HIGH-SENSITIVITY C-REACTIVE PROTEIN, POSSIBLE BIOMARKER FOR DEPRESSION IN ELDERLY POPULATION

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Abstract

Context. The immune system has an important role in the etiology of depression, through the pro-inflammatory cytokines and acute phase protein mechanisms. In elderly people, frequent association between depression and medical conditions leads to a difficult psychiatric diagnosis, becoming necessary to determine a specific biological marker for this category of population. C-Reactive Protein (CRP) did not prove to have a high level of validity, but higher levels of high-sensitivity C-Reactive Protein (hs-CRP) were found to be associated both with cardiovascular disease and depressive disorder, through a bidirectional relationship.

Objectives. To investigate the possible association between a major depressive episode and levels of inflammatory markers among a population of elderly.

Subjects and methods. A prospective study on a sample of 82 individuals aged over 65 years, who presented for laboratory evaluations in an outpatient setting. They were recorded socio-demographic and clinical data; depression and anxiety were assessed using the Hospital Anxiety and Depression Scale. Blood samples were collected and analyzed according to the protocol of the study.

Results. Anxiety, identified in 57 persons, was not statistically significant correlated with the levels of inflammatory markers. For depressive disorder (37 subjects), both CRP and hs-CRP were significantly higher, with an almost medium effect size.

Conclusions. The high levels of CRP and hs-CRP are associated with the presence of depression in elderly patients, but not with the anxiety. Further and complex studies need to validate these findings on this group of age.

Key words: biomarker, depression, elderly.

INTRODUCTION

The elderly population is increasing fast and it is estimated to reach 1.5 billion people above 65 years or older (16 percent of the world's population) by 2050

(1). One of the problems of the old age population is a high prevalence of depression and anxiety, with a lifetime prevalence for major depression of 16.52%, fact pointed out by some studies grouped in a meta-analysis (2).

The immune system plays a role in the etiology of depression and this was first recognized almost three decades ago (3). Increased levels of pro-inflammatory cytokines and changes in the acute phase protein response were frequently recorded (4). Elevated levels of C-reactive protein (CRP) were observed in some studies with depressive people, even though CRP is not a specific marker (5, 6). In this context, to find a specific and reliable biological marker for depression becomes of paramount importance for the elderly, where clinical diagnosis is usually difficult.

The clinical diagnosis of depression in old age is notoriously more difficult than in the younger population, because with old age comes a high association of depression with somatic and/or psychiatric illnesses which dramatically complicates the clinical picture and overlaps with the symptoms of depression (like insomnia, decrease of appetite or energy). These are present on a regular basis in the somatic illnesses of the elderly but are also criteria for diagnosing depression. Having a specific biological marker for elderly depressed patients would therefore be of tremendous importance for a better clinical diagnosis of the illness and for a better understanding of the biological underpinnings of depression. However, that is not a simple thing to do, because most of the somatic illnesses of elderly people (cardiovascular and/or metabolic diseases) are also associated with an increased level of the inflammatory markers (7) which make even more difficult to identify such a specific biological marker in the old age population.

Only a few studies and meta-analysis suggest

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that systemic inflammation might precede the onset of depression in the elderly (8). The research that aimed to reveal the association between CRP and the level of severity for depressive symptoms were also few, with inconsistent results (9), or confirming a significant association between CRP level and the presence of depression (9, 10). The relationship between inflammation and depression was explained through the reducing of glucocorticoid signaling by inflammation which would lead to abnormal brain functioning, especially to the onset of depressive symptoms (11), but also other biological mechanisms may operate as well. Environmental stress especially during childhood activates the release of norepinephrine in the brain and increases its level in the amygdala, hippocampus, and other brain regions. Afterwards, the paraventricular nucleus (PVN) of the hypothalamus synthesizes corticotrophin-releasing hormone (CRH), that binds to corticotropin-releasing hormone 1 (CRH1) and CRH2 receptors in the anterior pituitary. Then ACTH is secreted in the circulation and increases the release of glucocorticoids (GC) in the adrenal glands. Consequently, feedback mechanisms are initiated: GCs bind to glucocorticoid receptors (GR) of the hippocampus, the PNV and the anterior pituitary gland and thus inhibit the further release of CRH. In depression the sensitivity of the GR is impaired leading to a reduced negative feedback mechanism and subsequently to a central hypersecretion of CRH and an increased production of GCs. That also might result in relative glucocorticoid resistance in such states (11). The newer assays like high-sensitivity CRP (hs-CRP) has been endorsed as a marker for morbidity and mortality for cardiovascular disease (12); also major depression is associated with a 64% increased risk of having an elevated hs-CRP level, a phenomenon which is more frequent among men than women (burnout, depression, and anxiety are differentially associated with microinflammation biomarkers, dependent on gender), and more so in men older than 70 years (13-19). Thus, the mechanism is not clear, because it is affected by many biological variables (male/female, young/elderly, different hormonal stages, race), several hormonal hypotheses were suggested (13-19).

