Immunohistochemical examination of recurrence and giant cell tumor specificity, is it correlated? cohort retrospective study

Achmad Jadi Didy Surachman*, Achmad Fauzi Kamal, Inawati Wanandi Septelia, Nurjati Chairani Siregar, Rahyuussalim, Roswita Harahap Alida, Soemarko Sumaryani Dewi

ABSTRACT

Introduction: GCT is a benign tumor that has an aggressive local character. GCT recurrence after disinfection still often occurs. The reasons for treatment failure and triggering a recurrence are believed to be the role of the accumulation of stem cells in promoting tumor initiation and recurrence.

Methods: Study Cohort Retrospective cohort in patients who had undergone surgery with a diagnosis of GCT at RSUPN Dr. Cipto Mangunkusumo in 2017-2022. The subjects were separated into two groups, recurrence patients and non-recurrence patients. The tissue biopsy will be assessed with ALCAM+, Oct4, and Stro-1 biomarkers for tumor stem cells.

Results: A total of 38 patients were diagnosed with Giant Cell Tumor. There were 31.6% of recurrent patients with an age range between 31-40 years and 41-50 years. The most common location is the Distal Radius with a score of 41.7% in recurrent patients and 38.5% in non-recurrent patients. Based on the data, the expression of ALCAM+, Oct4, and Stro-1 did not have significant differences between the two groups. The Recurrence group in p63 was expressed more than in the Non-Recurrence group p=0.002. In CD147, the Recurrence group was also expressed more than in the Non-Recurrence group p=0.003.

Conclusion: Increased expression of ALCAM+, Oct4, and Stro-1 occurred in Recurrence and Non-Recurrence patients, although Stro-1 appeared to be more sensitive in detecting GCT progression, but was not statistically different. CD147 and p63 are statistically different between recurrence and non-recurrence.

Keywords: Giant Cell Tumor, Progressivity Giant Cell Tumor, Recurrence.


INTRODUCTION

GCT is a benign tumor that has an aggressive local character. Giant Cell Tumor recurrence after disinfection still often occurs. Based on data from the World Health Organization (WHO), GCT has a percentage of around 5% of all bone tumors and 20% of benign bone tumors.1-3

The annual incidence reaches 1-6 cases per 10 million population outside Indonesia, around 1 sufferer per 1 million population in the United States,3 Western Australia, Japan, and Sweden.5,6 The prevalence of bone GCT is in young adult patients in the 3rd and 4th decades reaching 80% at the age of 30 to 40 years,7 less than 3% occur under the age of 14 years, and about 13% occur over the age of 50 years. Bone GCT affects women more often than men with a ratio of 1.5:1.7,8

GCT is a tumor that has local recurrence properties. Treatment failure is a trigger that is believed to be a factor in GCT tumor recurrence. The reasons for treatment failure and triggering a recurrence are believed to be the role of the accumulation of stem cells in promoting tumor initiation and recurrence. Tumor Stem Cells contribute to the formation of a GCT development.9 Histologically, bone GCT cells are composed of mononuclear stromal cells and multinuclear giant cells that show osteoclast-like giant cell activity. In 1940 this tumor was called part of the osteoclast neoplasm, with the designation osteoclastoma.9

Several biomarkers can be used to detect bone GCT peaks such as ALCAM+, Oct4, and Stro-1.8,10,11 Activated leukocyte cell adhesion molecule (ALCAM+) is a transmembrane receptor that appears on the cell surface, has another name CD166. The appearance of ALCAM+ on the cell surface is a sign of cell proliferation towards malignancy and is involved in T-cell activation, hematopoiesis, development, inflammation, and transendothelial migration in neutrophils. This protein is expressed in many tumors.10,12-17

ALCAM+ was identified in active leukocytes, hematopoietic stem cells, myeloid progenitors, neuronal cells, mesenchymal stem cells, stromal bone, and osteoblastic cells which assist the process of hematopoiesis. ALCAM+ is found in intercellular bridges that act as anti-angiogenesis targets. Transendothelial migration of activated monocytes was inhibited by ALCAM+ antibodies. These findings indicate that ALCAM+ plays a role as a molecular process in regulating diapedesis.10,12-17
Figures 1. GCT features A. Typical features of large osteoclasts and many mononuclear cells, some show mitotic activity. B. Vascular lumen with a mixture of spindle and giant cells.

Octamer binding transcription factor 4 (Oct4) is a gene transcription factor formed by the Pou5f1 gene. Pou5f1 is located on human chromosome 6, part of POU (Pit, Oct, Unc), which is a group of DNA-binding proteins. During the development of the Oct4 blastocyst, it was also found in large quantities. In the process after embryo implantation into mice, Oct4 also shows a role in cell differentiation. Oct4 will decrease during the differentiation process and the gestation process on the 8th day Oct4 will reappear in undifferentiated embryonic stem cells, carcinoma cells, and embryonic primordial cells. The tumorigenicity, metastasis, and recurrence process after chemoradiotherapy has been associated with a spike in Oct4 in every tumor. Oct4 can be found in every undifferentiated cell, so it can play an important role in assessing tumor cell resistance.

