International Journal of Research in Medical Sciences
Kawiyana KS et al. Int J Res Med Sci. 2016 Jun;4(6):2006-2010
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20161750

Research Article

High expression levels of bone morphogenetic protein-2 and p-glycoprotein related with progressivity in patient with osteosarcoma

Ketut Siki Kawiyana, Henry Ricardo Handoyo*, I Gede Eka Wiratnaya, I Ketut Suyasa, K. G. Mulyadi Ridia

Department of Orthopaedi and Traumatology, Faculty of Medicine Udayana University/Sanglah General Hospital, Bali, Indonesia

Received: 18 March 2016
Accepted: 22 April 2016

***Correspondence:**
Dr. Henry Ricardo Handoyo,
E-mail: henryricardohandoyo@gmail.com

ABSTRACT

Background: Osteosarcoma progression characterized by the presence of metastasis dan local recurrence. Several studies focused on bone morphogenetic protein-2 (BMP-2) and P-glycoprotein as protein that influence the progression of osteosarcoma, which are characterized by metastasis and local recurrence.
Methods: This study was a cross sectional analytic study. This study looks at the relationship level of BMP-2 and P-glycoprotein with metastasis and local recurrence in osteosarcoma. Twenty three research subjects are paraffin blocks of osteosarcoma patients and secondary data was conducted through medical records to determine metastasis and local recurrence that occurs in these patients.
Results: The result showed significant correlation between BMP-2 and P-glycoprotein with metastasis and local reccurence.
Conclusions: BMP-2 and P-glycoprotein positivity as determined by immunohistochemistry, was a strong correlate of more rapid disease progression. It should be taken into consideration to identify a subgroup of osteosarcoma patients with poor outcome at the time of diagnosis. So it might be an important marker in planning innovative chemotherapeutic regimens.

Keywords: BMP-2, Metastasis, Local Recurrence, Osteosarcoma, P-glycoprotein

INTRODUCTION

Osteosarcoma is the most common primary malignant bone tumor in children.[1] It is characterized with production of osteoid matrix and osteocyte with malignant spindle cell. The peak incidence is at 2nd decade of life. Osteosarcomas arise from epiphyseal growth plate of the long bone.[2]

Majority of osteosarcoma is high grade and had tendency of lung metastasis. Osteogenicity level of osteosarcoma depends on metastasis and local reccurence. Metastatic process can be evaluated from clinical and radiographic evaluation.[3] Survival rate for non-metastatic osteosarcoma is 65%, and 10-20% for metastatic osteosarcoma.[4-6] To date, the most powerful predictors of outcome have remained the ability to detect metastatic disease at diagnosis and the histopathologic response of the tumor to preoperative chemotherapy.

Presently, 80% of patients who do not have distant metastases at initial diagnosis will become long-term survivors. Unfortunately, this means that approximately 20% of patients who do not present with metastases at diagnosis will not survive. This group of patients appears to be resistant to current treatment as attempts to intensify therapy after surgery for patients with a poor

histopathologic response has not significantly improved survival rates. It is these patients that are in the greatest need of additional clinically relevant markers for prognosis and who can be most helped by molecular analysis.[6]

BMP-2 (Bone Morphogenetic Protein-2) is a multifunction cytokine.[7] Its examination in osteosarcoma is still in controversy. Bone Morphogenetic Protein (BMPs) belong to transforming growth factor β superfamily.[8] Increase of BMP level can be seen in normal bone healing process, and malignancy, such as osteosarcoma. Study by Raida, BMP-2 induce tumor angiogenesis.[9,10] Study by Wang also show that BMP-2 can slow down cell tumorigenesis of osteosarcoma by suppression effect or eliminated bone tumor cell.[11]

P-glycoprotein is a member of the ATP binding cassette (ABC) superfamily of transporters, a large group of proteins that mediate the selective movement of solutes across biological membranes.[12] The relationship between P glycoprotein expression and malignancy is controversial. In particular, among solid tumors, detectable levels of expression of P-glycoprotein have been associated with a significantly poor prognosis in osteosarcoma.[13,14] However, based on several experimental observations, since the mid 1970's it has been postulated that cells overexpressing P-glycoprotein are less aggressive than their non P-glycoprotein-expressing counterparts.[15]

Because of the controversy of BMP-2 and P-glycoprotein role in osteosarcoma, this study looks at the relationship between level of BMP-2 and P-glycoprotein with progressivity in osteosarcoma which is evaluated from its metastatic and local reccurence.

METHODS

This was a cross sectional analytic study which was done from December, 2014 until March 2015 and the data was analysed at April 2015.

