The Correlation of Tc-99m MIBI Scintigraphy and Histological Response in Determining the Percentage of Tumor Necrosis in Osteosarcoma after Preoperative Chemotherapy

Thipachart Punyaratabandhu MD*, Surang Wittayawongsaruji MD*,
Samart Rajchadara MD**, Voranuch Thanakit MD***,
Supichai Charoenvareekul MD*, Narin Khunkitti MD*,
Thawee Songpatanasilp MD*

** Musculoskeletal Oncology Unit, Department of Orthopaedics*
*** Nuclear-medicine Unit, Department of Radiology, Phramongkutklao Hospital*
**** Department of Pathology, Faculty of Medicine, Chulalongkorn University*

Purpose: *The most important prognostic factor of osteosarcoma is the percentage of tumor necrosis. Only well trained and experienced musculoskeletal pathologists can count and assess the percentage of tumor necrosis from the resected specimens. The purpose of the present study was to characterize the correlation of Tc-99m MIBI scintigraphy for assessing the percentage of tumor necrosis using postoperative histology as a gold standard.*

Material and Method: *During September 2002 to August 2004, nine consecutive patients with the diagnosis of conventional osteosarcoma were included in the present study. The osteosarcoma protocol comprises 3 courses of doxorubicin and cisplatin preoperatively followed by wide resection of tumors and another 3 courses of postoperative chemotherapy. The Tc-99m MIBI scintigraphy examination was carried out before commencing chemotherapy and after completing preoperative chemotherapy. The tumor uptake was measured using the tumor to the background ratio (TBR). Comparison of TBR before and after preoperative chemotherapy was used to calculate the alteration ratio which was reported in terms of percentage. All of the resected specimens were sent to the pathological department. The histological assessment of the response to chemotherapy was performed using the standard technique according to the current practice of osteosarcoma from the Mayo Clinic. The percentages of tumor necrosis from histology were also reported.*

Results: *The analysis of the correlation between the reduction of tracer uptake and the postoperative histological response was performed. The correlation and linear regression analysis showed that the two methods had a significant correlation ($R = 0.75$) regression coefficient (1.172) with $p = 0.020$ and 0.043, respectively.*

Conclusion: *The present study showed that Tc-99m MIBI scintigraphy had a significant correlation with percentage of tumor necrosis from histology. This technique can be used to predict the response of osteosarcoma after preoperative chemotherapy.*

Keywords: *Osteosarcoma, Tumor necrosis, Tc-99m MIBI scintigraphy*

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Correspondence to: Punyaratabandhu T, Musculoskeletal Oncology Unit, Department of Orthopaedics, Phramongkutklao Hospital, Bangkok 10400, Phone: 0-2644-4940.

Osteosarcoma is the most common primary malignant primary bone tumor excluding multiple myeloma. Before 1970, the treatment was performed through amputation alone. A treatment consequence revealed that over 80 % of the patients died within 5 years. Nowadays, the improvment of chemotherapy and imaging studies as well as advanced surgical techniques have significantly increased the five-year survival rate. The standard treatment protocol consists of preoperative chemotherapy 3 courses (cisplatin and doxorubicin) followed by a limb salvage surgery, as well as another 3 courses of postoperative chemotherapy. The study revealed an increase of 70 % of five-year survival among patients without metastasis tumor[1].

A response to the treatment of osteosarcoma through chemotherapy is one of the significant prognostic factors influencing the outcome of treatment. However, the most important factor for non-metastasis tumor is the percentage of tumor necrosis after preoperative chemotherapy[2] which was calculated by well - trained and experienced pathologists that are also specialists in their particular fields .Thailand like other countries is still short of these groups of pathologists.

Due to a shortage of musculoskeletal pathologists, there are many studies which have attempted to calculate the percentage of tumor necrosis through other methods as an alternative, such as Thallium scan and Gallium scan. Thallium scan is found to be a good alternative method that can access the percentage of tumor necrosis[3]. However, it is still very expensive whereas the radioactive, substances have to be imported.

A subsequent study revealed that Tc-99m MIBI scintigraphy, which had a similar property to Thallium - 201, could be used to assess tumor viability as well as tumor necrosis for the treatment of bone and cartilage tumor[4] including viability and metabolic activity of tumor cell. Thus, it could be used to assess the recurrence of bone tumor and investigate the percentage of tumor necrosis[5].

