



## Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: [www.bioscmed.com](http://www.bioscmed.com)

### Differences in p53 Expression in High-Grade Conventional Osteosarcoma and Giant Cell Tumor of Bone

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#### ARTICLE INFO

##### Keywords:

Conventional osteosarcoma  
High-grade  
Giant cell tumor  
Expression p53

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All authors have reviewed and approved the final version of the manuscript.

<https://doi.org/10.37275/bsm.v6i15.680>

#### ABSTRACT

**Background:** Osteosarcoma is the most common primary tumor of bone. Generally, patients with osteosarcoma have a poor prognosis. So it is very important to be able to diagnose as early as possible. Histopathologically, osteosarcoma has a variety of types similar to non-malignant lesions, such as giant cell tumor of bone. Therefore, caution is needed in diagnosing because if it is misdiagnosed, it will result in different management. This study aims to look at differences in p53 protein expression in high-grade conventional osteosarcoma and giant cell tumor of bone. **Methods:** This observational analytic study used 30 samples from tissue biopsies/surgeries diagnosed with conventional high-grade osteosarcoma and 30 samples of giant cell tumor of bone at the Anatomical Pathology Installation of Dr. Saiful Anwar General Hospital Malang, Indonesia, between 2018-2022. p53 expression was examined using the immunohistochemical staining method. Positive if stained with brown on the nucleus of the cell, the percentage of 500 malignant cells was calculated in the large field of view of the objective lens (x40) randomly. Univariate and bivariate data analysis was performed using SPSS. **Results:** The average p53 protein expression in conventional high-grade osteosarcoma was 80.0% ± 26.2, with the lowest value being 19% and the highest value being 100%. Whereas in giant cell tumor of bone, the average p53 expression was 1.7% ± 4.3, with the lowest value being 0% and the highest value being 17.6%. Statistical analysis using the Mann-Whitney test showed that p53 expression in conventional high-grade osteosarcoma and giant cell tumor of bone showed a significant difference (p=0.000). **Conclusion:** There is a significant difference between p53 expression in high-grade conventional osteosarcoma and giant cell tumor of bone.

#### 1. Introduction

Osteosarcoma is the most common primary tumor of bone.<sup>1,2</sup> Historically, the incidence rate has been reported to be higher in men than in women, with an incidence rate of 5.4 cases per 1 million men per year and 4 cases per 1 million women per year.<sup>3</sup> Osteosarcoma has a bimodal age distribution, most common at ages 14 and 18 years, with a second peak at over 40 years of age.<sup>1</sup> In Indonesia, especially at Dr. Saiful Anwar General Hospital Malang, osteosarcoma is the most common primary malignant tumor of bone,

mostly males with the most age between 14-15 years, and the most common location is on the extremities.<sup>4</sup> Osteosarcoma patients have a poor prognosis. Even though the primary tumor has been surgically resected, the patient remains at high risk of developing pulmonary metastases.<sup>5</sup> So, it is very important to be able to diagnose this osteosarcoma as early as possible before metastases occur. Meanwhile, in Indonesia, most osteosarcoma patients come late due to the low early detection rate of osteosarcoma.<sup>6</sup> This delay, of

course, will worsen the prognosis and complicate its management. In addition, there are also difficulties in differentiating between osteosarcoma and other benign tumors which have similar clinical, radiological, and histopathological features. For this reason, high diagnostic accuracy is needed to establish the diagnosis of osteosarcoma so that patients can be treated quickly with appropriate therapy to increase the survival rate.

