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G/C -

ABSTRACT This study aimed to evaluate the association of plasma MIF level and –173 G/C single nucleotide polymorphism of the MIF gene with the occurrence, severity and mortality of sepsis patients. A study was conducted in adult surgical intensive care units of Zagazig University Hospitals, Egypt on 25 patients with sepsis, 27 with severe sepsis and 28 controls. Gram-negative bacilli were the most common isolates in both severe sepsis (63.0%) and sepsis (56.0%) patients. A highly statistically significant difference was found in MIF levels between sepsis cases and controls and a statistically significant difference as regards MIF level in different genotypes of the studied groups. MIF level was significantly associated with mortality in sepsis cases. High MIF levels and MIF –173G/C gene polymorphism are powerful predictors of the severity of sepsis and its outcome.

Pertinence fonctionnelle et pronostique du polymorphisme du gène – 173 G/C du facteur d'inhibition de la migration des macrophages chez des patients atteints de septicémie admis dans des unités de soins intensifs en Égypte

RÉSUMÉ La présente étude visait à évaluer l'association entre le taux facteurs d'initiative de lamigration des macrophages (MIF) plasmatique et le polymorphisme du nucléotide simple – 173 G/C du gène MIF et l'occurrence, la sévérité ainsi que le taux de mortalité chez les patientsprésentant une septicémie. Une étude a été menée dans des unités de soins intensifs en chirurgie pour adultes de l'hôpital universitaire de Zagazig, (Égypte) auprès de 25 patients atteints de septicémie, de 27 patients atteints d'une septicémie sévère et de 28 témoins. Des bacilles à Gram négatif étaient les isolats les plus fréquents dans les cas de septicémie sévère (63,0 %) et de septicémie (56,0 %). Une différence statistiquement très importante a été observée entre les taux du facteur d'inhibition de la migration des macrophages des cas de septicémie et des témoins tandis qu'une différence statistiquement significative a été notée entre le taux MIF des différents génotypes des groupes étudiés. Le taux du facteur d'inhibition de la migration des macrophages était nettement associé à la mortalité dans les cas de septicémie. De forts taux MIF et le polymorphisme du gène – 173G/C du MIF sont de puissants facteurs prédictifs de la sévérité de la septicémie et de son issue.

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Introduction

Sepsis is defined as a host's response to infection resulting from an imbalance between systemic pro-inflammatory reactions and an excessive anti-inflammatory response (1). Up to now there have been no published data about the incidence of sepsis in developing countries. Sepsis scores have been graded based on the international sepsis definitions as: local infection, bacteraemia, systemic inflammatory response syndrome, sepsis, severe sepsis and septic shock (2). Mortality rates related to sepsis and its complications are high: 20% for sepsis, 40% for severe sepsis and more than 60% for septic shock. Even those who recover may have some

permanent organ damage (1).

Bacterial infections are by far the most common causes of sepsis. Bacterial products such as lipopolysaccharides, lipoteichoic acid or cytokine receptors, including tumour necrosis factor-a and interleukin-1 via Toll-like receptors (TLR), enhance nuclear activation of nuclear factors and transcription of genes encoding expression of cytokines, chemokines, adhesion molecules, apoptotic factors and other mediators of inflammation and coagulation (2). Among the proinflammatory molecules is macrophage migration inhibitory factor (MIF), which has emerged as an important effector molecule of the innate immune system in response to infection (3). It enables macrophages, the forefront of the host antimicrobial defences, to sense invading Gram-negative bacteria and mount an innate immune response. Given that it is a pivotal regulator of innate immune responses by regulating the expression of the TLR4-LPS (Toll-like receptors–lipopolysaccharide) complex to bacterial infections, MIF appears to be a perfect target for novel therapeutic interventions in patients with severe sepsis (4).

High blood levels of MIF in children and adults with Gram-negative sepsis are associated with disease severity parameters and early mortality (5). Consequently, the MIF gene has been a candidate gene for investigation in inflammatory disease, and studies focusing on elucidation of MIF gene expression have been undertaken. A single nucleotide polymorphism (SNP) was identified in the untranslated 5´ region of the MIF gene at position –173 consisting of a G to C transition (6).

The aim of this study was to detect the incidence of sepsis in adult surgical intensive care units (ICUs) of Zagazig University Hospitals, Egypt. In addition, we aimed to evaluate the association of plasma level of MIF and –173 G/C single-nucleotide polymorphism of the MIF gene with occurrence, severity and suspected mortality of sepsis.

Methods

Study design and sample

This study was conducted in adult surgical ICUs of Zagazig University Hospitals, in Zagazig, Egypt (tertiary care hospital 2100 beds) from April 2009 to November 2011. The ICUs had annual numbers of admissions ranging from 940–1000.

The cases were sepsis patients in whom the severity of sepsis was assessed according to Acute Physiology and Chronic Health Evaluation (APACHE) II (2). The controls were those with fever of non-infectious origin who were selected to match the sepsis patients for age and sex.

Patients were excluded in case of death within 6 h of inclusion, age under 18 years or using anti-inflammatory agents, corticosteroid therapy or other sepsis-modifying agents.

