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Elevated serum levels of proinflammatory cytokines potentially correlate with depression and anxiety in colorectal cancer patients in different stages of the antitumor therapy

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ABSTRACT

Depression and anxiety, the most important psychological disorders in cancer patients, have now been considered as psychoneuroimmunological disorders, in which peripheral immune activation, through the release of proinflammatory cytokines, is implicated in the variety of behavioral, neuroendocrine and neurochemical alterations associated with these disorders. Along with the tumor itself, cancer treatment can also contribute to exacerbate the production of proinflammatory cytokines. This study aimed to investigate whether proinflammatory cytokine levels are related to depression and anxiety in CRC patients in different stages of the antitumor therapy We evaluated 60 patients in three stages of antitumor therapy (Pre-chemotherapy, Under-chemotherapy and Post-chemotherapy, n = 20 in each group) and 20 healthy volunteers by the Hospital Anxiety and Depression Scale (HADS). Serum levels of cytokines were measured by CBA. Depression and/or anxiety were found at clinically relevant levels in CRC patients during all antitumor therapy. Patients in pre-chemotherapy group exhibited the highest concentrations of pro-inflammatory cytokines and the lowest levels of IL-10. In latter stages of treatment, cytokines reached levels similar to the control group. Correlation analysis between HADS score and cytokine serum levels revealed positive associations of anxiety and/or depression with IL-1β, IL-6, IL-8, and TNF-α, and a negative correlation with IL-10, suggesting that cytokines are involved in the pathophysiology of these psychological disorders in CRC patients. A better understanding of the molecular mechanisms involved in these psychological disorders will allow the design of new therapeutic strategies to assist in alleviating such symptoms in cancer patients.

1. Introduction

Although the advances in chemotherapy at recent decades, colorectal cancer (CRC) is still the third most frequent cancer and the fourth deadliest in the world [1]. In Brazil, National Cancer Institute estimated that in 2016, approximately 18,000 women would be affected by CRC, surpassing for the first time the number of cases of cervical cancer, trailing only breast tumors. Among men, 17,000 new cases were estimated, a number surpassed only by lung and prostate tumors [2].

Cancer patients suffer from high emotional distress and experience a variety of affective states, including depression and anxiety, which are considered the most important psychological disorders in these individuals [3–5]. These symptoms closely interact with biologic stressors such as pain and physical symptom burden [6], usually affecting cancer progression, survival, and patient's quality of life [7,8]. Thus, the identification and proper management of these disorders is an important issue in oncology practice [9].

In addition to the obvious emotional and psychosocial dimensions of depression in cancer, evidence suggests that biological mechanisms may also be important [10]. Depression and anxiety have now been considered as psychoneuroimmunological disorders, in which peripheral immune activation, through the release of proinflammatory cytokines, is implicated in the variety of behavioral, neuroendocrine and neurochemical alterations associated with these psychological disorders

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[11,12]. The demonstration of a possible contribution of the immune system to the development of depression is likely to open new avenues in psychopathology.

Besides being strongly associated with depression, inflammation plays crucial roles in all stages of tumor development [13]. In tumor microenvironment, cells like infiltrated inflammatory cells, endothelial cells, tumor-associated fibroblasts and, mostly, epithelial cells including tumor cells produce proinflammatory cytokines [14]. Along with the tumor itself, cancer treatment can also contribute to exacerbate the production of proinflammatory cytokines. Tissue destruction by surgery, chemotherapy or radiotherapy leads to damage-associated molecular patterns (DAMPs) on damaged tissue, which bind to pattern recognition receptors (PRRs) on leukocytes triggering the expression of the transcription factor nuclear factor-κ-β (NFκβ) and the production of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, interferon-α (INF-α), and tumour necrosis factor-α (TNF-α) [15]. Experimental studies have further demonstrated that proinflammatory cytokines may be mediators of cognitive changes associated with cancer treatment, and the fluctuations of circulating cytokines have been suggested to mediate 'sickness behavior' in patients with cancer [16]. Despite the presence of high levels of proinflammatory cytokines in patients with CRC [17,18], research into the involvement of these molecules in the pathophysiology of depression and anxiety in these subjects is still in its infancy. In this study, we investigated whether proinflammatory cytokine levels are related to depression and anxiety in CRC patients in different stages of the antitumor therapy. An integral understanding of the molecular mechanisms involved in these disorders will allow the design of therapeutic interventions that lead to an improved quality of life and overall survival of CRC patients.

