Level of Vascular Endothelial Growth Factor, Carcinoma Embryonic Antigen, Calcium and Phosphorus in Blood Serum in Different Types of Bone Tumors

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Abstract

Background: The extent of angiogenesis seems to be an essential prognostic factor in many solid tumors of children and adults. Vascular endothelial growth factor (VEGF) is a proangiogenic cytokine that is highly expressed in many solid tumors often correlating with a poor prognosis. **Aim**: The aim of the study was to assess the angiogenesis on the basis of serum VEGF levels and also to compare the concentrations in serum of carcinoma embryonic antigen, oxyproline, calcium and phosphorus in different types of bone tumors. **Patients and Methods**: The research was conducted on 66 patients and also 22 healthy individuals, who were accepted to our clinic. Patients were divided into three groups: primary bone cancer (n = 22), bone metastasis (n = 22), and benign bone tumors (n = 22). **Results**: VEGF, CEA and oxyproline concentrations were increased in both primary bone tumors and bone metastasis in comparison with healthy individuals and patients with benign tumors ($p < 0.05$). However, we found statistically significant higher phosphorus levels in all patient groups ($p < 0.05$), and higher calcium only in the bone metastasis group as compared with controls ($p < 0.05$). A significant positive correlation existed between VEGF and CEA. **Conclusions:** High VEGF levels, as a marker of angiogenesis, were associated with primary bone tumors and bone metastasis. The calcium and phosphorus content, in addition to VEGF, CEA and oxyproline levels, could be useful in prognosis and differentiation of the bone tumors. Future studies in larger series are needed to confirm these data.

Key words: bone tumors, angiogenesis, vascular endothelial growth factor, cancer embryonic antigen, oxyproline, calcium, phosphorus.

Уровень сосудистого эндотелиального фактора роста, ракового эмбрионального антигена, кальция и фосфора в сыворотке крови при различных типах опухолей костей

Степень ангиогенеза представляется важным прогностическим фактором для многих солидных опухолей у детей и взрослых. Сосудистый эндотелиальный фактор роста (VEGF) является проангиогенным цитокином, который выявяается во многих солидных опухолях и часто приводит к отрицательным прогнозам. Целью исследования была оценка ангиогенеза, основанная на определении в сыворотке крови уровня VEGF, а также сывороточных концентраций ракового эмбрионального антигена (РЭА), оксипролина, кальция и фосфата в различных типах костных опухолей. В исследование было включено 66 больных, а также 22 здоровых человека. Пациенты были разделены на три группы: с первичными костными опухолями (n = 22), с костными метастазами (n = 22) и с доброкачественными опухолями костей (n = 22). Концентрация VEGF, РЭА и оксипролина в сыворотке крови пациентов с первичными и метастатическими опухолями костей была больше, в сравнении с показателями у контрольной группы и у больных с доброкачественными опухолями костей ($p < 0,05$). Однако, мы обнаружили статистически значимое увеличение уровня фосфора во всех группах ($p < 0,05$), а кальция только у пациентов с костными метастазами по сравнению с контрольной группой ($p < 0,05$). Существует значимая положительная корреляция между уровнями VEGF и РЭА. В заключение необходимо отметить, что высокий уровень VEGF в качестве маркера ангиогенеза был выявлен в первичных опухолях костей и при костных метастазах. Определение содержания кальция и фосфора, в дополнение к оценке уровней VEGF, РЭА и оксипролина, может оказаться полезным в оценке прогноза и дифференциации опухолей костей.

Ключевые слова: опухоль кости, ангиогенез, сосудистый эндотелиальный фактор роста, раковый эмбриональный антиген, оксипролин, кальций, фосфор.

