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Original Article

Thrombin generation in children with febrile neutropenia

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ABSTRACT

Background. Febrile neutropenia (FN) is a common and serious complication in patients with leukemia. Hemostasis and inflammation are two interrelated systems in response to infection. We aimed to investigate the course of thrombin formation in febrile neutropenia attack of children with acute lymphoblastic leukemia (ALL).

Methods. Thrombin generation was monitored in children treated for ALL at diagnosis of febrile neutropenia (FN) (t_0), at 48th hour of FN (t_1) and after recovery from neutropenia (t_2).

Results. Twenty-nine patients and 50 healthy children as control were enrolled into the study. Mean endogenous thrombin potential (ETP) and mean peak value of thrombin results at t_1 were significantly higher than at t_0, t_2 and control groups, respectively. A positive but statistically nonsignificant correlation between ETP values at t_1 and duration of neutropenia was observed.

Conclusion. Although thrombin generation is enhanced both due to chemotherapy or malignancy itself, our results revealed that thrombin formation also increased in neutropenic infection of children with leukemia.

Key words: acute lymphoblastic leukemia, febrile neutropenia, thrombin generation.

Thrombin, which has a direct role in promoting and regulating clot formation, is also the key component of innate immunity. It stimulates a variety of responses by endothelial cells, including cell surface expression and secretion of cellular adhesion molecules.[1-4] Binding thrombin to protease-activated receptors (PAR) causes an increase in the production of cytokines and growth factors, ultimately macrophage activation, neutrophil infiltration and expression of proinflammatory cytokines.[5,6] It is known that bacterial infection and lipopolysaccharides could stimulate monocytes or vascular endothelial cells to express tissue factor (TF) a transmembrane glycoprotein that activates the extrinsic coagulation cascade.[7]

The aim of this cross-sectional study was to evaluate the alterations of thrombin formation during febrile neutropenia (FN) in patients with acute lymphoblastic leukemia (ALL).

Material and Methods

The study group included children diagnosed with ALL who were treated between January to August 2016 in our hospital. Healthy children who were admitted to the hospital for routine control consisted the control group. Febrile neutropenia was diagnosed and treated according to the guidelines by Turkish Pediatric Hematology Association and was classified

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as clinically or microbiologically documented infection or fever of unknown origin.[8] After the onset of FN attack, cultures were immediately obtained from peripheral blood and catheter lumens, and broad-spectrum antibiotics were initiated. Chemotherapy blocks were interrupted to all patients during the FN. Blood cultures were repeated once a day for the first 5 days of FN period whereas those placed in Bactec (Blood culture system, Becton Dickinson Diagnostic Instrument Systems) for at least 10 days. Newly diagnosed patients received ALL-BFM 2009 protocol and patients with relapsed ALL were treated with REZ BFM 2002 protocol.[9,10] Demographics, diagnosis, clinical and laboratory data of patients' were obtained from their medical records.

Venous blood sample was drawn into the standard tubes containing sodium citrate (3,2% trisodium citrate) for protrombin time (PT), activated thromboplastin time (aPTT) and for thrombin generation tests (TGT). Blood samples of patient group were collected at diagnosis of FN (t_0), at the 48th hour of FN (t_1) and immediately after recovery from neutropenia (t_2).

Samples for TGT analysis were immediately centrifuged at 3309g for 25 minutes in order to obtain platelet poor plasma (PPP) and PPP stored at −80°C until analysis. Thrombinoscope (BV, Maastricht, the Netherlands), which was calibrated and automated thrombogram device was applied with fluorogenic method using commercial kits for TGT. The maximum concentration of thrombin formed was defined as the 'thrombin peak' and the area under the curve represented the "endogenous thrombin potential (ETP)". "Lag time" referred the time from the start of analysis to time that thrombin started to generate. 'Time to thrombin peak' was the time from the start of thrombin generation until the maximum thrombin value was obtained. 'Thrombin tail' described the time, when the curve reaches the end.

The study was performed in accordance with the Declaration of Helsinki and approved by the local Ethical Committee. Informed written consent was obtained from all parents or guardians before the study. (Ethics committee approval: Pediatrics Hematology Oncology Training and Research Hospital of Ankara Health Sciences University, 01.18.2016, 2016/010)

Statistical analysis was performed with SPSS 15.0 statistical package program. Kolmogorov-Smirnov test was used to determine the normality of values. The variables of groups were compared with Mann-Whitney U test. Pearson's correlation analysis was performed to compare the relation between two continuous variables. The p- value <0.05 was considered significant.

