Correlation of Serum Alkaline Phosphatase, Lactate Dehydrogenase, C-Reactive Protein, Blood Deposition Rate, B-Hcg Expression and Tumor Volume to Lung Metastasis Risk in Osteosarcoma Patients

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Abstract

Osteosarcoma is the most common type of sarcoma that found in bone. The survival rate in osteosarcoma patients was less than 20.00% at the end of 1980 but is currently increasing to over 70.00% for nonmetastatic patients. The objective **t**o analyze the correlation between Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP), C-Reactive Protein (CRP), Blood Deposition Rate (LED) , hCG expression and tumor volume with pulmonary metastases in osteosarcoma patients. The subjects were osteosarcoma patients that the data taken from January 2015 to December 2016. The subjects will be clinical, radiological (plain, CT scan thorax, MRI) examinations, serum marker tests consisting of ALP, LDH, CRP and LED and histopathologic staining IHC β-hCG. The study design was cross-sectional, while the statistical analysis used was normality test, Spearman correlation, chi-square test and logistic regression. The results majority of subjects were male (52.00%) and aged 11-20 years (85.00%). The tumor volume (p = 0.07), LDH levels (p = 0.07), and ALP levels (p = 0.016) were statistically significant for pulmonary metastases with moderate correlation (r = 0.587; 0.587; 0.53). The tumor volume was the most sensitive biomarker to diagnose lung metastasis in osteosarcoma patients (p = 0.171). Conclusion among other protein biomarkers, tumor volume was most sensitive in diagnosing pulmonary metastases. The volume of the tumor will increase the risk of pulmonary metastases in osteosarcoma patients.

Keywords: *Serum Alkaline Phosphatase, Lactate Dehydrogenase, C-Reactive Protein, Blood Deposition Rate, B-Hcg Expression And, Tumor Volume To Lung Metastasis.*

Introduction

Osteosarcoma is a malignant neoplasm and is the most common type of sarcoma found in bone [1]. Before the discovery of chemotherapy regimens in the late 1980s, survival rates in patients were less than 20.00%, and up to now have increased to over 70.00% for nonmetastatic patients. Currently, osteosarcoma diagnostics have been developed using biomarkers such as Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR), Beta human chorionic gonadotropin (β-hCG), and tumor volume [2].

ALP is a member of zinc metalloproteinase that is produced and located in the membrane cell of osteoblasts. Osteoblast cell transformation in osteosarcoma impairs strict control of proliferation and progressively causes constant increases in ALP levels [3]. The association between total ALP activity and the clinical condition of osteosarcoma patients has been known for more than 50 years. However, studies related to the role of ALP levels against prognostic osteosarcoma are still inconsistent [4].

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LDH is an enzyme involved in anaerobic metabolism of neoplasms. Many retrospective studies of serum LDH as prognostic factor survival rates in patients with osteosarcoma. However, the results are not convincing. Some studies show poorer results in patients with high LDH levels, while others are not [4].

CRP is a systemic biomarker widely used to diagnose acute and chronic inflammation. The measurement of serum CRP is a simple, inexpensive, and available method in day-to-day practice. It can serve as an additional prognostic predictor for survival and post-care monitoring in cancer patients [5].

Erytrocyte sedimentation rate (ESR) is one of the most common laboratory tests used in determining a progression in malignancy. Earlier research mentioned that in the case of patients with ESR increases by 25.00% suffered malignancy, but did not have significant statistics with the incidence of malignancy [6].

Human chorionic gonadotropin (hCG), a heterodimer protein comprising non-covalent bonds between the α and β subunits, usually secreted by the placenta placenta placenta. Several reports indicate that serum β-hCG and tissue levels are independent prognostic factors for adverse outcomes in some types of tumors [7]. However, negative correlations were also found, with no association between β-hCG levels and survival of patients (Connelly, Johnston et al. 1993). Therefore researchers were interested in analyzing serum levels of LDH, ALP, CRP, LED and β-hCG as biomarkers to pulmonary metastases in osteosarcoma patients.

Method

The subjects were osteosarcoma patients that data was taken from January 2015 to December 2016. The inclusion criteria were patients with a clinical and radiological diagnosis of an osteosarcoma and an age less than 25 years. Exclusion criteria were patients who refuse care and research.