2. Material and methods

2.1. Study design

A total of 60 patients of both genders aged over 18 years old was selected by convenience sampling at the Clinical Hospital of the Faculty of Medicine of Ribeirão Preto. The minimum sample size (n = 20) was estimated according to cytokine plasma levels found in a pilot trial conducted by our group (SD = 0.3897114320), and those reported in previous studies [19–23]. The Control group was composed of 20 healthy volunteers free of any psychiatric or immune system disease. Eligible participants for the others groups were those diagnosed with colorectal cancer. Pre-chemotherapy group: patients who underwent surgical resection and who did not start adjuvant therapy (n = 20); Under-chemotherapy group: patients undergoing chemotherapy for about 3 months (n = 20) and Post-chemotherapy group: patients who have completed adjuvant chemotherapy regimen about 6 months ago (n = 20). Patients in pre-under-post-chemotherapy groups presented in clinical stage III (local tumor in colon or rectum that is larger than 5 cm in diameter and/or has spread to regional lymph nodes) according to staging system of the American Joint Committee on Cancer/Union for International Cancer Control [24]. All patients received adjuvant chemotherapy with capecitabine (2000 mg/m^2/day for 14 days orally every 21 days) and oxaliplatin (130 mg/m^2 intravenous D1 every 21 days) [25] regimen, according to the therapeutic scheme adopted in the hospital. The exclusion criteria were as follow: (a) individuals with a history of autoimmune or chronic inflammatory disease, active infectious conditions, renal disease or diabetes mellitus; (b) individuals who were previously received or are receiving radiotherapy or chemotherapy; (c) use of immunosuppressive drugs; (d) patients diagnosed with schizoaffective disorder, bipolar disorder, or panic disorder, and (e) individuals with cognitive impairment that prevents them from understanding the study design and answer the questionnaire. Hospitalization records were monitored seeking for volunteers who met the criteria. The first twenty hospitalized patients who met the proposed criteria to each specific group were evaluated during all the study period. Socio-demographic data included age, gender, education and marital status.

2.2. Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Only patients who agreed and signed the informed consent form participated in the study. The study was approved by the Ethics Committee of Ribeirão Preto College of Nursing.

2.3. Measures

Depression and anxiety were measured using the Brazilian-Portuguese validated version [26] of the Hospital Anxiety and Depression Scale (HADS) [27]. It consists of 14 items and contains two subscales: anxiety and depression. Each item is rated on a four-point scale, giving maximum scores of 21 for both subscales. Scores of 11 up to 21 on each subscale are considered to be a significant case of psychological morbidity, while scores of 8–10 represent the "borderline" and 0–7 normal. For the combined anxiety and depression score (total HADS score), a cut-off score of 19 was used to identify patients with severe affective psychopathology [28]. Blood samples (8 mL) were collected from all study participants in the morning, at the same range of hours (more or less 1-h difference) with vacuum tubes (Vacutainer-Becton Dickinson, Franklin Lakes, USA) and were allowed to clot for 1 h ± 5 min at 37 °C. After centrifugation at 1000*g* for 10 min at 4 °C, the serum was stored at −80 °C until use.

2.4. Cytokine analysis

The concentrations of IL-1β, IL-6, IL-8, IL-10, IL-12, TGF-β, and TNF-α were measured by Cytometric Bead Array (CBA) kits according to the manufacturer's instructions (BD Biosciences, San Diego, USA). CBA was performed with a BD™ FACSCanto flow cytometer. Quantitative analysis was done using FCAP Array™ Software.

2.5. Statistics

Data were analyzed using GraphPad Prism6 software (La Jolla, CA). To analyze the hypothesis of equal means between the groups, Kruskal-Wallis one way ANOVA followed by Dunn's multiple comparison posthoc test were used. The results were expressed as mean ± standard deviation (SD). Pearson correlation was performed to verify the correlation between variables. The significance level used for the tests was 5%.

3. Results

3.1. Demographic characteristics

Demographic characteristics of the study participants are shown in Table 1. Twenty healthy volunteers of both genders comprised the control group (mean age of 47.8 years, SD = 9.0, 60% female). Most of them were married (80%) and had secondary education (65%). Pre-chemotherapy group was composed of patients who underwent surgical resection but did not start adjuvant therapy (mean age of 56.8 years, SD = 7.2, 55% female). Almost half of them were married and had primary education. Patients undergoing chemotherapy (at about 3 months after starting treatment) formed the Under-chemotherapy group (mean age of 56.4 years, SD = 8.6, 50% female). The majority was married and half of them had secondary education. The Post-chemotherapy group consisted of patients who finished adjuvant chemotherapy about 6 months ago (mean age of 59.9 years, SD = 8.7, 50%