Results

Twenty-nine children with ALL and 50 healthy children enrolled in the study. The sex ratio and mean age of patients and control group were 1.6, 1.7 and 8.3 ± 4.8 years, 8.8 ± 4.6 years, respectively. The groups were comparable with respect to age and sex (p>0.05). Treatment phase of ten patients were induction/reinduction. Ten patients were at consolidation phase and two patients were at maintenance phase of ALL-IC BFM 2009 protocol. Seven patients were followed as relapsed ALL. Twenty patients were in hospital at the onset of FN and the remaining patients admitted to hospital less than 24 hours after FN developed. Fifteen of 29 (51.7%) patients' neutrophil count was ≤ 100/mm^3 and 14 patients' (48.3%) neutrophil count was 100-500/mm^3. All patients had subcutaneous port catheter. The period between the last day of chemotherapy and development of FN was 4.2 ± 2 days (min-max 1-9 days). The period between the onset of FN and the recovery of neutropenia was 20.2 ± 11 days (min-max: 7-47 days). At the time of recovery of neutropenia all patients were in good condition and afebrile. Any complication was not noted and patients were continued on their chemotherapy schedule after FN attack.

Microbiologically documented infections were noted in eight of 29 (27.5%) febrile neutropenia attacks. Peripheral blood or catheter cultures revealed coagulase negative *Staphylococcus*, *Enterococcus* and *Stenotrophomonas maltophilia* in six, one and one patients, respectively. Severe sepsis and septic shock were not detected in any patient.

Protrombin time and aPTT tests were within normal ranges in both FN and CGs. Endogenous thrombin potential values at t_0 were higher than the CG, however it is not statistically significant (p>0.05). Mean ETP values of patients at t_1 were significantly higher than t_0, t_2 and CG (p<0.001). Mean ETP value at t_2 was higher than CG, but this difference was not statistically significant (p >0.05) (Table I). Mean thrombin peak at t_1 was also significantly higher than t_0 (p=0.002) and t_2 (p=0.001), respectively. Endogenous thrombin potential and mean thrombin peak values of patients and CG are shown in Table I. There was no significant difference between all groups in lag time, time to thrombin peak and thrombin tail times (p>0.05).

Absolute neutrophil count at t_0, t_1 and t_2 were 127 ± 133/mm^3 (min-max 0-400/mm^3), 134 ± 149 /mm^3 (min—max 0-600/mm^3) and 1748 ± 1075 /mm^3 (min-max 800-4100/ mm^3), respectively. Although not significant, a negative correlation between absolute neutrophil count and ETP at t_1 and t_2 was noted (p>0.05). A positive but statistically nonsignificant correlation between ETP at t_1 and neutrophil recovery duration was noted (p>0.05).

The t_0, t_1 and t_2 ETP values of relapsed patients were comparable with the newly diagnosed leukemia patients (p>0.05). However, significantly higher ETP level at t_1 (3042 ± 1629 nmol x minute) was noted in patients receiving induction/reinduction therapy than that of the other patients at t_1 (1784 ± 496nmol x minute) (p: 0.001). Endogenous thrombin potential and mean thrombin peak values were not different in patients that had microbiologically documented infections or not (p>0.05).

Discussion

Thrombin generation assays (TGA) have been used since the early 1950s.[11] The latest generation of the TGA calculate the parameters stemming from the thrombogram.[12] The area under the curve, defined as endogenous thrombin potential (ETP) and the peak value representing the highest thrombin concentration that can be generated, are two important parameters of the thrombogram.[13] Presence of hypocoagulability leads to low peak and low ETP whereas hypercoagulability leads to high peak and high ETP.[14]

Hemostasis and inflammation are tightly linked which have great influence on each other.[15] During infection, the damage to the endothelium and activation of leukocytes result in an increase in tissue factor expression and activation of the coagulation cascade. Thrombin is a multifunctional protein involved in coagulation, anticoagulation, platelet activation,

Table I. Mean neutrophil, ETP and thrombin peak values of patients with febrile neutropenia and control group.

