), whose p-tau/t-tau value exceeded 10 suggesting technical problems with the sample. With outliers excluded, a Mann-Whitney U test performed on the data revealed that AD subjects had significantly elevated p-tau/t-tau levels at three of the four phosphorylation sites tested, namely S396, S404 and the combination S400,T403, T404 site, U(45, 45) = 768.00, U(19, 18) = 95.00 U(46, 45) = 780, respectively, $P < .05$ for all comparisons (Fig. 2).

3.2. Round two results

Two hundred other subjects were included in the second round of analysis. Eight subjects were excluded because their t-tau levels were undetectable by Western blot, two subjects were excluded on receiving a final diagnosis not within criteria of any of the groups, and one sample was excluded due to the subject having given a saliva sample twice. A total of 189 saliva samples were analyzed in round two. Demographic data for subjects included in the analysis are shown in Table 2.

Similar to round one data, the Shapiro-Wilk tests for normality suggested that the data from each site did not have a normally distributed population, $P < .05$ for the data of each phosphorylation site. Data for phosphorylation site(s) were therefore analyzed using nonparametric tests.

A two-tailed Kruskal-Wallis test was performed on the p-tau/t-tau ratio at S396. Data revealed a significant diagnostic group effect, Chi^2 (3) = 12.973, $P < .05$ (Fig. 3).

Table 1
Demographic information for round one data

Subjects	N*	F:M	Median age (IQR)
AD	46	22:24	80 (9)
NEC	47	32:15	73 (6)
MCI	55	32:23	78 (14)

Abbreviations: AD, Alzheimer's disease; NEC, normal elderly control; MCI, mild cognitive impairment; IQR, interquartile range.

*For S404, part of the Western blot was technically uninterpretable, and therefore there was a reduced sample size for this site for this round of data (n = 19, 20, 16, for AD, NEC, and MCI, respectively).

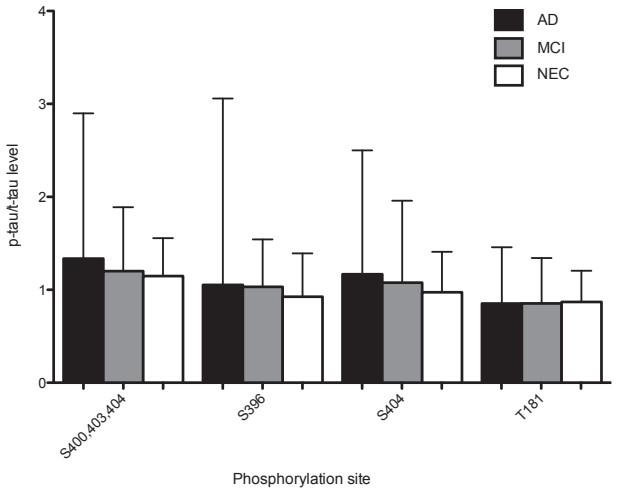

Fig. 1. Round one data. Median p-tau/t-tau levels in AD (n = 46 for all sites, except S404 where n = 19), MCI (n = 55 for all sites, except S404 where n = 16) and NEC (n = 47 for all sites, except S404 where n = 20) at each phosphorylation site. Error bars = IQR. All sites no significant (n. s.) difference ps > 0.05. Abbreviations: AD, Alzheimer's disease, MCI, mild cognitive impairment; NEC, normal elderly control; p-tau, phosphorylated tau; t-tau, total tau; IQR, interquartile range.

Subsequent pairwise comparison tests, conducted using Mann-Whitney tests with a Bonferroni-Holmes correction, indicated that the median p-tau/t-tau ratio at S396 for both the AD and FTD groups were significantly increased compared with the NEC group, $P < .05$ for each comparison. No other pairwise contrast was significant ($P > .05$ for all comparisons).

