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Impact of chronic *Helicobacter pylori* infection on Alzheimer's disease: preliminary results

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Abstract

Recent case-control studies reported an association between *H. pylori* infection and Alzheimer's disease (AD). Our aim was to compare cognitive impairment, neuroinflammation, and cerebrovascular lesion load in a group of AD patients according to their *H. pylori* status. For the 53 AD patients included, we assessed: clinical data (vascular comorbidities and cognitive assessment), biological data (especially fibrinogen, homocysteine levels, apolipoprotein E4 genotype; cerebrospinal fluid [CSF] total tau protein [Tau], phospho-tau$_{181}$ protein [pTau$_{181}$]), and amyloid beta peptide levels, serum/CSF-cytokines (interleukin [IL]-1β, IL-6, IL-8, tumor necrosis factor [TNF]-α) and pepsinogen I/pepsinogen II (PgI/PgII) ratio, and cerebrovascular lesion load (magnetic resonance imaging [MRI] fluid-attenuated inversion recovery [FLAIR] with the Fazekas and Schmidt scale). *H. pylori* infection was diagnosed by enzyme-linked immunosorbent assay (ELISA) and immunoblot test. *H. pylori* infection was associated with a decreased Mini Mental State Examination (MMS) ($p = 0.024$), and higher CSF pTau$_{181}$ ($p = 0.014$) and tau ($p = 0.021$) levels. A decreased PgI/II ratio (i.e., an increased gastric atrophy) was associated with the infection ($p = 0.005$). Homocysteine levels were positively correlated to Fazekas score ($r = 0.34$; $p = 0.032$) and to *H. pylori* immunoglobulin (Ig)G levels ($r = 0.44$; $p = 0.001$). Higher CSF cytokine levels (IL-8, $p = 0.003$; TNF-α, $p = 0.019$) were associated with the infection, but systemic inflammation results were controversial. Finally, in multivariate analysis, a lower MMSE score (odds ratio [OR], 0.83 [0.72–0.97]; $p = 0.017$), plasma IL-1β level (OR, 0.31 [0.11–0.87]; $p = 0.025$), an increased gastric atrophy, i.e., a lower PgI/PgII ratio (OR, 0.63 [0.43–0.93]; $p = 0.020$) were still associated with the infection. AD patients infected by *H. pylori* tended to be more cognitively impaired. Studies are needed to attest to the impact of *H. pylori* infection on AD course, especially on cerebrovascular lesions and neuroinflammation.

Keywords: Helicobacter pylori infection; Alzheimer's disease; CSF biomarkers; cerebrovascular lesions

1. Introduction

Alzheimer's disease (AD) is the main cause of dependency and disability in the elderly, and its incidence is currently increasing at a rate of 220,000 new cases per year in France (Helmer et al., 2006). No curative treatment for dementia is available, and most of the currently identified risk factors such as age, sex, and genetic factors like apolipoprotein E allele ε4 are not subject to intervention (Farrer et al., 1997). It has therefore become urgent to identify risk factors on which interventions would be possible. As an attempt to determine the etiology of late-onset AD and to search for new treatment, several groups have investigated

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the association between various infectious agents and AD. The first agent concerned was herpes simplex virus type 1 (HSV-1). HSV-1 may persist in a quiescent but persistent form known as latent infection, notably in the peripheral nervous system rather than in the central nervous system, and the mechanism for how HSV-1 may reactivate in the hippocampus remains unknown (Itzhaki et al., 1997). However the involvement of HSV is still debated. Wozniak and Itzhaki provided evidence that supports a causal role for HSV-1 in AD. The cascade of events leading to AD might involve reactivation of latent HSV-1 in the brain. Infection would cause both direct and indirect damage, inflammatory-mediated damage, and in apolipoprotein E allele ε4 carriers the damage would be greater, possibly through greater viral replication and spread, eventually leading to AD (Wozniak and Itzhaki, 2010).