Time	Mean neutrophil values (mm^3)	ETP (nmolxminute)	Mean thrombin peak (nmol/L)
T_0	127 ± 133 (0-400)	1460 ± 619	274 ± 118
T_1	134 ± 149 (0-600)	2131 ± 1080 *	408 ± 252**
T_2	1748 ± 1075 (800-4100)	1475 ± 552	292 ± 126
Control Group		1260 ± 267	212 ± 101

* Mean ETP values of patients at t_1 were significantly higher than t_0, t_2 and CG (p <0.001)
** Mean thrombin peak at t_1 was also significantly higher than t_0 (p= 0.002) and t_2 (p= 0.001)

endothelial activation, production of growth factors and proliferation of both smooth muscle cells and fibroblasts.[16] It is also a mediator of inflammation in diseases such as cancer.[17] On the other hand, thrombin leads to activation of the protein C system that has anti-inflammatory effects.

Infection is a major cause of morbidity and mortality during the neutropenic phase after intensive cytotoxic therapies for malignancies. Complex procoagulant and anticoagulant alterations in the bacterial infection modifies the inflammatory response.[18] A few studies, with a limited number of patients, have previously described TGA changes in sepsis with inconsistent results.[19-22] Mesters et al.[23] have evaluated prospectively coagulation measurements in patients with severe chemotherapy-induced neutropenia and reported that in neutropenic patients with septic shock has been associated with increased thrombin generation. Prothrombin fragment 1+2 was used as a marker of thrombin generation in that study.

In another study, though the patients were not neutropenic, initial thrombin generation in patients with sepsis had been noted to predict the development of multiorgan dysfunction and poor outcome.[19] Another study revealed no difference in ETP values in patients with sepsis, and the authors noted limited use of ETP in clinical practice.[20] A recent study that compared results in patients with severe sepsis to healthy controls have disclosed a significant difference regarding peak thrombin, lag time and time to thrombin peak. Thrombin peak has been higher in survivors compared to non-survivors at all time points. The lag time and time to thrombin peak has been shorter in non-survivors. No significant difference in ETP between survivors and non-survivors of sepsis has been noted. They reported that thrombin peak shows a positive correlation with survival and the ETP does not have any prognostic importance.[22] Picoli-Quaino et al.[23] investigated hypercoagulability status with TGA in adult patients with ALL during febrile neutropenia episode. In this study, TGA was performed at baseline, at the time of fever onset, and 48 hours thereafter. An increase in the time to peak thrombin, reflecting an impairment in TG generation, was observed during the first 48 hours of sepsis compared with baseline samples.[24]

Our results showed that thrombin generation was increased during the course of febrile neutropenia. Mean ETP and mean peak thrombin levels at t_1 are significantly higher than at t_0, and t_2. Also, there was a positive but statistically nonsignificant correlation between high ETP values at t_1 and duration of recovery from neutropenia. Duration and severity of neutropenia are important parameters of morbidity and mortality in patients with FN. Although all patients completed their treatment without any complication, neutrophil recovery time was longer in patients whose ETP values were high at t_1. Markers of inflammation are increased during chemotherapy. Platelet-derived, endotelial-derived, and tissue factor-positive microparticle levels are increased in children with ALL at diagnosis and after prednisone and L-asparaginase administration although tissue factor pathway inhibitor increases during the induction chemotherapy in leukemia patients at the same time.[25] Corticosteroids and L-asparaginase are widely used in induction and reinduction phase of ALL patients. As expected, the ETP value at t_1 of our patients in induction/reinduction was significantly higher than other patients. Although mean ETP and mean peak thrombin levels were particularly higher at the 48th hour than at diagnosis of FN and after recovery of neutropenia, it is not possible to define the main cause. Infection or previous chemotherapeutics might be related to thrombin generation.

To our knowledge, our preliminary study, which investigates the alterations of thrombin generation with TGA in children with ALL during FN attack, is unique in the English literature. Thrombin generation could not be evaluated in FN attacks in different chemotherapy phases of the same patient; this is a limitation of the cohort. Moreover, the

patient group was heterogeneous that consisted of children with leukemia who were at different stages of chemotherapy protocol. Another limitation was not assessing the TGT before FN attack of the patients and comparing the thrombin generation to the healthy children. Comparing thrombin generation during febrile neutropenia attack (both t_0 and t_1) to afebrile and normal absolute neutrophil count period (t_2) of the same patient may have improved this limitation.

In conclusion, thrombin generation increase during febrile neutropenia. Further studies in larger groups for the clinical significance of thrombin formation in pediatric leukemia patients with FN are warranted.

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