Serum endocan levels in children with febrile neutropenia

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Abstract

Endocan is an endotelial cell specific molecule; previous studies have shown that serum endocan levels increased in cancer and sepsis and are also related to the severity of sepsis. There are no clinical study about serum endocan levels in children with febrile neutropenia. The aim of this study was to evaluate serum endocan levels in pediatric leukemia patients with febrile neutropenia (n=33) and compare them with children with leukemia without fever (n=33) and also with healthy children (n=24). The median serum endocan level in the first group (children with febrile neutropenia) was statistically significantly higher compared to the leukemic children without febrile neutropenia and also control group (P<0.01 for both). No difference was determined between the serum endocan levels of the leukaemia patients without febrile neutropenia and the healthy control group (P>0.05). Serum endocan levels were also similar with febrile neutropenia due to bacterial causes comparing with the idiopathic febril neutropenia. The results of this study showed increased serum endocan in children with leukemia during the febrile neutropenia episode, and no changes of serum endocan levels in children without leukemia without infection/fever. The monitoring of a series of serum endocan levels would be helpful for the course of febrile neutropenia.

Introduction

Neutropenia-associated infections which develop secondary to chemotherapy are oncology emergencies in children with malignancy. The most significant feature of these patients is that there are no (or very few) symptoms or signs of inflammation and fever in patients with neutropenia may herald a life-threatening infection.[1] While the source of fever can be defined clinically or microbiologically as infections in only 30-50% of neutropenic patients, in other cases the source can not be determined. Therefore, there is a need for specific, swift and highly effective indicators which will be able to show infection at the early stage.[2]

Endocan (endothelial cell specific molecule 1, ESM1), which is found in the proximal part of the long arm of the 5th chromosome (5q11.2), is the product of a single gene called *esm.*[3] It was first defined in human umbilical vein endothelial cell cultures, but almost immediately endocan production was shown with extensive human endothelial cell culture panels such as dermal microvascular endothelial cells, coronary and pulmonary arteries and fat tissue capillaries.[3] Endocan has been reported to be specific predominantly to endothelial tumour cells and during angiogenesis, it has been shown to be expressed specifically from endothelial cells. Endocan has been defined as one of the key molecules playing a role in angiogenesis, lymphogenesis and the development of cancer.[3] Endocan is also a molecule specific to endothelial cells which stimulate synthesis by lipopolysaccharide which is a significant mediator of the gram negative bacteria wall, which plays a role in the pathogenesis of sepsis.[4] In clinical studies, the serum endocan level has been shown to be correlated to the development and severity of a sepsis.[4-6]

Although there are studies on the serum endocan level in adult acute myeloid leukemia cases,[7,8] in a detailed review of literature, no clinical study could be found related to endocan in febrile neutropenia patients. The aim of this study was to evaluate serum endocan levels in pediatric leukemia patients with febrile neutropenia.

Materials and Methods

The study was planned to include patients aged between 1 month and 18 years with a diagnosis of acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) who had experienced a febrile neuropenic attack and were followed-up in the Pediatric Haematology Oncology Department of Eskisehir Osmangazi University Faculty of Medicine, Turkey. Two control groups were formed, one of ALL and AML patients with no neutropenia or fever. Second control group included age and sex matched healthy children. This study was approved by the Ethics Committee of Eskisehir Osmangazi University Faculty of Medicine (26.09.2012/275).

According to the Turkish Paediatric Febrile Neutropenia Guidelines, diagnosis of febrile neutropenia is made when the absolute neutrophil count is 500/mm^3 or lower or in cases when it is 1000/mm^3 or less and it is expected to fall below 500/mm^3 within 24-48 hours, one temperature measurement from the axilla of ≥38°C or ≥37.5°C for at least a period of an hour. Informed consent was obtained from the parents of all the patients. A detailed systemic examination was made of each patient. Samples were obtained from the patients for haemogram, peripheral smear, C-reactive protein, procalcitonin. Blood, nasopharyngeal, faeces and urine samples were obtained for microbiological evaluation with culture. In addition, chest X-ray were taken and when necessary, plain paranasal sinus X-ray. A record was made for each patient of chemotherapy, antibiotic, antiviral, and antifungal treatments received, any treatments related to accompanying diseases and how many days the fever had been ongoing. In patients who experienced more than one febrile neutropenic attack throughout the study period, each attack was evaluated separately. The treatment and monitoring of the patients was managed by the clinicians and where it was deemed necessary by the febrile neutropenia guidelines, examination was made by high resolution chest tomography, paranasal sinus tomography or abdominal ultrasonography. In patients experiencing a febrile neutropenic attack, when the fever increased again after a decrease of at least 3 days, it was accepted as a new attack. Chemotherapy protocol of ALL patients includes prednisolone/dexamethasone, vincristine, daunorubicine, L-asparaginase, methotrexate, mercaptopurine ane 6-thioguanine and chemotherapy protocol of AML patients incluedes cytosine arabinoside, mitoxantrone, vincristine, and cyclophosphamide.

