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Leptin, hsCRP, TNF-α and IL-6 levels from normal aging to dementia: Relationship with cognitive and functional status

Carolina A. Magalhães [a], Cláudia N. Ferreira [b], Cristina M.G. Loures [a], Vanessa G. Fraga [a], Amanda C. Chaves [a], Ana Carolina R. Oliveira [c], Leonardo C. de Souza [d], Elisa de P.F. Resende [d], Karoline C. Carmona [d], Henrique C. Guimarães [d], Marco T.G. Cintra [d], Igor N. Lanna [d], Danielle A.G. Zauli [e], Maria A. Bicalho [d], Maria G. Carvalho [a], Lirlândia P. Sousa [a], Paulo Caramelli [d], Karina B. Gomes [a,c,*]

[a] Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Colégio Técnico, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[c] Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[d] Departamento de Clínica Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[e] Setor de Pesquisa e Desenvolvimento (P&D), Instituto Hermes Pardini, Vespasiano, Minas Gerais, Brazil

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ABSTRACT

Cognitive impairment, including mild cognitive impairment (MCI) and dementia, compromises the patients' cognitive abilities and, to different extents, to carry out daily activities, accompanied by personality and behavioral changes. Studies suggest that leptin, an adipokine, has a neuroprotective role against Alzheimer's disease (AD) and that cytokines are associated with inflammatory processes and dementia. This study aimed to evaluate serum leptin, hsCRP, IL-6 and TNF-α levels in a cognitive continuum group from normal to demential status, and to assess whether they correlates to Mini-Mental State Examination (MMSE) and Functional Assessment Staging (FAST) scores. Forty-three participants were included, of whom 12 with probable AD, 18 with MCI and 13 with no objective cognitive decline. Serum leptin and hsCRP levels were evaluated by immunoturbidimetric method, and IL-6 and TNF-α by ELISA. Higher TNF-α levels were found in individuals with FAST stages 1/2 and normal scores evaluated by MMSE. hsCRP levels were inversely correlated with FAST stages. No association with function or global cognition was observed for leptin and IL-6 levels. However, women presented higher leptin serum levels than men while lower leptin and IL-6 levels were observed in individuals aged ≥59 years. Our results suggest that TNF-α is associated with cognitive and functional decline and that inflammation could be a substrate of cognitive impairment at early clinical stages of dementia.

1. Introduction

A group of different disorders that usually compromise memory, besides other cognitive functions, may cause dementia. Alzheimer's disease (AD) is the most frequent cause of dementia, presenting significant and progressive cognitive and functional decline, often accompanied by personality and behavioral changes [20]. AD neuropathology is characterized by altered formation of amyloid-β (Aβ) plaques and neurofibrillary tangles composed by hyperphosphorylated tau protein [44].

A wide degree of impairment in both functional and cognition capabilities underlies the clinical profile of AD. From normal aging, the term "subjective cognitive decline" (SCD) is characterized by a self-perceived decline, but without objective evidence of cognitive decline on standard neuropsychological tests. This condition may represent a pre-symptomatic stage of mild cognitive impairment (MCI), which has been reported to occur 15 years prior to MCI [37,9]. On the other hand, MCI refers to cognitive impairment, particularly of episodic memory regarding recent events, in subjects who maintain preserved function in daily activities and do not fulfill the diagnostic criteria for dementia [35,14]. MCI may follow different trajectories: it may represent a transient condition where the patient recovers the normal cognitive condition; may remain stable; or still may progress to a pattern of cognitive loss resulting in a state of dementia, mainly AD [47]. Studies indicate that 10–20%

* Corresponding author at: Faculdade de Farmácia, Universidade Federal de Minas Gerais, Avenida Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais 31270-901, Brazil.
E-mail address: karinabgb@ufmg.br (K.B. Gomes).

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of individuals with 65 years or more present MCI [39] and the risk AD development is about 10–15% per year [36].

A progressive deficit in functional activities is expected with the evolution of cognitive impairment, because it requires memory storage and executive function processes [42]. The Functional Assessment Staging – FAST is a measure of the degree of impairment in functional capabilities, which has been correlated with cognitive impairment [38,42]. Several neuropsychological batteries are useful for cognitive screening, and the Mini-Mental State Examination (MMSE) is the most widely used of such scales and allows a good evaluation of global cognitive functioning [6].

Evidence from clinical studies suggests that inflammatory mechanisms play an important role in the pathophysiological process that leads to cognitive impairment and dementia. The cytokines may participate in cognitive processes by influencing neuronal plasticity, neurogenesis and neuromodulation [32,8] but their dual role on neurodegeneration and neuroprotection has been discussed widely.

Leptin is one of the most abundant adipokines produced by adipocytes, together with cytokines such as tumor necrosis factor TNF-α, IL-6 and IL-1. Leptin shows pro-inflammatory actions similar to those of the acute phase reactants, as C-reactive protein (CRP), and upregulates the secretion of inflammatory cytokines like TNF-α and IL-6. In addition, TNF-α increases the expression of leptin mRNA in the adipose tissue [34]. Leptin is actively transported across the blood-brain barrier and acts on hypothalamic modulation of feeding behavior and energy expenditure [7,3]. The levels of leptin in AD patients have been exploited; however, results remain inconclusive [29].

