Increased IL-1β levels are associated with an imbalance of “oxidant/antioxidant” status during Behçet’s disease

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INTRODUCTION

Behçet’s disease (BD) is a multisystem disease. Mouth sores, genital ulcers and cutaneous lesions hallmark this chronic inflammatory disorder. It may involve ocular, vascular, digestive and neurologic manifestations [1, 2]. BD occurs in the Silk Road, spreading from Eastern Asia to the Mediterranean Basin including Algeria [3]. In contrast, the etiology of BD remains uncertain and misunderstood. Many studies stipulated that, besides many clinical characteristic resemblances, the enhanced inflammatory response and over-expression of pro-inflammatory cytokines such as TNF-α, interleukin (IL)-6, IL-8 [4] and IL-1β [5] in BD patients are essential features that might provide an “auto-inflammatory” concept for BD, which shares with other auto-inflammatory diseases [6, 7]. IL-1β is a potent pro-inflammatory cytokine. It is first produced as a pro-IL-1β (biologically inactive protein). Then, it is turned into a mature and biologically active IL-1β via caspase-1, after being itself activated by a multicomplex protein labeled inflammasome [8]. Several studies demonstrated that IL-1β plays a pivotal role in combatting the microbial invaders and pathogens. However, it has been shown that dysregulation and/or inappropriate or prolonged production of IL-1β are implicated in many pathological conditions, and could be linked to a number of auto-inflammatory disorders [9, 10]. It is known that neutrophils are a capital component of the innate immune system. They constitute the first line of defense against pathogens. They neutralize invaders by phagocytizing and eliminating them via different mechanisms. However, their dysfunction may lead to defective and unreliable reactions into the body. Numerous studies have indicated that BD patients displayed increased and enhanced phagocyte functions [11]. Activated neutrophils and monocytes generate reactive oxygen and nitrogen

ABSTRACT. Background: Behçet’s disease is a multisystem disease. It stands at the crossroad between the autoimmune and auto-inflammatory disorders. In this study, we sought to address a relationship that might exist between interleukin-1β (IL-1β) and the oxidants/antioxidants markers in Behçet’s patients. Methods: Behçet’s disease patients (n = 78: active stage, n = 28; inactive stage, n = 50) and 41 healthy controls have been included in our study. In this context, we investigated the plasma levels of IL-1β and the nitrosative/oxidative markers: nitric oxide (NO), advanced oxidative protein products (AOPP) and fatty acids peroxidation-malondialdehyde (MDA). The antioxidant system was assessed by measuring the plasma level of superoxide dismutase (SOD) activity. The Mann-Whitney’s U and Pearson’s correlation tests were used for statistical analyses. Results: Our case-control study showed that patients in active stage displayed higher plasma levels of IL-1β, NO, AOPP and MDA versus healthy controls and patients in inactive stage. Patients in active stage showed significantly lower SOD levels related to patients in inactive stage and healthy controls respectively, whereas patients in inactive stage showed statistically insignificant SOD level versus healthy controls. Correlation studies showed a significant positive correlation between IL-1β and AOPP, IL-1β and NO, and negative correlation between IL-1β and SOD among Behçet’s disease patients. In addition, we showed positive correlation between AOPP and NO, AOPP and MDA and negative correlation between NO and SOD, AOPP and SOD in Behçet’s disease patients. Conclusion: Interestingly, our study revealed that IL-1β levels increased and correlated with an imbalance of oxidants/antioxidants system, especially during active stage of Behçet disease. Collectively, our study indicates a possible link between IL-1β production and nitrosative/oxidative markers during Behçet’s disease. Exploiting this relationship might provide valuable outputs in the follow-up and prognosis of Behçet’s disease with a potential therapeutic value.

Key words: Behçet’s disease, IL-1β, oxidative/nitrosative markers, activation loop
species ROS/RNS such as peroxynitrite (ONOO\(^{-}\)), radical hydroxyl (HO\(^{\cdot}\)), superoxide (O\(^{2-}\)) and hydrogen peroxide (H\(_{2}\)O\(_{2}\)). They react with proteins and lipids triggering their oxidation and leading to severe inflammatory reactions and tissue damages. Some authors stipulated that proteins and lipids oxidation products contribute to BD pathogenesis [12, 13]. Antioxidant enzymes are the defense system, which neutralize the oxidative stress effects. Superoxide dismutase (SOD) is one of those antioxidant machinery enzymes. Its role resums in scavenging superoxide and inhibiting the formation of peroxynitrite and, thereby, preventing tissue injury. Some authors reported that weaknesses in SOD activity and decrease in its plasma levels may accentuate the oxidative disorder and disrupt the balance in favor of the oxidative disorder [14]. The present study was carried out to study the possible relationship between the pro-inflammatory cytokine IL-1β and the “oxidants/antioxidants” markers in the Algerian BD patients, referring to their disease activity and clinical manifestations; and to determine whether these markers could serve as additional activity markers and a possible target for therapeutics in BD.

