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Early diagnosis of mild cognitive impairment and Alzheimer's disease based on salivary lactoferrin

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Abstract

Introduction: The Alzheimer's disease (AD) process is likely initiated many years before clinical onset. Biomarkers of preclinical disease are critical for the development of disease-modifying or even preventative therapies. Current biomarkers for early disease, including cerebrospinal fluid tau and amyloid-β (Aβ) levels, structural and functional magnetic resonance imaging, and the use of brain amyloid imaging, are limited because they are very invasive or expensive. Noninvasive biomarkers may be a more accessible alternative, but none can currently detect preclinical AD with the required sensitivity and specificity.

Methods: Here, we show a novel, straight-forward, and noninvasive approach for assessment of early stages of cognitive decline. Salivary samples from cases of amnestic mild cognitive impairment (aMCI) and AD, and neurology controls were analyzed.

Results: We have discovered and validated a new single saliva biomarker, lactoferrin, which in our cross-sectional investigation perfectly discriminates clinically diagnosed aMCI and AD patients from a cognitively healthy control group. The accuracy for AD diagnosis shown by salivary lactoferrin was greater than that obtained from core cerebrospinal fluid (CSF) biomarkers, including total tau and CSF $A\beta_{42}$. Furthermore, salivary lactoferrin can be used for population screening and for identifying those underdiagnosed subjects with very early stages of mild cognitive impairment and AD.

The present publication has been prepared on the basis of the results obtained in the biomedical research project signed between the Geroa Diagnostics S.L. entity and the Hospital 12 de Octubre Biomedical Foundation under the title "search of new salivary biomarkers in Alzheimer's disease and other neurodegenerative diseases: possible diagnostic application." The research was carried out in accordance with the scientific-technical specifications provided by Geroa Diagnostics S.L. The results of this project belong to Geroa Diagnostics S.L., who holds the exclusive ownership of the intellectual property rights that protect them, with the exception of the ones inherent to the author/inventor. This publication has been executed with the prior authorization of the rights titleholder Geroa Diagnostics S.L.

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Conclusion: This biomarker may offer new insights in the early diagnostics for AD.

Keywords: Noninvasive biomarkers; Saliva; Lactoferrin; Alzheimer's disease; Mild cognitive impairment; Dementia; Diagnosis

1. Background

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the aging population, and because of the increase in longevity, the prevalence of AD is expected to raise dramatically. Because AD process is probably initiated many years before the clinical onset [1], biomarkers of preclinical disease are critical for the development of disease-modifying or even preventative therapies [2]. Unfortunately, current biomarkers for early disease, including cerebrospinal fluid tau and amyloid-β (Aβ) levels [3], structural and functional magnetic resonance imaging [4], and the use of brain amyloid imaging or inflammaging [5,6], are limited because they are either invasive, time consuming, or expensive. Therefore, detecting AD at the earliest possible stage is vital to enable trials of disease modification agents and considerable efforts are being invested in the identification.

Saliva testing is currently used in areas of toxicology, endocrinology, infectious diseases, and forensics, with established diagnostic tests available for alcohol detection, HIV infections, hormonal analyses, and drug testing. Because saliva collection is noninvasive and relatively stress free, saliva can serve as a potential alternative and universal diagnostic fluid. Identification of Aβ and tau [7,8], or α-Syn and DJ-1 [9] in human saliva, proteins that are critically involved in AD and Parkinson's disease (PD), respectively, support the potential diagnostic value of saliva for neurodegenerative diseases.

A history of systemic infection is a known risk factor for AD [10–12]. Brain infections with bacteria or viruses are implicated in AD pathogenesis [13], but the impact of antimicrobial peptides on disease outcomes has not been sufficiently explored. Saliva is one of the body's first lines of defense due to its composition of antimicrobial proteins. Lactoferrin, one of the major antimicrobial peptides in saliva, represents an important defensive element by inducing a broad spectrum of antimicrobial effects against bacteria, fungi, protozoa, viruses, and yeasts [14–17], through its ability to decrease bacterial growth, biofilm development, iron overload, reactive oxygen formation, and regulating the inflammatory response [18,19].

The primary aim of our study was to investigate the potential of an AD diagnostic biomarker in saliva. We first carried out an AD diagnostic cross-sectional study and enrolled 274 participants at the Neurology Service at the Hospital Universitario 12 de Octubre (Madrid, Spain). We defined four groups of subjects according to their cognitive status: amnestic mild cognitive impairment (aMCI), AD, PD, and cognitively healthy control group. We discovered in this first diagnostic training study that saliva lactoferrin, an iron- but also Aβ-binding [20,21] glycoprotein, was strongly correlated with AD. We secondly validated the saliva lactoferrin as AD biomarker in two new blinded and independent cohorts. Finally, salivary levels of lactoferrin were examined in two independent longitudinal cohorts composed of healthy nondemented individuals.

2. Methods

2.1. Subjects and clinical classification

For the cross-sectional study, we included four groups of donors in the training study: (n = 80) AD patients; (n = 44) aMCI patients; (n = 59) PD patients; and (n = 91) elderly

Table 1
Demographic and clinical characteristics of subjects from first training study

Variable	Control	aMCI	AD	PD	*P* value
n (F/M)	91 (59/32)	44 (25/19)	80 (49/31)	59 (32/27)	ns
Age (years)	73.7 ± 6.88	75.16 ± 5.13	76.2 ± 5.33**	69.5 ± 8.6**	<.01
MMSE score	29 ± 0.8	26.8 ± 1.16***	19.25 ± 1.76***	NA	<.001
CDR score	0	0.5	≥1	NA	
APOE ε4 carriers	12.9%	42.1%**	45.9%**	NA	<.01
Education					
Can read and write	22.2%	36%	38.5%	NA	
Primary studies	33.3%	36%	33.3%		
Secondary studies	44.4%	28%*	28.2%*		<.05

Abbreviations: aMCI, amnestic mild cognitive impairment; AD, Alzheimer's disease; PD, Parkinson's disease; F, female; M, male; ns, not significant; MMSE, Mini–Mental State Examination; NA, not applicable; CDR, Clinical Dementia Rating.

NOTE. Data are expressed as mean ± SD. *P < .05 versus control group; **P < .01 versus control group; ***P < .001 versus control group.