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# Relationship of Survivin Expression with Histopathological Grade and Events of Metastasis to Omentum in Serous Carcinoma of Ovary

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**Abstract**

Ovarian cancer is the third most common cancer in Indonesia after cervical and breast cancer with the increasing incidence and mortality. The purpose of this study was to determine the relationship of survivin expression with the degree of histopathology and the incidence of metastasis to the omentum using immunohistochemical examinations. This is a cross-sectional study using 72 paraffine blocks of serous carcinoma of ovary without and with metastasis to omentum in Makassar, Indonesia during 2015-2018. Survivin was positive in tumor cells with a different degree based on the grade of histopathology and the incidence of metastases to the omentum. There was a significant difference between survivin expression with the degree of histopathology and the incidence of metastases to the omentum in serous carcinoma of ovary (p = 0.000).

***Keywords:*** survivin; serous carcinoma; ovary; metastasis.

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## 1. Introduction

Based on data from the American Cancer Society, in 2013 in the United States, the number of new cases of ovarian cancer was around 3% or 22,240 cases from 805,500 new cases of malignancy in female reproductive organs, and there were about 5% or 14,030 cases of which died from 273,430 cases of death due to cancer in women [1]. In 2015, the number of new cases of ovarian cancer was around 2.62% or 21,290 of 810,170 new cases of female reproductive organ malignancy, and about 5% or 14,180 cases of 277,280 cases of cancer deaths in women [2]. Ovarian cancer increases rapidly in women aged 50-55 years and slowly in women aged over 50 years [3]. In 2010 the Indonesian Cancer Foundation noted that there were 1156 cases of ovarian cancer and ranked eighth in the most common malignancies in Indonesia. In 2013, ovarian cancer ranked third after cervical and breast cancer [4]. More than 60% of new cases and around 70% of cancer deaths in the world each year occur in Africa, Asia, and Central and South America. It is estimated that annual cancer cases will increase from 14 million in 2012 to 22 million in the next two decades. Ovarian cancer metastasis is the spread of cancer cells to a place that is not directly related to the primary tumor. The path of spread or metastasis can occur through 3 pathways; direct seeding on the surface or cavity, lymphatic pathways, and hematogenous pathways. Ovarian cancer patients usually come in advanced stages (with metastasis) which have poor therapy outcomes and high mortality rates. Survivin plays a role in tumorigenesis through a variety of mechanisms, including angiogenesis and cell motility which have a role in invasion and metastasis. Based on these, we did a study to determine survivin expression with grade and event of metastasis in serous carcinoma as the most common type of ovarian cancer.

## 2. Methods

### *2.1. Case Selection*

This research is a cross-sectional study using paraffine blocks of serous carcinoma of ovary without and with metastasis to omentum in Anatomical Pathology Laboratories of DR. Wahidin Sudirohusodo Hospital, Hasanuddin University Hospital, and Sentra Diagnostik Patologia, Makassar from 2015-2018. Samples were grouped in low-grade non-metastasis, high-grade non-metastasis, low-grade metastasis, and high-grade metastasis based on WHO classification.

### *2.2. Survivin Immunohistochemistry*

Immunohistochemical staining used the standard method of protocol from Biocare using concentrated polyclonal survivin antibody with dilution 1:50. The results of immunohistochemical staining were scored manually using a light microscope by two pathologists. The interpretation of the score is calculated based on the staining intensity and percentage of the tumor cells using Allred-like score. (Score 0-1 = negative, 2-3 = weak positive, 4-5 = moderate positive, 6-7 = strong positive, and 8-9 = very strong positive).