The research team aimed to study the correlation of Tc-99m MIBI scintigraphy and histological response in determining the percentage of tumor necrosis in osteosarcoma after preoperative chemotherapy so that the consequence would be used as preliminary data to further develop a practical substitutive examination for a pathological examination.

Material and Method

During September 2002 to August 2004, a prospective study was done. There were 9 patients (7 males and 2 females) with an average age of 19.7 years range from 12 - 50 years. All the patients were diagnosed to have conventional osteosarcoma.

Sample Size

Even though osteosarcoma is the most frequently found, its incidence is very rare among the population. Statistically, the proportion is 2 cases per 100,000 population.

A calculation of sample sizes uses the following formula[6].

$$N = \frac{\{Z_{\alpha} + Z_{\beta}\sqrt{1 - r^2}\}^2}{r} + 2$$

Where r = correlation coefficient = 0.998 (the parameter "r" is derived from the reference 7)

$$N = \frac{\{1.96 + 1.28\sqrt{1 - (0.998)^2}\}^{(2)}}{0.998} + 2$$

$$= 6.128 \text{ rounded off to } 7 \text{ cases}$$

Thus, based on the calculation of sample size, however, in the present study, a sample size consisting of 9 patients was applied.

Inclusion Criteria

1. A patient who is diagnosed to have conventional osteosarcoma without metastasis.

2. Has never been treated with chemotherapy or radiation.

3. Must receive 3 courses of preoperative chemotherapy and within an identified time.

4. Written consent to participate in the study in written.

5. Understands and cooperates in an examination.

Exclusion Criteria

1. A patient with extra skeletal osteosarcomas.

2. A patient with a record of being sensitive to radioactive substances.

3. A patient with a serious condition of kidney failure.

4. A patient with low - grade osteosarcoma.

5. A pregnant patient.

The present study began with a selection of patients susceptible of osteosarcoma through a physical examination and plain radiography. Then, magnetic resonance imaging was performed to locate the extension of the tumor for the planning of operative treatment. Subsequently, there was a bone scan to find disease staging, as well as an open biopsy to corroborate the diagnosis of actual osteosarcoma prior to a selection of patients as a case study. Afterwards , the first round of Tc-99m MIBI scintigraphy was carried out before commencing chemotherapy (Tc-99m MIBI scintigraphy of 25 mCi was injected into the vein and then waited for 10 minutes before scanning) to find out the consequence and collect data derived before the treatment in order to be used to calculate the first tumor count per background ration (TBR_1). Next, chemotherapy was started with the osteosarcoma protocol consisting of three courses of doxorubicin of 25 mg/m^2/day for 3 days and cisplatin of 100 mg/m^2. Each course had a 3 - 4 week lapse as the recommendation regimen of European Osteosarcoma Intergroup (EOI)[8]. The second Tc-99m MIBI scintigraphy was performed after completing preoperative chemotherapy within 1 - 2 days before the operation. The collected data was used to calculate the second tumor count per background ration (TBR_2). Then, the consequence of an alteration of TBR_1 derived before chemotherapy and TBR_2 received after chemotherapy was used to calculate the percentage of tumor necrosis, which was called an alteration ratio. Limb salvage surgery was then performed. The resected specimens were sent for a histological examination and the percentage of tumor necrosis after preoperative chemotherapy was calculated. The alteration ratio or percentage of tumor necrosis derived from an approach of Tc-99m MIBI scintigraphy was used to find its correlation with the percentage of tumor necrosis from histology through nonparametric correlation analysis and regression analysis.

The Histological Assessment of the Percentage of Tumor Necrosis

(Using an approach of the Mayo Clinic[9])

1. Soak the slice of bone tumor in 10% formalin buffer.

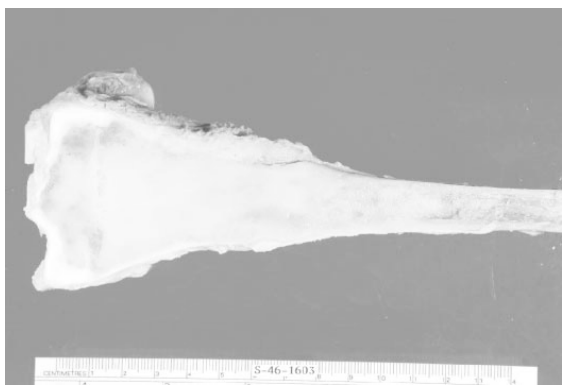
2. Record the specimens derived, measure their size, examine and ink for margin.