Introduction

Bone cancer is an uncommon cancer type that begins in bone. Bone cancer can begin in any bone in the body, but most commonly it affects the long bones of the arms and legs [1]. Several types of bone cancer exist and some types of it occur primarily in children, while others affect mostly adults [1, 2]. The most common symptom of bone cancer is pain; other symptoms may vary depending on the location and size of the tumor [1]. The four most common types of primary bone cancer are: Multiple Myeloma, Osteosarcoma, Ewing's sarcoma and Chondrosarcoma. There are also many types of benign bone tumors [1-3]. Primary bone cancer accounts for less than 0.2% of all cancers and approximately 1% of all diagnosed adult solid malignancies, although its incidence approaches 20% in pediatric cancers [2].

Bone metastasis are the tumors that begin elsewhere in the body and spread (metastasize) into the bone [1]. Breast, lung, and prostate cancers account for 80% of all bone metastasis [1, 4]. Intractable pain and pathological fractures are the major complications of bone metastasis and can significantly affect the quality of life of the patients [1, 4].

The growth and dissemination of bone tumors depend on angiogenesis [5]. There are many markers related to the tumor angiogenesis including vascular endothelial growth factor (VEGF). It is a homodimeric heparin-binding protein (34-42 kDa), which induces formation of new blood vessels (angiogenesis) [6, 7]. VEGF-mediated capillary invasion is an essential signal that regulates growth plate morphogenesis and triggers cartilage remodeling [5]. Thus, VEGF is an essential coordinator of chondrocyte death, chondroclast function, extracellular matrix remodeling, angiogenesis and bone formation in the growth plate [6, 8].

Carcinoma embryonic antigen (CEA) is one of the first tumor markers used in the monitoring patients with metastatic disease during active therapy [9]. It is a glycoprotein which traditionally has been used to monitor gastrointestinal and breast malignancies [9]. However, an increasing CEA is a marker suggesting the failure of the treatment [10].

We aimed in this study to assess the angiogenesis by measuring the level of serum VEGF and to compare the concentrations of serum CEA, oxyproline, calcium and phosphate in primary bone tumors, bone metastasis and benign bone tumors.

Material and methods

The subjects of the study were 66 patients admitted to the new oncology hospital of the Azerbaijan Medical University from May 2008 till December 2009. The patients were divided into three experimental groups. In the first group 22 patients with primary bone cancer were included, Osteosarcoma (n = 12), Ewing's sarcoma (n = 8) and Chondrosarcoma (n = 2). Their age ranged from 11 to 46 years (mean, 18.8 years). In the second group 22 patients with bone metastasis were included, aged 42 to 61 years (mean, 51 years). In the third group comprised 22 patients with benign bone tumors, aged 11 to 51 years (mean, 19.2 years). The control group included 22 healthy volunteers. Their age ranged from 18 to 56 years (mean, 22.8 years), see tab. 1.

Table 1

General characteristic of controls and bone cancer patients

Variables	Controls	Primary bone cancer	Bone metastasis	Benign bone tumors
1. Total number (n)	22	22	22	22
2. Age in years (mean)	22.8	18.8	51	19.2
3. Sex:				
Males	14 (64%)	14 (64%)	10 (45%)	15 (68%)
Females	8 (36%)	8 (36%)	12 (55%)	7 (22%)

Sample collection and preparation: Fasting blood samples (12 hours fasting) were collected from the patients and controls into test-tubes without additive and allowed to coagulate for 30 min. Serum was obtained by centrifugation (1500 g) for 15 min at room temperature, and stored at –20° C.

Measurement of VEGF: Quantification of VEGF was performed using commercial quantitative immunoassay kits for human VEGF (The IBL's, Human VEGF ELISA kit; R&D systems, Germany). The amount of VEGF was expressed in pg/mL.

Measurement of CEA: CEA assay was done using commercial quantitative immunoassay kits for human CEA, (The CEA ELISA kit; R&D systems, Germany). Values of CEA were expressed in ng/mL.

Measurement of calcium and phosphorus: The determination of calcium and phosphate was performed using commercial quantitative kits from Human Diagnostic (Germany), on HOSPITEX biochemical analyzer.

