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The influence of RANKL/osteoprotegerin on the prognosis of childhood acute lymphoblastic leukemia

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ABSTRACT

Aims: The aim is to investigate the association of the osteoprotegerin (OPG)/ soluble(s) receptor activator nuclear kappa B ligand (RANKL) with the prognosis of children with acute lymphoblastic leukemia (ALL).

Methods: Patients with the diagnosis of ALL between the years 2008-2010, were enrolled in the study. Demographic characteristics and complete blood count findings, treatment responses, as well as OPG and sRANKL levels were evaluated on admission and at the end of induction treatment.

Results: Mean serum levels of OPG and sRANKL on admission were 38,6 ±19,03 ng/ml and 0,22±0,24 ng/ml, respectively. Whereas, at the end of induction, serum levels were measured as 35,5±40,8 ng/ml for OPG and 0,033±0,056 ng/ml for sRANKL. A statistically significant decrease was determined for sRANKL at the end of induction therapy, compared to admission (p:0,000).

Conclusion: In the current research, OPG and sRANKL are increased at diagnosis of childhood B-ALL. Whereas, no significant relationship between the OPG and sRANKL levels and the disease prognosis was determined.

Keywords: Acute lymphoblastic leukemia, osteoprotegerin, bone remodeling, prognosis

INTRODUCTION

Leukemia is the most common childhood cancer. A significant survival advantage has been achieved in recent decades with advances in leukemia treatment and supportive care. Therefore, long-term toxicities and providing these patients with a life without morbidity have gained importance. Bone metabolism is one of the most important systems to be protected. In childhood, while the target bone mass has not been reached, multiagent chemotherapy and the disease itself cause serious bone loss. Of course, providing this protection can only be possible with a better understanding of bone metabolism and determination of metabolic points to be intervened.

Normal physiological bone remodeling has become more understandable with recent studies on this era. The most significant identified pathway involved in bone remodeling consists of osteoprotegerin (OPG) - receptor activator nuclear kappa B (RANK) and receptor activator nuclear kappa B ligand (RANKL). Bone mass homeostasis is achieved with the balance between these three molecules.[1-3] In the present study, the levels of OPG and soluble (s) RANKL are evaluated both on admission and at the end of induction, to determine any significant association between the levels and the prognosis of the disease.

METHODS

The study was carried out with the permission of Erciyes University Faculty of Medicine Ethics Committee (Date: 08.10.2010, Decision No: 2010/120). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The current research has been planned as a cross-sectional prospective study evaluating the patients who had been admitted to Erciyes University Faculty of Medicine Department of Pediatric Hematology and Oncology between 2008-2010 with the diagnosis of acute lymphoblastic leukemia (ALL). Enrolled patients have been evaluated in terms of; the demographical data (sex, age, risk group), immunophenotype, laboratory values

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both on admission and end of induction, involvement of central nervous system, bone mineral density on admission, and treatment response, relapse, and outcome. Continuous data were compared by the Student t test if normally distributed, if not were compared by the Mann-Whitney U test. Also proportions were compared with x^2 test and Fisher exact test. A p value <0.05 was considered as statistically significant. Results were analyzed using SPSS version 22.0 software.

RESULTS

Of the 45 patients enrolled in study 20 of them were female and 25 were male. The median and mean age on admission was 6 years (range between 1.8-18 years) and 7,5 years (±4,5 years), respectively. The laboratory evaluation of the patients on admission is available in **Table 1**. Turkish National ALL (TRALL) protocol was initiated for the patients. According to this protocol, 14 patients (31.1%) were assigned to the standard risk group, 20 patients (44.4%) were to the medium-risk group, and 11 patients (24.4%) high-risk group. On admission, 6 patients (13.3%) had central nervous system involvement. Five patients (11.1%) had poor steroid response on day 8. Also, on day 15th, 3 patients (6.6%) had a positive minimal residual disease in bone marrow evaluation on flow cytometry. All patients achieved remission on day 33rd. Six patients (13.3%) relapsed, 6 patients died owing to progressive disease and 2 patients died due to infectious complications. Mean serum levels of OPG and sRANKL on admission were 38,6 ±19,03 ng/ml and 0.22±0.24 ng/ml, respectively. Whereas, at the end of induction, serum levels were measured as 35,5±40,8 ng/ml for OPG and 0.033±0.056 ng/ml for sRANKL. A statistically significant decrease was determined for sRANKL at the end of induction therapy, compared to admission (p:0.001). However, the decline in OPG was not significant (p:0.69). There was no difference in OPG and sRANKL levels in terms of risk groups. The mean OPG level on admission was 37.6±20.9 ng/ml for the patients admitted with an LDH level of <1000 U/L, whilst the level was 68.1±50.1 ng/ml for patients whose LDH levels were >1000 U/L. The difference between the two groups in terms of OPG levels was statistically significant (p:0.09). Besides the sRANKL level on the day, 33rd was significantly lower in patients with an LDH level below 1000 U/L, which refers to a low tumor burden (p:0.04). The mean OPG and sRANKL levels for these groups and p levels are available in **Table 2**. The mean OPG and sRANKL levels on admission and at the end of induction had no significant difference between the patients who relapsed and not relapsed.