Another major infectious pathogen incriminated was *Chlamydia pneumoniae* as up to 90% of AD brain biopsy specimens may be positive for *C. pneumoniae* as detected by polymerase chain reaction (PCR), especially in brain regions that exhibit AD pathology (Balin et al., 1998). However, in an interventional study in 100 patients, Loeb et al. (2004) could not prove a definite *C. pneumoniae* involvement in AD pathophysiology.

Recent observations also showed that several types of spirochetes, including *Borrelia burgdorferi* and oral *Treponema* may be involved in the pathogenesis of AD, but results remain controversial (Miklossy, 2008).

More recently, an association between AD and *Helicobacter pylori* infection was reported. *H. pylori* infection is a chronic infection usually acquired in childhood and which remains for life when no specific treatment is given. An increase in *H. pylori* prevalence with age is explained by changes in socioeconomic conditions. In fact, studies reported that when cohorts of 70-year-old subjects born in 1901 or 1902 and 1922 were compared, the latter cohort showed a significantly lower *H. pylori* positive serology. In Western Europe there is still more than a third of the population older than 60 years who are infected. *H. pylori* is a heterogenous bacterial species. Genomic studies have shown that some strains may harbor a pathogenicity island namely *cag* (*cag* PAI), which encodes a type IV secretion system and 1 of its effectors, CagA, which triggers a strong inflammatory response (Backert and Selbach, 2008).

Recent data suggested that *H. pylori* infection plays a role in extradigestive diseases (Figura et al., 2010) and in AD but controversial results persist. Indeed, in addition to 2 case-control studies pointing out an association between *H. pylori* infection and AD (Kountouras et al., 2006; Malaguarnera et al., 2004), an interventional study has shown that *H. pylori* eradication positively influences AD manifestations, especially cognitive decline (Kountouras et al., 2009). On the contrary, Shiota et al. (2011) showed negative results, but the authors based their diagnosis on antibody tests on urine samples which has been shown to be unreliable (Leodolter et al., 2003).

Preliminary results of a cohort study conducted in our laboratory concluded that *H. pylori* infection was a significant risk factor for developing AD (submitted for publication). We hypothesized that *H. pylori* infection could act as a trigger in the clinical revelation of AD or in the accumulation of AD lesions via cerebral hypoperfusion due to atherosclerosis, or via an exacerbation of neuroinflammation.

In order to test this hypothesis, we have compared, in this study, the cognitive impairment, cerebrospinal fluid (CSF)-β amyloid$_{1-42}$ (Aβ_{1-42}), -total tau (Tau), and -phosphorylated tau (pTau$_{181}$) proteins, cerebrovascular lesions assessed by brain magnetic resonance imaging (MRI), and markers of neuroinflammation in a group of AD patients according to their *H. pylori* status.

2. Methods

2.1. Study population

Since 2003, serum and CSF samples from 213 demented patients were collected in Lyon, France by NeuroBioTec, CRB HCL (Hospices Civils de Lyon, France). Written informed consent allowing research, including genetic research, was obtained from each patient participating in the study. The study was approved by the local Ethics Committee (CPP).

2.1.1. Inclusion criteria

Among the 213 patients, we selected those who responded to criteria for the diagnosis of AD, i.e., the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984), associated with the presence of medial temporal lobe atrophy on MRI (Fox and Schott, 2004) and abnormal CSF biomarkers. We chose, after a review of the literature and following the manufacturer's recommendations the association of the following cutoff values: Aβ_{1-42} concentration $<$ 600 pg/mL, Tau concentration $>$ 300 pg/mL, pTau$_{181}$ concentration $>$ 60 pg/mL, Innotest amyloid tau index (IATI): Aβ_{1-42}/(240 + 1.18[Tau]) $<$ 0.8 (Hulstaert et al., 1999).

2.1.2. Exclusion criteria

In order to exclude hypothyroidism and Creutzfeldt-Jakob disease, specific clinical signs were collected as well as thyroid-stimulating hormone (TSH) (Architect i2000SR TSH Assay; Abbott, Princeton, NJ, USA) and CSF-protein 14-3-3 (Western Blot immunoassay antibody K19 β anti 14.3.3; Signet Laboratories, Dedham, MA, USA) measurements. Presumed AD cases without CSF samples were excluded. Non-AD dementia were also excluded.