Leukaemia patients without a raised tem-

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perature or neutropenia with normal physical examination results and for whom infection was excluded by laboratory tests, were included in the first control group. Previously healthy children with no complaints serve as the second control group. On the first day of febrile neutropenia, 2 cc blood was taken into sterile tubes, centrifuged at 4000 rpm for 10 minutes, then the serum was separated and all samples were stored at –80°C until examination. The endocan levels were assayed with the Human Endocan immunoassay LIK-1205 kit (Lunginnov, Lille, France). All the stored serums were thawed at the same time and mixed by shaking, then before applying 100 microlitre dilution to the serum samples, they were tested with the Sandwich ELISA protocol. They were then processed for 30 minutes with the kit at 450 nanometer wavelength. The results were obtained as mean optic density change from the milli-absorbance unit/min and by comparing with the standard kit curve provided by the manufacturer's computer software, units were changed to ng/mL. According to the manufacturer's guidelines, values over 0.3 ng/mL were evaluated as positive.

Statistical analysis

Statistical evaluation of the data was made with SPSS for Windows 16 (SPPSS Inc., Chicago, IL, USA) software program. Data were shown as mean ± standard deviation (SD). For the comparison of data with the one way ANOVA test and Bonferroni post hoc analysis were used and in the evaluation of the correlation between parameters, the Spearman correlation test was used. A value of $P<0.05$ was accepted as statistically significant.

Results

The total 33 febrile neutropenic children, the 33 leukaemia patients without fever and 24 healthy children have been enrolled. In the febrile neutropenic children, the mean age was 72.2±52.8 months (range, 6-192 months), in the leukaemia patients without fever 87.7±41.0 months (range, 24-204 months), and in the healthy control group, 84.8±64.2 months (range, 12-192 months). Of the febrile neutropenic patients, 23 (70%) were diagnosed as ALL, 8 (24%) as AML and 2 (6%) as biphenotypic leukaemia and the leukaemia patients without febrile neutropenia were 25 (78%) ALL, 5 (15%) AML and 2 (7%) biphenotypic leukaemia. In the etiology of the febrile neutropenia attacks, 15 (45%) were determined as bacterial infection, 4 (12%) as invasive fungal disease and in the remaining 14 (43%), as no microorganism was shown, they were accepted as idiopathic febrile neutropenia. Of the febrile attacks, the absolute neutrophil count was determined as ≤500, >100/mm^3 in 11 (33.3%) and as ≤100/mm^3 in 22 (66.7%). In 30 (90.9%) of the febrile attacks, there was thrombocytopenia (<150,000/mm^3). During the attacks, mean CRP as 5.05±5.1 mg/dL (min: 0.341, max: 20.7) and median procalcitonin as 4.45 ng/mL (min: 0.05, max: 94.9). In cases where the fever and infection continued despite antibiotic therapy, the antibiotic treatment was changed and mean 4±2.84 (min: 2, max: 10) types of different antibiotics were used. With antibiotic treatment, the fever decreased in mean 2.5±0.94 days. In 3 cases where the fever could not be brought under control, there was a need for additional antibiotics and/or antifungal agents. The mean period of antibiotic use was 14.6 days (range, 7-38 days). Mortality was seen in 3 (9%) patients who developed febrile neutropenia attacks. In the current study, the serum endocan levels of the patient group with febrile neutropenia ranged from 0.094 to 0.943 ng/mL and the median was determined as 0.241 ng/mL. In the group of leukaemia patients without febrile neutropenia, the serum endocan levels ranged from 0.089 to 0.314 ng/mL and the median was determined as 0.127 ng/mL. The median serum endocan levels of the febrile neutropenia paients were determined to be statistically significantly high compared to those of the leukaemia patients without febrile neutropenia ($P<0.01$). In the control group of healthy paediatric subjects, the serum endocan levels ranged from 0.089 to 0.314 ng/mL with a median of 0.119 ng/mL. The median serum endocan level of the febrile neutropenia patients was determined to be also statistically significantly high compared that of the control group ($P<0.01$). No difference was determined between the serum endocan levels of the leukaemia patients without febrile neutropenia and the healthy control group ($P>0.05$) (Table 1 and Figure 1). No statistically significant difference was determined in terms of endocan, CRP or procalcitonin between patients showing micro-organisms in culture and those with no micro-organism ($P>0.05$). Three patient who were died in febrile neutropenia group have higher serum endocan levels, above 0.3 ng/mL.

