Brief Report

Plasma Levels of Monocyte Chemotactic Protein 3 and Beta-Nerve Growth Factor Increase with Amnestic Mild Cognitive Impairment

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A number of studies have investigated peripheral inflammatory indices, including plasma cytokines and related molecules according to subtypes of dementia, but not in mild cognitive impairment (MCI). In this study, we used multiplex cytokine assay to assess the plasma levels of 22 cytokines in patients with MCI subtyped as amnestic and non-amnestic, according to cognitive features. When comparing the levels of plasma growth factors, chemokines and cytokines, plasma levels of monocyte chemotactic protein 3 (MCP-3), and beta-nerve growth factor (β-NGF) in these two groups, they were found to be significantly higher in amnestic MCI patients than in non-amnestic MCI patients, after adjusting for age and gender. This suggests that plasma MCP-3 and β-NGF may be useful in differentiating subtypes of MCI. *Cellular & Molecular Immunology*. 2009;6(2):143-147.

Key Words: mild cognitive impairment, cytokine, plasma, inflammation, biomarker

Introduction

The upregulation of cytokines, chemokines, acute phase proteins, activated complement factors, and free radicals has been clearly established in Alzheimer's disease (AD) (1-3). Amyloid deposition in the AD brain elicits a range of reactive inflammatory responses, including astrocytosis, microgliosis, the upregulation of proinflammatory cytokines, complement activation, and acute phase reactions (4). Activated microglia and inflammatory mediators are found in close association with or within the amyloid plaques, indicating important interactions (5). The overproduction of brain cytokines might contribute to the pool of peripheral cytokines, *via* a spread-out from the central nervous system (CNS) (6). On the other hand, peripheral cytokines might affect human brain functions by crossing the blood-brain barrier and interacting with the CNS (7). Thus, it seems clear that the CNS is able not only to produce cytokines, but also to contribute to the pool of peripheral cytokines (8).

Mild cognitive impairment (MCI) is an evolving concept defined as a predementia syndrome and has been subtyped according to cognitive features, clinical presentation, neuroimaging findings, and genetic features (9). Until now, the MCI classification system has been descriptive, particularly in cases where the etiology is unknown or the outcome uncertain. According to the proposed clinical subtypes of MCI, it is conceivable that they differ in both etiology and outcome. Amnestic MCIs are considered to have a high likelihood of progressing to AD, while non-amnestic MCIs are assumed to convert more frequently to non-AD dementia (10, 11). A better understanding of the preclinical stage of other dementia syndromes is needed.

The measurement of a single cytokine does not allow us to reach to any conclusions *vis-à-vis* disease-dependent effects. The fluorescent-bead-based detection assay has a clear advantage above the conventional ELISA, which is able to detect large numbers of analytes simultaneously; the former therefore provides a powerful tool for profiling multiple cytokines (12). The volume required to detect all analytes would be sufficient to test only a single cytokine by ELISA (13).

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A variety of possible biomarkers have been examined in AD patients, in order to establish them as disease markers and obtain insights into the pathophysiology of AD (14). Recently, several growth factors, cytokines, and chemokines were found to be differentially regulated in patients with AD (15-17). This raises the question as to whether these markers differentiate subtypes of MCI that would progress into different types of dementia. In the present study, we evaluated the plasma levels of growth factors, chemokines, and cytokines; to do so, we used a multiplex analysis of elderly patients affected by MCI, and compared them on the basis of subtype.

Materials and Methods

Subjects

The study sample consisted of 54 community-dwelling older adults aged over 60 years and the main sources of study sample were the community elderly mental health centers. Twenty-five individuals were excluded those who had difficulty in communicating with the investigators due to visual or hearing impairments; those taking anti-depressants, sedatives, or other psychiatric drugs; those under treatment for epilepsy or other psychiatric diseases; those under chemotherapy; and those who had a history of cerebro-vascular disease. We also measured the height, weight, body mass index (BMI), blood pressure, fasting blood sugar, total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol of all subjects. All subjects provided written consent to perform the procedure and be referred to the hospital. The study was approved by the Severance Mental Health Hospital Institutional Review Board.

