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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Can chitotriosidase be a surrogate marker for invasive fungal disease?

La chitotriosidase peut-elle être un marqueur possible d'une mycose invasive ?

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Summary
Background. — Chitotriosidase (CHT) enzyme has been known to be secreted from the activated macrophages. We infer with these data that CHT activity is an indicator for the defence.
Methods. — In this study, we evaluated CHT levels in both neutropenic and non neutropenic patients. CHT enzyme activity was measured and compared to each other groups.
Results. — Chitotriosidase levels were found to be significantly higher in neutropenic patients with candidemia.
Conclusion. — In the comparison between neutropenic and non neutropenic patients, there was a significant difference for CHT levels. The use of this enzyme as a surrogate marker for candidemias were evaluated in neutropenic and non neutropenic patients.

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[3] Contribution to the manuscript: co-author, language support and study supervision.
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MOTS CLÉS
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Résumé La chitotriosidase (CHT) est connue pour être sécrétée par les macrophages activés. Dans cette étude nous avons évalué le taux de CHT chez des patients neutropéniques et non neutropéniques. Le taux de CHT a été comparé à d'autres groupes de patients. Les résultats ont montré que le taux de CHT est significativement différent selon les groupes de patients étudiés. La chitotriosidase peut être utilisée comme un marqueur possible des candidoses invasives.

Introduction

Morbidity and mortality from invasive fungal infections have increased dramatically over the past two decades, especially in immuno-compromised hosts [1]. The inadequacy of serodiagnosis to facilitate preemptive therapeutic approaches in the management of fungal disease stresses the importance of newer diagnostic tools. Actually, chitotriosidase (CHT) is an active chitinase, specifically expressed by activated phagocytes. It is a chitin-fragmenting enzyme that has a role in the defence against chitin containing pathogens [2]. Due to the chitin layer present in the fungal membrane, CHT can be over expressed during the course of invasive fungal disease (IFI) and might be a specific marker in the diagnosis of IFIs [3]. In sarcoidosis, which is a granulomatous disease, the CHT levels were found to be increased and thus, the fungi have been accused for the etiological diagnosis [4,5]. Accordingly, Tercelj has reported a marked decrease in CHT levels during the course of sarcoidosis with antifungal treatment [6]. Currently, there are several reports on the interrelations of CHT with the metabolic, bacterial and protozoal infections [7,8]. But to the best of our knowledge, there is no report in the literature on the efficacy of this enzyme in the detection of invasive fungal diseases.

Material and methods

In this study, we evaluated CHT levels in both neutropenic and non neutropenic patients. Blood culture negative neutropenic patients (N) (n = 37, 25—69 years old, mean 43 $\pm$ 12 years, 23 males and 14 females), neutropenic patients with candidemia (NC) (n = 10, 18—77 years old, mean 49 $\pm$ 16 years, seven males and three females), neutropenic patients with bacteremia (NB) (n = 6, 29—79 years old, mean 53 $\pm$ 17 years, five males and one female), non neutropenic patients with candidemia (NNC) (n = 11, 28—63 years old, mean 40 $\pm$ 10 years, eight males and three females), non neutropenic patients with bacteremia (NNB) (n = 6, 22—67 years old, mean 39 $\pm$ 14 years, five males and one female), control group (C) (n = 11, 19—52 years old, mean 31 $\pm$ 10 years, nine males and two females) were compared to each other. None of the patients were receiving antifungals when the blood samples were taken. In the group N and NC, the blood samples were obtained on the first, fifth, and tenth days after chemotherapy. Only one blood sample was obtained for the other groups. The sera were stored at 80 °C. The CHT evaluations were made from tenth-day samples in group N and NC patients. CHT enzyme activity was measured according to the method described by Guo et al. [9].

Subjects

Overall, 81 adult human subjects were included in this study. Neutropenic patients had non-hodgkin's lymphoma [22], Hodgkin's lymphoma [9], multiple myeloma (n = 8), leukemia (n = 5), sarcoma (n = 4) and testicular cancer (n = 3). Non neutropenic patients, who were admitted to the intensive care unit, included those with respiratory, urinary and abdominal complaints (Table 1). Neutropenic patients renal, cardiac, liver, and pulmonary function tests were performed and those with normal organ functions were performed high dose chemotherapy (HDC) and stem cell transplantation (SCT). A detailed history of patients was taken. Proper and complete examination was done, dental care was performed by a dentist and all patients were examined by an ear, nose, and throat specialist to exclude any infection site before the transplantation procedure. Serum CHT activities were determined before the initiation of HDC and during the neutropenic period after haemopoietic stem cell reinfusion. One sample before HDC and three samples on +1, +5, +10th day after stem cell reinfusion. All patients were screened with daily fever measuring, daily total white blood cell count and culture samples were taken on days of fever $>$ 38,3 °C or any clinical finding of sepsis during the neutropenic period after stem cell reinfusion for 10 days. Informed consent was obtained from each patient and the study was approved by the Ethical Committee of Gulhane Military Medical Academy according to the ethical guidelines of the 2004 Declaration of Helsinki.

Sample analysis

Advia 120 Automated Hematology Analyzer Venous blood samples were withdrawn between 07:00 and 08:00 a.m. Blood samples were obtained in an EDTA-containing tube for CHT activity measurement. Plasma and packed cells were separated for centrifugation at 1500×g for 10 minutes and frozen for CHT activity determination. CHT enzyme activity was measured according to the method described by Guo et al. Briefly, 5 μL of plasma was incubated with 100 μL of 22 μmol/L 4-methylumbelliferryl-β-D-N-N- N″-triacetylchitotriosidase (Sigma M-5639; Sigma-Aldrich ChemieGmBH, Taufkirchen, Germany) in McIlvain's phosphate-citrate buffer; pH = 5.2, for 1 hour at 37.0 °C in the dark. The reaction was terminated by adding 120 μL 0.5 mol/L Na_2CO_3—$NaHCO_3$ buffer, pH = 10.7. In the quantitative method, the fluorescence of four methylumbelliferon was read in a Microfluor2® plate by a fluorimeter (BIO-TEK SynergyHT; Biotek Instruments Inc., Winooski, VT) (excitation: 360, emission: 450 nm). The CHT activity was expressed as nanomols of substrate hydrolyzed per milliliter per hour (nmol/mL per hour). Reference range of plasma CHT (4—195 nmol/mL per hour) was used as described by Guo et al. [9].