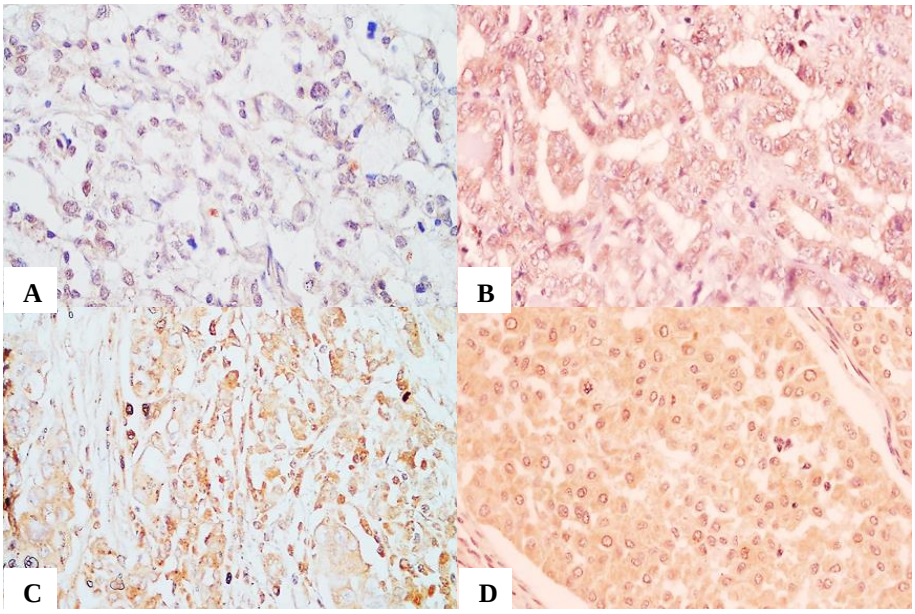


**Figure 1:** Positive Survivin Expression. (A) Weak, (B) Moderate, (C) Strong, (D) Very Strong. (400X)

### *2.3. Data analysis*

The data were analyzed statistically using the SPSS (Statistical Package for Social Sciences) version 20.0 (IBM Company). The relationship of survivin expression with the degree of histopathology and the incidence of metastasis to the omentum of serous carcinoma of ovary was analyzed with Oneway Anova and Chi-Square tests.

## 3. Results

There were 72 samples of serous carcinoma of ovary, consisted of 9 samples (12.5%) of low-grade non-metastasis, 15 samples (20.8%) of high-grade non-metastasis, 12 samples (16.7%) of low-grade metastasis, and 36 samples (50%) of high-grade metastasis. (Table 1) The samples' age was ranged from 23 to 72 years old with a mean and median 49 years old. (Table 2)

**Table 1:** General Characteristics

| Characteristics | | n | % |
|---|---|---|---|
| Serous Carcinoma of Ovary | Low-grade non-Metastasis (LGNM) | 9 | 12.5 |
| | High-grade non-Metastasis (HGNM) | 15 | 20.8 |
| | Low-grade Metastasis (LGM) | 12 | 16.7 |
| | High-grade Metastasis (HGM) | 36 | 50.0 |
| Survivin Expression | Weak | 4 | 5.6 |
| | Moderate | 10 | 13.9 |
| | Strong | 32 | 44.4 |
| | Very Strong | 26 | 36.1 |

**Table 2:** Age Characteristic

| | Mean | Median | Minimum | Maximum |
|---|---|---|---|---|
| Age (Years) | 49.25 | 49 | 23 | 72 |

Samples of serous carcinoma of ovary with low-grade non-metastasis, high-grade non-metastasis, low-grade metastasis, and high-grade metastasis had age range 44-67, 32-64, 28-68 and 23-72 years old with mean 54, 48, 47 and 49 years old. These data showed no significant difference in grade and event of metastasis with age (Oneway Anova Test; p = 0.375 (p> 0.05)). (Table 3)

**Table 3:** Comparison between Serous Carcinoma of Ovary's Grade and Event of Metastasis to Age Characteristic

| Age | Serous Carcinoma of Ovary | | | | p-value |
|---|---|---|---|---|---|
| | LGNM | HGNM | LGM | HGM | |
| Mean | 54.66 | 48.00 | 47.50 | 49.00 | 0.375* |
| Median | 53.00 | 47.00 | 47.50 | 49.50 | |
| Minimum | 44.00 | 32.00 | 28.00 | 23.00 | |
| Maximum | 67.00 | 64.00 | 68.00 | 72.00 | |