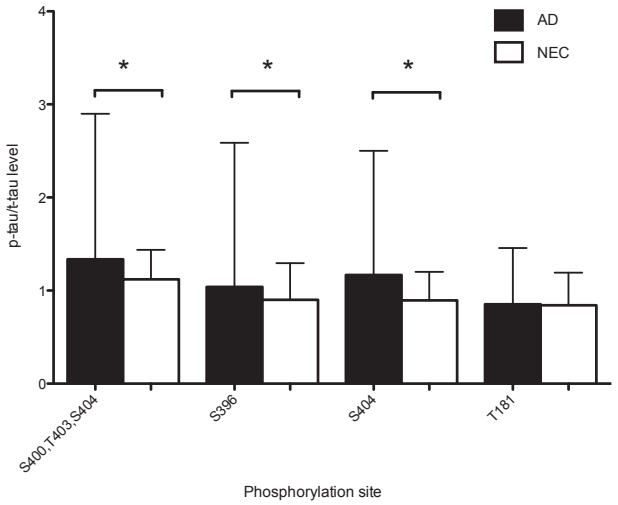

Fig. 2. Round one data. Median p-tau/t-tau levels in AD (n = 46 for S400, 403, 404, and T181, n = 45 for S396, and n = 19 for S404) and NEC (n = 45 for S400, 403, 404, and S396, n = 46 for T181, n = 18 for S404) at each phosphorylation site, with outliers (>10 p-tau/t-tau level) excluded. Error bars = IQR. *$PS < 0.05$. Abbreviations: AD, Alzheimer's disease; NEC, normal elderly control; p-tau, phosphorylated tau; t-tau, total tau; IQR, interquartile range.

Table 2
Demographic information for round two data

Subjects	N	F:M	Median age (IQR)
AD	41	24:17	80 (8)
NEC	44	30:14	72 (7)
FTD	16	5:11	71.5 (10)
NEUR	12	7:5	55 (11)
YN	76	45:31	32 (22)

Abbreviations: AD, Alzheimer's disease; FTD, frontotemporal dementia; NEC, normal elderly control; NEUR, neurology; YN, young normal; IQR, interquartile range.

A two-tailed Kruskal-Wallis test performed on the p-tau/t-tau ratio at S404 revealed a significant diagnostic group effect, $Chi^2(3) = 15.900$, $P < .05$ (Fig. 4). Subsequent pairwise comparison tests, conducted using Mann-Whitney tests with a Bonferroni-Holmes correction, indicated that the median p-tau/t-tau ratio level at S404 in the FTD group was significantly greater than the NEC group, $P < .05$. No other pairwise contrast was significant ($P > .05$ for all comparisons).

3.3. Sensitivity and specificity

We looked only at the AD and NEC groups to establish measures of sensitivity and specificity of salivary p-tau as a biomarker. Patients with AD had most significantly elevated p-tau/t-tau ratios compared with NEC at S396. Seventy-five percent of AD subjects demonstrated a ratio greater than a cutoff ratio measure of 0.96, whereas this was approximately the median level for NEC subjects (1.00). We examined AD and NEC groups of the round 2 data to establish measures of sensitivity and specificity in the standard fashion. Using a cutoff level of 1.0, the p-tau/t-tau ratio at S396 had a sensitivity of 73% and specificity

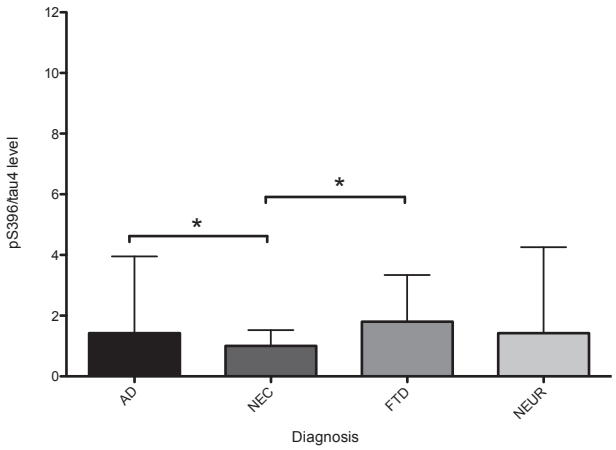

Fig. 3. Round two data. Median p-tau/t-tau ratios for S396 in AD (n = 41), NEC (n = 44), FTD (n = 16) and NEUR (n = 12) subjects. Error bars = IQR. *$PS < 0.05$ (with Bonferroni-Holmes correction). Abbreviations: AD, Alzheimer's disease; FTD, frontotemporal dementia; NEC, normal elderly control; NEUR, neurology; p-tau, phosphorylated tau; t-tau, total tau; IQR, interquartile range.

