ORIGINAL ARTICLE

# LEVELS OF PROINFLAMMATORY CYTOKINES IL-17 AND IL-23 IN PATIENTS WITH ALZHEIMER'S DISEASE, MILD COGNITIVE IMPAIRMENT AND VASCULAR DEMENTIA

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**Olga E. Dubenko[1], Olha S. Chyniak[2], Olexander O. Potapov[2]**
[1]KHARKIV MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KHARKIV, UKRAINE
[2]SUMY STATE UNIVERSITY, SUMY, UKRAINE

## ABSTRACT

**The aim:** To research differences of interleukin (IL)-17 and IL-23 serum levels in patients with Alzheimer's disease, vascular dementia and mild cognitive impairment.

**Material and methods:** Serum levels of IL-17 and IL-23 were measure by ELISA for 15 patients with Alzheimer's disease, 14 with vascular dementia, 30 with mild cognitive impairment and 30 control individuals without cognitive impairment.

**Results:** Serum concentrations of IL-17 were significantly higher in Alzheimer's disease patients (P=0.0023) than control, in vascular dementia no significant differences (P=0.4154). Level of IL-23 was significantly higher than control in Alzheimer's disease patients (P=0.0170) and vascular dementia (P=0.0002), but in Alzheimer's disease it was in 12.5 time higher. In total mild cognitive impairment patients no significant differences in interleukin concentration with control, but significant differences observed for amnestic form in IL-17 (P=0.0436) and IL-23 (P=0.0019).

**Conclusions:** IL-17 and IL-23 level significant higher in Alzheimer's disease patients compared with control and vascular dementia. From mild cognitive impairment levels of detectable interleukins was higher in amnestic form that may be early marker of progression in Alzheimer's disease.

**KEY WORDS:** Alzheimer's disease, vascular dementia, interleukins

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## INTRODUCTION

Among old-related dementia, Alzheimer's disease (AD) is the most common and characterized by a progressive and irreversible deterioration of cognitive and function abilities [1]. Dementia was name major neurocognitive disorder (NCD) in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [2]. Mild NCD is a diagnostic category in DSM-5 added to recognize the substantial clinical need of individuals living with this disorder, which might also be termed mild cognitive impairment (MCI). Mild NCD possible is preddementia stage in AD but not always a precursor of major NCD. AD is a multifactorial etiopathogenesis disorder and neuroinflammatory processes are a central feature in which microglia are over-activated, resulting in increased production of pro-inflammatory cytokines. Evidence suggests that different cytokines, including interleukins (IL) IL-6, IL-10, IL-12, TNF-α and TGF-β are actively participated in AD pathogenesis [3]. IL-17 and IL-23 augmented in AD patients upon stimulating of cell with Aβ in vitro and play role in AD-associated neuroinflammation [4].

## THE AIM

The aim of this study to research differences of interleukin (IL)-17 and IL-23 serum levels in patients with Alzheimer's disease, vascular dementia and mild cognitive impairment.

## MATERIALS AND METHODS

The study involved 59 patients with cognitive impairment (43 men and 46 women, average age – 66.8 ± 8.4 years), of which 29 has major NCD and 30 mild NCD. 15 (25.4%) patients with major NCD meet to updated criteria for clinical practice proposed for the diagnosis of Alzheimer's disease at the Alzheimer's Association of the National Institute of Aging [5, 6], 14 (23.7%) – meet to criteria probable vascular dementia (VD) according to the NINDS-AIREN [7]. 30 patients with mild NCD was divide to amnestic MCI (aMCI) – 9 (15.25%) patients if they had impairment in the memory domein and nonamnestic MCI (naMCI) – 21 (35.59%) if they had impairment in any 1 or more of the nonmemory cognitive domain. There is no patients with early-onset dementia or MCI or family history of AD.

Inclusion criteria were: the objective confirmation of cognitive impairment according to clinical and neuropsychological tests based on criteria of propable AD, probable VD and MCI, presence signs of cerebrovascular and neurodegenerative brain damage according to clinical and neuroimaging methods. Exclusion criteria were: severe somatic diseases, other mental disorders, traumatic brain damage and brain tumors, infections, epilepsy, Parkinson's disease, demyelinating and inherited degenerative diseases, alcohol consumption, intake of drugs that reduce

