

DESCRIPTION

In vitro Diagnostic Method for Alzheimer's disease, based on the Albumin Redox level in the cerebrospinal fluid

- 5 The present invention relates to the sector of clinical diagnosis, and relates in particular to an *in vitro* method for diagnosing Alzheimer's disease (AD) based on determining the redox state of albumin in cerebrospinal fluid (CSF), in particular the content of mercaptoalbumin (HMA), which is the reduced form of albumin containing cysteine Cys-34 in the form of a free thiol group.
- 10 AD is a progressive neurodegenerative disease, typically characterised by loss of short-term memory and other mental abilities (such as higher cognitive abilities) as the neurons die and various areas of the brain become atrophied. The disease tends to have an average duration of approximately 10 years from diagnosis, although said duration can vary in direct proportion to the severity of the disease at the time of diagnosis.
- 15 AD is the most common form of dementia; it is incurable and terminal, and occurs more frequently in persons aged over 65 years although it can also, in rare cases, develop as early as the 40s.

In general, the initial symptom is the inability to acquire new memories, but it tends to be confused with aging- or stress-related behaviour. When AD is suspected, the clinical diagnosis procedures available while the patient is living have up to now been based on guidelines and criteria established by the National Institute of Neurological Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984), mostly based on clinical opinion according to the results of neuropsychological tests, reports from the patient's family and friends and a general neurological assessment. The neurological examination can also include a study of brain images (neuroimaging).

25 There is currently no definitive diagnostic method for AD, apart from post-mortem histological analysis of the patient's brain.

Summary

30 In one aspect, the present invention refers to an *in vitro* method for diagnosing Alzheimer's disease (AD), comprising the following steps a) determining the content of mercaptoalbumin (HMA) in a sample of cerebrospinal fluid (CSF); and b) comparing the content determined in a) with the content of HMA in CSF in healthy subjects. In another aspect, the present invention refers to an *in vitro* method for diagnosing Alzheimer's disease (AD), comprising the following steps a) determining the content of mercaptoalbumin (HMA) in a sample of cerebrospinal fluid (CSF) and in a sample of blood or plasma from a patient; b) determining a difference or ratio between the content of HMA in the sample of CSF and the content of HMA in the sample of blood or plasma determined in step a); and c) comparing the difference or ratio determined in step b) with the corresponding difference or ratio in healthy subjects.

40 It is, however, known that human albumin is a non-glycosylated 66-kDa protein. Quantitatively, it is the most important protein in the blood plasma and its concentration in normal plasma is between 35 and 50 g/L, constituting up to 60% of total plasma proteins (Peters T. J.: All About Albumin; Biochemistry, Genetics, and Medical Applications. Academic Press, San Diego, 1996). Similarly, albumin is also the most abundant protein in the CSF, constituting around 67% of total proteins, with a concentration of around 200 mg/L (Edward J. Thompson, 2005, The roster of CSF proteins, PROTEINS OF THE CEREBROSPINAL FLUID: Analysis and Interpretation in the Diagnosis and Treatment of Neurological Disease, 2nd Ed. London, UK: Elsevier Academic Press.: 13-31).

50 The structure of human albumin consists of a polypeptide of 585 amino acids and approximately 67% alpha helices having no beta sheets (Otagiri M., Chuang V.T. Pharmaceutically important pre- and posttranslational modifications on human serum albumin. Biol Pharm Bull 2009; 32:527-534). Human albumin contains 6 methionine residues and 35 cysteine residues, the latter being involved in the formation of 17 disulfide bonds. Cys-34 is the only free cysteine in the whole molecule. Human albumin has specific antioxidant functions owing to its ability to bind to multiple ligands and its radical capture properties, both being closely related to its structure.

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