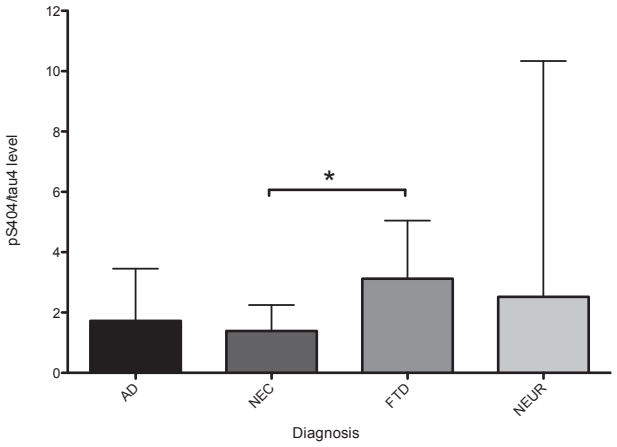

Fig. 4. Round two data. Median p-tau/t-tau ratios for S404 in AD (n = 41), NEC (n = 44), FTD (n = 16), and NEUR (n = 12) subjects. Error bars = IQR. *$PS < 0.05$ (with Bonferroni-Holmes correction). Abbreviations: AD, Alzheimer's disease; FTD, frontotemporal dementia; NEC, normal elderly control; NEUR, neurology; p-tau, phosphorylated tau; t-tau, total tau; IQR, interquartile range.

of 50%. Using the same cutoff level, the p-tau/t-tau ratio at S404 had a sensitivity of 83% and a specificity of 30%.

3.4. Correlations and further analyses

3.4.1. Age and sex analysis

Given the significant difference in our AD and NEC subjects at S396 and S404, we compared p-tau/t-tau ratio levels of NEC (old normal) and YN subjects, at these two sites. Two-tailed Mann-Whitney U tests performed on the data at each site revealed that age group in cognitively normal individuals did not differentially influence either S404 levels, U (44, 76) = 1542.000, or S396 levels, U (44, 76) = 1413.000, $P > .05$ for all comparisons.

There was an unequal female to male ratio in our groups (Table 2). A two-tailed Mann-Whitney U test performed on the data revealed that p-tau levels were not different in male vs. female sex at either S396 location, U (30, 14) = 158.000, or S404 location, U (30, 14) = 192.000, $P > .05$ for all comparisons.

3.4.2. CSF correlation

A Spearman correlation (n = 12) showed CSF p-tau/t-tau ratio and salivary p-tau/t-tau ratio at T181 were not significantly correlated, $r_s = 0.168$ $P > .05$. Furthermore, CSF p-tau/t-tau and salivary p-tau/t-tau at sites S396 and S404 were not correlated, $r_s = 0.357$ and $r_s = 0.070$ respectively, $P > .05$ for all comparisons.

3.4.3. Hippocampal volume correlation

A Spearman correlation (n = 12) showed that salivary p-tau/t-tau ratio at S396 was not significantly correlated with either left or right hippocampus volume, $r_s = -0.413$ and $r_s = -0.483$, respectively, $P > .05$ for each comparison. Likewise, a Spearman correlation (n = 12) showed that salivary p-tau/t-tau ratio at S404 was not significantly correlated with either left or right hippocampus volume, $r_s = 0.049$ and $r_s = -0.133$, respectively, $P > .05$ for each comparison.

3.4.4. Episodic memory scores

Spearman correlations for round one data showed that salivary p-tau/t-tau ratio at S396 (n = 21) and S404 (n = 8) were not significantly correlated with MoCA scores, $r_s = -0.125$ and $r_s = -0.109$, respectively, $P > .05$ for each comparison. As well, Spearman correlations showed that salivary S396 (n = 21) and S404 (n = 6) levels were not significantly correlated with logical memory 2 scores of the Wechsler Memory Scale, $r_s = -0.165$ and $r_s = -0.058$, respectively, $P > .05$ for each comparison. Finally, Spearman correlations showed that salivary S396 (n = 23) and S404 (n = 8) levels were not significantly correlated with the clock drawing test scores, $r_s = -0.099$ and $r_s = -0.600$, respectively, $P > .05$ each comparison.

