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Basal inflammation and innate immune response in chronic multisite musculoskeletal pain

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ABSTRACT

Dysregulation of the immune system may play a role in chronic pain, although study findings are inconsistent. This cross-sectional study examined whether basal inflammatory markers and the innate immune response are associated with the presence and severity of chronic multisite musculoskeletal pain. Data were used on 1632 subjects of the Netherlands Study of Depression and Anxiety. The Chronic Pain Grade questionnaire was used to determine the presence and severity of chronic multisite musculoskeletal pain. Subjects were categorized in a chronic multisite musculoskeletal pain group (n = 754) and a control group (n = 878). Blood levels of the basal inflammatory markers C-reactive protein, interleukin-6, and tumor necrosis factor-alpha were determined. To obtain a measure of the innate immune response, 13 inflammatory markers were assessed after lipopolysaccharide (LPS) stimulation in a subsample (n = 707). Subjects with chronic multisite musculoskeletal pain showed elevated levels of basal inflammatory markers compared with controls, but statistical significance was lost after adjustment for lifestyle and disease variables. For some LPS-stimulated inflammatory markers, we did find elevated levels in subjects with chronic multisite musculoskeletal pain both before and after adjustment for covariates. Pain severity was not associated with inflammation within chronic pain subjects. An enhanced innate immune response in chronic multisite musculoskeletal pain may be examined as a potential biomarker for the onset or perpetuation of chronic pain.

1. Introduction

Chronic pain, defined as pain lasting longer than 3 months [37], is prevalent with worldwide population estimates of approximately 10% [50]. Chronic pain usually presents at multiple body locations, typically in the musculoskeletal system [7,10,21]. Compared to single-site pain, multisite musculoskeletal pain has been associated with a greater negative impact on patients' functioning [45] and disability [21,22], and an increased risk of depressive and anxiety disorders [16]. Interventions for chronic pain are, at best, moderately effective [27,57], and although studying the etiology of chronic pain has gained more attention, its underlying biological mechanisms are only partially understood.

Whereas acute pain can often be attributed to damage of peripheral structures, chronic multisite musculoskeletal pain is likely to be a result of amplification of nociceptive transmission that can occur without any nociceptive input [37,44] (ie, "central sensitization" [58]). In central sensitization, dysregulation of cytokine levels may play a role in initiating or perpetuating pain [12,47]. Animal studies suggest that proinflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) induce central sensitization and contribute to pain hypersensitivity [23,41]. Therefore, inhibition of proinflammatory cytokines might be beneficial for the treatment of chronic pain [56].

Although studies on inflammation in chronic multisite musculoskeletal pain are rare, some studies have investigated cytokine levels in fibromyalgia patients. A recent systematic review

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indicated elevated IL-1, IL-6, and IL-8 levels in blood serum and normal levels of other cytokines such as TNF-α in fibromyalgia patients [46]. Some studies found unaltered [46] or reduced levels [48] of antiinflammatory markers IL-4 and IL-10 in blood serum of patients with chronic widespread pain. Although studies on C-reactive protein (CRP) in chronic multisite musculoskeletal pain are currently lacking, elevated levels of CRP in fibromyalgia have been demonstrated [6,30,62]. In addition, increased inflammatory levels have previously been associated with higher pain severity in fibromyalgia [30].

Some researchers suggest that immune disturbances may be revealed only after stimulation of the immune system [24]. Alterations in basal inflammatory levels may be harder to detect because they generally have low values and show circadian rhythmicity and large variability [46]. Some studies propose that in fibromyalgia, the innate capacity of the immune system to produce cytokines is disturbed [5,28]. One study among 110 fibromyalgia patients found reduced responses of 7 different cytokines to a mitogen challenge of peripheral blood mononuclear cells, compared to 90 healthy controls [5]. Other studies demonstrated enhanced immune responsiveness to stimulation in fibromyalgia patients [18,24,54]. For example, after lipopolysaccharide (LPS) stimulation, increased proinflammatory IL-1β release by peripheral blood mononuclear cells was found in 19 chronic pain patients, compared with 11 pain-free controls [24].

The few previous studies mostly focused on basal inflammatory levels in fibromyalgia, only assessed a small range of cytokines, have had small sample sizes (n < 140 [46], with the exception of 2 studies [3,65]; n = 425 in largest study [3]), or insufficiently controlled for covariates that may influence immune activity [5,24,29,46]. Therefore, this cross-sectional study investigated the association between basal and LPS-stimulated inflammatory markers, and the presence and severity of chronic multisite musculoskeletal pain while controlling for sociodemographics, lifestyle and disease variables, depression and anxiety, and medication intake. We hypothesize that elevated levels of inflammatory markers are associated with the presence and severity of chronic multisite musculoskeletal pain.