Measurement of oxyproline: The determination of oxyproline content was performed using colorimetry with dimethyl-aminobenzaldehyde after oxidation by chloramine B. The absorbance was read at 550 nm.

Statistical Analysis: Statistical analysis was determined using STATGRAPHICS plus 5.1 statistical package software (STATPOINT TECHNOLOGIES, INC., USA) and Microsoft Excel. The experimental data were expressed as mean ± standard deviation (SD). The value of $p < 0.05$ was considered significant.

Results

Sixty-six patients with bone tumors were enrolled into this study. A summary of descriptive statistics for the results of the serum levels of VEGF in the examined blood samples are presented in tab. 2.

Table 2

Levels of serum VEGF in the examined blood samples of the patients with bone tumors and healthy controls[a]

Parameter	Controls (n = 22)	Primary bone cancer (n = 22)	Bone metastasis (n = 22)	Benign bone tumors (n = 22)
VEGF (pg/mL)	214.00 ± 41.7	698.22 ± 72.1*,†	481.53 ± 67.4*,§	230.23 ± 34.6**
Minimum	63.41	307.32	281.19	77.23
Maximum	267.23	1205.21	825.30	294.76

[a]Values are expressed as mean ± SD,
* $p < 0.05$; ** $p > 0.05$; as compared with controls;
† $p < 0.05$; as compared with bone metastasis and/or benign bone tumors;
§ $p < 0.05$; as compared with primary bone cancer and/or benign bone tumors.

The results revealed that the levels of serum VEGF were significantly higher in both primary bone cancer and bone metastasis patient groups as compared with those of the controls ($p < 0.05$). However, the difference between the levels of VEGF in the benign bone tumor patient group is not significant as compared with the control ($p > 0.05$). Furthermore, the differences in VEGF levels between all investigated groups were significant ($p < 0.05$). Thereby, the serum VEGF levels were higher as compared to controls in patients with primary bone cancer by 226.27% or 3.26 fold ($p < 0.05$), in patients with bone metastasis by125.01% or 2.25 fold ($p < 0.05$) and in patients with benign bone tumors by 7.58% or 1.08 fold ($p > 0.05$). The serum VEGF levels in patients with primary bone cancer were higher as compared to the rest of the experimental groups. In this group VEGF levels were higher by 45.0% or 1.45 fold ($p < 0.05$) than in patients with bone metastasis and by 203.27% or 3.03 fold ($p < 0.05$) than in patients with benign bone tumors (fig. 1).

The results of the assay of CEA serum levels in the examined blood samples are summarized in tab. 3.

The levels of serum CEA were found to be significantly higher in both primary bone cancer and bone metastasis patient groups than in those of the controls ($p < 0.05$). However, there is no significant difference between the level of CEA in the patients with benign bone tumors as compared to the controls ($p > 0.05$). Furthermore, there were significant di-

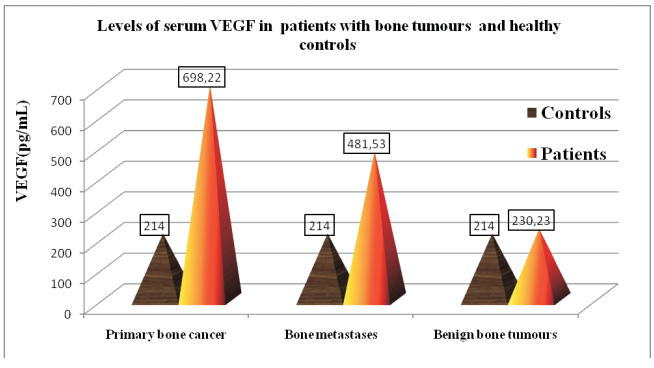

Fig. 1. Levels of serum VEGF in patients with bone tumors and healthy controls.