4. Discussion

Our analysis of salivary p-tau/t-tau ratio levels revealed a significant difference between AD subjects and cognitively healthy elderly subjects at S396, along with several other positions. There was no such elevation in young or old normal subjects or in subjects with other neurological diseases. There was also a significant increase in the p-tau/t-tau ratio in FTD subjects at the same site and at S404. The p-tau/t-tau ratio increase in AD subjects, however, did not correlate with direct or indirect brain damage measures reflecting AD—namely CSF tau levels and decreased hippocampal volume. Nor did it correlate with impairment on neuropsychology tests. Notably, at all the sites and in all diagnostics groups we considered, there was large variation in p-tau/t-tau levels. The relatively low sensitivity and specificity of p-tau/t-tau levels to distinguish AD and NEC reflects a variability which severely limits the utility of the test as a diagnostic biomarker for clinical use.

An unexplained finding was that only one (or two) out of the four sites examined showed a significant difference in p-tau/t-tau levels between AD and NEC. Surprisingly, T181, one of the most studied CSF phosphorylation sites, did not show a difference between the two groups. Furthermore, no significant correlation between the CSF p-tau/t-tau ratio (using T181) and salivary p-tau/t-tau was found. One possible reason that not all of our sites showed a significant difference is that some sites may simply be better peripheral markers than others. More information on upstream mechanisms that result in salivary p-tau is needed to help understand why some sites are better than others.

The most problematic finding for the use of this test as a biomarker was the variability in phosphorylation level of the AD group. This limited the sensitivity to no more than 83% and 73% at the most significant phosphorylation sites. A

portion (approximately 1/3) of AD subjects did not have elevated p-tau/t-tau in their saliva. Although the overlaps and limited sensitivity limit the use of salivary tau as a biomarker, our findings do suggest that it represents a peripheral manifestation of AD. While not the first to find markers outside of the brain cavity, our study adds to the literature of peripheral manifestations of AD. Our findings and those of others question the notion of AD as strictly a disease of the brain.

Several explanations for the variability of p-tau/t-tau ratio levels in AD and controls are worth considering. A small proportion of individuals clinically diagnosed as probable AD may lack tau pathology itself [29]. Another explanation may be that even for those AD subjects (the large majority) who showed elevated tau levels in the brain, a proportion of these individuals failed to express the tau peripherally in salivary gland tissue. Alternatively, it is possible that abnormal tau is present in most of the salivary tissue but secreted in saliva only in a subgroup of AD individuals. Finally, while we believe that we controlled for other technical issues related to saliva collection, there may have been unknown technical limitations which impaired our measurements nevertheless.

Despite an overall p-tau/t-tau ratio level that was lower in NEC than AD subjects, a high level was found in some of our NEC subjects. This resulted in a low specificity-50% and 30%, for the p-tau/t-tau ratio at pS396 and pS404, respectively. NFTs can be present in elderly subjects without dementia, although neocortical NFTs are mostly absent [30–32]. In our study, the elderly subjects recruited were volunteers, many having been previously involved in research, and had on average greater than 15 years of formal education. Therefore, they may have some capacity to compensate for accumulation of neurofibrillary pathology.

Further study is needed to determine the stability of salivary tau to assess its utility as a biomarker. There may be diurnal variation, although this is unproven. In our study, to minimize these potential effects, we collected saliva in the morning. Furthermore, future research would be needed to determine day to day variability of salivary tau in an individual. In addition, other factors may affect quantification of salivary tau, such as the types of tubes used for collection. In this study, we have included our methodology for quantifying tau, while minimizing degradation after collection. CSF tau has good reproducibility within a site, but there is some variability in measurements between centers [2,4]. It is possible that a similar phenomenon would be seen with salivary tau.