Table 1. Demographical and laboratory findings and serum endocan levels of study groups.

	Children with febrile neutropenia (n=33)	Leukemia group without fever/neutropenia (n=33)	Healthy children (n=24)	P values
Age, months	72.2± 52.8	87.7±41.0	84.8±64.2	ns
Gender, boys/girls	20/13	20/13	12/12	ns
White blood cell, mm^3	1818±773	5861±3052	10,521±3051	P1<0.001 P2<0.001 P3<0.001
Absolune neutrophil count, mm^3	138±223	3158±392	6935±3542	P1<0.001 P2<0.001 P3<0.01
Platelet count, mm^3	67,468±11,717	244,382±105,285	305,658±103,927	P1<0.001 P2<0.001 P3<0.05
Serum CRP levels, mg/dL	5.05±5.1	0.36±0.10	0.35±0.02	P1<0.001 P2<0.001 P3>0.05
Serum endocan levels, ng/mL	0.241 (0.094-0.943)	0.127 (0.089-0.314)	0.119 (0.089-0.314)	P1 <0.01 P2<0.0 P3>0.05

Values expressed as mean ± standard deviation except serum endocan levels, shown as median (minimum-maximum). ns: not significant, CRP: C-reactive protein, P1, children with febrile neutropenia *vs*. leukemia group without fever/neutropenia; P2, children with febrile neutropenia *vs*. healthy control; P3, leukemia group without fever/neutropenia *vs*. healthy control.

Discussion and Conclusions

The results of this study showed that the serum endocan levels of paediatric leukaemia patients with febrile neutropenia were determined as statistically significantly high compared to the group of paediatric leukaemia patients without febrile neutropenia and the healthy control group. Previous studies have shown that serum endocan levels are increased in cancer, sepsis, coronary artery disease and hypertenison.[3-5,9,10] In cases with malignancy, especially for solid tumors, the increase in serum endocan levels has been shown to increase the spread of the disease, is related to a poor prognosis and it could be used in the evaluation of vascularity following surgical treatment (the removal of angiogenic tissue).[11-14] Although there have been studies related to the serum endocan level in adult cases of acute myeloid leukaemia,[7,8] to the best of our knowledge, this is the first clinical study in worldwide literature to evaluate serum endocan levels in childhood leukaemia and febrile neutropenia. In a previous study, as it was shown that serum endocan levels in 40 untreated AML patients were significantly high compared to those of a control group, it was determined that the serum endocan levels which dropped in severe neutropenia which develops because of chemotherapy or after intensive chemotherapy, increased together with bone marrow regeneration. However, during complicated bacterial infections, the level of serum endocan has been observed to increase.[8] In a study of 17 adult acute myoblastic leukaemia patients, it was shown that serum endocan levels were affected by ATRA treatment given for a 2-day period.[7] In the present study, although the serum endocan levels of the children with febrile neutropenia were higher than those of the children without febrile neutropenia and of the healthy control group children, similar results were obtained for the control group and the leukaemia patients with no infection attack. This can be explained by the administration of chemotherapy in the continuing treatment of leukaemia cases without febrile neutropenia and that the number of cases was limited. However, the evaluation of serum endocan levels can be considered useful in increased fever caused by infection during febrile neutropenia. Serum endocan levels increase in septic patients and this increase has been found to be associated with the severity of the disease.[4,5]

