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CLINICAL STUDY

Serum osteopontin and CD44 levels in lymphoreticular malignancies in children

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Abstract: *Objective:* Osteopontin (OPN) is an adhesive glycoprotein that interacts with a variety of cell surface receptors, including several integrins and CD44. OPN is expressed and secreted by numerous human malignancies. CD44 play an important role in tumor growth and metastasis. We aimed to evaluate serum levels of osteopontin and CD44 in patients with lymphorethicular malignancies in childhood.
Methods: We studied serum levels of CD44 and OPN levels of 54 patients (26, 18 and 10 patients with non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL) and acute lymphoblastic leukemia (ALL), respectively) at the diagnosis.
Results: The mean levels of OPN were significantly higher in patients (5.42±8.24 ng/ml) than in controls (3.89 ±1.96 ng/ml). The mean levels of CD44 levels were also significantly higher in patients (3.82±2.31 ng/ml) than in controls (1.96±0.62 ng/ml), and significantly higher in the advanced stages than in early stages. The mean levels of the CD44 in NHL, HL and ALL were 3.49±2.00, 3.56±1.74, and 5.15±3.50 respectively. Serum OPN and CD44 levels were found to be increased in parallel (p=0.003). A more advanced disease and/or poor prognostic factors were seen in 9 patients who had both serum CD44 and OPN levels higher than 2SD of the control.
Conclusion: Elevated levels of both CD44 and OPN at the diagnosis may predict an unfavorable outcome in childhood leukemias and lymphomas *(Tab. 2, Fig. 3, Ref. 44)*. Full Text in PDF *www.elis.sk*.
Key words: osteopontin, CD44, lymphoma, leukemia, children.

Osteopontin (OPN) is an arginine-glycine–aspartate containing adhesive glycoprotein first identified in bone and subsequently detected in many other tissues, including dentin, cartilage, kidney, and vascular tissues. OPN is involved in diverse biologic processes, including inflammation, leukocyte recruitment, wound healing, and cell survival (1 – 4). In addition to these pathologic processes, OPN might have a protective role in interactions between epithelial surfaces and the external environment (5). OPN is expressed and secreted by numerous human cancers and functions in cell adhesion, chemotaxis, macrophage-directed interleukin 10 (IL-10) suppression, stress-dependent angiogenesis, prevention of apoptosis, anchorage-independent growth of tumor cells by regulating cell–matrix interactions and cellular signaling through binding with integrin and CD44 receptors (6). The CD44 glycoproteins are ubiquitously expressed cell-surface adhesion molecules that mediate cell-matrix and cell-cell interactions (7). CD44 may play an important role in tumor growth and metastasis. Many of the primary carcinoma specimens examined expressed high levels of CD44 (8). Serum CD44 concentration is found to be correlated with tumor metastasis and tumor burden, and surgical resection of tumors resulted in decreases in serum CD44 levels (9). In this study, we aimed to measure serum levels of osteopontin and CD44 in patients with lymphorethicular malignancies in childhood and to determine the relation of osteopontin levels with disease stage.

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Materials and methods

Patients

We studied the serum levels of CD44 and OPN in 54 patients with non-Hodgkin's lymphoma (NHL, 26 patients), Hodgkin's lymphoma (HL, 18 patients) and acute lymphoblastic leukemia (ALL, 10 patients). The blood samples were obtained at diagnosis in division of the Pediatric Hematology and Oncology at Ondokuz Mayis University and Dr. Sami Ulus Children's Hospital, then centrifuged and the supernatant was stored at –80 °C until the analysis. Stage III and IV NHL and HL and all leukemia were defined as advanced disease. Age and sex-matched 20 healthy children were enrolled to the study as the control group. Informed consent was obtained for all children from their parents.

Detection of serum CD44

Serum soluble CD44 (sCD44) was measured with an enzyme-linked immunosorbent assay (CD44 ELISA; Bender Med Systems, Vienna, Austria). *The Human sCD44std ELISA BMS209/2®* is the enzyme-linked immunosorbent assay for the quantitative detection of human sCD44std. The anti-human sCD44std coating antibody

is adsorbed onto micro wells. Human sCD44std present in the sample or standard binds to antibodies adsorbed to the micro wells. The *HRP-conjugated* anti-human sCD44std antibody is added and binds to human sCD44std captured by the first antibody. Following incubation, unbound HRP conjugated anti-human sCD44std is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of human sCD44std present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 6 human sCD44std standard dilutions and human sCD44std concentration is determined.