Tumor stem cells create several processes of tumor formation by initiating a cell environment, multipotency, rapid growth, and resistance to a drug as evidenced by the emergence of Stro-1 antigen expression. Cells having positive Stro-1 antigen is a sign that can be found in bone GCT. This antigen is triggered by tumor stem cell stem which helps a cell to have a differentiation pattern into osteogenic and adipogenic cells.

This study aims to assess the origin of bone Giant Cell Tumors and reverse with recurrence of Giant Cell Tumor patients who have undergone rehabilitation through histopathological, radiological, and biomarker examinations.

METHODS

The research method in this study was a Cohort Retrospective in patients who had undergone surgery with a diagnosis of GCT at RSUPN Dr. Cipto Mangunkusumo in 2017-2022. The research subjects were separated into two groups, namely Recurrence patients and Non-Recurrence patients. The tissue biopsy will be assessed with ALCAM+ (DAKO, Carpinteria, CA), Oct4 (Dako Agilent Technologies, Santa Clara), and Stro-1 (R&D System, Minneapolis, MN) biomarkers for tumor stem cells. Immunohistochemical results were assessed by identifying well-stained tumor areas and then taking 3-5 photos with an objective lens magnification of 40x using a Leica ICC 50 HD Microscope. The photos were processed using Image J processor software. This study has calculated the minimum sample size which is 38 patients.

Inclusion and Exclusion Criteria

The inclusion criteria in this study are: 1) GCT patients who have gone through the stages of diagnosis through clinical, radiological, MRI, and core biopsy (histopathology) descriptions, 2) primary GCT grade 2, 3) GCT patients who have no recurrences, 4) bone GCT patients receiving treatment, both extended curettage and en bloc resection. Meanwhile, the exclusion criteria in this study are: 1) lost to follow-up, 2) damaged tumor tissue that cannot be examined biomarkers, 3) and grade 1 GCT patients

Analysis of Biomarker

The immunohistochemical measurement method that can be used in interpreting the results of histochemical biomarker staining to be used is a semi-quantitative method using the ImageJ application.

Data analysis

Data processing uses the Statistical Package for the Social Sciences 26 (SPSS) program. Expression positivity data is presented in the form of numerical data which is further categorized. The statistical assessment used a comparative hypothesis test to determine differences in the expression of biomarkers in each group using the Shapiro Wilk Test.

RESULTS

A total of 38 selected samples are the patients with a diagnosis of Giant Cell Tumor in 2017-2022 at RSUPN Dr. Cipto Mangunkusumo. The subjects were then separated into two groups, namely recurrence patients and Non-Recurrence patients. There is a distribution value of 31.6% which is patient recurrence, with the highest age range between 31-40 years and 41-50 years.

Based on gender characteristics, women were found more often in Non-Recurrence cases (61.5%), while men with 58.3% were mostly in recurrence cases. As for the distribution based on tumor location, GCT is more common in the Distal Radius with a value of 41.7% for recurring patients and 38.5% for Non-Recurrence patients Table 1.

From a total of 38 patients with GCT stage 2, 12 patients had recurrences, and 26 patients without recurrences. Of the patients who had recurrences, the highest score in strong positives was in the Stro-1 biomarker compared to ALCAM+ and OCT4 p=0487. ALCAM+ and OCT4 had more moderate positive scores than
Table 1. Patients Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recurrence (n=12)</th>
<th>Non Recurrence (n=26)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
<td>Frequency</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-20 years old</td>
<td>1</td>
<td>8.3</td>
<td>2</td>
</tr>
<tr>
<td>21-30 years old</td>
<td>2</td>
<td>16.7</td>
<td>16</td>
</tr>
<tr>
<td>31-40 years old</td>
<td>4</td>
<td>33.3</td>
<td>3</td>
</tr>
<tr>
<td>41-50 years old</td>
<td>4</td>
<td>33.3</td>
<td>3</td>
</tr>
<tr>
<td>51-60 years old</td>
<td>1</td>
<td>8.3</td>
<td>3</td>
</tr>
<tr>
<td>61-70 years old</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 70 years old</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>58.3</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>41.7</td>
<td>16</td>
</tr>
<tr>
<td>Tumor Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radius distal</td>
<td>5</td>
<td>41.7</td>
<td>10</td>
</tr>
<tr>
<td>Femur distal</td>
<td>3</td>
<td>25.0</td>
<td>8</td>
</tr>
<tr>
<td>Tibia distal</td>
<td>1</td>
<td>8.3</td>
<td>2</td>
</tr>
<tr>
<td>Tibia proximal</td>
<td>2</td>
<td>16.7</td>
<td>3</td>
</tr>
<tr>
<td>Fibula proximal</td>
<td>1</td>
<td>8.3</td>
<td>0</td>
</tr>
<tr>
<td>Humerus proximal</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
</tr>
<tr>
<td>Ulna distal</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>Fibula</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>Metatarsal</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>Surgical Methods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>En Bloc Resection</td>
<td>1</td>
<td>3.6</td>
<td>2</td>
</tr>
<tr>
<td>Extended resection</td>
<td>11</td>
<td>39.3</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 2. Mean Value from each Biomarker

