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Abstract

A very significant challenge in Alzheimer's disease today is the discovery of suitable technologies for detection of the disease that are easy to use, cost effective and non-invasive. In this study we demonstrate that saliva biomarkers are a suitable option for detection of the disease. Accordingly, we collected saliva samples by three different methods from matched age and gender of Alzheimer patients and normal healthy subjects. Salivary β -40, β -42, IGF-I, IGF-II, alpha amylase, IL-1 β , and TNF-alpha levels were analyzed. Of the three methods, passive drooling was found to be the best saliva collection method for analysis of biomarkers of Alzheimer's disease. There were significant differences in the salivary biomarkers evaluated between patients and controls. Our results confirm that β -40, β -42, IGF-I, IGF-II, alpha amylase, IL-1 β , and TNF-alpha are appropriate diagnostic biomarkers for Alzheimer's disease.

Introduction

Alzheimer's disease [AD] is a lethal neurodegenerative disorder that presently affects approximately 10.6 million people in the USA and Europe, with predictable estimates reaching epidemic proportions of nearly 15.4 million afflicted by the year 2030 (1). AD leads to a decrease in cognitive function, loss of memory and other effects. It has been reported that more than US\$183 billion was spent on AD patients in 2011 in the USA alone new estimates that project cost increases to as high as US\$1 trillion by the year 2050 (1). For this reason there is an urgent need for early detection methodologies and effective treatment regimens. Confirmation of AD is only currently possible by postmortem analysis of brain specimens of dementia-afflicted subjects. The clinical detection of AD is based on a clinical examination which includes a battery of laboratory tests, functional neuro-imaging tools such as functional MRI, PET etc, and neuropsychological evaluation by a range of different

methods. Clinical symptoms only appear after the onset of disease (2-4). Certain β -amyloids (β) such as β 10, β 12 etc., hyperphosphorylated tau protein, α -synuclein, ubiquitin, apolipoprotein E, alpha antichymotrypsin and others have been shown to be pathological indicators of AD (2-6).

β 40, β 42, apoE-2, apoE-3, apoE-4, interleukin-6, transforming growth factor β 1, monocyte chemo attractant protein-1, interferon α , interleukins-2, -3, heparin binding growth-associated molecule, macrophage inflammatory protein-1 β , interleukin-8 receptor B, nitric oxide synthase, macrophage-colony stimulating factor, fibroblast growth factor-9, interferon γ -inducible chemokine IP-10, vascular endothelial growth factor and others have also been proposed as biomarkers for AD based upon cerebrospinal fluid analysis (2-9). Different plasma and serum based biomarkers such as β 40, β 42, IgG, α -synuclein, phenylalanine, ITIH4, Gpx3, CDK5, TNF- α , total β and others have also been observed to exhibit positive diagnostic properties (2,5, 10-13). Although there has been progress and advancements in the field of biomarkers for AD as mentioned above, and these have resulted in complete databases with protocols and published methods, AD biomarker research remains a relatively undeveloped area. Presently, there are no valid non-invasive biomarkers identified in patient samples that may be used with high sensitivity and specificity to diagnose AD. All of the above mentioned biomarker studies rely on invasive specimens, are expensive, require special training and can lead to possible infection.. To overcome these challenges, the healthcare system requires a technology which is inexpensive, non-invasive, cost effective and easy to use. Recent advances include reports that saliva based technologies can meet these market requirements.

Very few studies are available on salivary biomarkers for AD and only a few suitable salivary biomarkers have been characterized up until now that have been validated for AD (14). In an important study five salivary biomarkers have been proposed for the diagnosis of AD (15), so we undertook further research necessary to study these specific salivary biomarkers for AD in addition to the effect of different

saliva collection methods on the recovery of these biomarkers.

Materials And Methods

Fifteen (15) Alzheimer's disease (AD) patients and 10 non-demented controls without neurological disease were selected for this study. Informed consent was taken from each subject. Ethical permission was taken according to the Helsinki guidelines. All AD patients were diagnosed by using established literature methods (16-18). The matched age and gender control group consisted of family members of the AD patients who were selected and given clinical, cognitive and function examinations; however in this control group no neuro-imaging analyses were performed. Unstimulated saliva samples were taken from subjects in each group using simple "passive" drooling and also by two stimulated methods using commercially available saliva collection devices (Salivette® polyester roll device and the Salivette® cotton roll device, Sarstedt). The salivary biomarkers analyzed included IL-1 β , (Luminex, USA); alpha amylase (Salimetrics, USA), A β -40, A β -42 (Biosource International, Invitrogen), IGF-I and IGF-II RIA (Van Wyk and Underwood antibody). Each were evaluated as described in previous work (15).