Detection of human osteopontin in serum

Assay Designs' human OPN TiterZyme® Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determination of OPN in human biological fluids. The kit uses a monoclonal antibody to human OPN immobilized on a microtiter plate to bind the human OPN in the standards or sample. A recombinant human OPN Standard is provided in the kit. After a short incubation, the excess sample or Standard is washed out and a biotinylated monoclonal antibody to human OPN is added. This antibody binds to the human OPN captured on the plate. After a short incubation, the excess antibody is washed out and Streptavidin conjugated to Alkaline Phosphatase is added, which binds to the biotinylated monoclonal human OPN antibody. An excess conjugate is washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 405 nm. The measured optical density is directly proportional to the concentration of human OPN in either standards or samples.

Statistical analyses

The data were analyzed on the SPSS 9.05 packed program for windows. The mean levels of osteopontin and sCD44 levels of the groups were compared to the Mann-Whitney U test. The Pearson correlation test was used for searching a correlation between sCD44, osteopontin levels and the other parameters including serum LDH levels, age, subtypes of the disease (leukemia, non-Hodgkin's and Hodgkin's lymphoma), white blood cell count and primary site of the disease.

Results

The mean levels of sCD44 levels were 3.82 ± 2.31 and 1.96 ± 0.62 ng/ml in patients and controls, respectively, and the difference between them was significant (p = 0.01) (Fig. 1, Tab. 1). On the other hand, the mean levels of OPN were 5.42 ± 8.24 and 3.89 ± 1.96 ng/ml in patients and controls, respectively, and the difference between them was not significant (p = 0.214) (Fig. 2). The mean levels of the sCD44 in ALL, NHL and HL were 5.15 ± 3.50, 3.49 ± 2.00 and 3.56 ± 1.74, respectively. The mean levels of OPN in ALL, NHL and HL were 7.24 ± 7.13, 5.95 ± 8.52 and 3.63 ± 1.86, respectively (Tab. 1). When we looked at the levels of the subgroups of the disease, sCD44 levels in NHL, HL and ALL levels were significantly higher than the levels of control group

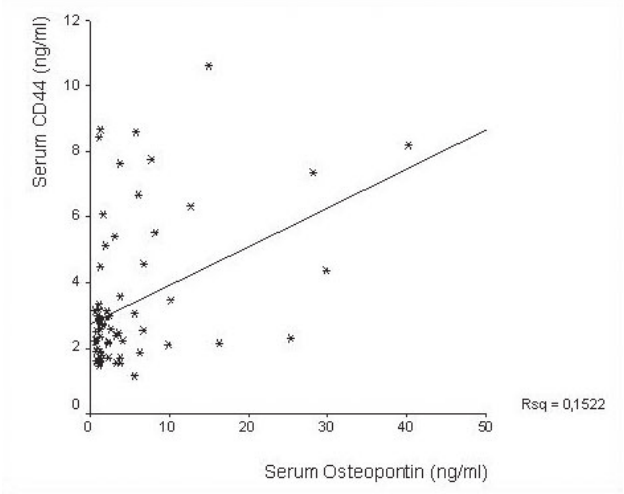

Fig. 1. Box plots of serum CD44 levels of the groups. CD44 levels in NHL, HL and ALL were significantly higher than in the control group (p values were 0.001, 0.001 and 0.022, respectively).

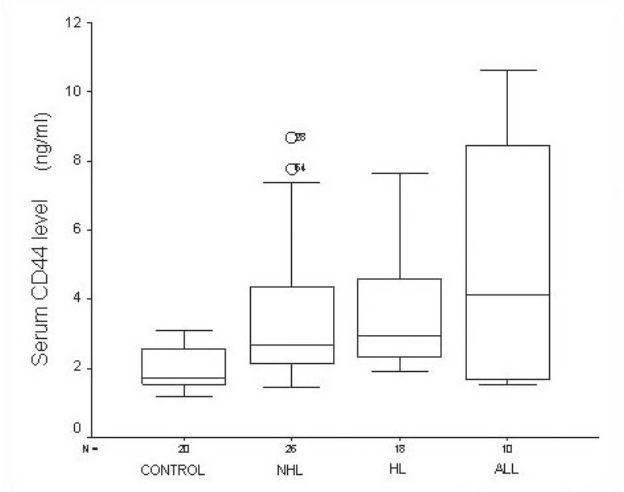

Fig. 2. Box plots of serum osteopontin levels of the groups. OPN levels in the same groups were not significantly higher than in the control group (p values were 0.297, 0.105 and 0.286, respectively).

