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Serum chitotriosidase levels in cancer patients undergoing high dose chemotherapy and stem cell transplantation

M. OZTURK, E. ÖZKAN[1], V. KESIK[2], S. OZAYDIN[3], O. BABACAN[2], H. YAMAN[4], E.O. AKGÜL[4], S. KILIC[5], F. ARPACI[3], A. OZET[3], I. KURT[4], S. DEMIRBAS[6], E. ARSLAN[6], D. TOK[7]

Department of Medical Oncology, Gulhane Military Medical Academy, Ankara, Turkey
[1]Department of Biochemistry, Ankara Occupational Diseases Hospital, Ankara, Turkey
[2]Department of Pediatric Oncology, Gulhane Military Medical Academy, Ankara, Turkey
[3]Department of Medical Oncology, Military Medical Academy, Ankara, Turkey
[4]Department of Biochemistry, Gulhane Military Medical Academy, Ankara, Turkey
[5]Department of Public Health, Gulhane Military Medical Academy, Ankara, Turkey6
[7]Department of Internal Medicine, Gulhane Military Medical Academy, Ankara, Turkey
[7]Department of Infectious Diseases, Gulhane Military Medical Academy, Ankara, Turkey

Abstract. – OBJECTIVE: Human chitotriosidase (ChT) is an active chitinase expressed by activated phagocytes. Increased ChT activity has been reported in systemic *Candida albicans* infections and in Gram-negative and Gram-positive bacterial infections, indicating that an increase in ChT activity reflects phagocyte activation. The aim of this study was to determine the changes in serum ChT activity in patients who underwent high dose chemotherapy (HDC) and stem cell transplantation (SCT), who are at an increased risk for fungal and bacterial infections due to depression of the immune system during the neutropenic period.

PATIENTS AND METHODS: A total of 55 SCT patients were included in the study. Serum ChT activity was determined before the initiation of HDC and during the neutropenic period after hematopoietic stem cell reinfusion on post-transplant first, fifth and tenth days.

RESULTS: Chitotriosidase levels before transplantation were significantly lower than the results at first, fifth and tenth days post-hematopoietic stem cell reinfusion.

CONCLUSIONS: Although the number of neutrophils was low, ChT enzyme activity was high in newly produced granules of neutrophils. Chitotriosidase may be supplemented as a drug for preventing and treating infections in the near future.

Key Words:
Chitotriosidase, Chemotherapy, Stem cell transplantation, Infection.

Introduction

Human chitotriosidase (ChT) is an active chitinase, specifically expressed by activated phagocytes. It is a chitin fragmenting enzyme that has a role in the defense against chitin containing pathogens. Chitin is synthesized and incorporated as a structural component of the protective coat of fungi, protozoa and nematodes[1]. Increased ChT activity has been reported in systemic *Candida albicans* infection[2]. Increased activity was also detected with Gram-negative and Gram-positive bacterial infections, indicating that an increase in ChT activity is not a specific response to fungal infections but rather reflects phagocyte activation[3]. Additionally, it is shown that activated macrophages have increased ChT activity[4]. ChT activity has been proposed as a biochemical marker of macrophage accumulation in several lysosomal diseases, especially in Gaucher's disease[5]. It is also used as a marker of recovery in transplanted patients with thalassemia[6].

Patients who undergo high dose chemotherapy (HDC) and stem cell transplantation (SCT) are at an increased risk for fungal and bacterial infection due to depression of the immune system during the neutropenic period. Increased permeability of mucosal barriers, long-term need for central vascular access, parenteral nutrition, use of broad-spectrum antibiotics, and extensive use of

Corresponding Author: Oguzhan Babacan, MD; e-mail: oguzhanbabacan@yahoo.com

corticosteroids increases the risk of infection. Changes in the gastrointestinal flora due to HDC protocols may result in increased fungal colonization. The aim of this study was to determine the changes in serum chitotriosidase activity in patients who underwent HDC and SCT.

Patients And Methods

Patients

A total of 55 SCT (46 autologous and 9 allogeneic) patients, 37 men (67%) and 18 women (33%) were included in the study. The most frequent three diagnoses were non-Hodgkin's lymphoma (36%, n = 20), Hodgkin's lymphoma (13%, n = 7), and multiple myeloma (13%, n = 7). Other malignancies were sarcoma, leukemia, and testicular cancer patients (38%, n = 21). BEAM (n = 22), ICE (n = 14), melphalan 200 mg/m^2 (M200) (n = 7), and TBI + cyclophosphamide (TBI+C) (n = 12) were used as conditioning regimens. Renal, cardiac, liver, and pulmonary function tests were performed and those who had normal organ function received HDC and 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. Four peripheral blood serum samples were collected from each patient for serum ChT activity samples. Serum ChT activity was determined before the initiation of HDC (8 days before stem cell infusion) and during the neutropenic period after hematopoietic stem cell reinfusion on post-transplant day 1, 5, and 10. 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 local Ethical Committee according to the ethical guidelines of the 2004 Declaration of Helsinki.