FTD subjects in our study were found to have higher p-tau/t-tau levels than NEC subjects, although there was still considerable variation (Figs. 3 and 4). Not all FTDs are tauopathies, which may have contributed to variability. Interestingly, there was less variation in the FTD group than that in the AD group. Although this test would not be ideal for differentiating AD and FTD, the use of different isoform-specific antibodies could create for a more specific test to distinguish between these subjects. Even CSF tau may have limited value for discriminating FTD from AD or healthy aging due to pathological heterogeneity of FTD's many subtypes [33]. Further work should look at specific subtypes of FTD and abnormal salivary tau.

As presented in the introduction, brain tau pathology is correlated with decreased hippocampal and medial temporal volumes [35,36] as well as decreased episodic memory [37–39]. We therefore expected salivary tau to correlate with these in a similar fashion. Although we found a decrease in hippocampal volume in AD subjects and there was a trend in the correct direction (decreased volume should correlate with increased salivary p-tau/t-tau), the correlation was not significant (Table 3). Salivary p-tau/t-tau did not correlate significantly with logical memory 2, clock drawing test, or MoCA scores at either S396 or S404. Athena diagnostics, where CSF samples were analyzed, uses phosphorylation at T181 as the exclusive site for measuring p-tau/t-tau, and so we used this same site of salivary tau to look for a correlation [34]. No significant correlation was found between CSF p-tau/t-tau and salivary p-tau/t-tau (Table 3). At present, we have no good explanation for the lack of these expected correlations. Again, unexplained variability in secretion of p-tau from salivary glands may simply have obscured these physiological correlations.

Biomarkers for AD in peripheral tissues have been studied previously, with literature support for significant biological changes appearing in nonneural tissues like fibroblasts, blood, and buccal cells [42]. For example, tau was found to be elevated in buccal cells of AD subjects [42]. Further research into tau and its presence and phosphorylation in salivary glands is required. Shi et al. [6] identified several possible explanations for the mechanism by which tau gets into saliva. Because the salivary glands are near the central nervous system, one idea is that tau is released from nerves that innervate the salivary glands [6]. Another suggestion is that tau is expressed and secreted by acinar epithelial cells of the salivary glands [6], supported by the fact that tau mRNA has been found in salivary glands [41]. Further work is needed to establish the mechanism for which tau and p-tau end up in saliva.

Table 3
Correlations with other measures

Measure	Salivary site used for correlation	N	r_s (all n.s. $P > .05$)
CSF p-tau/t-tau	T181	12	0.168
Left hippocampal volume	S396	12	−0.412
Right hippocampal volume	S396	12	−0.483
Logical memory II score (Wechsler memory scale)	S396	21	−0.165
Clock drawing task	S396	23	−0.099
Montreal cognitive assessment	S396	21	−0.125

Abbreviation: CSF, cerebrospinal fluid.

Acknowledgments

This study was funded by grants from the Garfield-Weston Fund and by the Canadian Institutes for Health Research (CIHR) to H.C. The authors gratefully acknowledge support from Shelley Solomon (recruitment and testing), Chris Hosein (recruitment), Shrisha Mohit (collection and processing), Dr. Jim Nikelski (MRI analysis), Dr. Joe Rochford (statistical advice), Dr. Ana Velly (statistical advice), Dr. Nora Kelner (neuropsychology advice), and Kathy De Sousa (consent form translation). Many thanks to Jessica Hier, Jessica Wang, and Rebecca Kucharsky Hiess for their help in collection and processing of samples. Thank you to Randi Pilon and Dr. Tyler Roncero for providing the MRIs. Thank you to all staff of the Memory and Neurology clinics at the Montreal JGH for allowing us to collect samples for the project. This study was approved by the Research Ethics Board of the Jewish General Hospital (January 21, 2015).

RESEARCH IN CONTEXT

1. Systematic review: An ideal biomarker does not exist for Alzheimer disease (AD) but salivary tau levels seem promising, and are inexpensive and noninvasive. It is unknown whether it is feasible, sensitive, or specific.
2. Interpretation: In a large sample of subjects with Alzheimer's disease and mild cognitive impairment, salivary tau phosphorylation site levels established by Western blot showed abnormally increased levels in AD at certain phosphorylation sites (particularly S396) compared with normal elderly controls, but the sensitivity and specificity were not robust enough to serve as a clinical test. About a third of AD subjects failed to show this elevation of salivary tau.
3. Future directions: Assessment of diurnal variability and replicability of salivary tau is needed. Potential search for clinical subgroup is warranted.