Results

Salivary A β -40, A β -42, IGF-I and IGF-II were not detected using the Salivette® cotton roll based device in either of the patient groups. Levels of alpha amylase, IL-1 β and TNF-alpha levels were significantly lower in samples collected using the Salivette® cotton roll collection device in comparison to samples collected using the Salivette® polyester roll collection device. In each case the recoveries of the various biomarkers are lower than using the passive drooling technique in both groups (Table -1). Levels of salivary A β -40, A β -42, IGF-I, IGF-II, alpha amylase, IL-1 β , and TNF-alpha were all found to be significantly different in AD patients in comparison to normal, healthy controls (Table-1).

Discussion

Cotton based saliva collection methods have been confirmed to affect the levels of detectable biomarkers in saliva, such as A β -40, A β -42, IGF-I and IGF-II rendering these biomarkers undetectable in saliva.

Further in this study we show that cotton based saliva collection leads to a decrease in levels of alpha amylase, IL-1 β and TNF-alpha, supporting earlier evidence from previous studies (19, 20). We postulate that the "cotton interference effect" may be due to the formation of a bond between certain salivary biomarkers and cotton fiber used to collect the specimens. We found significant differences in salivary A β -40, A β -42, IGF-I, IGF-II, alpha amylase, IL-1 β , and TNF-alpha levels in AD patients compared to normal healthy controls, supporting amyloid, inflammation and the infectious concept of AD pathology (2-13). We believe these biomarkers are suitably disposed to be used for the early diagnosis of AD. The fact that salivary levels of the biomarkers A β -40, A β -42, IGF-I and IGF-II were not detectable using cotton based collection devices in both groups also supports a previously reported study (21). A further conclusion of this brief study is that passive drooling represents the most appropriate of the three methods of saliva collection tested for the detection of AD biomarkers as well as neurological biomarkers in oral fluid [saliva] specimens.

Saliva as a convenient bodily fluid has unique advantages over serum, blood and CSF due to its non-invasive properties, ease of handling, simplicity and minimal training requirements. In addition saliva sampling is highly cost effective for the screening of large population (22) and deserves to find greater application in the future.

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Illustrations

Illustration 1

Table 1 - Salivary Biomarkers Levels in AD Patients and Normal Healthy Controls

| Salivary Biomarkers | (Normal Healthy Patients) | | | AD Patients | | |
|----------------------|---------------------------|------------------------|---------------------------|------------------|------------------------|------------------------------------|
| | Whole Saliva | Stimulated Saliva | | W h o l e Saliva | Stimulated Saliva | |
| | | Salivette® cotton roll | Salivette® polyester roll | | Salivette® cotton roll | S a l i v e t t e ® polyester roll |
| Aβ-40 (pg/ml) | 30.8(6.7) | - | 21.7(4.8) | 22.1 (15.2) | - | 17.2(11.3) |
| TNF-α (pg/ml) | 66.4 (34.2) | 44.3(22.4) | 55.8 (31.3) | 208.3(67.2) | 176.3(46.8) | 189.2(34.3) |
| IL-1-β (pg/ml) | 44.6 (32.2) | 35.6(26.8) | 34.8 (21.3) | 156.3(34.7) | 104.8(12.3) | 132.3(41.2) |
| Aβ-42 (pg/ml) | 3.8 (2.3) | - | 3.2 (2.1) | 8.5(1.3) | - | 7.3 (3.2) |
| IGF-I (ng/ml) | 2.4 (1.3) | - | 2.1 (1.3) | 1.1 (0.8) | - | 0.8 (0.5) |
| IGF-II (ng/dL) | 3.5 (1.8) | - | 3.1 (2.2) | 2.3 (1.2) | - | 2.1 (1.3) |
| Alpha-amylase (U/ml) | 19.6 (8.9) | 14.3(8.4) | 15.9 (8.2) | 34.1(11.5) | 21.2(11.3) | 27.3(12.2) |

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