**Table I.** Comparison of characteristics between patients with AD, VD and MCI

| characteristics | Major NCD (n=29) | Mild NCD (MCI) (n=30) | p value | AD (n=15) | VD (n=14) | p value |
|---|---|---|---|---|---|---|
| Mean age (y) | 67.5±0.6 | 65.6± 0.8 | 0.0638 | 67.9±0.8 | 67.0±0.3 | 0.3145 |
| Male/female | 14/15 | 18/12 | 0.1640 | 5/10 | 9/5 | 0.0960 |
| Arterial hypertension | 20 | 17 | 0.3290 | 6 | 14 | <0.0001 |
| Smoking | 19 | 11 | 0.0270 | 5 | 14 | <0.0001 |
| Diabetes mellitus | 13 | 2 | 0.0001 | 3 | 10 | 0.0050 |
| Ischemic heart disease | 22 | 5 | <0.0001 | 8 | 14 | 0.0030 |
| Acute ischemic events in anamnesis | 14 | 5 | 0.0090 | 0 | 14 | <0.0001 |
| Mean MMSE score | 20.2±1.64 | 25.2±0.85 | 0.0083 | 18.8±0.56 | 21.7±0.69 | 0.0028 |
| Mean MoCA test | 18.1±1.67 | 24.2±0.86 | 0.0018 | 16.6±0.50 | 19.7±0.61 | 0.0005 |
| Mean FAB test | 11.7±0.77 | 14.3±0,9 | 0.0327 | 12.4±0.50 | 11.1±0.36 | 0.0468 |
| Mean HIS score | 5.6±1.58 | – | – | 2.4±0.50 | 9.1±0.77 | <0.0001 |

cognitive function, taking corticosteroids, severe post-stroke deficite, inability to have sufficient verbal contact.

For record vascular risk factors the patient's medical history and medication use was obtain. Hypertension was define by casual blood pressure ≥140/90 mmHg or current use of antihypertensive drugs, diabetes was define by fasting glucose ≥ 7 mmol/l or use glucose-lowering agents.

The control group consisted 30 subjects (mean age 65.7 ± 0.9) without cognitive deficit and serious illnesses. No significant differences observed for age, gender, education level between patients groups and control subjects.

All patients were examine to a comprehensive neuropsychological examination using the following tests and scales: Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Frontal Assessment Battery (FAB), Hachinski's Ischemic Scale – (HIS). The severity of cognitive impairment determined by the Clinical Dementia Rating (CDR). In addition, all patients evaluated using Magnetic Resonance Imaging (MRI).

Serum levels of cytokines of IL-17 and IL-23 was assayed using sandwich ELISA on "Chem Well 2900" immunoanalyzer (Awareness Technology, USA). Test systems using Bender Medsystems, Australia (IL-17 and IL-23) were assay in according with the manufactures instructions.

The work performed in accordance with the principles of the World Health Association Helsinki Declaration "Ethical Principles of Medical Research with Human Involvement as Object of Study" Order of the Ministry of Health of Ukraine No. 690 (dated September 23, 2009). Before inclusion in the study, patients and their relatives were inform with the study protocol and signed voluntary informed consent.

The IBM Statistical Package was use to perform statistical analyses. The level of significance was defined as $p<0.05$. $\chi^2$ test were conducted to compare clinical characteristics and Kruskal-Wallis test was applied to compare the concentration of the IL between different group.

## RESULTS

In our study the vascular risk factors that associated with cognitive impairment was higher in patients with major NCD compared with mild NCD. However, in group with VD the incidence of arterial hypertension, smoking, congestive heart failure, diabetes mellitus and anamnesis of acute ischemic events was significantly higher compared with patients with AD (table 1).

The mean scores of MMSE and MoCA test was significantly lower in patients with AD comparable with VD (p=0.0028; p=0.0005), particular in subtest orientation (3.4±0.51 vs 4.8±0.34, p=0.0226), delayed recall (1.8±0.4 vs 2.9±0.2, p=0.0108). Mean HIS score was higher in VD patients.

The detectable serum levels of IL-17 and IL-23 in patients with major NCD, AD and VD presented in table 2.

Levels of detectable interleukins were significantly higher in patients with AD comparable with VD (P=0.0481). IL-17 level was in 10 time higher in AD patients comparable control (p=0.0023). In patients with VD no significant differences with control (p=0.4154), but individual values in patients with VD was significantly greater than normal.

IL-23 level also was significant higher in AD patients than in control group (p=0.0170) and significant differences observed between patients with AD and VD (p=0.0027). Level of IL-23 was in 42 time higher comparable with control and in 12.5 time higher comparable with VD patients. This result confirm that elevate concentration of IL-17 and IL-23 high specific for AD.

When comparison the IL-17 and IL-23 concentration in patients with total mild NCD and control no significant differences were found (p=0.1215; p=0.4733) (table 3). However when compared patients with aMCI and naMCI significant differences found in IL-17 between aMCI and control (p=0.0436).