3. Cut off the tissue that is not tumor.

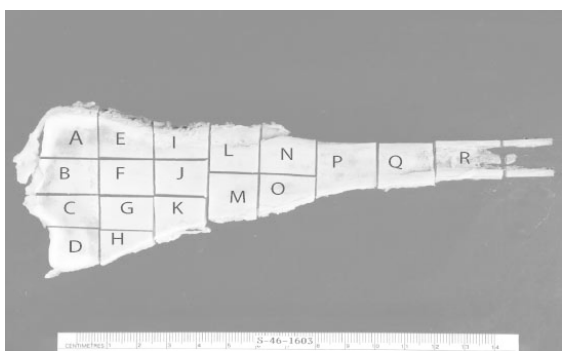
4. Cut the bone in half using a band saw, going along the middle line of the bone to get most of the tumor diameter on both sides.

5. Cut the bone horizontally in parallel to the first piece with a width of 4 millimeters. Wash and slightly brush it to eliminate the bone powder.

6. Take a photo of the bone piece and print out to be used for cutting the slice of bone tumor into pieces, and then labeling them in the grid diagram.

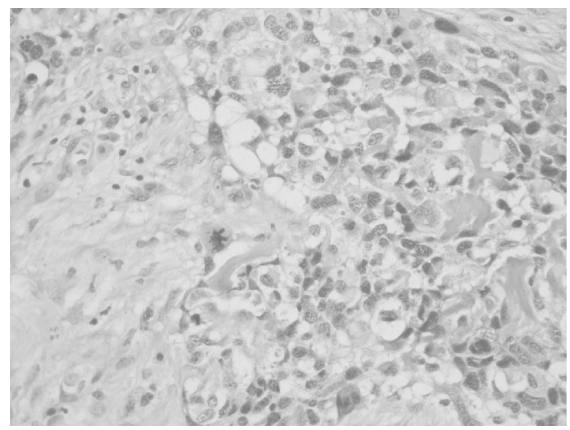


A



B

Fig. 1 Specimen was cut and label in grid diagram



A

B

Fig. 2 Histological finding before and after receive chemotherapy

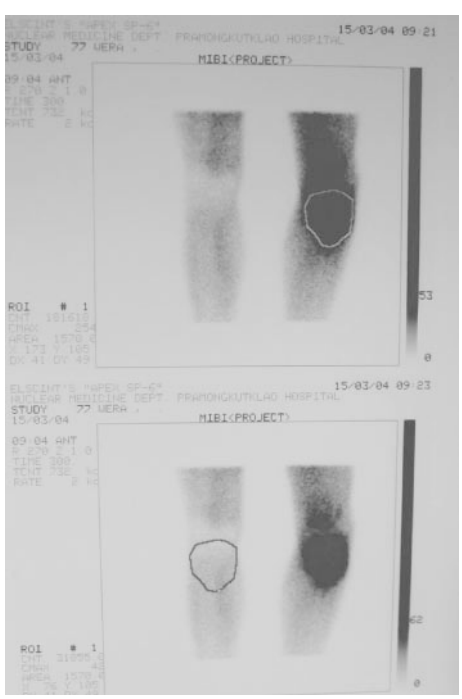


Fig. 3 Show the outline of tumor lesion for counting tumor per pixel (T) and background per pixel (BG) from the opposite distal femur