Table 1. Laboratory evaluation of patients on admission

	Mean (±SD)	Median (min-max)
White blood cell	88,387/mm³ (±161,262)	15,150/mm³ (870-741,660)
Absolute neutrophil count	2,264/mm³ (±3,970)	600/mm³ (14-20,300)
Hemoglobin	7.9 g/dl (±2.9)	8 g/dl (2.2-13.9)
Platelet	101,000/mm³ (±103,000)	53,000/mm³ (3,000-409,000)
Lactate dehydrogenase	955 U/L (±1.177)	400 U/L (172-5,133)
Erythrocyte sedimentation rate	38.9 mm/h (±32.6)	26 mm/h (3-120)

Table 2. OPG and RANKL levels grouped by tumor burden

	Low tumor burden (LDH <1000 U/L) (n:29)	High tumor burden (LDH>1000 U/L) (n:16)	p value
OPG1	37.6 ng/ml (±20.9)	68.1 ng/ml (±50,7)	0,009
OPG2	37.7 ng/ml (±46.7)	29.2 ng/ml (±9.2)	0,666
RANKL1	0.19 ng/ml (±0.2)	0.4 ng/ml (±0.52)	0,101
RANKL2	0.02 ng/ml (±0.03)	0.07 ng/ml (±0.1)	0,040

OPG1: OPG levels on admission, OPG2: OPG levels on day 33. RANKL1: RANKL levels on admission, RANKL2: RANKL levels on day 33.

DISCUSSION

Acute leukemias emerge with a clonal expansion and maturation arrest at a specific stage of myeloid or lymphoid lineage, accounting the 97% of all childhood leukemias. The most common acute leukemia in childhood is ALL, of which 85% are B-cell acute lymphoblastic leukemia (B-ALL). The peak incidence of ALL is between 2 and 5 years of age.[4] Monitoring minimal residual disease and determining the exact risk group of the patient is vital to ensure the patients have the required treatment. In order to provide this, different methods are being investigated for minimal residual disease monitoring.[5,6] Owing to the multiagent chemotherapy protocols, central nervous system-directed regimens, and better management of side effects, pediatric ALL patients have achieved a 5-year overall survival rate of 90%.[7] Despite this excellent survival rate, long-term morbidities constitute the most important challenge.[8]

Skeletal comorbidities are one of the most important long-term toxicities of ALL. Therefore, providing a healthy bone metabolism to long-term survivors is vital. To implement a targeted and beneficial therapy, the underlying mechanisms of bone destruction should be understood better. Corticosteroids are well-known drugs with the effects of osteopenia and reduced bone mass. However, the previous studies demonstrated that at the time of diagnosis, before the initiation of steroids; bone pain, vertebral fractures, and low bone mineral density can be detected in patients with B-ALL, both in the standard and high-risk groups.[9,10] Thus, B-ALL-mediated bone destruction is accused.[11]

B-cells originate from bone marrow and the development of them rely on growth factors produced by bone marrow stromal cells.[12] Besides, multiple factors and mechanisms, such as vitamin D3, parathyroid hormone, estrogen, cytokines, and mechanical stress influence normal bone remodeling. As mentioned earlier, RANK-RANKL and OPG systems play a major role in remodeling by providing a balance between osteoclastogenesis and remodeling of bone.[1,2,13] RANKL, which is a member of the tumor necrosis factor (TNF) superfamily, when binds to the cognate receptor RANK, initiates the differentiation, activation, and survival of osteoclasts and bone remodeling. Whilst, OPG binds RANKL, prevents the RANK-RANKL, and afterward inhibits the RANKL-mediated bone resorption and osteoclastogenesis. In recent studies, cancer-induced signals are demonstrated to alter the balance between these molecules, by activating RANKL and inhibiting the OPG. Tumor-induced bone resorption results in releasing of growth factors from the bone niche which helps the tumor grow.[13,14] The mechanism is well-known and studied for solid tumors and myeloma.[14,15]

As well, the mechanism of bone destruction caused by tumor cells is described in B-ALL cells by using a mouse model.[13] Rajakumar et al.[13] exhibited both the mouse and human leukemic cells create bone demolition in the absence of steroids or any other chemotherapeutic agents. The destruction is caused by the increased RANKL expression by the leukemic cells.[13] In the present study, at the end of induction therapy, sRANKL levels decreased significantly compared to admission. This result can be ascribed to the decreased tumor burden with the induction treatment. Similar to this result, sRANKL levels are expected to be higher on admission if the tumor burden is high, as displayed in the current study. However, it was not statistically significant, which can be attributed to the small number of enrolled patients. On the other hand, sRANKL levels were significantly lower in the presence of a low tumor burden.

The present study, evaluating the value of sRANKL and OPG, has certain limitations. First of all, the number of enrolled patients is scarce. Therefore, the comparison between the groups may not be resulted in statistically significant. The other limitation is the short-term observation period of the patients. As the tumor burden has been speculated to have a major role in the levels of sRANKL, longer observation periods are needed to ascertain the exact result of ALL treatments to reduce the tumor burden.

In mice studies, treatment with RANKL antagonists (recombinant OPG) has protected from increased bone destruction even in the absence of chemotherapy. Therefore, the inhibition of this altered expression of RANKL may be a therapeutic target for protecting the bone even in the presence of a high tumor burden.[16]

CONCLUSION

The data of the present study suggest that OPG and sRANKL are increased at diagnosis of childhood B-ALL, related to the microenvironment of the initial leukemic cell burden. However, no obvious relation with prognosis is demonstrated. Further studies with large numbers of patients to highlight the clear effect of these molecules and their value in targeted therapy in childhood ALL are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Faculty of Medicine Ethics Committee (Date: 08.10.2010, Decision No: 2010/120).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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