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Increased DNA damage and increased apoptosis and necrosis in patients with severe sepsis and septic shock

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ABSTRACT

Purpose: Reactive oxygen species (ROS) has a key role in the pathogenesis of sepsis. We wanted to evaluate ROS-associated lymphocyte necrosis and apoptosis.
Materials and methods: A total of 51 patients were included in the study, 29 in the patient group and 22 in the control group. Blood samples were taken from patients in the patient group during severe sepsis or septic shock, then again once they had recovered. Oxidative DNA damage was evaluated by 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels. Peripheral blood lymphocytes from patients were evaluated with a microscope immediately. The rate of apoptosis and necrosis of lymphocytes were evaluated according to the number of cells in the peripheral
Results: The level of 8-OHdG increased with severe sepsis or septic shock. There were significant differences between the pre- and post-treatment values for apoptotic cell frequency (4.21 ± 3.15 vs. 3.82 ± 3.07, $P < 0.05$) and necrotic cell frequency (4.75 ± 3.61 vs. 4.09 ± 3.37, $P < 0.05$). Apoptosis and necrosis was increased during severe sepsis and septic shock, and apoptosis increase also continued after recovery, but necrosis decreased following disease recovery.
Conclusıons: In patients with severe sepsis or septic shock, apoptosis and necrosis were increased along with increased 8-OHdG level.

1. Introduction

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. Neutrophil dysfunction occurs through pathogens in the sepsis. Neutrophils secrete ROS, proteases and pro inflammatory mediators as exaggerated [2]. ROS neutrophils are also secreted in healthy humans. However, sepsis is also exaggerated [3]. If ROS occur at a low level, cell survival effect occurs. However, if it occurs at a high level, damage to the cells may occur [4]. Sepsis is also linked to LPS because of the occurrence of excess ROS [5]. This oxidative damaging that leads to tissue damage [2-6]. ROS can damage both purines and pyrimidines in the DNA and in the free cellular nucleoticle pool [7,8]. The most significant damage to DNA involves single-strand breaks (SSB) and double-strand breaks (DSB). The DNA repair mechanism is activated upon recognition of DNA damage, which attempts to repair the DNA of the cell [9]. The enzymes of the repair mechanism aim to clear 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is produced as a result of oxidative damage to DNA. Therefore, 8-OHdG can be used as a biomarker of oxidative DNA damage or oxidative stress. The cell continues to replicate even if the oxidative damage is irreparable, allowing continuation of the damage [10]. The damaged DNA in these irreparable cells, produced as the result of DNA damage, can also trigger apoptosis [11]. Ischemic damage is dependent on whether apoptosis or necrosis occurs in the cell, the extent of the oxidative stress, the amount of ATP in the cell, and its oxidant/antioxidant status [12]. There are several pathways that can induce apoptosis in lymphocytes, however, the specific underlying pathways are not completely understood [13]. In this study, we evaluated patients with severe sepsis and septic shock with suspected ROS-induced organ failure. We used a cytokinesis-block micronucleus cytome (CBMN cyt) assay, a comprehensive test used to determine chromosomal DNA damage, and measured 8-OHdG levels to determine oxidative DNA damage. The CBMN cyt assay is a sensitive assay that measures chromosomal DNA damage biomarkers, and is also a comprehensive technique for measuring cytostasis (proportion of mono-, bi- and multinucleated cells) and cytotoxicity (apoptotic and necrotic cell ratio) in lymphocytes [13-15]. To our knowledge, our study is the first to use the CBMN cyt method in sepsis. The exact

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mechanism of apoptosis in sepsis remains unknown [13]. Therefore, we also attempted to investigate whether a correlation exists between ROS and DNA damage, apoptosis and necrosis.

2. Materials and methods

2.1. Subjects

Our study was prospectively conducted in the tertiary medical and surgical intensive care units of the Faculty of Medicine at Erciyes University. Approval was obtained from the ethics committee (no. 2016/298), and the study was conducted in accordance with the Declaration of Helsinki and local laws, depending on which afforded greater protection to the patients. After approval by the ethics committee, consent was obtained from the first-degree relatives of the patients diagnosed with severe sepsis and septic shock according to the criteria detailed in the guidelines of our clinic between 1 January 2015 and 1 January 2016 [16]. The first blood samples were collected while the patients had severe sepsis or septic shock. The second blood samples were collected after recovery from severe sepsis or septic shock. Recovery was considered as the absence of any remaining organ failure. The control group was matched to the patient group for gender and age.

2.2. Figure 1

For each patient, the demographics, age, body weight, height, gender, baseline Apache II score and vasopressor use was recorded, together with the WBC count, procalcitonin (PCT), C-reactive protein (CRP) and blood pH in the first and second blood samples, lactate level and Sequential Organ Failure Assessment score (SOFA) in the first blood sample, duration of hospitalization in the intensive care unit, duration of overall hospitalization, intensive care mortality, hospital mortality, site of the intensive care unit, and the first blood withdrawal time (Fig. 1).