Mild cognitive impairment diagnostic criteria

We ultimately enrolled 29 MCI patients. The diagnostic criteria for MCI included the following: (1) subjective memory complaints that were corroborated by an informant, (2) objective impairments that were at least 1.0 standard deviation point below the age-adjusted mean for at least one of the neuropsychological tests assessing four cognitive domains (i.e., memory, language, visuospatial functioning, and frontal lobe function) Verbal and visuospatial memory were assessed by the Seoul Verbal Learning Test (SVLT) for delayed recall and Simple Rey Figure Test (SRFT) for delayed recall, respectively. In addition, language and visuospatial function were determined by the Korean version of the Boston Naming Test (K-BNT) and the copy score of the Simple Rey Figure Test, respectively. Frontal lobe function was assessed by contrasting program, go-no go, fist-edge-palm, Luria loop, semantic and phonemic Controlled

Table 1. Demographic characteristics and dementia-related scale scores of participants

	Amnestic MCI (n = 15)	Non-amnestic MCI (n = 14)	*t* or chi	*p*
Age	71.5 ± 5.6	71.5 ± 6.1	-0.015	0.988
Gender Male (%)	5 (33.3%)	4 (28.6%)	0.077	0.782
Female (%)	10 (66.7%)	10 (71.4%)		
Educational level (y)	4.7 ± 4.6	8.2 ± 6.2	-1.767	0.088
K-MMSE	23.3 ± 3.7	24.3 ± 5.0	-0.620	0.540
K-BNT	9.9 ± 3.3	8.9 ± 3.5	0.793	0.435
SRFT copy	14.3 ± 3.3	13.8 ± 3.7	0.452	0.655
SRFT delayed recall	6.9 ± 3.1	11.6 ± 4.1	-3.535	0.001
SVLT delayed recall	3.1 ± 2.1	6.9 ± 2.0	-4.913	0.0001
Stroop color reading	9.9 ± 6.5	10.6 ± 5.7	-0.281	0.781
Height (cm)	155.4 ± 13.4	159.3 ± 8.6	-0.782	0.444
Weight (kg)	54.7 ± 9.4	60.0 ± 8.4	-1.484	0.151
Body mass index (BMI)	22.8 ± 3.7	24.5 ± 3.5	-1.020	0.321
Systolic blood pressure (mmHg)	131.5 ± 23.0	122.7 ± 11.9	1.143	0.265
Diastolic blood pressure (mmHg)	76.9 ± 11.1	75.5 ± 8.2	0.363	0.720
Fasting blood sugar	116.4 ± 32.6	134.9 ± 82.8	-1.017	0.322
Total cholesterol	202.4 ± 39.5	224.8 ± 38.3	-1.462	0.157
HDL cholesterol	48.4 ± 13.1	54.8 ± 12.9	-1.253	0.222
LDL cholesterol	120.9 ± 41.5	134.9 ± 43.0	-0.848	0.405
Triglyceride	165.6 ± 81.9	175.2 ± 78.4	-0.304	0.764
Hachinski ischemia scale	1.4 ± 1.3	0.4 ± 1.0	1.803	0.089
S-GDS-K	6.6 ± 3.3	3.8 ± 3.7	1.921	0.068
CGA-NPI	1.9 ± 3.0	1.4 ± 2.2	0.578	0.568

MCI, mild cognitive impairment; K-MMSE, Korean version of Minimental State Examination; K-BNT, Korean version of Boston naming test; SRFT, simple Rey figure test; SVLT, Seoul verbal learning test; S-GDS-K, Korean version of Short form Geriatric Depression Scale; CGA-NPI, Korean version Caregiver-administered Neuropsychiatric Inventory.

Oral Word Association Test (COWAT) and stroop color reading, (3) activities of daily living were preserved, and (4) the subject was not demented. MCI patients were classified as amnestic MCI if they had prominent memory impairment, either alone or with other cognitive impairments, or as non-amnestic MCI if a single nonmemory domain was impaired alone or in combination with other nonmemory deficits.

Bioplex cytokine assay

We measured interleukin-2 receptor alpha (IL-2Rα), IL-3, IL-12, IL-16, IL-18, cutaneous T-cell-attracting chemokine (CTACK), growth-regulated protein-alpha (GRO-α), hepatocyte growth factor (HGF), intercellular adhesion molecule-1 (ICAM-1), interferon-alpha2 (IFN-α2), leukemia inhibitory factor (LIF), monocyte chemotactic protein 3 (MCP-3), macrophage colony-stimulating factor (M-CSF), migration inhibitory factor (MIF), monokine induced by gamma interferon (MIG), beta-nerve growth factor (β-NGF), stem cell factor (SCF), stem cell growth factor-beta (SCGF-β), stromal cell-derived factor-1 alpha (SDF-1α), tumor necrosis factor-beta (TNF-β), TNF-related apoptosis-inducing ligand (TRAIL) and vascular cell adhesion molecule-1 (VCAM-1) levels *via* a bioplex cytokine assay (Human Group II Plex Panel 171-A11123, Bio-Rad, Veenendaal, The Netherlands). The detection range was 0.49-32,000 pg/ml.