Sample Analysis

White blood cell (WBC) counts were assayed using an automated hematology system (ADVIA 120, Bayer Corp., Tarrytown, NY, USA). 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 measurements. Plasma and packed cells were separated after centrifugation at 1500xg for 10 minutes and frozen for ChT activity determination. ChT activity was measured according to the method described previously by Hollak et al[5]. The intra and inter-assay coefficient of variation (CV%) for WBC at the low levels were 2.8% and 4.4%, respectively. Briefly, 5 μL of plasma was incubated with 100 μL of 22 μmol/L 4-Methylumbelliferyl β-D-N,N′,N′-triacetylchitotrioside hydrate (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 Na2CO3 - NaHCO3 buffer, pH=10.7. In the quantitative method, the fluorescence of 4 methylumbelliferon was read in a Microfluor 2® plate by a fluorimeter (BIO-TEK SynergyHT; Biotek Instruments Inc., Winooski, VT, USA) (excitation: 360, emission: 450 nm). ChT activity was expressed as nanomols of substrate hydrolyzed per milliliter per hour (nmol/mL/h). Reference range of plasma ChT (4-195 nmol/mL/h) was used as described by Guo et al[7].

Statistical Analysis

SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were presented as median (min-max), frequency, and percents. Repeated measures of serum ChT were compared with the Friedman test. Bonferroni corrected Wilcoxon signed ranks test was used for post-hoc analysis. A p value of < .05 was accepted as statistically significant.

Results

Serum ChT levels decreased during the follow-up period (before transplant to 10th day post-transplant) (Table I, Figure 1). There was no significant difference with regard to stages, gender or treatment ($p > 0.05$). There was no significant difference in the ChT levels of patients using granulocyte colony stimulating factor for more than or less than 10 days or those with antifungal therapy for more than or less than 10 days ($p > 0.05$). However, the ChT levels before transplantation were significantly lower than the results of first, fifth and tenth days ($p < 0.05$). Mean fever values reached 38°C at 5th and 6th day after stem

Table I. Serum chitotriosidase levels and ChT /WBC count.

	Pre-transplant	Post-transplant day 1	Post-transplant day 5	Post-transplant day 10
Serum total ChT (nmol/mL/h)	26.6 (2.0-183.9)[a]	7.3 (0.0-181.5)[a]	4.8 (0.0-133.1)[a, b]	9.7 (0.0-54.7)[a, b]
ChT/WBC count (X1000)	5.3 (4.0-36.8)[c]	6.1 (0.0-145.2)[d]	24.2 (0.0-169.4)[c, d]	19.5 (0.0-338.7)[c]

Data were given as median and range. [a]$p < 0.001$ for pre-transplant versus day 1, day 5 and day 10; [b]$p < 0.001$ for day 5 versus day 10; [c]$p < 0.001$ for pre-transplant versus day 5 and day 10; [d]$p < 0.001$ for day 1 versus day 5.

cell reinfusion, where the WBC count levels decreased to 200 cells/mm^3 (Figure 2). Although serum total ChT levels decreased during the follow-up period from pre-transplant to post-transplant day 10, ChT activity per WBC count was increased (Table I).

Discussion

Patients with cancer undergoing hematopoietic SCT are at an increased risk of bacterial and fungal infections due to prolonged neutropenia, disruption of cellular and humoral immune systems and deterioration of mucosal barriers in general. Increased ChT activity was reported in systemic *Candida albicans* and in Gram-negative and positive bacterial infections, indicating that an increase in ChT activity reflects phagocyte activation towards the chitin barrier of the fungi, protozoa and nematodes [2,3]. Although the level of ChT activity is known for several diseases, the plasma ChT activity level is not known for patients without infection. We aimed to evaluate plasma ChT levels in patients with cancer undergoing hematopoietic SCT who have a decreased number of cells producing the enzyme.

Neutrophils and macrophages, which are the main cells of the innate immune system phagocytes, kill pathogens by recognizing the pathogen cell-wall constituents, such as lipopolysaccharides, glucans, mannans, mannoproteins and chitin. Although it is well known that human ChT is a chitin fragmenting enzyme playing a role in several biological processes, including defense against chitin-containing pathogens such as fungi, its biological role is still unclear[3,8]. Neutrophilic (polymorphonuclear neutrophils) progenitors synthesize the 50-kDa ChT form and store the enzyme in specific granules. The enzyme is secreted following stimulation with granulocyte macrophage colony-stimulating factor (GM-CSF). In addition, macrophages are the main source of ChT and one of the primary defenses against fungal intruders. They degrade both colloidal chitin and chitin in the cell wall of *Candida albicans*[1]. GM-CSF also induces expression of ChT in macrophages that constitutively secrete the enzyme and partly accumulate it in their lysosomes[9]. The authors suggested that GM-CSF administration has beneficial effects in individuals suffering from invasive fungal infections[8].

