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Identification of metabolic changes in dementia patients using FTIR

Jéssica Lopes[1], Marta Correia[2], Rafaela Silva[1], Ilka Martins Rosa[3], Ana Gabriela Henriques[3], Ivonne Delgadillo[4], Odete A.B. da Cruz e Silva[3], and Alexandra Nunes[1*]

[1] Department of Medical Sciences, iBiMED, University of Aveiro, Aveiro, Portugal
[2] Department of Health Promotion and Noncommunicable Disease Prevention, Cardiovascular Research Group, R&D Unit, National Health Institute Dr. Ricardo Jorge, Lisboa, Portugal
[3] Department of Medical Sciences, Neurociences and Signalling Group, iBiMED, University of Aveiro, Aveiro, Portugal
[4] Department of Chemistry, QOPNA, University of Aveiro, Aveiro, Portugal

* Corresponding author: alexandranunes@ua.pt

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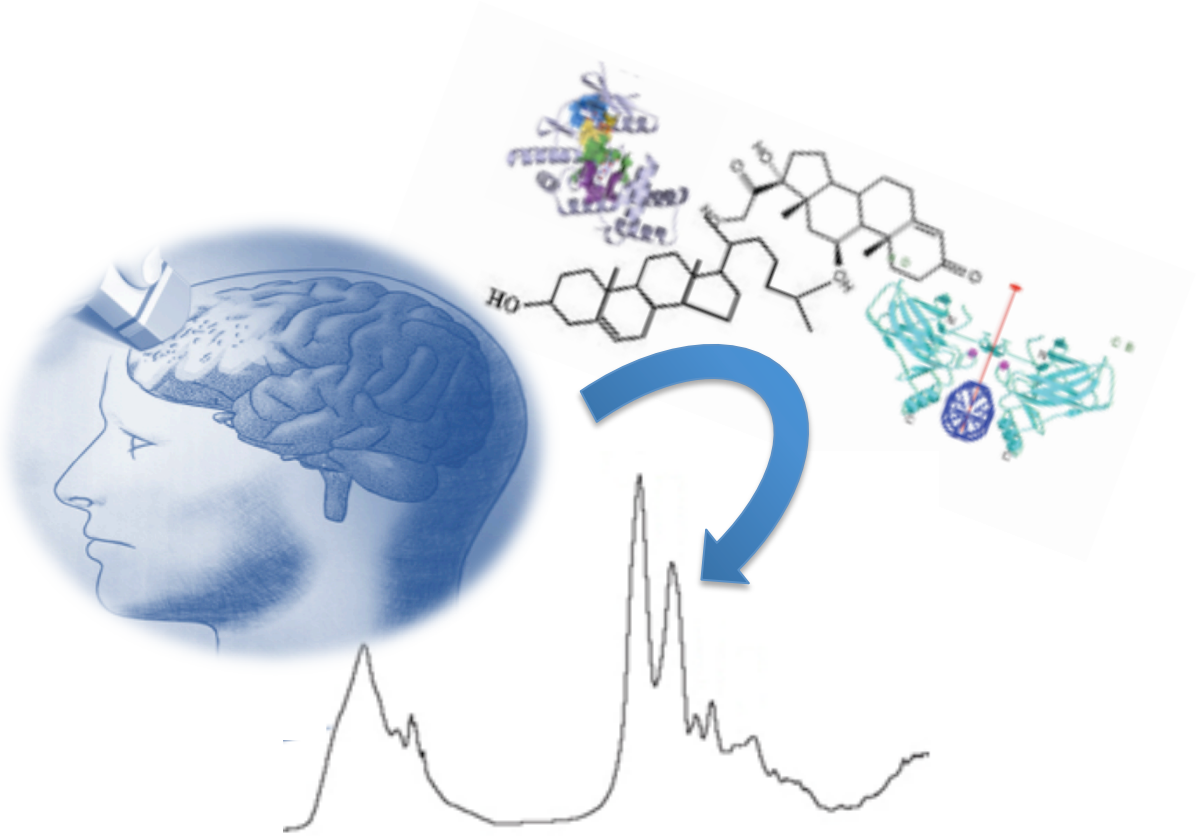