Some other studies highlighted that depressive disorder is cross-sectional associated with higher hs-CRP levels (21-24) which would rather suggest a state-like situation, but this association persisted especially among women which would suggest a more trait-like condition. Thus, it was reported an apparent 44% increased risk for major depressive disorder among

women without positive history for depression but with an elevated level of hs-CRP (25).

Based on these data, it was suggested that increased levels of peripheral and central levels of pro-inflammatory cytokines may be generated by psychological stress through the interventions of cytokine-producing cells which leads to the activation of the sympathetic nervous systems and adrenergic receptors on macrophages (26). The inflammatory processes may be the outcome of stress (becoming well-recognized risk factors for depression) probably due to inflammatory mechanisms which are increasing the intensity of oxidative stress and HPA axis activity as suggested by data (27-29).

The relationship between depression and inflammation was proved to be a two-way street because the psychiatric disorder enhances the production of inflammatory cytokines (30-32) and, conversely, cytokines induce depressive symptoms (33, 34).

OBJECTIVES

The objective of the study was to investigate if there is an association between the major depressive episode symptoms (MDE) and the level of inflammatory markers – fibrinogen, CRP, and hs-CRP – among the elderly.

MATERIAL AND METHODS

Design

The data analyzed refers to a population of elderly patients (age ≥ 60 ; N=82) who presented for laboratory evaluations between 1st February and 30th June 2016 in the outpatient clinic of “Ana Aslan” National Institute of Gerontology and Geriatrics (“Ana Aslan” NIGG), Bucharest, Romania. For all participants, were recorded socio-demographic (gender, age, marital status, environmental origin, level of education, socio-economic status), and clinical (medical conditions) data. This study was approved by the Ethical Committee of the University of Medicine and Pharmacy of Craiova, Romania, and had a collaboration protocol with the outpatient clinic “Ana Aslan” NIGG. Details of the study were explained to the patients and a written consent was obtained from all participants.

Psychometric tests

To evaluate the levels of depression and anxiety we used the Hospital Anxiety and Depression Scale

(HADS). HADS is a self-rating scale with 14 items, 7 attributed to depression and 7 to anxiety symptoms. On each item, the patient can score from 0 to 3 and this means that a person can score between 0 and 21 for either anxiety or depression; cut-off point of ≥ 8 for anxiety or depression (35). For depression (HADS-D) has a specificity of 0.79 and a sensitivity of 0.83. For anxiety (HADS-A) has a specificity of 0.78 and a sensitivity of 0.9 (36).

Blood Sample Collection and hs-CRP Assays

Before the screening visit, a 12-hour-fasting blood sample was collected between 7 and 10 am. Blood collection and laboratory test fasting blood was collected via venipuncture in BD Vacutainer® (New Jersey, USA) tubes. Blood samples were centrifuged at $1000\times g$ for 12 min, and serum was separated. Serum was isolated and refrigerated at -20°C . The measurement of hs-CRP was performed in batches. Inter-assay and intra-assay coefficients of variation for hs-CRP were compliant with the CDC-accepted ranges. In addition, we excluded 66 (3% of total) CRP values greater than 10 mg/L from this analysis because such elevated values are likely to be caused by acute infection or underlying medication problems (37).