The ChT activity was lowered and then increased through post-transplant day 1 to 10 in our study. The increase was used as an engraftment marker in patients with thalassemia undergoing transplantation[6]. We also found that increased ChT/WBC activity indicating increased ChT activity in newly produced neutrophilic granules. Di Rosa et al[4] demonstrated increased ChT activity during monocyte-macrophage.

The ChT activity per WBC count increased in the post-transplant day 5th day, this might be due to neutropenic fever and the higher probability of sepsis in this study (Figure 2). Klostergaard et al[10] found no significant association between CHIT1 polymorphism and sepsis or death caused

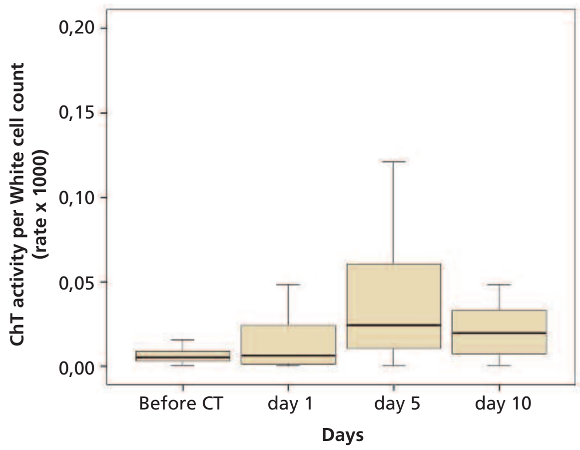

Figure 1. Serum chitotriosidase enzyme levels per white blood cell count before and during stem cell transplantation.

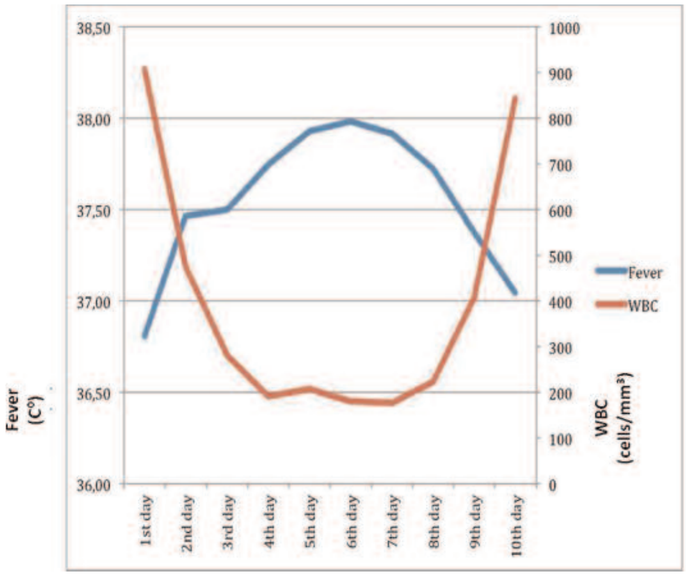

Figure 2. Fever increases maximum on days where white blood cell count drops minimum. However, serum ChT activity/WBC count rates on these days were protected.

by sepsis in patients with AML receiving high dose chemotherapy.

Although there was a large decrease in the number of leukocytes (as well as neutrophils), the ChT activity increased nearly 5 fold in comparison to the beginning of the SCT in our study. This finding reveals that newly synthesized neutrophils that are clearly known to have large and visible granules secrete much more ChT than older neutrophils. In comparison to WBC count, the ChT level was at the highest level on post-transplant day 7, which was the nadir for WBC. Secretion of the enzyme might be augmented by the granulocyte colony stimulating factors applied. In a recent study, the antifungal properties of chitotriosidase and lysozyme indicated were found similar *in vitro*[11]. This finding reveals that in situations like neutropenia where ChT activity is lowered, lysozyme activity also provide protection from fungal infections.

Conclusions

The patients undergoing hematopoietic SCT for various types of cancer have augmented ChT activity in comparison to WBC count. Granulocyte colony stimulating factor application may be responsible for the augmentation of enzyme activity. This finding demonstrates normal defense mechanisms against fungi. However, it should be kept in mind that deterioration in various parts of the immune system can exist in the presence of bacterial and fungal infections. This study showed that total ChT was decreased during the neutropenic period after HDC but ChT activity in newly neutrophils was clearly high. The evaluation of ChT enzyme replacement therapy and methods for proliferating young neutrophils earlier and in high amounts for preventing and treating infections in those patients may be goal for future studies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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