(p values were 0.001, 0.001 and 0.022, respectively). On the other hand, OPN levels of the same groups were not significantly higher than the levels of control group (p values were 0.297, 0.105 and 0.286, respectively) (Tab.1).

Serum CD44 levels were significantly higher in advanced stage of the disease than in early stages of lymphomas (4.04 ± 2.08 and 2.43 ± 0.52) (p = 0.05). Serum OPN levels were also higher in advanced stage of the disease than in early stages (6.01 ± 8.12 and 3.05 ± 4.34) but not statistically significant (p = 0.061) (Tab. 1). The levels of OPN correlated with sCD44 (p = 0.003) (Fig. 3). The levels of sCD44 and OPN correlated with serum LDH levels but not with other parameters including age, subtypes of the disease (leukemia, non-Hodgkin's and Hodgkin's lymphoma), white blood cell count and primary site of the disease.

Both sCD44 and OPN were detected above 2SD of the control group in 9 patients (ALL: 2, NHL: 5 and HL: 2 patients).

Tab. 1. Serum levels of CD44 and osteopontin of the patients and control groups.

	Control (Mean±SD)	Patients (Mean±SD)		p
CD44	1.96±0.62	Total	3.82±2.31	0.010
		NHL	3.49±2.00	0,001
		HL	3.56±1.74	0,001
		ALL	5.15±3.50	0,022
Osteopontin	3.89±1.96	Total	5.42±8.24	0.214
		NHL	5.95±8.52	0.297
		HL	3.63±1.86	0.105
		ALL	7.24±7.13	0.286

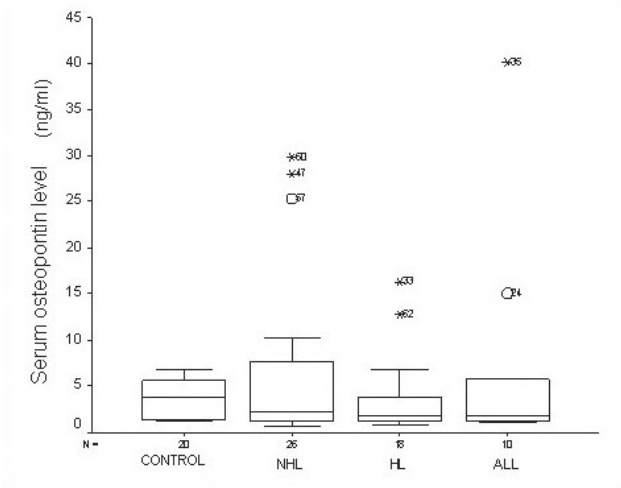

Fig. 3. Comparison of serum CD44 and osteopontin levels. The levels of OPN correlated with CD44 (p=0.003).

One of the ALL patients relapsed in CNS and bone marrow and subsequently died. The other also had CNS disease but achieved a complete remission. Two cases with NHL presented with bone marrow involvement. Furthermore, two other cases of NHL presented with bulky masses: one of them had vena cava superior syndrome and the other one with large abdominal masses had an intestinal perforation (Tab. 2).

Discussion

Osteopontin is an acidic extra cellular matrix cell adhesion protein that is relatively abundant not only in bone matrix, plasma, urine, and milk, but is also found in malignant tissue. OPN is a multifunctional phosphoprotein secreted by many cell types including osteoclasts, epidermal cells, activated immune cells such as T cells, natural killer cells, macrophages, Kupffer cells, and tumor cells. Phosphorylation, glycosylation and calcium modifications allow intact and fragmented OPN to direct a variety of diverse responses including tissue remodeling, inflammation and cell survival (2–4, 10, 11).