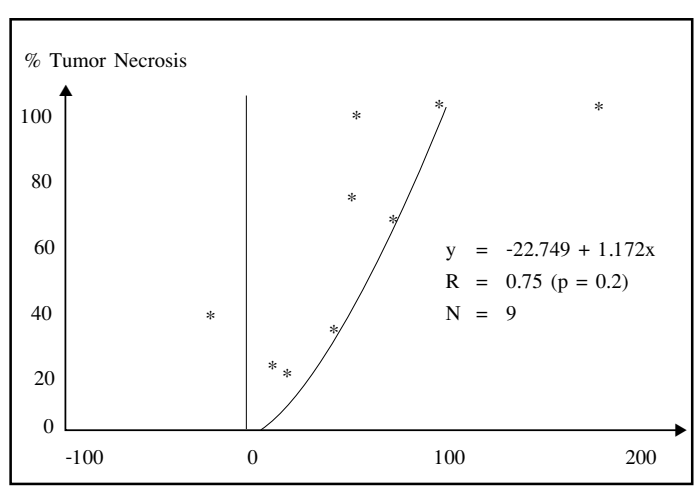


Fig. 4 Correlation between alteration ratio (ALT_RTIO) and percentage of tumor necrosis (PECT NECR)

Table 1. The details of osteosarcoma patients: consists of location, stage, treatment and percentage of tumor necrosis from histological compare to alteration ratio from Tc-99m MIBI scintigraphy after preoperative chemotherapy

No.	Age (yr)	Gender	Location	Staging (Enneking)	% Tumor Necrosis (Histological response)	Treatment	Alteration Ratio (Tc-99m MIBI)
1	50	M	distal femur	IIB	20%	Wide resection + osteochondral allograft	8.86%
2	15	M	distal femur	III	68%	Wide resection + osteochondral allograft	57.74%
3	9	M	proximal tibia	IIB	97%	Wide resection + intercalary allograft + fusion knee	171.17%
4	21	M	proximal humerus	IIB	98%	Wide resection+ osteochondral allograft	43.71%
5	20	F	distal femur	IIB	55%	Wide resection + endoprosthesis	-15.4%
6	16	M	distal femur	IIB	18%	Wide resection + intracalary allograft + knee fusion	11.3%
7	15	F	distal femur	IIB	95%	Wide resection + osteochondral allograft + ACL & PCL reconstruction	94.5%
8	20	M	proximal tibia	IIB	70%	Wide resection + osteochondral allograft + ACL & PCL reconstruction	37.3%
9	12	M	distal tibia	III	32%	Below knee amputation	33.92%

7. Soak the bone piece in nitric acid of 5 % for the decalcification. After 24 hours, press them slightly with a razor blade to check whether the bone is soft enough to be cut. If not, keep on soaking it and check every 6 -8 hours, generally not more than 48 hours. When the bone is soft enough, wash it by having tap water flow through it for 30 minutes to get rid of the excessive acid.

8. Cut the bone tumor vertically and separate them into small pieces and put them in a small box of 1.5 x 1.5 centimeters and label them A, B, C,..., respectively so that they corresponded to the position in the photo, and then recorded them in a table with the space provided in the photo as in a grid diagram (Fig. 1).

9. Cut the boundary of the tumor to see whether it has a free tumor margin.

10. Count the tumor necrosis from each slide and label it in the photo, and record the outcome of an assessment of tumor necrosis of all resected specimens as the percentage of tumor necrosis (Fig. 2).

An Assessment of the Percentage ofTumor Necrosis through Tc-99m MIBI scintigraphy

(Use the same calculation principle as that of renal scan.)

1. Define the region of interest of the tumor and find the measurement of tumor (T) in that region.

2. Find the measurement of the background (BG) at the surrounding tissue from the area of the same size at the lump of hand bone or leg bone on the opposite side (Fig. 3).

3. Since the amount of isotope has to be counted for all 3 dimensions, the image of Tc-99m MIBI scintigraphy recorded has only 2 dimensions, virtually there will be an overlapping of the isotope amount both in the front and back lines for the tumor lesion that has been recorded. Thus, the measurement of the background (BG) of the tissue from the area of the same size at the lump of hand bone or leg bone on the opposite side of the tumor area has to be subtracted from the measurement of tumor (T) at the region of interact so as to get the measurement of real tumor excluding other unrelated tissue.