No significant differences in serum concentration of IL-23 observed in total mild NCD patients and control, but significant differences was found between aMCI patients

**Table II.** Serum levels of the IL-17 and IL-23 in patients with AD, VD and control

| Interleukin concentration, pg/ml Mean±SD | Total major NCD n=29 | AD n=15 | VD n=14 | Control n=30 | P value |
|---|---|---|---|---|---|
| IL-17 | 13.11±5.11 | 22.44±8.92 | 3.11±1.35 | 2.10±0.56 | $P_1$=0.0335<br>$P_2$=0.0023<br>$P_3$=0.4154<br>$P_4$=0.0481 |
| IL-23 | 35.75±15.2 | 64.33±22.41 | 5.14±1.62 | 1.53±0.20 | $P_1$=0.0265<br>$P_2$=0.0170<br>$P_3$=0.0002<br>$P_4$=0.0027 |

$P_1$ – differences between major NCD and control
$P_2$ – differences between AD and control
$P_3$ – differences between VD and control
$P_4$ – differences between AD and VD

**Table III.** Serum levels of the IL-17 and IL-23 in patients with mild NCD and control

| Interleukin concentration, pg/ml Mean±SD | Total mild NCD N=30 | aMCI n=9 | naMCI n=21 | Control n=30 | P value |
|---|---|---|---|---|---|
| IL-17 | 4.04±1.10 | 4.36±0.61 | 3.90±0.58 | 2.10±0.56 | $P_1$=0.1215<br>$P_2$=0.0436<br>$P_3$=0.0344<br>$P_4$=0.6411 |
| IL-23 | 1.84±0.38 | 2.80±0.17 | 1.43±0.21 | 1.53±0.20 | $P_1$=0.4733<br>$P_2$=0.0019<br>$P_3$=0.7376<br>$P_4$=0.0004 |

$P_1$ – differences between mild NCD and control
$P_2$ – differences aMCI and control
$P_3$ – differences naMCI and control
$P_4$ – differences between aMCI and naMCI

and control (p=0.0019) and aMCI and naMCI patients groups (p=0.0004). Concentration of IL-23 was significantly higher in patients with aMCI compare with naMCI (p=0.0004). Such differences confirm that aMCI may be early stage of AD and elevation of serum concentration IL-17 and IL-23 in patients may be addition markers of risk progression aMCI in AD.

## DISCUSSION

Alzheimer's disease (AD) is a neurodegenerative disorder that is the most common cause of dementia. AD is characterize by two core pathologies, the presence of β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs). A number of investigations initially demonstrated that in addition to Aβ plaques and NFT, the brains of patients with AD exhibited evidence of a sustained inflammatory response [8]. This chronic neuroinflammation is attributed to activated microglia cells and the release of numerous cytokines. Many studies now point to the involvement of neuroinflammation playing a fundamental role in the progression of the neuropathological changes that are observe in AD [9, 10]. Such overproduction of IL-6 leads to chronic neuroinflammation and neurodegeneration [11]. IL-1 is a proinflammatory cytokine that is upregulated early in AD development and are considere crucial for β-amyloid plaque deposition. IL-1β is similarly elevated in both MCI and AD patients compared with controls, suggesting that increased IL-1β production begins early and remains elevated as the disease progresses. Specific IL-1β polymorphisms resulting in higher IL-1β production are linked to increased AD risk [12]. The participant of IL-10 that play anti-inflammatory and neuroprotective role in nervous system also investigated in AD [13]. The role of IL-17 and IL-23 is less elucidate. Research demonstrated that IL-23/T17 axis plays a role in AD-associated neuroinflammation and IL-17 in the production of Th17 [14]. In vitro studies suggest that IL-23 might promote Th17 development, stimulate Th17 expansion and prolong IL-17 production [15]. In previous study are observe the elevation of IL-18, IL-23 and IL-17 levels in Chinese patients with AD and differences between males and females [16]. In this study, we compared serum level IL-17 and IL-23 in patients with clinical diagnosis AD and VD. Our results suggest that in AD patients interleukins significantly increase that reflect increase of inflammatory response, which could contribute to the development of neurodegeneration in AD.

Patients with aMCI are consider to be at high risk for AD [17]. Routine use of biomarkers such cerebrospinal fluid $A\beta_{1-42}$ still obstacle for identify the disease etiology [18] and search new biomarkers for it identify and early therapeutic intervention is important aim. In our study IL-17 and IL-23 was statistically significant higher in aMCI patients comparable with control group.

## CONCLUSIONS

IL-17 and IL-23 level significant higher in Alzheimer's disease patients compared with control and vascular dementia. Levels of detectable interleukins was higher in aMCI compared with control and significant differences between aMCI and naMCI groups was demonstrate for IL-23. Future investigation may be elucidate a potential role of this interleukins as additional biomarkers for early predict of progression aMCI in Alzheimer's disease.

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**ORCID and contributionship:**
*Olga E. Dubenko: 0000-0002-4911-5613* [A,D,F]
*Olha S. Chyniak: 0000-0002-6008-1039* [B,C,D]
*Olexander O. Potapov: 0000-0002-9683-3685* [B,E]

**Conflict of interest:**
*The Authors declare no conflict of interest.*

**CORRESPONDING AUTHOR**
**Olga E. Dubenko**
Kharkiv medical academy of postgraduate education
58 Amosova st., 61176 Kharkiv, Ukraine
tel:+30 050 6601442
e-mail:olgadubenko05@gmail.com

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