Multiple and complex mechanisms are involved in the role of OPN in cancer (12–17). Osteopontin interacts with a variety of cell surface receptors, including several integrins and CD44. Binding of osteopontin to these cell surface receptors stimulates cell adhesion, migration, and specific signaling functions (6, 9). CD44 is a glycoprotein present in a wide variety of non-neoplastic and neoplastic cells (9, 18, 19). CD44 isoforms` major physiological role is to maintain organ and tissue structure via cell-cell and cell-matrix adhesion, but certain variant isoforms can also mediate lymphocyte activation and homing, and the presentation of chemical factors and hormones (29). CD44 may play an important role in tumor growth and metastasis. Many of the primary carcinoma specimens examined expressed high levels of CD44. CD44 is highly expressed in many tumors and is correlated with the tumor biological behavior including tumorigenesis, growth, metastasis and prognosis (20–22). Overexpression of OPN has been found in a variety of cancers, including breast (23), lung (24), colorectal (25), stomach (26), ovarian (27), liver (28) and prostate cancers (29). Moreover, OPN is present in elevated levels in the blood and plasma of some patients with metastatic cancers. Recently, some authors suggested that OPN levels in the blood or tumors of cancer patients might provide useful clinical information for the patient prognosis (27, 30, 31) and stage of disease (32). In our study, the mean levels of OPN were significantly higher in patients than in controls and also higher in advanced stage of the disease than in early stages, but not statistically significant. This result may be due to the small number of patients

Over expression of CD44 is associated with an aggressive behavior, dissemination, advanced stages and poor prognosis of human non-Hodgkin's lymphomas, Hodgkin's and nodal diffuse lymphomas (19, 33–36). Serum CD44 levels were significantly declined in HL and NHL patients, who were in a complete remission. Therefore, it may be useful as a marker of treatment response (19). High serum CD44 levels were also associated with a high tumor tissue expression of CD44 in patients with HD and BL. In

Tab. 2. Characteristics of patients with advanced disease whose CD44 and OPN were detected above 2SD.

Age	Disease	Cytology	Stage	Bone marrow involvement	CNS involvement	WBC	LDH	Current status	Relaps	CD44	OPN
15	ALL	pre-B ALL		+	+	15500	1597	Ex (died of disease)	1	10.618	15.00
3	ALL	pre-B ALL		+	+	13700	377	alive	0	8.170	40.06
15	ALL	pre-B ALL		+	-	39200	4579	alive	0	8.583	5.76
6	NHL	pre-B ALL		+	-	10300	2980	alive	0	7.344	28.05
10	HL		IV					alive	0	6.663	6.10
11	NHL		III					alive	0	4.355	29.79
10	Hodgkin	NS	IV	-	-	3600	181	alive	0	6.306	12.74
7	NHL	Burkitt lymphoma	III	-	-	11500	1324	alive	0	3.451	10.27
3	NHL	T Cell LBL	III	-	-	13100	1700	alive	1	5.517	8.26

addition, patients with higher levels of serum sCD44 had a poorer outcome and survival than those with lower sCD44 levels in HL and NHL groups (19, 36). CD44 overexpression has been showed to be associated with poor prognosis in a number of hematological malignancies, such as acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, lymphoma, and multiple myeloma (37 – 39). Tacyildiz et al (36) detected that serum CD44 levels were significantly higher in pediatric patients with HL, NHL and Burkitt's lymphoma (BL) compared to the control group. In our study, the mean levels of sCD44 levels in patients were significantly higher than in the control group. Also, the serum CD44 levels were significantly higher in advanced stages of the disease than in early stages. The levels of CD44 and OPN correlated with serum LDH levels.

Yang et al (40) found that OPN combined with CD44 might be of value as an unfavorable prognostic factor for patients with hepatocellular carcinoma. It has been reported that OPN expression in human cancer cells increases CD44 surface expression (41–43). Furthermore, Marroquin et al (44) reported that OPN increases plasma membrane CD44 expression and cell adhesion by binding to its $\alpha_v\beta_3$-integrin receptor in RAW 264.7 murine leukemia cells and suggested that OPN may promote tumor metastatic behavior by CD44 expression. We found that OPN and CD44 levels were elevated in childhood lymphoreticular malignancies. Also, both CD44 and OPN levels were detected above 2SD in 9 patients with advanced disease in our study (Tab. 2).

As a result, elevated levels of both CD44 and OPN at the diagnosis may give more information on prognosis in childhood leukemias and lymphomas. Additional studies including larger series of patients and monitoring of these levels in the progress of the disease are needed to confirm whether these markers give more information about unfavorable outcome of these cancers.

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