Data collection

Blood samples of 9 mL were collected from patients within 24 h of onset of illness and from controls into lithium heparin tubes; 8 mL blood was used for blood culture, 0.5 mL for DNA extraction and 0.5 mL for plasma separation.

Blood culture

Blood samples of 8 mL were inoculated into blood culture bottles (Egyptian

Diagnostic Media) then incubated at

37 °C for 7–14 days. Subcultures were done every 48 hours on blood, chocolate and MacConkey agar (Oxoid) plates both aerobically and anaerobically using BD GasPack EZ anaerobic container (Becton and Dickinson). Bacterial isolates were identified on the basis of colonial morphology, microscopic examination of Gram-stained films of the different colonies and conventional biochemical reactions for either Gram-positive or -negative bacteria (7).

Determination of plasma MIF

To avoid the possible influence of MIF released from lysis of erythrocytes during blood clotting, circulating MIF levels were determined in plasma rather than serum samples (8). In addition, visibly haemolysed samples were excluded from measurements. Samples were stored at –80 ºC until the concentrations were determined by enzyme-linked immunosorbent assay (human MIF ELISA, RayBiotech) according to the manufacturer's recommendations. The limit of detection was 6 pg/mL.

Genomic DNA extraction

DNA was extracted using QIAamp DNA blood mini kit (Qiagen GmbH) according to the manufacturer's recommendations.

Detection of MIF –173G/C gene polymorphism

Detection of MIF –173G/C gene polymorphism was performed using the tetra-primer amplification refractory mutation system (ARMS) method (9). The primers (Sigma-Aldrich Chemie) designed in this study were as follows: forward inner primer for the G allele, 5´-AGCCGCCAAGTGGAGAACTGG-3´; reverse inner primer for the C allele, 5´-AGCCCGGCGCACCGCTCCTAG-3´; forward outer primer, 5´-CAGTGCGTGCAGTGGAATGAAC-3´; reverse outer primer, 5´-TGGGGAAGTCACCGCCTGCCT-3´. A polymerase chain reaction (PCR) assay was done using PCR-gold master-mix beads (Bioron): 50 µL reaction contained 2.5 U taq DNA polymerase, 250 mM each dNTP, 10 mM tris-HCL (pH 9.0), 30 M KCl, 1.5 mM MgCl2, to which 70 ng template DNA, 0.20 pmol concentration of outer primers, 1.0 pmol concentration of inner primers, then distilled water was added to a total volume of 50 µL. The reaction was performed in a thermal cycler (Biometra) using the amplification programme: initial denaturation at 94 ºC for 5 min, followed by 33 amplification cycles, each consisting of denaturation at 94 ºC for 50 s, annealing at 60 ºC for 50 s and extension at 72 ºC for 50 s and final extension at 72 ºC for 5 min. Each run of PCR amplification included negative controls and included no template DNA to avoid false positive results caused by possible contamination. The amplified products were separated by electrophoresis on 2.0% agarose gel stained with ethidium bromide and visualized under an ultraviolet transilluminator (Biometra) with a 100-bp DNA molecular weight marker (Sigma-Aldrich Chemie) (Figure 1).

Ethical considerations

The study was reviewed and approved by the review board of the research ethics committee, Faculty of Medicine, University of Zagazig. Informed consent was obtained from all participants after explanation of the procedure and the purpose of the study.

Statistical analysis

All patients' data were tabulated and processed using SPSS, version 12.0. Quantitative variables were expressed by mean and standard deviation (SD) and then compared using the Mann–Whitney U-test for comparing 2 independent variables and the Kruskal–Wallis analysis for more than 2 independent variables. Qualitative variables were expressed by frequency and percentages and compared using the chi-squared test or Fischer exact test when appropriate. Correlations between variables were assessed using Spearman analysis. Differences were considered significant at P-value

Results

Out of 459 patients admitted 52 (11.3%) were shown to have septicaemia; 25 were classified as sepsis and 27 as severe sepsis.

Table 1 showed the characteristics of the study groups. There were no significant differences in the age and sex of patients and controls. Patients with severe sepsis had significantly higher

mean APACHE scores (19.5) than those with sepsis (16.5) (P = 0.01). The overall mortality rate for cases was 19/52 (36.5%) and the greater severity of illness in the severe sepsis group was reflected in a statistically significantly higher mortality rate during the time of stay in the ICU (55.6%) compared with the sepsis group (20.0%) (P = 0.003). Concerning risk factors, older age

(> 60 years), hospitalization more than 48 h, use of instrumentation, presence of polytrauma and history of diabetes mellitus were all statistically significantly different between the patient and control groups.

Table 2 shows that there was no statistically significant difference regarding the rate of microbial isolation between sepsis patient groups. Gram-negative bacteria were isolated from 63.0% and 56.0% of severe sepsis and sepsis patients respectively. However, the rates of leukocytosis (P

Figure 2 shows that mean plasma MIF levels were significantly higher in sepsis patients than in controls, with the highest levels in the severe sepsis group [severe sepsis 12 855 (SD 2823) pg/mL, sepsis 9036 (SD 1623) pg/mL and controls 2207 (SD 823) pg/mL] (P