Statistical analysis

Descriptive analyses for amnestic MCI and non-amnestic MCI are done using the χ^2 test and independent sample *t* test. The plasma cytokine levels of amnestic MCI subjects and non-amnestic MCI patients were compared *via* an independent sample *t* test. Of them, MCP-3, β-NGF, and TNF-β-all of which showed significant differences between the two groups-were compared using a univariate analysis of covariance. SPSS 12.0 for Windows was used in all statistical analyses, and the significance level was $p < 0.05$.

Results

Table 1 presents the demographic factors, K-MMSE, K-BNT, SRFT copy, SRFT delayed recall, SVLT delayed recall, Stroop color reading, height, weight, BMI, S-GDS-K, and CGA-NPI of the amnestic MCI and non-amnestic MCI groups. We also compared vascular risk factors such as blood pressure, fasting blood sugar, lipid profiles and Hachinski ischemia scale of both groups, but no significant differences

Table 2. Plasma cytokine levels using bioplex assays, according to MCI subtype

Plasma cytokine levels (pg/ml)	Amnestic MCI (n = 15)	Non-amnestic MCI (n = 14)	*t*	*p*
CTACK	454.5 ± 96.5	403.2 ± 96.1	1.433	0.163
GRO-α	103.2 ± 50.3	75.8 ± 36.8	1.665	0.107
HGF	290.5 ± 80.0	247.7 ± 85.7	1.391	0.176
ICAM-1	16035.9 ± 5222.0	12993.8 ± 3578.2	1.769	0.089
IFN-α2	503.5 ± 155.4	438.7 ± 156.1	1.120	0.272
IL-2Rα	480.1 ± 124.0	421.2 ± 151.8	1.149	0.260
IL-3	118.8 ± 27.4	101.2 ± 26.7	1.754	0.091
IL-12	986.7 ± 255.6	935.8 ± 311.6	0.482	0.633
IL-16	269.7 ± 146.4	278.3 ± 270.1	0.107	0.916
IL-18	39.5 ± 14.8	31.2 ± 14.0	1.552	0.132
LIF	35.4 ± 15.8	27.7 ± 14.3	1.232	0.231
MCP-3	99.6 ± 24.9	77.6 ± 17.7	2.731	0.011
M-CSF	32.2 ± 12.3	26.9 ± 12.2	1.176	0.250
MIF	1410.2 ± 746.4	1431.2 ± 1020.7	0.064	0.950
MIG	553.6 ± 352.2	447.7 ± 248.1	0.930	0.361
β-NGF	5.4 ± 1.9	3.9 ± 1.3	2.329	0.028
SCF	42.7 ± 9.9	42.7 ± 15.0	0.010	0.992
SCGF-β	2123.8 ± 1326.2	1771.6 ± 928.4	0.823	0.418
SDF-1α	334.0 ± 73.9	296.9 ± 75.7	1.335	0.193
TNF-β	10.8 ± 5.5	5.4 ± 2.0	2.256	0.041
TRAIL	271.5 ± 110.5	251.8 ± 107.7	0.486	0.631
VCAM-1	16769.1 ± 2326.5	15215.5 ± 4452.9	1.189	0.245

IL-2Rα, interleukin-2 receptor alpha; CTACK, cutaneous T-cell-attracting chemokine; GRO-α, growth-regulated oncogene-alpha; HGF, hepatocyte growth factor; ICAM-1, intercellular adhesion molecule-1; IFN-α2, interferon-alpha2; LIF, leukemia inhibitory factor; MCP-3, monocyte chemotactic protein 3; M-CSF, macrophage colony-stimulating factor; MIF, macrophage migration inhibitory factor; MIG, monokine induced by gamma interferon; β-NGF, beta-nerve growth factor; SCF, stem cell factor; SCGF-β, stem cell growth factor-beta; SDF-1α, stromal cell-derived factor-1alpha; TNF-β, tumor necrosis factor-beta; TRAIL, TNF-related apoptosis-inducing ligand; VCAM-1, vascular cell adhesion molecule-1.