Osteosarcoma often shows a variety of histopathological features, so it is grouped into several subtypes. Of the several types of osteosarcoma, the most common type is high-grade conventional osteosarcoma.<sup>1</sup> Histopathologically, high-grade conventional osteosarcoma has a variety of types that are similar to non-malignant lesions, such as giant cell tumors of bone, especially in variants with many mitoses. Therefore caution is needed in diagnosing giant cell tumors of bone.<sup>7</sup> This is because if it is misdiagnosed, it will result in different management. The p53 tumor suppressor gene is the most frequently mutated gene in human cancer. More than 70% of malignant tumors have mutations in p53, including osteosarcoma.<sup>8</sup> Although the exact etiology of osteosarcoma is still unclear, there is an association between an increased incidence of osteosarcoma and several genetic disorder syndromes, as in Li-Fraumeni syndrome, where in this syndrome, there is a mutation of the TP53 gene.<sup>1</sup> This study aims to look at differences in p53 protein expression in high-grade conventional osteosarcoma and giant cell tumor of bone. Until now, there has been no study in Indonesia regarding differences in p53 expression in high-grade conventional osteosarcoma and giant cell tumor of bone.

## 2. Methods

This study used an analytic observational research design. This research was approved by the Research Ethics Committee of Dr. Saiful Anwar General Hospital Malang Indonesia with ethical approval number no. 400/194/K.3/102.7/2022. The research samples were all paraffin block biopsy/surgery specimens

diagnosed with conventional high-grade osteosarcoma and giant cell tumor of bone at the Anatomical Pathology Laboratory of Dr. Saiful Anwar General Hospital Malang Indonesia in 2018-2022, who met the inclusion and exclusion criteria. A total of 30 tissue samples were for diagnosis of Conventional Osteosarcoma High Grade and a total of 30 tissue samples for diagnosis of giant cell tumor of bone, so there are 60 samples in total.

The paraffin block cut (5um thickness) was placed on a glass slide that had been coated with poly-L-Lysine, then deparaffinized by inserting the glass slide into xylol and alcohol solution. Then soaked in peroxide block solution for 25 minutes, followed by DIVA solution, before being put into a decloaking chamber at 90° for 45 minutes. The staining was continued by soaking in PBS solution for 5 minutes, then incubating the primary antibody for 60 minutes, then administering the polymer. The next step was giving DAB chromogen and counterstaining using hematoxylin for 2 minutes and lithium carbonate. The next process is clearing into a xylol solution which is then followed by mounting. To see the expression of p-53, p-53 antibodies were used with a ratio of 1:100 (Biocare Medical, APA111 AA).

Calculation of p53 expression was carried out using a light microscope, counted in cells that were immunohistochemically stained with brown on their cell nuclei from 500 cells divided into 5 large fields of view (x400) randomly. The mean of cells stained positively is expressed as a percentage. Data analysis was performed with the help of SPSS software version 26. Univariate analysis was performed to display the frequency distribution of the study subject's characteristic data. Bivariate analysis was performed to determine differences in p-53 expression between the two groups using the Mann-Whitney test with  $p < 0.05$ .

## 3. Results

The study sample consisted of 31 (51.6%) males and 29 (48.33%) females with a minimum age range of 10 years and a maximum age of 66 years. The

characteristics of the study sample can be seen in table 1. In conventional high-grade osteosarcoma, most patients were aged 11-20 years, 13 (43.33%), with an average age of 27.8 years, a median age of 23 years, and some the sample size is male as much as

20 (66.667%). Meanwhile, in the giant cell tumor of bone, the highest number of patients aged 31-40 years was 10 (33.33%) with an average age of 35 years, the median age was 32 years, and the sex of the majority was male, with 20 samples. (66,67).

Table 1. Characteristics of research samples.

		High-grade conventional osteosarcoma (%)		Giant cell tumor of bone (%)	
<b>Age</b>	0-10	1	(3.33)	0	(0.00)
	11-20	12	(40.00)	2	(6, 67)
	21-30	7	(23.33)	9	(30.00)
	31-40	2	(6.67)	10	(33.33)
	41-50	4	(13.33)	5	(16.67)
	51-60	4	(13.33)	3	(10.00)
	61-70	0	(0.00)	1	(3.33)
<b>Gender</b>	Male	20	66.67	31	(36.67)
	Female	10	(33.33)	29	(63,33)