**PATIENTS AND METHODS**

**Patients**

This study has included BD patients (n = 78: active stage ABP [active BD patients], n = 28; inactive stage IBP [inactive BD patients], n = 50) and 41 sex- and age-matched healthy controls. They were received at the Bab-el-oued CHU hospital in Algiers. Diagnosis of BD was established referring to the international study group (ISG) and the patients were divided into two distinguished categories depending on the presence of active and inactive manifestations. ABP have responded to one major criterion (recurrent oral ulcerations) and two minor criteria from the four ones, i.e., recurrent genital ulceration, eye lesion, skin lesions and positive pathergy test. All the patients were under colchicine at 1 mg/day and prednisone at 1 mg/kg per day and, in some cases, Aspegic\(^{\circ}\) was given at 100 mg/day. All our subjects provided informed consent and the local Ethics Committee approved the study (table 1).

**Samples and blood collection**

The blood samples were collected into the heparinized tubes, and placed immediately on ice to preserve their biological integrity. After that, we centrifuged the blood at 1,000 rpm for 10 minutes at 4°C, separated the plasma and stored it at −80°C until analysis.

**Reagents**

Almost chemical reagents and ready-to-use kits were purchased from Sigma-Aldrich, unless otherwise specified in the text.

**IL-1β levels measurement**

The IL-1β plasma levels were measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (Biotechnne). Absorbances were read at 450 nm and IL-1β levels quantified based on a standard curve first established. The sensitivity of the kit is less than 1 pg/mL of IL-1β.

**Nitric oxide estimation**

Nitric oxide (NO) levels in plasma were quantified by taking the concentration of nitrite as a measure of NO production as described by Touil-Boukoffa et al. [15]. This method consists of mixing 100 μL of serum with 50 μL of GRIESS B reagent (0.5% N-1-naphthylethylenediamine in 20% HCl) and 50 μL of a GRIESS A (5% sulphanilamide in 20% HCl). This mixture solution was then incubated at a room temperature for 15 minutes in darkness. Absorbance was determined with a spectrophotometer at 543 nm. The concentrations were calculated from the standard sodium nitrite [NaNO\(_{2}\)] curve.

**Advanced oxidative protein products levels determination**

The determination of the advanced oxidative protein products (AOPP) plasma levels was carried out referring to Witko-Sarsat et al. [16]. Briefly, 200 μL of plasma diluted 1:5 was transferred in PBS, or chloramine-T standard solutions (0 to 100 μmol/L) in each well of a 96-well microtiter plate, followed by 20 μL of acetic acid. We then added 10 μL of 1.16 M potassium iodide (KI). The reaction was stopped by adding 20 μL of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm in a microplate reader (Perkin Elmer) against a blank containing 200 μL of PBS, 10 μL of KI and 20 μL of acetic acid. The chloramine-T absorbance at 340 nm was linear (R\(^{2}\) = 0.99) within the range of 0 to 100 μmol/L. We used the chloramine-T standard curve to express the AOPP concentrations in μmol/L of chloramine-T equivalents.

**Malondialdehyde levels measurement**

The measurement of the malondialdehyde (MDA) plasma levels was performed following the thioarbituric acid reactive substances (TBARS) assay. The MDA assay kit (Sigma-Aldrich) was used. According to the manufacturer’s instructions, we established the MDA standard curve. The fluorescence intensities (λex = 532 nm/λem = 553 nm) were measured using the microplate reader (Perkin Elmer). The amount of MDA present in the samples has been quantified based on the MDA standard curve.

**Superoxide dismutase activity estimation**

SOD activity estimation has been carried out on the basis of a nitroblue tetrazolium NBT technique, consisting of quantifying formazan dye produced by tetrazolium salt after the reduction of anion superoxide (O\(^{2-}\)). The SOD assay kit (Sigma-Aldrich) was used. Absorbance was read at 450 nm using the microplate reader (Perkin Elmer). SOD activity was quantified according to the formula provided by the manufacturer’s procedure.

**Statistical analysis**

Our results were expressed as mean ± standard deviation (SD) and analyzed statistically by the Mann-Whitney’s U test. Correlation analyses were performed with the