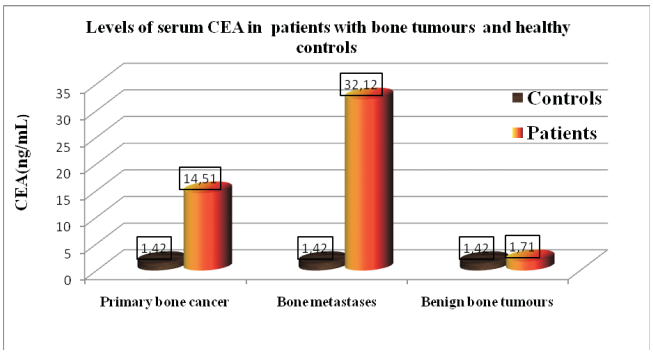

Fig. 2. Levels of serum CEA in patients with bone tumors and healthy controls.

fferences in CEA levels between all experimental groups ($p < 0.05$), see fig. 2.

The serum CEA levels as compared with the control values were higher in the patients with primary bone cancer by 921.83% or 10.22 fold ($p < 0.05$), in patients with bone metastasis by 2161.97% or 22.62 fold ($p < 0.05$), and inpatients with benign bone tumors by 20.42% or 1.2 fold ($p > 0.05$).

The serum calcium, phosphate and oxyproline levels in the examined blood samples are summarized in tab. 4.

The results revealed that the levels of oxyproline were significantly higher in primary bone cancer and bone metastasis patients groups than those of the controls ($p < 0.05$).

Table 3

Levels of serum CEA in the examined blood samples for bone tumors patients and healthy controls[a]

Parameter	Controls (n = 22)	Primary bone cancer (n = 22)	Bone metastasis (n = 22)	Benign bone tumors (n = 22)
CEA (ng/mL)	1.42 ± 0.11	14.51 ± 3.13*	32.12 ± 6.54*,†	1.7 1± 0.09**
Minimum	0.94	2.71	3.81	43.23
Maximum	2.18	29.87	62.77	294.76

[a]Values are expressed as mean ± SD,
* $p < 0.05$; as compared with controls; ** $p > 0.05$; as compared with controls;
† $p < 0.05$; as compared with primary bone cancer and/or benign bone tumors.

However, there was no significant difference between the oxyproline levels in the patients with benign bone tumors as compared to the controls ($p > 0.05$). The levels of oxyproline were significantly lower in benign bone tumor patient group as compared with both primary bone cancer and bone metastasis patient groups ($p < 0.05$). Thus, the levels of serum oxyproline, as compared to the control levels, were higher in primary bone cancer patients by124.47% or 2.24 fold ($p < 0.05$), in patients with bone metastasis by 94.86% or 1.95 fold

Table 4

Levels of oxyproline, calcium and phosphorus in the examined blood samples of the patients with bone tumors and healthy controls[a]

Parameter	Controls (n=22)	Primary bone cancer (n=22)	Bone metastasis (n=22)	Benign bone tumors (n=22)
Oxyproline (ng/mL) Range	122.6 ± 9.88 (112-163)	275.2 ± 12.31* (167-429)	238.9 ± 10.54* (134-340)	136.7 ± 9.23**,† (129-170)
Calcium (mg %) Range	8.13 ± 1.41 (7.8-8.9)	7.89 ± 1.25** (7.2-8.7)	9.53 ± 1.43* (8.2-10.1)	8.01 ± 1.19** (7.4-9.0)
Phosphorus (mmol/L) Range	1.11 ± 0.19 (0.89-1.32)	1.63 ± 0.21* (1.41-1.93)	1.57 ± 0.14* (1.34-1.92)	1.69±0.31* (1.44-1.95)

[a] Values are expressed as mean ± SD;
* $p < 0.05$; as compared with controls; ** $p > 0.05$; as compared with controls;
† $p < 0.05$; as compared with primary bone cancer and/or bone metastasis.