Serum hs-CRP was measured on the Konelab 30i by using an immunoturbidimetric CRP and C-reactive protein (latex) high sensitivity method. Konelab 30 is made by Thermo Fisher Scientific Group (USA) and it is a random-access clinical chemistry system for routine and special chemistries, including specific proteins, electrolytes, therapeutic drugs, and drugs of abuse tests. Specimens were initially analyzed using the high-sensitivity assay, which has a range of 0.1–20 mg/L. Specimens with results above 20 mg/L were re-analyzed using the CRP assay, which has a range of 3–480 mg/L. The long-term inter-assay coefficient of variation is 510% at 1 mg/L and 55% at 5 mg/L. Medication used was classified as current if used regularly at the time of assessment for non-steroidal anti-inflammatory drugs (NSAIDs, including aspirin), oral glucocorticoids, antidepressants, oral contraceptives and hormone therapy. Exposure to diseases, including pernicious anemia, cancer and systemic lupus erythematosus, was documented by self-report. Cases of rheumatoid arthritis were identified from specialist registers in the region (37).

Fibrinogen level was measured by using the PT-derived Fibrinogen Assays which is a simple and inexpensive test and is widely used in laboratories across Romania. The PT is determined by optical

density change for a range of plasma dilutions with known fibrinogen levels and the optical change for each different fibrinogen level is plotted as a calibration curve. A PT is performed on the patient's platelet poor plasma and the fibrinogen derived from the change in optical density compared to the calibration curve (37).

Technicians blinded to the patients' clinical data collected random blood samples on the same day as registration before patients answered the self-administered survey using the HADS scale.

Statistical analysis

Descriptive functions were used for presenting the demographic values. Because the values of all biological markers of interest (fibrinogen, CRP, hs-CRP) were abnormally distributed, we used a non-parametric test to compare the subjects with depressive symptoms with the subjects without depressive symptoms and the subjects with anxiety symptoms with the subjects without anxiety symptoms. We used for this comparison Mann-Whitney U Test. Therefore, the values of fibrinogen, CRP and hs-CRP will be presented by the medians and mean ranks instead of mean. Effect size (r) was determined with the formula $r = Z / \sqrt{N}$ (where N = is the total number of participants in a pair of groups) and we used Cohen's effect size estimates (0.2 = 'small', 0.5 = 'medium' and 0.8 = 'large' effect size) (51). All statistical analysis was performed with Statistical Package for Social Sciences for Windows, version 22 (SPSS Inc., Chicago, IL, USA).

RESULTS

The study sample included 82 elderly people, with a mean age of $71.6 (\pm 7.51)$ years, and the following characteristics: 52 (63.4%) women, 76 (92.7%) living in urban areas, and almost all subjects, 81 (98.8%) being diagnosed with at least one medical condition. Based on the values obtained on HADS-anxiety (HADS-A) subscale and HADS depression subscale (HADS-D) we created 2 groups:

- subjects with anxiety symptoms ($N=57$), out of which 36 females (63.16%) – scores ≥ 8 on HADS-A subscale;
- subjects with depressive symptoms ($N=37$), out of which 22 women (59.46%) – scores ≥ 8 on.

HADS-D subscale

HADS scale is associated with good specificity and sensitivity (depression (HADS-D) has a specificity of 0.79 and a sensitivity of 0.83 and for anxiety

(HADS-A) has a specificity of 0.78 and a sensitivity of 0.9) (36).

For the people with anxiety symptoms vs. people without anxiety symptoms we did not find any statistically significant results for any of the evaluated inflammatory markers (Table 1).

For the patients with depressive symptoms both CRP and hs-CRP were significantly higher compared to people without depressive symptoms, with an almost medium effect size (Table 2).

This analysis found a relationship between higher levels of high-sensitivity C-reactive protein (hs-CRP) and increased risk for depressive symptoms in elderly adults, which is in accord with the data from some previous studies (4). That may be important as hs-CRP represents a vulnerability marker for cardiovascular diseases too (7).

Regarding the putative major bias determined by having a somatic illness associated with an increased level of inflammatory markers, our cohort had a “self-randomization” procedure because virtually all patients had a somatic disease (98.8%) and, therefore, there are no differences regarding the proportion of somatic illnesses in the anxiety symptoms group, the depressive symptoms group or individuals without mental health disorder.