The study design was cross-sectional. Subjects will be taken history data (age and sex) and physical examination. Subjects also performed laboratory tests in the form of LED, CRP, ALP, LDH. The LED was measured using the Westergren method with a normal value of 15-20 mm. Normal ALP values for males were 45-115 U/l and females 37-98 U/l. The normal standard CRP was ≤10 mg/l with the Immunoturbidimetric Assay method. The normal value of LDH was 100-190 units/l using the Beckman Unicel® DxC 800 Synchron method. The subjects also performed the radiological examination with MSCT thorax (metastatic thorax) and MRI (Volumetric tumor) [8].

The subjects will also be examined histopathologically starting with immuno histochemical imaging on the biopsy specimen. The tissue block was cut with a thickness of 6 micrometers and placed on glass slides, followed by depolarization with xylene and rehydration gradually using an alcohol solution and washed in Tris-buffered saline solution with Tween 20. The specimen block was then immersed in the target retrieve solution, and placed in the hot water bath for 20 minutes and cooled for 20 minutes. Then incubated with β-hCG antibody for 30 min at room temperature on moisture chamber.

Data analysis using test of normality test then continued with correlation test with spearman. Statistical analysis of β-hCG using chi-square test. The quantitative data groups include tumor volume, ALP, LDH, CRP and LED correlated with data groups with nominal data scale, i.e., pulmonary metastases using logistic regression and followed by multivariate analysis to determine which variables are most sensitive as lung metastasis biomarkers. Statistical analysis program using SPSS (SPSS, Inc., Chicago, IL).

Results

Characteristics of subjects

The majority of subjects were male (52.00%) and aged over 11-20 years (85.00%). In the histopathology sample, 17 patients were obtained from open biopsy preparation and post-amputation tissue. 1 patient died before the procedure was performed and 2 patients lost to follow-up and decided not to continue treatment. Out of the 17 specimens obtained, 2 preparations were excluded because the lysis cells were painted (Table 1).

Additionally, 73.00% subjects showed positive results of expression of β-hCG with the majority of subjects of 64.00% with osteosarcoma chondroblasts type. The majority of subjects who expressed negative β-hCG of 50.00% with chondroblastist osteosarcoma type. Out of the 11 positive subjects expressing β-hCG, there were 64.00% of subjects who did not have metastasis to the lungs otherwise negative subjects β-hCG, 50.00% of subjects each metastasized to the

lungs and did not have metastases to the lungs (Table 1).

Bivariate Research Analysis

The tumor volume (p = 0.07), LDH level (p = 0.07), ALP level (p = 0.016) was statistically significant for pulmonary metastases with moderate correlation (r = 0.587; 0.587; 0.53). While CRP levels, plasma levels, and β-hCG expression did not have significant statistics with pulmonary metastases (p = 0.471; 0.447; 1.000) (Table 2).

Independent Logistic Regression Analysis of Independent Variables Research

The result of the analysis showed that p-value sub-variable of tumor volume (0.055), LDH (0.17) and ALP (0.136) had the p-value <0.25, so it entered the multivariate test. The smallest value of significance was obtained in tumor volume (p = 0.171). So the tumor volume was a predictor of the sensitivity factor to determine the presence of pulmonary metastasis (Table 2).

Table 1. Characteristics of Research Subject

Variables	Amount (N=20)	Percentage (%)
Sex		
Female	7	35.00
Male	13	65.00
Age		
0-10 and/or	1	5.00
11-20 and/or	17	85.00
21-30 and/or	2	10.00
β-hCG Expression		
Yes	11	55.00
No	4	20.00
Unevaluated	5	25.00
Lung Metastases		
Yes	7	35.00
No	13	65.00
Location of Tumor		
Distal Femur	13	65.00
Proximal Tibia	4	20.00
Distal Tibia	1	5.00
Proximal Fibula	1	5.00
Proximal Humerus	1	5.00
Type Osteosarcoma		
Chondroblastic	11	53.00
Fibroblastic	2	10.00
Osteoblastic	1	5.00
Fibroblactic dan Osteoblastic	2	10.00
Chondroblastic dan Osteoblastic	1	5.00
Drop out/Die	3	15.00

β -hCG=*Beta-Human chorionic gonadotropin*

Table 2. Correlation between Independent Variable and Lung Metastasis(N=20)