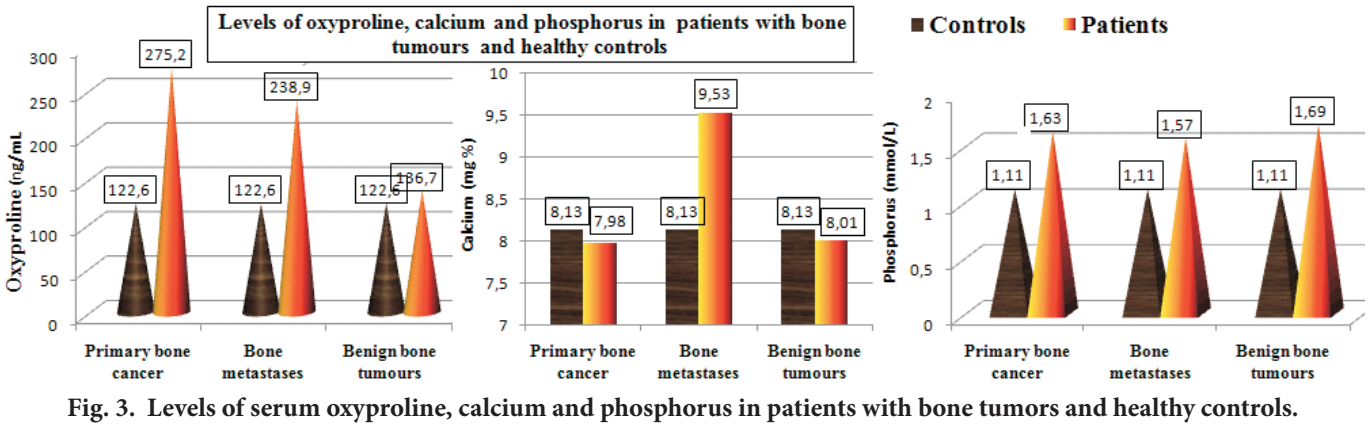

Fig. 3. Levels of serum oxyproline, calcium and phosphorus in patients with bone tumors and healthy controls.

($p < 0.05$) and in patients with benign bone tumors by11.50% or 1.13 fold ($p > 0.05$).

The levels of serum calcium were significantly higher only in bone metastasis patients group as compared to control levels by 17.22% or 1.17 fold ($p < 0.05$). The levels of serum calcium as compared to controls were lower in patients with primary bone cancer 2.95% or 0.03 fold ($p > 0.05$) and with benign bone tumors by 1.48% or 0.01 fold ($p > 0.05$).

The levels of serum phosphates were significantly higher in all investigated groups as compared with the control values ($p < 0.05$). The levels of serum phosphate as compared with the control values were higher in the patients with primary bone cancer by 46.85% or 1.47 fold ($p < 0.05$), in patients with bone metastasis by 41.44% or 1.41 fold ($p < 0.05$) and with benign bone tumors by 52.25% by 1.52 fold ($p < 0.05$), see fig. 3.

The correlation analysis revealed significant positive correlations between VEGF and CEA concentrations ($r = 0.511$, $p = 0.007$) and between VEGF and oxyproline concentrations ($r = 0.315$, $p = 0.014$) in the blood serum of the investigated patients.

Discussion

The bone is an unique and complex microenvironment that serves as a primary site for sarcomas [3] and a preferential secondary site for the metastasis of primary carcinomas such as breast, prostate and lung cancers [1, 4]. Little is known about the role of angiogenesis and proangiogenic factors, such as VEGF, in the development and biologic activity of malignant bone tumors [7]. VEGF stimulates not only angiogenesis, but also osteoclastic bone resorption and osteoblastic bone formation, so it could have multiple effects [11]. The level of circulating VEGF in patients with different tumor types may be a predictive index of tumor status and prognosis [12]. Several clinical studies have found that many osteosarcoma patients with pulmonary metastasis had primary tumors with high levels of VEGF expression [13]. In a study of bone sarcoma however, serum levels of VEGF were elevated in contrast to tissue levels in Ewing sarcoma [8, 14]. In the process of bone metastasis formation, an increase in bone resorption is a crucial step prior to the invasion of the bone. On the other hand a relation between tumor burden and circulating VEGF levels was shown by the rapid decrease of previously elevated concentrations of the compound after surgery [13-15]. Our data also show high levels of circulating VEGF in both primary bone tumors and bone metastasis.