2. Methods

2.1. Subjects

The current cross-sectional study was based on data from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing cohort study conducted among 2981 adults, who were between 18 and 65 years of age at the baseline assessment. Subjects were recruited from the general population (n = 564), general practices (n = 1610), and mental health care organizations (n = 807). People in different developmental stages of psychopathology, as well as controls with no psychiatric diagnosis, participated. The NESDA study contains a high proportion of subjects with chronic multisite musculoskeletal pain and provides a unique opportunity for rigorous control of relevant variables such as depressive and anxiety disorders. Further details of the NESDA study have been described elsewhere [35]. The research protocol was approved by the Ethical Committee of participating universities, and all respondents provided written informed consent.

From the initial 2981 respondents, 767 persons met the criteria for chronic multisite musculoskeletal pain, and 887 persons for the control group (criteria discussed in measurement section below). Of these 1654 subjects, 22 persons were excluded because no valid data on basal inflammatory markers were available, leaving a total of 1632 subjects for our analyses on basal inflammation (754 with chronic multisite musculoskeletal pain and 878 controls).

Data of LPS-stimulated inflammatory markers were available for 707 subjects (307 with chronic multisite musculoskeletal pain and 400 controls). Excluded persons (n = 925) from the LPS substudy did not differ in sociodemographic factors, basal inflammatory marker levels, and chronic multisite musculoskeletal pain, but more often had a lifetime depressive and/or anxiety disorder (76.6 vs 70.0%, $P = 0.004$) compared with included persons.

2.2. Chronic multisite musculoskeletal pain

Chronic multisite musculoskeletal pain was measured using the Chronic Pain Grade (CPG) [53], a reliable and valid measure of severity of chronic pain [14,43]. The CPG first inquires about the presence of pain in several locations (ie, the extremities [joints of the arms, hands, legs, or feet], back, neck, head, abdomen, chest, and the mouth and face [orofacial area]) in the prior 6 months. The subsequent questions in the CPG refer to the most painful location and include: 1) days in pain in the prior 6 months (scale 0-180); 2) pain at this moment (scale 0-10); 3) worst pain in the prior 6 months (scale 0-10); 4) average pain in the prior 6 months (scale 0-10); 5) disability days in the prior 6 months (scale 0-180); 6) disability in daily activities (scale 0-10); 7) disability in spare time, social life, and family activities (scale 0-10); and 8) disability in work (scale 0-10). According to the CPG protocol, 5 grades were categorized from these measures: grade 0 (pain free, no pain in the prior 6 months); grade I (low disability, low intensity); grade II (low disability, high intensity); grade III (high disability, moderately limiting); and grade IV (high disability, severely limiting).

Subjects were classified as having chronic multisite musculoskeletal pain if they had grade I, II, III, or IV and pain present in the extremities, and the back and the neck (n = 754). For reasons of clarity, we further refer to the chronic multisite musculoskeletal pain group as the chronic pain group. The control group consisted of people with grade 0 (n = 168) or with grade I and pain in, at most, 2 locations (n = 710). The remaining subjects who did not meet the criteria of the chronic multisite musculoskeletal pain group or the control group were not included in the present study (n = 1349).

To indicate pain severity, both pain intensity and pain disability were assessed in subjects with chronic multisite musculoskeletal pain. For assessment of pain intensity, questions 2, 3, and 4 of the CPG were used (see above) to yield a total pain intensity score (average of the 0-10 ratings of the 3 questions multiplied by 10, resulting in a 0-100 score) [53]. For assessment of pain disability in subjects with chronic multisite musculoskeletal pain, questions 6, 7, and 8 of the CPG were used (see above) to yield a total pain disability score (average of the 0-10 ratings of the 3 questions multiplied by 10 resulting in a 0-100 score) [53].

2.3. Immune system

Basal inflammatory markers included CRP and the proinflammatory cytokines IL-6 and TNF-α. Fasting blood samples were obtained in the morning between 8 and 9 am after overnight fasting and kept frozen at −80°C. CRP and IL-6 were assayed at the Clinical Chemistry department of the VU University Medical Center. Plasma levels of CRP were measured in duplicate by using high-sensitivity in-house enzyme-linked immunosorbent assay (ELISA) based on purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). Plasma IL-6 levels were measured in duplicate by a high-sensitivity ELISA (PeliKine CompactTM ELISA; Sanquin, Amsterdam, The Netherlands). Plasma TNF-α levels were assayed in duplicate at Good Biomarker Science, Leiden, The Netherlands using a high-sensitivity solid-phase ELISA (Quantikine HS Human TNF-α Immunoassay; R&D systems Inc, Minneapolis, MN, USA). Intra- and interassay

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