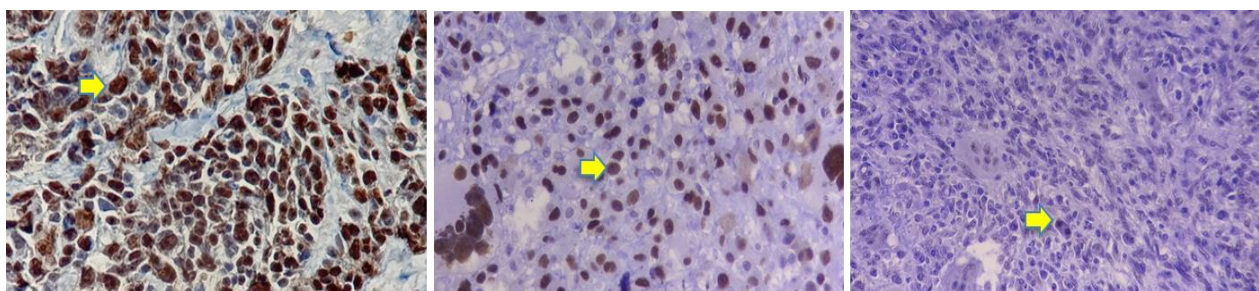


Figure 1. Results of immunohistochemical examination of p53 expression. (A) Expression of p53 protein in positive control preparation (Ca Mammae) 400x, (B) Expression of p53 protein in high-grade conventional osteosarcoma sample, magnification 400x. (C) Expression of p53 protein in conventional giant cell tumor of bone samples, 400x magnification.

The results of this study showed that the average p53 protein expression in high-grade conventional osteosarcoma was  $80.0\% \pm 26.2$ , with the lowest percentage value being 19% and the highest percentage value being 100%. Whereas giant cell

tumors of bone showed an average p53 expression of  $1.7\% \pm 4.3$ , with the lowest percentage value being 0% and the highest percentage value being 17.6% (Figure 2).

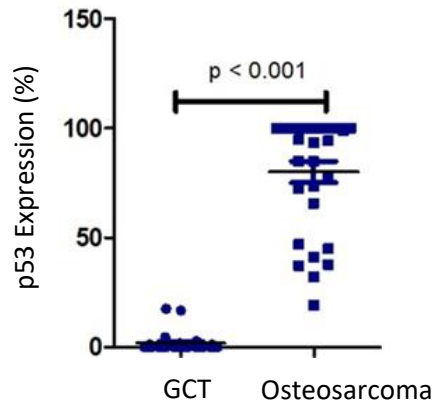


Figure 2. Percentage of p53 protein in the high-grade conventional osteosarcoma group. The high-grade conventional osteosarcoma group with an average of  $80.0\% \pm 26.2$ , and the conventional giant cell tumor of bone of  $1.7\% \pm 4.3$ . The difference test between the two groups showed a significant mean difference ( $p < 0.01$ ).

#### 4. Discussion

Based on age, most patients with conventional high-grade osteosarcoma were aged 11-20 years, as many as 12 (40%) patients, with an average age of patients 27.8 years and a median age of 23 years. This is in accordance with the literature, which states that osteosarcoma has a bimodal age distribution. The initial peak occurs in the 10 to 14-year age group, corresponding to the pubertal growth spurt. In the age range of 0 to 14 years, the incidence of osteosarcoma in all races and sexes is 4 cases per year per million people. And the second peak is in adults over 65 years.<sup>9</sup> Other literature also mentions that osteosarcoma is the most common malignancy in cases of pediatric bone malignancy.<sup>2</sup> It accounts for about 2.4% of all pediatric cancers, making it the eighth most common childhood malignancy.<sup>2</sup> The gender of people with the majority of high-grade conventional osteosarcoma patients in this study sample was male, with as many as 20 samples (66.67%). This is also in accordance with the literature, which states that the incidence of osteosarcoma has historically been reported to be higher in men than women, with an incidence rate of 5.4 cases per 1 million men per year and 4 cases per 1 million women per year.<sup>10</sup>