We could consider that, for our study sample of elderly people, inflammatory mechanisms emphasized by higher levels of oxidative stress, previously underlined (33, 34), and triggered by late life stress (or

triggering by itself a pathogenic mechanism implicated in depression) represents a risk factor, especially in the presence of comorbid medical conditions for depression in elderly patients. Our results regarding CRP and hs-CRP in depressive symptoms patients’ group are positive, even though there are somatic comorbidities in both control group and in depressive symptoms patients’ group.

Our data related to the levels of fibrinogen was not statistically significant, even though some other studies proved it to be a possible biological marker for depressive symptoms (4, 15).

DISCUSSION

Understanding the mechanisms underlying the onset of depression and identifying early markers that predict its occurrence in elderly patients could have major clinical implications in both the optimal management of depression and secondary prevention of cardiovascular diseases.

One of the advantages of our study stems in the fact that virtually all the people involved (98.8%) in study had a somatic illness, making the situation very naturalistic. The fact that in this situation we still obtained positive data for CRP and hs-CRP suggests two things: first, that these two markers may still be used as biological markers in elderly people with somatic illnesses (which represents the overwhelming majority of these people) and second, that inflammatory reaction

Table 1. Inflammatory markers in people with anxiety symptoms vs. people without anxiety

	Fibrinogen (mg/mL)		CRP (mg/mL)		hs-CRP(mg/mL)	
	People with anxiety (N=57)	People without anxiety (N=25)	People with anxiety (N=57)	People without anxiety (N=25)	People with anxiety (N=57)	People without anxiety (N=25)
Mdn	312	311	7	7	3.12	2.84
Mean rank	42.02	40.32	42.93	38.24	42.26	39.76
U (Z)	683 (-0.297)		631 (-0.955)		669 (-0.438)	
p	0.766		0.339		0.661	

Table 2. Inflammatory markers in people with depression symptoms vs. people without depression

	Fibrinogen (mg/mL)		CRP (mg/mL)		hs-CRP(mg/mL)	
	People with depression (N=37)	People without depression (N=45)	People with depression (N=37)	People without depression (N=45)	People with depression (N=37)	People without depression (N=45)
Mdn	312	311	7	7	4.34	2.82
Mean rank	42.19	40.93	48.08	36.09	48.36	35.86
U (Z)	807 (-0.238)		589 (-2.640)		578 (-2.367)	
p	0.812		0.008		0.018	
r			0.43		0.39	

in people with both depressive symptoms and somatic illnesses is bigger than the same reaction in elderly people with somatic illnesses only. So, the elderly with depressive symptoms and comorbidities may be at increased risk to produce a larger inflammatory reaction than the elderly people with only somatic illnesses.

Using the hs-CRP as a marker may help to identify people at risk for both cardiovascular illnesses and depression. The relationship between the three above variables is a complex one, since depression represents a risk factor for cardiovascular diseases and, reversely, and hs-CRP seems to be a marker for both conditions. However, the cross-sectional nature of our study does not allow us to clarify this relationship.

The negative results of our study (no differences regarding inflammatory markers in old people with anxiety symptoms compared to old people without anxiety symptoms) are also interesting. While there is an important genetic (38), symptomatologic (39) and treatment (40) overlap between anxiety and depression, there are also differences between the two conditions. Our study emphasizes some differences, at least regarding the inflammatory markers. The results of our study are suggesting that the inflammatory reaction of human organism is more important in depression comparing to anxiety. Direct comparisons of the inflammatory markers in people with anxiety and depressive symptoms are necessary before drawing solid conclusions.

LIMITATIONS

The main limitation of this research is represented by the rather small number of participants. Another important limitation is represented by the fact that people in our study are afflicted almost in totality (98.8%) with somatic illnesses. However, that may be in fact an advantage of our study because it is a way of self-randomization and permits a complete naturalistic design. One other limitation is that the patients with predominantly anxiety symptoms may well have some depression symptoms too. Therefore, we used a self-rating scale like HADS, followed by the psychiatric interview and diagnosis after the International Statistical Classification of Diseases and Related Health Problems 10th Revision criteria for MDD.

Also, the control group was included into the study and was represented by the patients without anxiety and depressive symptoms.

In conclusion, the high levels of CRP and hs-CRP are associated with the presence of depressive symptoms in elderly patients. Further and more

complex studies need to validate these findings on this group of age.

Conflict of interest

The authors declare that they have no conflict of interest.

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