<table>
<thead>
<tr>
<th>Patients n=38</th>
<th>Stadium GCT</th>
<th>Mean Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non Recurrence</td>
<td>Recurrence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=26</td>
<td>n=12</td>
</tr>
<tr>
<td>ALCAM+</td>
<td>2</td>
<td>1.69±0.47</td>
<td>1.67±0.49</td>
</tr>
<tr>
<td>OCT4</td>
<td>2</td>
<td>1.65±0.49</td>
<td>1.67±0.49</td>
</tr>
<tr>
<td>STRO-1</td>
<td>2</td>
<td>1.65±0.69</td>
<td>1.50±0.80</td>
</tr>
<tr>
<td>CD147</td>
<td>2</td>
<td>1.46±0.81</td>
<td>1.58±0.51</td>
</tr>
<tr>
<td>p63</td>
<td>2</td>
<td>1.96±0.87</td>
<td>2.25±0.45</td>
</tr>
</tbody>
</table>

GCT: Giant Cell Tumor, ALCAM: Activated Leucocyte Cell Adhesion, OCT 4: Octamer binding transcription 4

DISCUSSION

Tumor Stem Cells are stem cells that have undergone a shift in stem cell function, cancer stem cells were first proposed in the 1950s, strong evidence of the existence of cancer stem cells appeared in 1994 in a preliminary study by Dick et al. who demonstrated that CD34+/CD38 cells harvested from leukemia patients were sufficient to cause acute myeloid leukemia when inoculated into mice with severe combined immunodeficiency (SCID). TSC population is only about 1% of the total cell population that forms the tumor mass. SPK is thought to divide asymmetrically, producing identical stem cell daughters with more dissimilar cells. Stem cells like these are thought to play a role in initiating and maintaining tumor growth and if they are not completely eradicated, either by surgery or chemotherapy procedures, they will trigger local recurrences and distant metastases.

Reya et al. described cancer stem cells as a process of tumorigenesis or aberrant organogenesis processes. Cancer stem cells irregularly renew cells. Cells...
ALCAM+ moderate cytoplasmic staining in the Non-Recurrence group (A1) and ALCAM+ low cytoplasmic staining in the Recurrence group (A2), OCT4 found non-specific staining in both groups, Non-Recurrence group (B1) and Recurrence group (B2), Stro-1, Non-Recurrence group (C1) and Recurrence group (C2) groups found light cytoplasmic staining.

CD147 was found to have moderate membranous staining in the Non-Recurrence group (A1) and the Recurrence group (A2), p63 was found to have negative nuclear staining in the No Recurrence Data group (B1) and high nuclear staining in the group (B2).

CONCLUSION
The Increased expression of ALCAM+, OCT4, and Stro-1 occurred in Recurrence and Non-Recurrence patients although it seems that Stro-1 is more sensitive in detecting the progressivity of GCT, but it was not statistically different. CD147 and p63 were statistically different between Recurrence and Non-Recurrence. Theoretically, CD147 was more sensitive than ALCAM+ and OCT4 as the Prognostic Test and p63 was more subpopulation of bone GCT stromal cells. In this study, it was found that ALCAM+ exhibited properties similar to stem properties, when stromal cells with ALCAM+ were given to the sample in this study of mice with immunodeficiency, the stromal cells turned into multinucleus. These findings indicate that stromal cells are factors that promote GCT formation. Stromal cells originate from SPM cells from the bone marrow and appear during the early differentiation of osteoblasts. Zhou Z et al’s study also showed that the SPK population appeared in the stromal portion of the GCT. GCT bone stromal cells exhibit SPK properties and express ALCAM+ expression on the cell surface.

Oct4 is also expressed in cancer stem cells, conditions of high Oct4 expression will lead to tumorigenicity, metastasis, and recurrence. In general, Oct4 is highly expressed in rarely differentiated and undifferentiated cells. The differentiation itself will lead to a decrease in Oct4 expression, so that Oct4 can still be found in differentiated cells, but the expression is not high. Oct4 is also expressed in SPM, but not in high amounts. Oct4 indicates the ability of pluripotency in SPE (embryonic stem cells) to be expressed located in the nucleus.

Stro-1 is a cell derived from osteoblastic stem cells. Protein molecules of pre-osteoblastic origin such as ALP and COL1 are triggered from Stro-1. This process of osteoblastic cell formation triggers the multipotential nature of bone GCT. Expression of stem cell genes such as OCT4, and NANOG helps in tumor stem cell growth. Expression of these genes helps in the process of updating and reprogramming the characteristics of tumor stem cells.
sensitive than Stro-1 as the Diagnostic Test for the progressivity of GCT.

CONFLICT OF INTEREST
There is no conflict of interest in this study.

ACKNOWLEDGMENT
I would like to thank RSUPN Cipto Mangunkusumo for facilitating this study.

FUNDING SOURCES
None.

ETHICAL STATEMENT
This study has received the ethical clearance from local authorities.

AUTHOR CONTRIBUTION
All authors contributed equally to this study.

REFERENCES