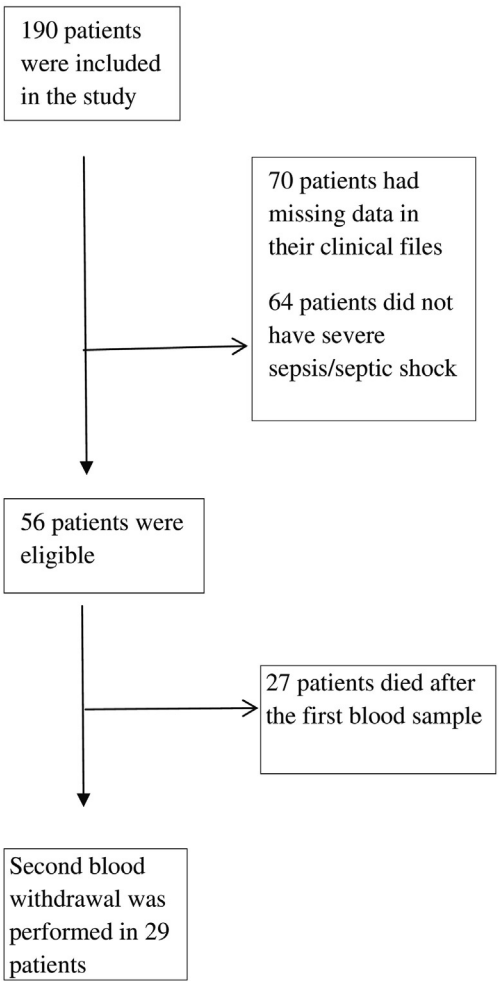

Fig. 1. Collection of patient data.

2.3. Whole-blood culture for human lymphocytes

After informed consent had been obtained, heparinized blood samples (3–4 ml) were obtained from sepsis patients pre-treatment and post-treatment, and from control subjects. Approximately 0.4 ml of each sample was immediately cultured in 5 ml of peripheral blood karyotyping medium supplemented with 2% phytohaemagglutinin-M (all from Biological Industries, Kibbutz Beit Haemek, Israel) for 72 h at 37 °C. Two parallel cultures of blood from each subject were used to determine intra-individual differences.

2.4. The cytokinesis-block micronucleus cytome assay

The cytokinesis-block micronucleus cytome (CBMN-Cyt) assay was performed as described by Fenech [14,15] with some modifications. After 44 h of incubation, cytochalasin-B (Sigma Chemical Co., St. Louis, MO, USA) was added to cultures to give a final concentration of 3 μg/ml in order to block cytokinesis. After 72 h, the cultures were treated with a hypotonic solution (0.1 M KCl) for 4 min, then fixed inmethanol/acetic acid (3:1) [15]. The fixed cells were spread onto glass slides and stained with 5% Giemsa for 10 min. All slides were coded and evaluated blinded. To determine intra-individual differences, slides for the two parallel cultures of one patient were also prepared and evaluated.

Published criteria for the selection of binucleated (BN) cells and identification of CBMN-cyt assay parameters were followed For each subject, the total number of micronuclei (MN), and frequencies of nucleoplasmic bridges (NPB) and nuclear buds (NBUD) were analyzed, and BN cells with two macronuclei surrounded by cytoplasm and a cell membrane, obtained from whole-blood cultures, were scored to determine genomic damage. The number of MN, NPB and NBUD was determined for 1000 BN cells per subject. The frequency of BN cells containing one or more MN was also determined. The number of necrotic and apoptotic cells was measured in 1000 mononucleated cells per subject to determine cytotoxicity, in accordance with the published CBMN cyt scoring criteria [15]. In addition, the number of mono-, bi-, tri- and tetra-nucleated cells per 1000 viable mononucleated cells was scored to determine the cytostatic effects and the rate of mitotic division in the peripheral blood lymphocytes of all subjects. The nuclear division index (NDI) was calculated as NDI = [(M1 + 2 M2 + 3 M3 + 4 M4) / N], where M1–M4 represents the number of cells with 1–4 nuclei and N is the total number of viable cells scored (excluding necrotic and apoptotic cells) [17].

2.5. Measurement of oxidative DNA damage

Heparinized blood samples (2–3 ml) were immediately centrifuged at 3000 rpm (1512 *g*) for 15 min at room temperature for analysis of 8-OHdG. The plasma was then stored in microtubes at –80 °C until analysis. The 8-OHdG concentration in plasma samples was measured using a commercial ELISA kit (NWK-8-OHdG02; Northwest Life Science Specialties, LLC, WA, USA), and the intra-assay coefficient of variation for the 8-OHdG assays was calculated to be 5.9%. Plasma 8-OHdG concentrations were expressed as ng/ml. Calibration, curve fitting and data analysis was performed according to the manufacturer's instructions.

2.6. Morphological analysis

The above-mentioned morphological staining procedure and scoring criteria for evaluation of the morphological changes in peripheral T lymphocytes were also applied to phytohemagglutinin-stimulated