A growing number of studies have searched reliable biomarkers that allow an early diagnosis of cognitive impairment, especially AD. In agreement with these studies, we aimed to investigate the leptin, hsCRP, TNF-α and IL-6 levels in a spectrum of cognitive decline from normal aging to demential group, correlating these markers to global cognitive performance and functional status. We hypothesized that leptin and cytokines levels are higher in pre-symptomatic stage of cognitive impairment, with a decrease in these levels with the onset of MCI and AD.

2. Material and methods

2.1. Subjects

Subjects were recruited between June 2014 and December 2016 among patients attending at the Geriatric and Neurology Outpatient Clinics of the Hospital das Clínicas, Federal University of Minas Gerais (UFMG), in Belo Horizonte, Brazil. The 43 participants were sequentially selected from those who sought out the outpatient clinic and were assessed by at least two team doctors. The patients were classified: 12 with probable AD, 18 with MCI, 7 with SCD and 6 with no objective cognitive or functional impairment. The diagnosis of AD was based on the clinical criteria for probable dementia due to AD according to recommendations from the National Institute on Aging and Alzheimer's Association – NIA-AA [31] and subjects with MCI were selected according to Petersen *et al.* recommendations [35].

Cognitive performance was evaluated according to four levels of educational attainment (illiterate; 1–3; 4–7; and >7 years of education). For the MMSE score, a reference cut-off was set at the 25th percentile, determined by the epidemiological survey of dementia in a Brazilian healthy community [21] and in accordance with the baseline performance from the participants that remained free of dementia in a 3-year follow-up [6]. The MMSE values were also normalized as z scores, from the standards of performance, according to four levels of formal educational attainment obtained from the whole sample from Caramelli et al. [6] study.

Patients with no functional and cognitive impairment were classified as FAST stage 1. Individuals with subjective cognitive complaints but without objective evidence of impairment were classified as FAST stage 2. FAST stage 3 included MCI patients. Patients with FAST stage 4, 5 and 6 had functional deficits that correspond to mild, moderate and severe dementia, respectively [38,42].

Exclusion criteria for both groups were age <50 years, autoimmune, kidney and liver disease, cancer (past six months), acute inflammatory disease, acute myocardial infarction or stroke (past five years), other dementia diagnoses, use of anticoagulant, steroidal and non-steroidal anti-inflammatory medications, except acetylsalicylic acid.

This study was approved by the ethics committees of the Federal University of Minas Gerais and was conducted according to the ethical guidelines of the Declaration of Helsinki. All participants provided written informed consent prior to entering the study.

2.2. Methods

Venous blood samples were obtained from each participant after eight hours fasting using tubes anticoagulant-free (Vacuette®). The samples were centrifuged at 1500×*g* for 20 min at 4 °C to obtain the serum. Aliquots were immediately processed or stored at −80 °C until the use.

The serum leptin levels were performed using an enzyme linked immunosorbent assay (ELISA) (Quantikine HS ELISA, R&D systems®, USA). The high sensitivity- CRP (hsCRP) levels were determined by immunoturbidimetric method (dCRP Latex, Beckman Coulter®, USA). The serum concentrations of IL-6 and TNF-α were determined using ELISA (Quantikine HS ELISA, R&D systems®, USA). The samples were analyzed in duplicate, with an intra-assay variation <5%. An internal quality control was used in all assays.

Body Mass Index (BMI) was measured by weight in kilograms divided by the square of the height in meters (kg/m^2) [48]. The abdominal circumference (AC) was measured and values for men ≥102 cm and women ≥88 cm were classified as increased [48]. The individuals were also classified according to age range: ≤59 years, 60–78 years, and ≥79 years.

A commercially kit (Biopur – Biometrix®, Brazil) was used for obtain genomic DNA from blood cells. The presence of the allele ε4 on *Apolipoprotein E* gene was evaluated by Polymerase Chain Reaction (PCR-RFLP), using the methodology described by Lara et al. [27].

2.3. Statistical analyses

Statistical analyses were performed using Statistical Package of the Social Sciences (SPSS) 13.0 version. The results were expressed as median (interquartile ranges) (all non-parametric variables). We performed the Mann-Whitney test for compare variables between two groups. A Kruskal-Wallis test, followed by Bonferroni correction, was applied to compare non-parametric variable between three groups. For categorical variables we used the chi-square test or Fisher test when appropriated. Correlation was assessed using the Spearman rank correlation test. For all analyses, we considered $p < 0.05$ statistically significant.

3. Results

Patients presented median age equal to 72.0 (12.0) years, of which 15 (35%) were men and 28 (65%) women. Regarding educational level, 2 patients (4.6%) were illiterate, 23 (53.5%) had 1–3, 8

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