4. Calculate the ratio of the measurement of tumor compared to the measurement of background of the tissue at the region of interest

in order to get the tumor count per background ratio (called TBR) or the ratio of tumor density compared to background of all tissue at the region of interest, using a calculation formula in item 5

5. Calculate TBR before chemotherapy (TBR_1) and TBR after chemotherapy (TBR_2) using the following formula[10].

$$\text{Tumor-to-Background Ratio (TBR)} = T - \frac{BG}{BG}$$

Where T = Count per pixel of tumor
BG = Count per pixel of background

6. Use TBR_1 and TBR_2 to calculate ΔTBR (TBR_1 - TBR_2) to find the percentage of tumor necrosis or reduction. A comparison is made before and after chemotherapy and the value derived is called "alteration ration" (%), which is the percentage of tumor necrosis after chemotherapy obtained through Tc-99m MIBI scan. A calculation uses the following formula.

$$\text{Alteration Ratio (\%)} = \frac{TBR_1 - TBR_2}{TBR_1} \times 100$$

TBR_1 = tumor per background ratio *before* pre-op chemotherapy

TBR_2 = tumor per background ratio *after* pre-op chemotherapy

Statistic Analysis

Linear regression and correlation analysis were applied to find the relationship between alteration ratio from MIBI scintigraphy and percentage of tumor necrosis. A p-value of less than 0.05 was considered to be statistically significance.

Results

The present study showed that eight patients had a positive alteration ratio of Tc-99m MIBI scintigraphy except for the fifth patient who had a negative ratio (- 15.4 %). Table 1 displays the percentage of tumor necrosis from histology including the alteration ratio derived from Tc-99 m MIBI scintigraphy.

The present study applies correlation analysis getting r= 0.75 and p = 0.02, as well as regression analysis getting β = 1.172 and p = 0.043. Therefore, the authors could identify the linear equation of the relationship between alteration ratio obtained from Tc-99m MIBI scan (X) and the percentage of tumor necrosis from histology (Y). Then, Y = -22.749 + 1.172 X as revealed in Fig. 4.

Discussion

For the current treatment of osteosarcoma, it has been found that the percentage of tumor necrosis is the most significant factor influencing the prognosis of a patient without metastases[2]. The histological assay for the percentage of tumor necrosis carried out by a well trained and experienced pathologist is considered as a gold standard.

Currently, the best assessment of the indicator of drug effectiveness is to examine and find over 98 %[1] of tumor necrosis. If the percentage of tumor necrosis is over 95, this indicates a favorable response to chemotherapy and, thus the chemotherapy may go on without having to change the regimen of the drugs after surgery. Nevertheless, if an unfavorable response is found, meaning that the percentage of tumor necrosis is less than 95, a consideration for a change to a new regimen of drugs after an operation to get rid of tumor has to be made[11]. Because there may be - chemo resistance or local recurrence of the tumor, particularly in the case of positive tumor margin.

A study through nuclear medicine can be conducted using the Gallium scan, Thallium - 201 scan and Tc-99m MIBI scintigraphy. The first two types of examination are very sensitive and thus

widely used. The study of musculoskeletal imaging[3] to diagnose and assess the treatment of a musculoskeletal tumor indicates that an assessment of the percentage of tumor necrosis though chemotherapy with Thallium - 201 scan is very useful. It has been found that nearly 100 % of patients with a significant decrease uptake of Thallium - 201 have a satisfactory response to chemotherapy while for patients with over 95 % of tumor necrosis, the uptake of Thallium - 201 is very little.

The study of tumor activity and an examination of the percentage of tumor necrosis normally uses Thallium - 201 since there is a good affinity for general tumor including osteosarcoma[3]. Nevertheless, Thallium - 201 can not be prepared in Thailand and has to be imported and so it is not convenient to use it since Thallium - 201 is not stable and its half life is 13 hours, which makes it difficult for practical use.

There has been a comparative study by Ashok et al[12] of examination consequences between Thallium - 201 and Tc-99m MIBI for patients with osteosarcoma. It was found that Tc-99m MIBI scintigraphy yields a better examination consequence in that little lesion can be found while it can 't be found by Thallium - 201. However, in terms of the treatment assessment through chemotherapy, it was found that both methods can effectively assess the treatment. The previous study[10] showed that there is a relationship between the alteration ratio of Tc-99m MIBI scintigraphy and the percentage of tumor necrosis in primary bone and soft tissue tumor.