In the currrent study, that the serum endocan levels of the leukaemia cases who died were determined as high. It may be supports the view of other malignacy studies that it could be an indicator of prognosis, however the number of cases were small for comparison. In another study of 36 patients being monitored in the intensive care unit for sepsis or septic shock, the serum endocan levels of 7 patients with systemic inflammatory response syndrome (SIRS) were found to be higher than those of a control group of 20 healthy subjects.[15] In addition, the measured serum endocan level of 12 patients who died after admittance to the intensive care unit were determined as higher than those of patients with 10-day survival in the intensive care unit. It is known that the infection agent can be shown microbiologically in lower percentage of febrile neutropenia patients, the most common infection agent has been observed to be bacteria.[1,2] Similar to other studies in literature of febrile neutropenia patients, in the current study, the etiology agent was determined in 43% (15 bacterial cuases, 4 invaisve fungal infection) of the cases with febrile neutropenia. No statistical difference was determined in the serum endocan levels between the cases determined with bacterial agent and those without.

Limitation of this study, this is a pilot study and the sample size is not enough to evaluate the microbiologically confirmed infection, or invasive fungal infections. Mortality rate is low and it is difficult to evaluate the correlation between the serum endocan levels and prognosis. We have no chance to show with our study protocol, changing serum endocan levels in febrile neutropenia in same patient during and after the febrile neutropenia episode. Further studies needed including paired comparison of two samples from each patient, one sample derive early during stable neutropenia and a later sample derived during febrile neutropenia to clarify whether the increased levels in their neutropenic patients are caused by the infection and/or to more accuartely quantify the alteration of the endocan level caused by the febrile neutropenia.

In neutropenic patients, clinical symptoms and findings are seen more often than expected in inflammation and infection and in most cases, the only symptom is fever. In this study, increased serum endocan levels have been observed in children with febrile neutropenia. Previous study showed that serum endocan levels increased during complicating bacterial infections before a decrease was seen during antibiotic therapy in adult patients with leukemia.[8] There is a need for further studies related to the effect of changes in blood endocan levels on the efficiency of treatment, and the prognosis.

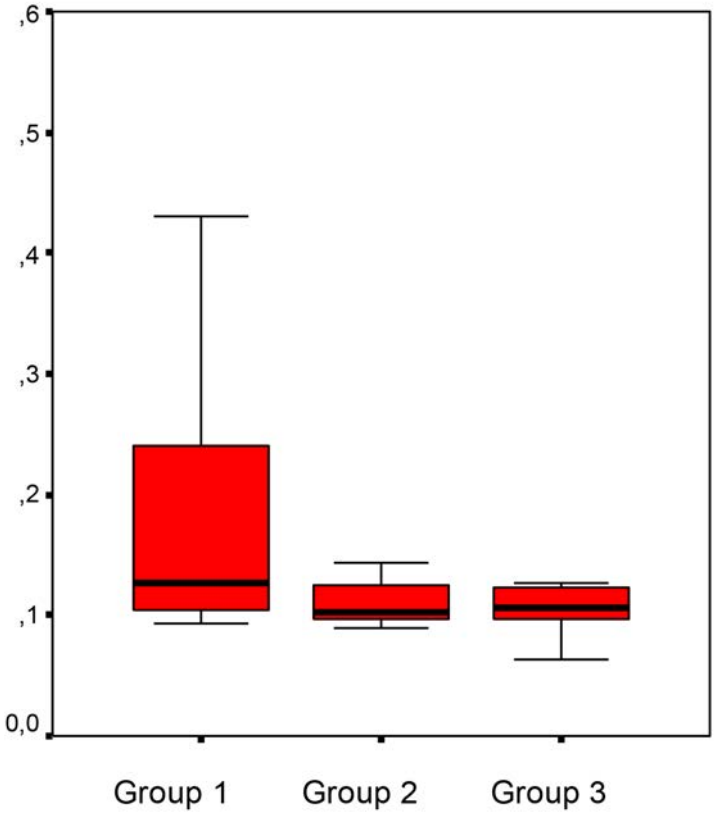


Figure 1. Serum endocan levels in study groups (group 1: children with febrile neutropenia; group 2: leukemia children without fever/neutropenia; group 3: healthy children).

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