References

[1] Kennard M. Diagnostic markers for Alzheimer's disease. Neurobiol Aging 1998;19:131–2.
[2] Blennow K, Zetterberg H. The application of cerebrospinal fluid biomarkers in early diagnosis of Alzheimer disease. Med Clin North Am 2013;97:369–76.
[3] Blennow K, Vanmechelen E, Hampel H. CSF total tau, AB42 and phosphorylated tau protein as biomarkers for Alzheimer's disease. Mol Neurobiol 2001;24:87–97.
[4] Hoglund K, Fourier A, Perret-Liaudet A, Zetterberg H, Blennow K, Portelius E. Alzheimer's disease – recent biomarker developments in relation to updated diagnostic criteria. Clin Chim Acta 2015;449:3–8.
[5] Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. Am Soc Exp NeuroTher 2004;1:213–25.
[6] Shi M, Sui Y, Peskin E, Li G, Hwang H, Devic I, et al. Salivary tau species are potential biomarkers for Alzheimer's disease. J Alzheimer's Dis 2011;27:299–305.
[7] Wang J, Schipper H, Velley A, Mohit S, Gornitsky M. Salivary biomarkers of oxidative stress: a critical review. Free Radic Biol Med 2015;85:95–104.
[8] Qureshi H, Pekeles H, Schipper H, Gornitsky M, Chertkow H, Paudel H. Development and validation of a salivary Tau biomarker in Alzheimer Disease. Alzheimers Dement 2015;11:510.
[9] Chertkow H, Feldman HH, Jacova C, Massoud F. Definitions of dementia and predementia states in Alzheimer's disease and vascular cognitive impairment: consensus from the Canadian conference on diagnosis of dementia. Alzheimer's Res Ther 2013;5:S2.
[10] Albert M, DeKosky S, Dickson D, Dubois B, Feldman H, Fox N, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:270–9.
[11] Peterson R, Smith G, Waring S, Ivnik R, Tangalos E, Kokmen E. Mild cognitive impairment. Arch Neurol 1999;56:303–8.
[12] Bang J, Spina S, Miller B. Frontotemporal dementia. Lancet 2015; 386:1672–82.
[13] de Souza L, Chupin M, Lamari F, Jardel C, Leclercq D, Colliot O, et al. CSF tau markers are correlation with hippocampal volume in Alzheimer's disease. Neurobiol Aging 2012;33:1253–7.
[14] Chiu M, Chen Y, Chen T, Yang S, Yang F, Tseng T, et al. Plasma tau as a window the brain-negative associations with brain volume and memory function in mild cognitive impairment and early Alzheimer's disease. Hum Brain Mapp 2014;35:3132–42.
[15] Aschenbrenner A, Balota D, Fagan A, Duchek J, Benzinger L, Morris J. Alzheimer disease cerebrospinal fluid biomarkers moderate baseline differences and predict longitudinal change in attentional control and episodic memory composites in the Adult Children study. J Int Neuropsychol Soc 2015;21:573–83.
[16] Pettigrew C, Soldan A, Moghekar A, Wang M, Gross A, O'Brien R, et al. Relationship between cerebrospinal fluid biomarkers of Alzheimer's disease and cognition in cognitively normal older adults. Neuropsychologia 2015;78:63–72.
[17] McKhann G, Knopman D, Chertkow H, Hyman BT, Jack CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging and the Alzheimer's Association workgroup on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–9.
[18] Rascuvsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 2011; 134:2456–77.
[19] Nasreddine ZS, Philips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53:695–9.
[20] Roalf D, Moberg P, Xie S, Wolk D, Moelter S, Arnold S. Comparative accuracies of two common screening instruments for classification of Alzheimer's disease, mild cognitive impairment, and healthy aging. Alzheimer's Dement 2013;9:529–37.
[21] Jessen F, Wolfgruber S, Wiese B, Bickel H, Mosch E, Kadusziewicz H, et al. AD dementia risk in late MCI, in early MCI, and in subjective memory impairment. Alzheimer's Dement 2014;10:76–83.
[22] Hesse C, Rosengren L, Andreasen N, Davidsson P, Vanderstichele H, Vanmechelen E, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci Lett 2001;297:187–90.
[23] Wechsler D. Whechsler Memory Scale - Third Edition, Adminitration and Scoring Manual. San Antonio, USA: The Psychological Corporation; 1997.