Variables	Spearman test (N=20)		Chi-Square Test	Logistic Regression			
	p-value	r	p-value	B	p-value	B	p-value
Volume Tumor	0.07	0.587	-	0.006	0.055*	0.003	0.171
LDH level	0.07	0.587	-	0.004	0.170*	-0.004	0.185
ALP level	0.016	0.53	-	0.003	0.136*	0.000	0.832
CRP level	0.471	-	-	0.366	0.340		
LED level	0.447	-0.180	-	-0.010	0.645		
Expression β- Hcg	-	-	1000	-0.560	0.635		

LDH= *Lactat dehydrogenase,* ALP= *Alkaline phosphatase*, CRP=*C-reactive protein,*

LED = Blood Endap rate, β -HCG =*Beta-Human chorionic gonadotropin*

**p-value*<0.25

Discussion

Assessment of tumor malignancy degree based on ALP gives a moderate correlation. The meta-analysis study showed that patients with high ALP values were significantly correlated with a high risk of having osteosarcoma metastasis at the time of diagnosis(Ren, Sun et al. 2015). Serum ALP in patients showed a higher value of metastatic events in patients with higher ALP levels in diagnosis initials than patients with low ALP levels.

Assessment of malignancy degree based on LDH examination in this study showed statistically significant with moderate correlation. This was consistent with a study showing that the percentage of patients with metastases, having twice the LDH (36.60%) higher than non-metastable (18.80%) patients [9].

Assessment of tumor malignancy degree based on LED rate gives negative correlation value. In this study of 20 patients only obtained a significant increase in LEDs in 1 patient (with> 4x normal values). In a previous study, the value of an increase in LEDs was only 25.00% of all patients with malignancy [6]. In general, the 2 main factors that play a role in increasing the LED was the aggregation of erythrocytes and hematocrit. Aggregation of erythrocytes was influenced by plasma protein levels resulting in decreased negative electrostatic pressure among red blood cells that causing aggregation and increased sedimentation. If variability was found in the composition and interaction of plasma proteins then different values will be obtained.

Assessment of malignancy degree based on CRP examination gives negative correlation. Inflammation was the first sign of initiation and progression of cancer, where there was a linkage between intrinsic factors (oncogenes, genome instability) and extrinsic factors (immune factors and tissue around the tumor) [10]. CRP was one single molecule to monitor acute phase reactions. CRP was a sensitive marker but not specific to inflammation. CRP was an independent prognostic factor for survival in high-grade osteosarcoma. The role of CRP as an acute phase protein and the prolonged time span between symptom emergence and diagnosis can be a factor in the absence of a positive correlation between CRP levels and the incidence of pulmonary metastases.

The significance of β-hCG IHC staining in tumor cells was unclear. In this study obtained a positive result 55.00% of the total samples performed β-hCG painting. A recent study showing 57.00% of a total of 49 patients had positive expression and no correlation between β-hCG expression and clinical outcome in patients [11]. . The results obtained can also be attributed to the limited

time span of the study, and the samples obtained within the timeframe are few. Further research was needed to find out the correlation of β-hCG expression with the outcome and pulmonary metastases with longer periods of time and a large number of samples.

The tumor volume was the best predictor of pulmonary metastases [12]. If after being given tumor chemotherapy increased or fixed, it can be said to have a therapeutic response is still bad. The tumor volume had a sensitivity value of 68.6% and an 80.2% specificity for detecting pulmonary metastases. Patients with a >15cm diameter tumor had a 3.4 times higher risk of death, whereas in patients with tumor diameter <15 cm the survival rate was better. In patients with small tumor, volumes showed a better response to therapy, with a positive predictive value of 88.00% [13].

Conclusion

However, serum CRP, LED, β-hCG expression did not have a statistically significant relationship with pulmonary metastases. What affects these three biomarkers did not correlate that can limited time span of research, samples, and plasma protein levels. The tumor volume was the best predictor factor against the risk of pulmonary metastasis in osteosarcoma patients. Further studies include larger and longer-term samples so that β-hCG expression may affect pulmonary metastases in osteosarcoma patients.

Conflict of Interest: There is no conflict of interest

Source of Funding: This study is self-funded

Ethical Clearance: This study was approved by Ethical Comission of Health Research Faculty of Medicine University of Airlangga

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