In the present study, the researcher team used Tc-99m MIBI scintigraphy to monitor the response to chemotherapy for osteosarcoma. Tc-99m MIBI (Tc-99m - hexalxis - 2 - methoxyisobuty lisonitril) is a radioactive pharmaceutical substance previously used to examine myocardial ischemia[13] and has an approximate property to Thallium - 201 but it is better in that it is convenient and easy to use. The uptake of this radioactive substance will pass through the cells by passive diffusion and go through the cell membrane to uptake within mitochondria of tumor cell. Generally, tumor cells have high metabolic activity, which makes it possible for an obvious viability examination. Nevertheless, the uptake of this radioactive substance in tumor cells will depend on the amount of blood flow. Thus, in the tumor with necrosis or ischemia, there is generally low uptake.

Advantages of Tc-99m MIBI scintigraphy

1. It is a radioactive pharmaceutical substance that has only a minimal side - effect and absorbs little amount of radioactivity.
2. It is easy for an examination and takes only a short time.
3. A patient doesn't have to stay in the hospital.
4. It is easy and rapid to interpret an examination consequence.

Disadvantages of Tc-99m MIBI scintigraphy

1. A patient may suffer from anaphylactic allergy but this is normally found in a few cases (2 - 3 cases out of 100,000 examination cases).
2. A pregnant patient can't obtain an examination.
3. An examination can be made only at an institution having a single photon emission computerized tomography (SPECT) scan.
4. It is difficult to examine pelvis bone and spine because there is disturbance from the gastrointestinal tract[5].

In the present study, there is a comparison of the percentage of alteration ratio of the uptake Tc-99m MIBI scintigraphy and the percentage of tumor necrosis from histology after chemotherapy. It is found that the alteration ratio of Tc-99m MIBI scintigraphy has a linear correlation with the percentage of tumor necrosis form histology.

The correlation is statistically significant. Based on the calculation, p = 0.02 and correlation coefficient (r) = 0.75 by correlation analysis. The linear equation of the relationship between alteration ratio derived from Tc-99m MIBI scintigraphy (X) and the percentage of tumor necrosis from histology (Y) is Y = -22.749 + 1.172 X. This may be applied in practice for an assessment of the response of osteosarcoma to chemotherapy using Tc-99m MIBI scintigraphy in place of a histological examination method. However, it needs a further research because the sample size used in the present study is too small to make a conclusion based on the relationship equation obtained.

Patient number five had the alteration ratio = - 15.4 %, which was negative. This was due to the fact that the tumor became much bigger after chemotherapy indicating that the tumor was resistant or poorly responded to chemotherapy, which also had a relationship with the percentage of tumor necrosis from histology which was little (55 %). Another patient (the third case) had an alteration ratio = 171.71 %, revealing that there was also the decrease of normal tissue apart from tumor cells, which also had a relationship with the percentage of tumor necrosis from histology which was high (97 %).

For clinical application, the sequential Tc-99m MIBI scintigraphy during chemotherapy should be made periodically and see the percentage of tumor necrosis obtained from Tc-99m MIBI scintigraphy to find how it responds to chemotherapy. This may help in treatment planning and adjusting the regimen for chemotherapy. If a patient has a favorable response to chemotherapy or there is over 95 % of tumor necrosis, it is appropriate for him to undergo limb salvage surgery.

At present, an issue of positron emission computed tomography scan (PET), which is metabolism imaging is of great interest, particularly in aspect of tumor and musculoskeletal oncology. Nevertheless, the advantage of PET scan for osteosarcoma is not apparent. It is found in various studies that Tc-99m MIBI scintigraphy has to be used in combination for a consequence interpretation[14]. Therefore, the diagnosis and an assessment of the treatment consequence through Tc-99m MIBI scintigraphy is still useful and can be effectively used clinically.

Conclusion

In conclusion, the Tc-99m MIBI scintigraphy may be used as a monitor of the treatment consequence of osteosarcoma after preoperative chemotherapy by assessing the percentage of tumor necrosis. It is inclined to be used for an assessment so as to adjust the regimen of chemotherapy and to formulate the plan for a limb salvage surgery.

The findings of the present study reveal that an examination through Tc-99m MIBI scintigraphy has statistically significant correlation with the percentage of tumor necrosis from histology with P = 0.02. This indicates that this method may be applied for an assessment of the response of osteosarcoma to preoperative chemotherapy as a replacement of a histological examination.