were observed. When the plasma levels of 22 cytokine were compared between the two groups, the plasma MCP-3, β-NGF, and TNF-β levels of the amnestic MCI group were found to be significantly higher than those of the non-amnestic MCI group ($t = 2.731$, $p = 0.011$; $t = 2.329$, $p = 0.028$; and $t = 2.256$, $p = 0.041$, respectively) (Table 2). After adjusting for age and gender, only the plasma MCP-3 and β-NGF levels in the amnestic MCI patients were significantly higher in comparison with those in the non-amnestic MCI subjects ($F = 7.274$, $p = 0.012$ and $F = 5.026$, $p = 0.034$, respectively).

Discussion

In order to examine the inflammatory markers of the MCI subtypes and determine a possible distinctive marker, we measured the plasma concentrations of 22 cytokines using multiplex assay. The results showed that β-NGF was higher in amnestic MCI patients, which is consistent with previous findings (14). The increase in NGF is specific to AD and depends on the extent of neurodegeneration, as expressed by the ratio of P-tau181/Aβ42 (14). NGF is the most potent trophic factor supporting the survival of cholinergic neurons; cholinergic neurons degenerate early in AD, possibly due to the defective retrograde transport of NGF to the basal nucleus of Meynert (18). It supports the view that increased NGF is a result of neurodegeneration in the cholinergic system, rather than a non-specific sign of brain damage following head injury (19).

Among the cytokines and chemokines studied, the plasma level of MCP-3 was significantly higher in amnestic MCI patients than that in non-amnestic MCI patients. MCP is a member of the CC chemokine family. It plays a relevant role in inflammatory processes, including atherosclerosis and neurodegenerative disorders (20). Microglia cells produce MCP (21) and stimulate astrocytes, which together participate in the degradation of Aβ peptides (22). MCP-1 upregulation has been demonstrated in neurodegenerative diseases such as AD (23), and there is evidence indicating that levels of MCP-1 in plasma could act as biomarkers in monitoring the inflammatory process of AD (17). Similarly, MCP-3 is the most potent chemokines capable of regulating macrophages and monocytes, and they have been shown to play an important role in recruiting and maintaining the inflammatory infiltration. MCP-3 is one of the 18 signaling proteins in blood plasma that can be used to classify Alzheimer's disease and control individuals with an accuracy of almost 90% (24).

In this study, we observed higher levels of MCP-3 in amnestic MCI patients in comparison with non-amnestic MCI patients; therefore, it is possible that plasma MCP measurements can predict the progression of the dementia subtype.

The limitations of our study are as follows. First, it was designed for a small sample size and employed a cross-sectional study methodology. Second, cytokine production is highly dependent on health status and varying levels may be due to genetic polymorphisms; to overcome this probability, we excluded from our study each person with another medical disease or even the slightest sign of infection, because any comorbidity can influence cytokine production. We were not able to sufficiently detect other cytokines and chemokines, because of the limitations of the bioplex cytokine assay in exclusively determining the protein-like immunoreactivity and thus its inability to suggest the absence of significant inflammation specific to MCI. Third, our study only described the difference of two cytokine production between amnestic and non-amnestic MCI patients. Functional study of MCP-3 and β-NGF could be performed to understand why the productions of these two cytokines were higher in amnestic MCI patients.

Nonetheless, there are several strengths to our study that merit comment. First, we demonstrate for the first time that plasma MCP-3 and β-NGF levels are significantly higher in amnestic MCI patients than those in non-amnestic MCI patients. Second, an overlapping set of 22 cytokines, as presented in this study and as found in MCI subtypes, may provide more information. Further investigation is required to determine the temporal relationship between these cytokines and MCI leading to AD, and vascular cognitive impairment (VCI) to vascular dementia (VD); by way of that investigation, we could come to better understanding the role of cytokines in this observation. A prospective study should be undertaken with sequential measurements, to determine changes in plasma cytokine levels.

In conclusion, our results show that plasma levels of MCP-3 and β-NGF are higher in patients with amnestic MCI, supporting the diagnostic usability of the MCP-3 and β-NGF in distinguishing patients with amnestic MCI from those with non-amnestic MCI. In the future, new diagnostic markers-for which researchers are currently searching-may provide an important tool in making early diagnoses of AD and other forms of dementia.

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