Biomarkers are widely used in oncology for prognostic or predictive purposes. An important area of biomarker use is the surveillance of cancer recurrence after treatment. However, for most solid tumors there are few blood-based biomarkers of the disease relapse, including CEA [9, 16]. CEA is synthesized during the development of the fetal gut, and is turned on in adults in case of intestinal carcinomas and other cancers [10]. For monitoring patients with metastatic disease during active therapy, CEA can be used in combination with diagnostic imaging [17]. The high levels of the CEA are predictive markers of metastasis into the axial skeleton, of multiple skeletal metastases and visceral metastasis [18]. Tumor markers such as CEA are useful as a screening test to distinguish skeletal metastasis of carcinoma from primary bone tumors or hematological malignancy from primary bone tumor [18]. CEA appears currently as the most useful biochemical marker for the prediction of the risk of recurrence of the disease and the assessment of the response to the treatment or it failure [19].

Bone is a living tissue and consists of appatite and various proteins that make it strong. Secondary bone cancer can alter the bone structure so that calcium is released from the appatite into the bloodstream [20]. However, the release of calcium from living bone by tumor cells is mediated partially through stimulation of the endogenous bone-resorbing systems [21]. The development of the bone tumors lead to abnormalities of the regulation of the bone calcium and phosphate content by PTH that will induce disturbances in the bone and mineral metabolism [20]. Alterations in calcium and phosphate balance should be restored with bisphosphonates before starting the therapy of the bone tumors [7]. Physicians could monitor a patient's calcium level over his or her lifetime and detect metastatic cancer in the early stages, when treatment would be more effective [22].

Oxyproline is produced by oxidation of proline [23]. It is one of the major collagen's amino acids, which enables it as a marker that reflects the catabolism of this protein [24]. Oxyproline content which correlates with the rise in serum antioxidant activity is observed in some malignant tumors [25].

Our data show high levels in serum calcium, phosphates and oxyproline in bone metastasis. In addition, high levels of both serum phosphorus and oxyproline were observed in primary bone cancer. However, in benign bone tumor only high levels of serum phosphorus were observed.

Conclusions

Our results suggest that angiogenesis indices (VEGF) may be potential prognostic markers of bone tumors and may be a potential target for their therapy. In addition, we can conclude that the calcium and phosphorus contents, in addition to VEGF, CEA and oxyproline levels, could be useful in prognosis and differentiation of the bone tumors. Future studies in larger series are needed to confirm these data.

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Lipid Peroxidation and Enzymatic Antioxidants in Ulcerative Colitis

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Abstract

Increased oxidative stress has been previously demonstrated in patients with inflammatory bowel disease. But this phenomenon has not been analyzed in the course of ulcerative colitis (UC). In this study we evaluated levels of malondialdehyde (the main product of lipid peroxidation), superoxide dismutase and catalase erythrocyte activities in 62 patients with active UC, in 22 patients after achievement of complete, endoscopic remission and in 52 control subjects. Significant increase of malondialdehyde in patients with active disease in comparison with control subjects, demonstrated in this study, suggests the presence of enhanced oxidative stress in active UC. Activation of enzymatic antioxidant system is characteristic of active UC, which is confirmed by an increase in superoxide dismutase and catalase erythrocyte activities in patients with active disease in comparison with control group. There is no significant difference in malondialdehyde and catalase erythrocyte activity between patients in remission of UC and the control subjects. The increase of conditional adaptive index in patients with active UC confirms large adaptive possibilities of enzymatic antioxidant system. The normal levels of malondialdehyde and catalase can be proposed as markers of complete disease remission in UC.

Key words: ulcerative colitis, oxidative stress, lipid peroxidation, malondialdehyde, superoxide dismutase, catalase, adaptive index.