Abstract: In order to improve early recognition of Alzheimer's disease, novel approaches for biomarkers identification, such as metabolomics, are been developed and the potential of Fourier Transform Infrared Spectroscopy (FTIR) in the clinical field is receiving particular attention. The present work aims to contribute to identification of the main pathological changes that occurred during neurodegeneration, by identifying plasma biochemical alterations that might be related to dementia and discriminate control from cognitive impaired samples, through FTIR analysis and multivariate analysis. Differences have been identified; plasma samples from cognitive impaired subjects presented a higher content of saturated lipids in relation to the unsaturated ones. It was also noticed the presence of carboxylic acids production of reactive carbonyls. Differences in protein conformation were also identified between control and disease samples and were mainly related with occurrence of protein aggregates. In conclusion, FTIR has potential to be applied in future not only for cognitive impairment diagnosis but also for identification of disease stage and prognostic evaluation, besides assessment of disease developing risk for control subjects.

Keywords: Dementia Diagnosis; Fourier Transform Infrared Spectroscopy; Multivariate Analysis; Plasma-Based Biomarkers; Metabolomics

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Introduction

Dementia is associated with neurodegenerative diseases, characterized by chronic and progressive cognitive decline. Typically dementia affects individuals 65 years old or more, but can affect younger individuals [1,2] and affects individuals all over the world; the last report of Alzheimer's Disease International, estimates that in 2015, 46.8% of the world population was living with dementia and that probably in 2030 the number will double to affect 74.7 million people. In Europe it is estimated that 10.5 million individuals develop some type of dementia [3,4].

Alzheimer's disease is the most common type of dementia and accounts for 60% of all dementia cases [3,4]. The diagnosis is mainly based in the identification of the cognitive disorder in patients who have already overt the advanced stage of dementia, when is too late for some kind of therapeutic adjustment [4]. Therefore, in order to improve early recognition of Alzheimer's disease and other dementias, novel approaches for biomarkers identification, such as metabolomics, are been developed and the potential of Fourier Transform Infrared Spectroscopy (FTIR) in the clinical field is receiving particular attention.

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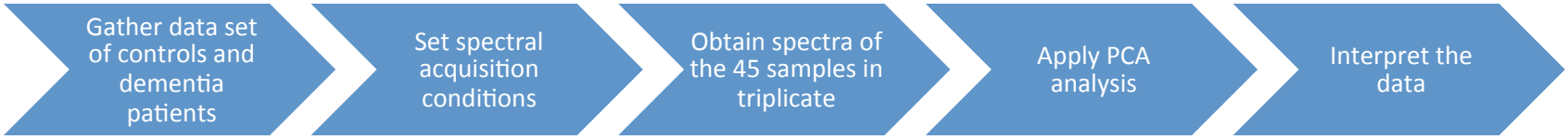
Materials and Methods



The inclusion criteria for the study group were individuals aged more that 50 years old and resident in the Aveiro region (Portugal). Exclusion criteria included individuals diagnosed with psychiatric disorders (excluding depression), aphasia, using illicit drugs, or unable to answer the questions in the structured interview. Cognitive evaluation of individuals was carried out at several Primary Health Care Centers in the Aveiro region. The project was approved by the ethics committee of the Regional Health Center (012 804 of April 04, 2012).

Data set include 45 samples, controls and putative dementia patients with the following characteristics:

Characteristics		Control	Putative AD
Gender	Male	5	4
	Female	20	16
Age	Mean	75	77
MMSE	+	0	20
	-	25	0
CDR	+	0	18
	-	25	2

AD: Alzheimer's Disease; MMSE: Mini-Mental State Examination; CDR: Clinical Dementia Rate.

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Materials and Methods

Plasma spectra of the selected volunteers (Table 1) were acquired with a Perkin-Elmer Spectrum BX FT-IRTM spectrometer in the range of 4000-900 cm^{-1}, at resolution of 8 cm^{-1} with 64 co-added scans.

Background single beam was performed against air and to spectra acquisition 8 µl of plasma of each sample was placed on the ATR crystal (diamond crystal)and the sample acquisition started after the drying process was complete. Each sample was analyzed in triplicate. All spectra are shown in Figure 1.