The samples were paraffin blocks from osteosarcoma patients who have been diagnosed by clinical, radiological and histopathological examination. The number of samples with cross sectional analytic study design is 23 samples.[16] Sample distributions according to tumor location as seen in table 1.

Immunohistochemical examination was performed using reagents BMP-2 (P275) pAb to detect the expression of BMP-2 and Anti P-glycoprotein Antibody [4E3](ab10333). The preparation was viewed under a microscope with 1000x magnification, 20 fields of view. The number of cells expressing BMP-2 was calculated and quantitatively assessed using IRS formula (immunoreactive score).[17]

RESULTS

This study had 23 patients as sample with the lowest age distribution was 8 years old and the highest was 38 years old with an average age of 20.35 years. While based on sex, total of 14 samples were male (60.9%) and 9 samples were female (39.1%). In this study there were 8 samples (34.8%) that experienced a local recurrence and 16 samples (65.2%) without local recurrence.

Table 1: Sample distribution according to tumor location.

Location	Amount	Percentage (%)
Scapula	1	4.3
Proximal humerus	1	4.3
Ilium	1	4.3
Distal femur	11	47.8
Proximal tibia	7	30.4
Proximal fibula	1	4.3
Distal tibia	1	4.3
Total	23	100.0

Table 2: T paired test between metastasis and local recurrence.

	SD	Pearson's Correlation	95% CI		P value
			Lower	Upper	
Metastasis and Local recurrence	1.894	0.671	0.224	1.86	0.015

Table 3: Relationship between BMP-2 levels with metastasis and local recurrence of osteosarcoma.

BMP-2 Expression	Metastasis (+) Local recurrence (+)	Metastasis (+) Local recurrence (-)	Metastasis (-) Local recurrence (+)	Metastasis (-) Local recurrence (-)	
Low	0 (0.00%)	1 (20.0%)	1 (20.0%)	3 (60.0%)	Pearson's Correlation (r=0.680)
Moderate	7 (38.9%)	6 (33.3%)	0 (0.00%)	5 (27.8%)	
Total	7 (30.4%)	7 (30.4%)	1 (4.3%)	8 (34.8%)	p=0.000

The results of immunohistochemical examination of BMP-2 of 23 samples were based on the calculation formula immunoreactive score (IRS) that showed as much as 5 samples (21.7%) with mild expression and 18 samples (78.3%) with moderate expression. The results of immunohistochemical examination of P-glycoprotein showed as much as 4 samples (17.4%) with mild expression, 11 samples (47.8%) with moderate expression and 8 samples (34.8%) with strong expression. The presence of metastasis and local recurrence were 7 samples (30.4%), metastasis but no local recurrence 7 samples (30.4%), no metastasis but local recurrence 1 samples (4.3%), no metastasis and no local recurrence 8 samples (34.8%).

Table 4: Relationship between P-glycoprotein levels with metastasis and local recurrence of osteosarcoma.

P-Glycoprotein	Metastasis (+) Local recurrence (+)	Metastasis (+) Local recurrence (-)	Metastasis (-) Local recurrence (+)	Metastasis (-) Local recurrence (-)	
Low	0 (0.00%)	3 (37.50%)	1 (12.50%)	4 (50.0%)	Pearson's Correlation (r=0.457)
Moderate	3 (30.0%)	4 (40.0%)	0 (0.00%)	3 (30.0%)	
Strong	4 (80.0%)	0 (0.00%)	0 (0.00%)	1 (20.0%)	
Total	7 (30.4%)	7 (30.4%)	1 (4.3%)	8 (34.8%)	p=0.028

Based on the results of parametric analysis with t-paired test, there was a significant relationship between metastasis variable and local recurrence with p=0.015 (p<0.05) and Pearson's Correlation r =0.671 (Table 2). There is significant correlation between BMP-2 level with metastasis and local recurrence with p=0.000 (p<0.05) and *Pearson's Correlation* r =0.680 (Table 3). For evaluation of P-glycoprotein level, there is significant correlation between P-glycoprotein level with metastasis and local recurrence with p=0.028 (p<0.05) and Pearson's Correlation r=0.457 (Table 4).

These results indicate that there was statistically significant relationship between BMP-2 and P-glycoprotein with metastasis and local recurrence in osteosarcoma.

DISCUSSION

The relationship between the levels of BMP-2 and P-glycoprotein with metastasis and local recurrence in osteosarcoma was done by analyzing the levels of BMP-2 and P-glycoprotein on immunohistochemical examination and associate it with the presence or absence of metastasis and local recurrence in patients with osteosarcoma.