In the giant cell tumor of a bone sample, the minimum age range was 11-20 years, and the maximum was 61-70 years, most patients aged 31-40 years were 10 (33.33%) patients, with an average age of patients 35 years, the median age at 32 years. These findings are in accordance with the literature, which states that epidemiologically, this type of benign tumor is the most common benign tumor that often occurs in the second and third decades of age, and the most common location is distal to the long bones of the lower extremities (distal femur, proximal tibia, distal radius, and proximal humerus) on mature bone and occasionally on the metaphysis, in some patients, it may also occur on immature bone. The incidence of giant cell tumors of bone in the axial bone most often occurs in the sacrum and vertebral bodies. Flat bone involvement is rare but can occur in the pelvis.<sup>1</sup> This aggressive tumor frequently appears in the metaphyseal region of long bones in individuals in the second, third, and fourth decades of life.<sup>7</sup> Patients with giant cell tumors of bone in this study were male, with as many as 31 (36.6%) patients. This is not in accordance with the literature, which states that most patients with giant cell tumors of bone are women.<sup>1</sup>

This study assessed the expression of p53 protein in tissues diagnosed with conventional high-grade

osteosarcoma and giant cell tumor of bone. The presence of mutations or LOH p53 is generally found in cases of malignancy, which shows overexpression in the immunohistochemical staining of p53. The p53 tumor suppressor gene is the most frequently mutated gene in human cancer. More than 70% of malignant tumors have mutations in p53, including osteosarcoma.<sup>8</sup> The p53 gene is a tumor suppressor gene located on chromosome 17p131. Under normal conditions, this protein has an important role in cell cycle regulation, apoptosis, and DNA repair. In cells that have lost or have mutations of TP53, DNA damage will not induce cell cycle arrest or DNA repair, and cells with genetic damage will continue to proliferate and then grow into malignant neoplasms.<sup>11</sup>

The results of this study showed that the average p53 protein expression in high-grade conventional osteosarcoma was 80.02% ± 26.22, with the lowest percentage value being 19% and the highest percentage value being 100%. Whereas in the giant cell tumor of the bone group, the average p53 expression was 1.69% ± 4.33, with the lowest percentage value being 0% and the highest percentage value being 17.6%. The results of the Mann-Whitney different test analysis show that the asymp. Sig (2-tailed) is 0.000. It can be concluded that the hypothesis is accepted. Thus it can be said that there is a significant difference between p53 expression in high-grade conventional osteosarcoma and p53 expression in giant cell tumors of bone. This is in accordance with some literature which states that about 90% of cases of osteosarcoma often have mutations in p53.<sup>1</sup>

Whereas in a giant cell tumor of bone, lower p53 expression was obtained. Previous studies have shown that p53 expression was statistically significantly higher in secondary than primary tumors in GCTB.<sup>12</sup> And in most cases of giant cell tumors of bone, the mutation that occurs is in the histone H3, a member of the 3A family (H3F3A) (G34W/V/R/L), which is also often used to rule out other differential diagnoses.<sup>13</sup> In some cases of giant cell tumors of bone, p53 mutations can also occur, although no significant relationship between the mutation and clinicopathological

parameters was found.<sup>14</sup> In a study conducted by Okubo et al., it was also explained that LOH at the p53 locus was detected in 8 out of 37 cases. Although this was not related to p53 overexpression in conventional GCTB, p53 mutations play a role in tumor recurrence and malignant transformation of GCTB through interaction with GPX-1.<sup>15</sup> In another study. It was found that p53 expression was stronger in secondary malignant GCTB than conventional GCTB and concluded that there was a role for p53 in the malignant transformation of GCTB.<sup>12</sup> This was also mentioned in a study conducted by Saito.<sup>16</sup> Therefore, the detection of p53 mutations and high GPX1 expression can help diagnose malignant transformation in GCTB.

## 5. Conclusion

There is a difference in the mean expression of p53 between high-grade conventional osteosarcoma and giant cell tumor of bone.

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