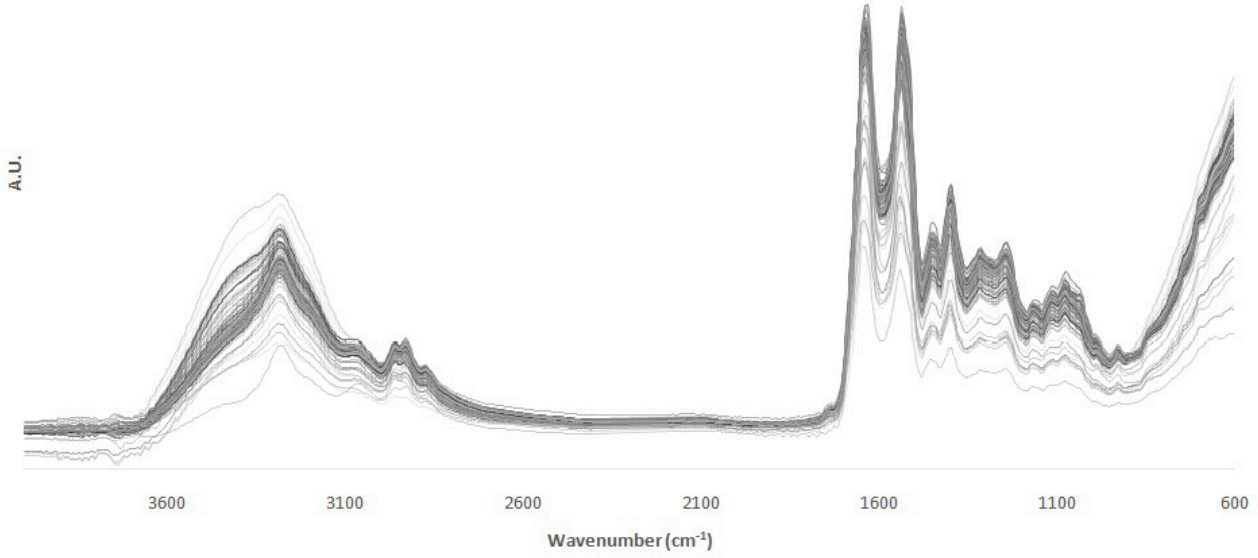

Figure 1. FTIR spectra of the plasma samples, both control and cognitive impaired patients.

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Results and discussion

Figure 2 shows a representative spectra of plasma samples data set where the corresponding assignments are placed near each wavenumber. It is possible to observe functional groups of the molecules present in plasma composition.