The results of this study were consistent with the study conducted by Raida M, et al, it was noted that BMP-2 can induce tumor angiogenesis.[9] It was also similar with the research of Luo X et al which stated that the BMP can induce tumor growth in osteosarcoma.[18] But the result of this study was different from the study conducted by Wang L et al that noted the use of BMP-2 may inhibit tumor cell osteosarcoma tumorigenesis by providing suppressive effect or even eliminate the tumor cells of the bone.[9] Another study conducted by Rici REG et al gives similar results to the study conducted by Wang, which is Rici REG et al found that modulating angiogenesis of osteosarcoma in dogs can be done with the administration of BMP-2 and mesenchymal stem cells.[19]

In particular, among solid tumors, detectable levels of expression of P-glycoprotein have been associated with a significantly poor prognosis in osteosarcoma which are similar with the results of this study.[13,14] Study conducted by Pakos EE et al stated that P-glycoprotein was not associated with the histologic response of patients with osteosarcoma to combination chemotherapy regimens. Conversely, P-glycoprotein positivity, as determined by immunohistochemistry, was a strong correlate of more rapid disease progression.[20] Italian/Scandinavian Sarcoma Study Group stated that in osteosarcoma patients treated with doxorubicin-based chemotherapy protocols, P-glycoprotein overexpression at diagnosis is an important adverse prognostic factor for outcome. P glycoprotein evaluation can therefore constitute the basis for stratifying, at diagnosis, osteosarcoma patients for whom alternative treatments may be considered.[21] Study by Pakbaz S et al showed that P-glycoprotein positivity was significantly associated with a higher incidence of adverse events and relapse rate.[22] Based on several experimental observations, since the mid 1970's it has been postulated that cells overexpressing P-glycoprotein are less aggressive than their non P-glycoprotein-expressing counterparts.[15]

However, there is still a lack of general consensus about the relative prognostic value of P-glycoprotein expression, since uncertain and sometimes conflicting data have been reported. Several factors may be responsible for these discrepancies but, in general, it has to be taken into account that the predictive value of this prognostic factor may be significantly influenced not only by the inherent biology of the tumour but also by the

treatment modalities. Therefore, the analysis of the predictive value of P-glycoprotein expression has to be considered in relation to both the clinicopathologic features and characteristics of each treatment protocol.[22]

At the time of diagnosis, approximately 80% of patients with osteosarcoma had undergone metastasis. However, the process of metastasis was clinically apparent only in 20% of patients with ostesarcoma. Metastasis occurs most often in the lung. Patients diagnosed with metastatic osteosarcoma had a poor prognosis. Survival of patients with osteosarcoma that had undergone metastasis was around 10-50%. Around 50% incidences of local recurrences occur within 12 months after termination of therapy, and only 5% of this incident comes after 5 years. In research conducted by Gelderbrom H et al, 564 patients with osteosarcoma, patients who experience a recurrence within two years after diagnosis had a worse prognosis compared with patients experiencing a recurrence after two years.[3] Patients with good histologic response to neoadjuvant chemotherapy had a better overall survival rate.

It is widely accepted that limb-sparing surgery generally results in a slightly higher local recurrence rate but improved overall survival with the use of adjuvant therapies. However, studies such as the one by Bacci G et al showed that all patients with local recurrence after limb salvage developed lung metastases at some stage in the course of disease. Regardless of treatment there was a 96% mortality in the local recurrence group, compared with 72% in those with metastases but no local recurrence.[23] Similarly, Briccoli A et al showed that the 5year survival in patients with local recurrence and lung metastases was 6% compared with 37% in patients with only metastases.[24] These results suggest that the combination of local recurrence and metastasis is worse than metastasis alone.

CONCLUSION

Osteosarcoma is a fascinating and complex disease, encompassing many different biological and clinical distinctions that have complicated the treatment and outcome of this disease. Despite its rarity, it has been important in the understanding of cancer and has played a role in the discovery of many of the important genes in cancer. The need for improvement in treatment is a clear call for new therapeutic targets and more clinical trials. The combination of new trials and new methods of analysis promises to open this difficult disease to new avenues of care.

From the data analysis that had been done in this study, it can be concluded as follows: expression level of BMP-2 and P-Glycoprotein associated with progressivity in patients with osteosarcoma, which are characterized by increasing metastasis and local recurrence rate.

From the conclusion of this study, we can use BMP-2 and P-Glycoprotein as an option indicator to see the probability of the appearance of metastasis and local recurrence in patients with osteosarcoma. If the expression level of BMP-2 and P-Glycoprotein higher, we can treat the patient more aggressive.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Kawiyana KS, Handoyo HR, Wiratnaya IGE, Suyasa IK, Ridia KGM. High expression levels of bone morphogenetic protein-2 and p-glycoprotein related with progressivity in patient with osteosarcoma. Int J Res Med Sci 2016;4: 2006-10.