[24] Butler M, Retzlaff P, Vanderploeg R. Neuropsychological test usage. Prof Psychol Res Pr 1991;22:510–2.
[25] Morris J, Swier-Vosnos A, Woodworth C, Glass Umfleet L, Czipri S, Kopald B. Development of alternative paragraphs for the logical memory subtest of the Wechsler Memory Scale-IV. Appl Neuropscyhol Adult 2014;21:143–7.
[26] Shulman K. Clock-drawing: is it the ideal cognitive screening test? Int J Geriatr Psychiatry 2000;15:548–61.
[27] Pinto E, Peters R. Literature review of the clock drawing test as a tool for cognitive screening. Dement Geriatr Cogn Disord 2009; 27:201–13.
[28] Shen L, Saykin A, Kim S, Firpi H, West J, Risacher S, et al. Comparison of manual and automated determination of hippocampal volumes in MCI and early AD. Brain Imaging Behav 2010;4:86–95.
[29] Galasko D, Hansen L, Katzman R, Wiederholt W, Masliah E, Terry R, et al. Clinical-neuropathological correlations in Alzheimer's disease and related dementias. Arch Neurol 1994;51:888–95.
[30] Haroutunian V, Purohit D, Perl D, Marin D, Khan K, Lantz M, et al. Neurofibrillary tangles in nondemented elderly subjects and mild Alzheimer disease. Arch Neurol 1999;56:713–8.
[31] Serrano-Pozo A, Frosch M, Masliah E, Hyman B. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 2011;1:a006189.
[32] Davis D, Schimitt F, Wekstein D, Markesbery W. Alzheimer neuropathologic alterations in aged cognitively normal subjects. J Neuropathol Exp Neurol 1999;58:376–88.
[33] Hampel H, Teipel S. Total and phosphorylated tau proteins: evaluation as core biomarker candidates in frontotemporal dementia. Dement Geriatr Cogn Disord 2004;17:350–4.
[34] Ewers M, Mattson N, Minthon L, Molinuevo J, Antonell A, Popp J, et al. CSF biomarkers for the differential diagnosis of Alzheimer's disease: a large-scale international multicenter study. Alzheimer's Dement 2015;11:1306–15.
[35] Apostolova L, Zarow C, Biado K, Hurtz S, Boccardi M, Somme J, et al. Relationship between hippocampal atrophy and neuropathology markers: a 7T MRI validation study of the EADC-ADNI Harmonized Hippocampal Segmentation Protocol. Alzheimer's Dement 2015; 11:139–50.
[36] Tarawneh R, Head D, Allison S, Buckles V, Fagan A, Landenson J, et al. Cerebrospinal fluid markers of neurodegeneration and rates of brain atrophy in early Alzheimer disease. JAMA Neurol 2015; 72:656–65.
[37] Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991;82:239–59.
[38] Visser P, Verhey F, Hofman P, Scheltens P, Jolles J. Medial temporal lobe atrophy predicts Alzheimer's disease in patients with minor cognitive impairment. J Neurol Neurosurg Psychiatr 2002;72:491–7.
[39] Shin J, Lee S, Kim S, Kim Y, Cho S. Multitracer PET imaging of amyloid plaques and neurofibrillary tangles in Alzheimer's disease. NeuroImage 2008;43:236–44.
[40] Brion J. Neurofibrillary tangles and Alzheimer's disease. Eur Neurol 1998;40:130–40.
[41] Conrad C, Vianna C, Freeman M, Davies P. A polymorphic gene nested within an intron of the tau gene: implications for Alzheimer's disease. PNAS 2002;99:7751–6.
[42] Francois M, Leifert W, Martins R, Thomas P, Fenech M. Biomarkers of Alzheimer's disease risk in peripheral tissues; focus on buccal cells. Curr Alzheimer Res 2014;11:519–31.