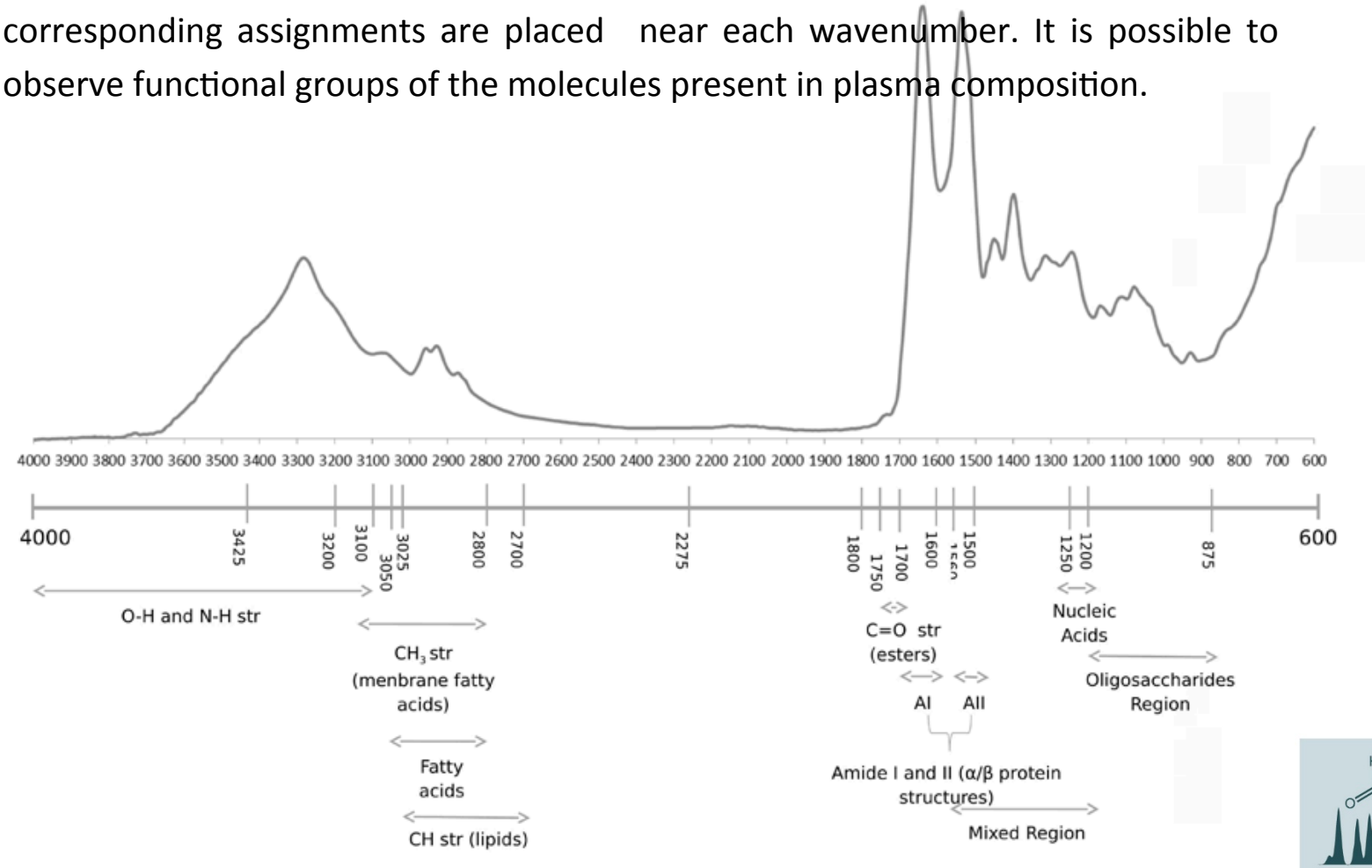

Figure 2. FTIR spectra example of the main spectroscopic regions and corresponding assignments in the range of 4000-600 cm^{-1} [5,6].

Results and discussion

PCA was applied to the mid-infrared spectra of plasma samples in order to extract the main sources of variability. Specific spectral regions were studied: 3500-2700 cm^{-1} (related to the presence of lipids (e.g., especially saturated fatty acids and phospholipids); 1800-1400 cm^{-1} (mainly related to protein conformation mode and to chemical properties of nucleic acids bases, fatty acids and carbohydrates) and 1200–900 cm^{-1} (dominated by the symmetric stretching of PO_2 groups in nucleic acids and polysaccharides and nucleic acids structural and functional information). Standard Normal Variate (SNV) was the pre-treatment applied to all spectral regions; the data set was auto-scaled, to equalize the spectra to the same scale (standardize) and decrease the differences among them (divided by the standard deviation). Results are shown in Figure 3.