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การศึกษาความสัมพันธ์ระหว่างวิธีการตรวจเทคนิเชี่ยมมีบี้สแกนและวิธีการตรวจทางพยาธิวิทยาในการหาเปอร์เซ็นต์การตายของเซลล์มะเร็งกระดูกออสติโอซาโคมาภายหลังการได้รับยาเคมีบำบัด

ทิพชาติ บุณยรัตพันธุ์, สุรังค์ วิทยาวงศรุจิ, สามารถ ราชดารา, วรนุช ธนากิจ, สุพิชัย เจริญวารีกุล
นรินทร์ คุณกิตติ, ทวี ทรงพัฒนาศิลป์

วัตถุประสงค์: จากการศึกษาพบว่าค่าเปอร์เซ็นต์การตายของมะเร็งกระดูกชนิดออสติโอซาโคมาหลังได้รับยาเคมีบำบัดมีความสำคัญที่สุดในการใช้พยากรณ์โรคของผู้ป่วยได้ การตรวจที่ใช้เป็นมาตรฐานคือการตรวจทางพยาธิวิทยาซึ่งต้องอาศัยพยาธิแพทย์เฉพาะทางที่ผ่านการฝึกอบรมมาแล้ว จึงจะสามารถตรวจได้ ซึ่งยังคงขาดแคลนมากในประเทศไทย การศึกษานี้ต้องการหาความสัมพันธ์ระหว่างวิธีการตรวจเทคนิเชี่ยมมีบี้สแกนและวิธีการตรวจทางพยาธิวิทยาในการหาเปอร์เซ็นต์การตายของเซลล์มะเร็งกระดูกออสติโอซาโคมาว่ามีความสัมพันธ์กันหรือไม่

วัสดุและวิธีการ: ทำการศึกษาในผู้ป่วยมะเร็งกระดูกชนิดออสติโอซาโคมาจำนวน 9 ราย ระหว่างเดือนกันยายน พ.ศ. 2545 ถึงเดือนสิงหาคม พ.ศ. 2547 ผู้ป่วยทุกรายจะได้รับยาเคมีบำบัดก่อนการผ่าตัดจำนวน 3 วงรอบ ประกอบด้วยยา doxorubicin และ cisplatin หลังจากนั้นจะทำการผ่าตัดเอากระดูกที่เป็นมะเร็งออกไปส่งตรวจหาค่าเปอร์เซ็นต์การตายของมะเร็งกระดูกชนิดออสติโอซาโคมาที่ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์จุฬาลงกรณ์ และให้ยาเคมีบำบัดอีก 3 ครั้งหลังการผ่าตัด การตรวจด้วยเทคนิเชี่ยมมีบี้สแกนจะทำ 2 ครั้ง ครั้งที่ 1 จะทำก่อนการให้ยาเคมีบำบัด และทำครั้งที่ 2 หลังจากได้ยาเคมีบำบัดครบ 3 วงรอบก่อนการผ่าตัด คำนวณหาค่า Tumor to Background Ratio (TBR) เปรียบเทียบครั้งที่ 1 และ 2 นำมาคำนวณหาค่า alteration ratio เป็นเปอร์เซ็นต์การตายที่ได้จากการตรวจเทคนิเชี่ยมมีบี้สแกน นำค่าที่ได้ไปเปรียบเทียบกับวิธีทางพยาธิวิทยาและหาว่ามีความสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติหรือไม่

ผลการศึกษา: จากการคำนวณเปรียบเทียบหาค่า correlation และ linear regression analysis พบว่าทั้งสองค่ามีความสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติ ที่ $P = 0.020$ และ 0.043

สรุป: การตรวจหาค่าเปอร์เซ็นต์การตายของมะเร็งกระดูกชนิดออสติโอซาโคมาโดยวิธีเทคนิเชี่ยมมีบี้สแกนมีความสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติกับการตรวจทางวิธีทางพยาธิวิทยาซึ่งเป็นการตรวจมาตรฐาน สรุปได้ว่าอาจจะนำมาทดแทนการตรวจทางวิธีทางพยาธิวิทยาได้ ซึ่งต้องการการศึกษาเพิ่มเติมต่อไป