Results and discussion

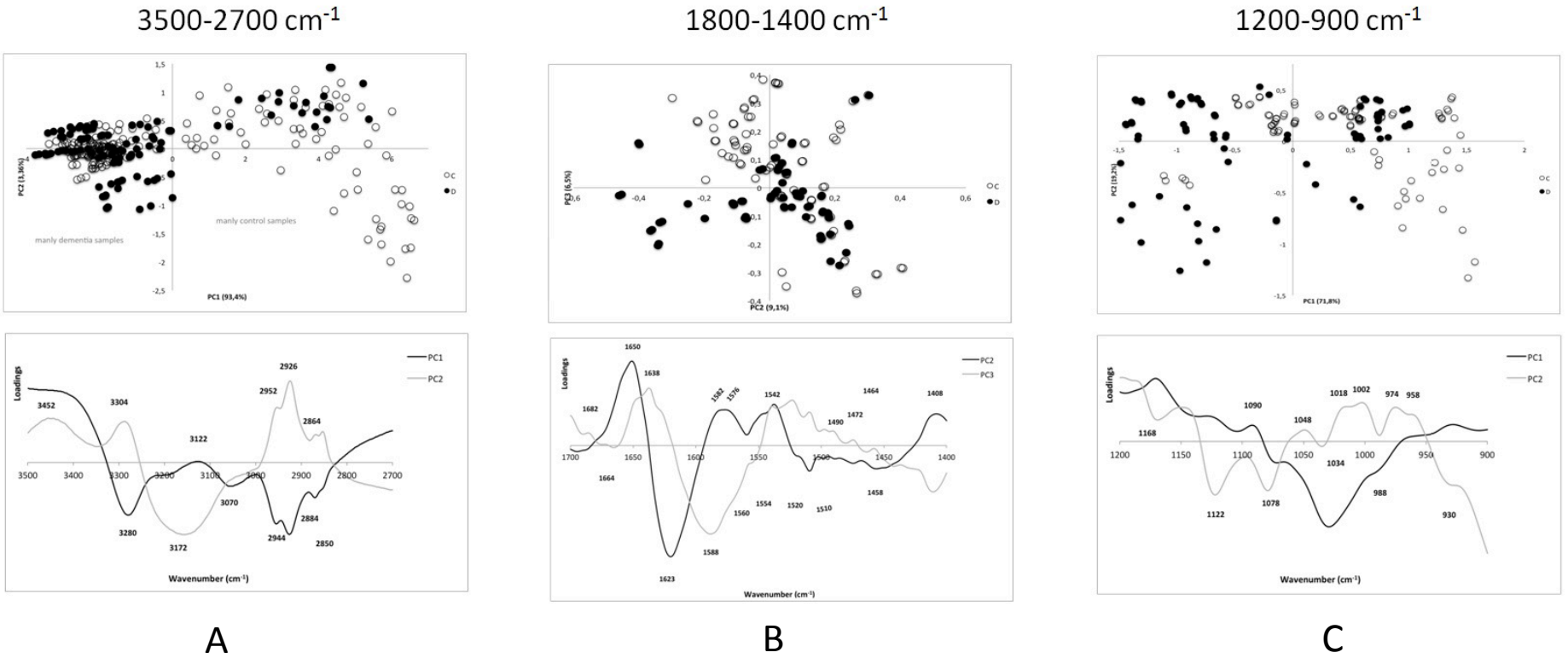

Figure 3. PCA scores scatter plot and corresponding PCA loadings profile of the three spectral regions studied by multivariate analysis of: 3500-2700 cm^{-1} (A) 1800-1400 cm^{-1} (B) and 1200-900 cm^{-1} (C) spectral regions. C corresponds to control samples and D to dementia/cognitive impairment samples. Loading profiles show maximum peaks wavenumbers [6].

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Results and discussion

In the scores scatter plot it is possible to observe samples subdivided into sub-groups, within control and putative AD groups. The cluster variability could be explained by some clinical factors that influence the results of this preliminary work, in particular the presence of several comorbidities, unknown and/or undiagnosed genetic predisposition factors, use of medication capable of affecting biochemical plasma levels, or the presence of a neurodegenerative disorder of mixed nature.

The loading profiles allow to extract information about the functional groups, and corresponding molecules, responsible for the discrimination observed in the scores scatter plot. The loading interpretation allow to extract differences between controls and putative dementia groups.

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Results and discussion

According to the interpretation of loading profile of PCA results (Figure 3):

- 3500-2700 cm^{-1}

  Plasma samples from cognitive impaired subjects presented a higher content of saturated lipids in relation to the unsaturated ones, which translates in high potential brain damage.

  It was also noticed the presence of carboxylic acids (related to lipid hyperoxidation), production of reactive carbonyls, and proteins structural and functional alterations.

- 1800-1400 cm^{-1}

  Differences in protein conformation were also identified between control and disease samples and were mainly related with occurrence of protein aggregates. Data suggests the presence of protein aggregates and the change in protein conformation for highly stable parallel β-sheet, which agrees with the presence of Aβ fibrils.

- 1200-900 cm^{-1}

  Some changes were also associated with oxidative cellular damage in disease samples.

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Conclusions

With this study it was possible to establish a method to identify the main metabolic changes that occur during neurodegeneration, by monitoring plasma biochemical alterations through FTIR analysis.

It was possible to reveal some common changes in dementia patients, suggesting that FTIR analysis can be used to build classification models that in the future may aid in the diagnosis of cognitive impairment or in the identification of disease or risk development.

Clinical trials with enlarged data sets and samples fully characterized would be necessary to validate the use of FTIR in dementia diagnosis and prognosis however FTIR showed potential to be applied in future for cognitive impairment diagnosis.

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