* Oneway Anova Test

Serous carcinoma of ovary had weak-strong positive survivin expression for low-grade non-metastasis, and high-grade non-metastasis, moderate-strong positive for low-grade metastasis, and strong-very strong positive for high-grade metastasis. Weak positive survivin expression was in non-metastasis (75% in low-grade and 25% in high-grade), and very strong positive was only in high-grade metastasis (100%). These data showed a significant difference of survivin expression with grade and event of metastasis (Chi-Square Test; p = 0.000 (p< 0.05)). (Table 4)

**Table 4:** Comparison between Survivin Expression with Serous Carcinoma of Ovary's Grade and Event of Metastasis

| Serous Carcinoma of Ovary | Survivin Expression | | | | | | | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | Weak | | Moderate | | Strong | | Very Strong | | |
| | n | % | n | % | n | % | n | % | |
| LGNM | **3** | **75** | **3** | **30** | **3** | **9.4** | 0 | 0.0 | 0.000* |
| HGNM | **1** | **25** | **6** | **60** | **8** | **25** | 0 | 0.0 | |
| LGM | 0 | 0 | **1** | **10** | **11** | **34.4** | 0 | 0.0 | |
| HGM | 0 | 0 | 0 | 0 | **10** | **31.3** | **26** | **100** | |

*Chi-Square Test

## 4. Discussion

Ovarian cancer usually occurs in women aged 45 to 65 years old. It is 3% of malignancies that occur in women and the fifth leading cause of death from cancer in the United States. According to The American Cancer Society, the number of new cases of ovarian cancer in 2013 in the United States was around 3% or 22,240 cases out of 805,500 new cases of cancer and 5% or 14,030 cases of them died from 273,430 cases of cancer deaths in women [1]. In 2015, the number of new cases of ovarian cancer was about 2.62% or 21,290 of 810,170 new cases and 5% or 14,180 cases of 277,280 cases of cancer deaths in women [2]. Based on research in histopathology, molecular and genetic, carcinogenesis of ovarian cancer is divided into 2 categories called type 1 and type 2. Low-grade serous carcinoma (type 1) is characterized by a lack of genetic mutations, mutations in ERBB2 occur in less than 5% of tumors. Mutations in KRAS and BRAF are found both in serous borderline tumors. This shows that KRAS and BRAF mutations occur early in tumorigenesis, even before the development of serous borderline tumors. Oncogenes such as KRAS, BRAF, and ERBB2 are regulators of mitogen-activated protein kinase (MAPK) and mutations in this gene result in the activation of the MAPK signal transduction pathway, which results in uncontrolled cell proliferation [5]. High-grade serous carcinoma (type 2) is usually found at an advanced stage and rapidly developed. At the molecular level, high-grade serous carcinoma shows TP53 gene mutations in 80% of cases and has a high Ki67 proliferation index (between 50-75%). Over-expression of HER2/neu is also found in 20-67% of cases, AKT activation in 12-30% of cases, and p16 inactivation in 15% of cases. About 10-15% of ovarian cancers are hereditary [5,6]. Table 2 shows that the average age of patients when diagnosed with ovarian cancer ranged from 23 years to 72 years with a median of 49 years. This is in line with epidemiological studies that state that ovarian malignancies occur in the fourth decade of a woman's life [8]. This can also be related to the incidence of menopause at that age. Menopause is defined as the permanent cessation of menstruation for 1 year and physically correlates with decreased estrogen secretion resulting from the loss of follicular function [9]. Menopause occurs in women aged between 45 and 55 years worldwide [10]. Table 4 shows a significant difference in survivin expression between groups with different grades and events of metastasis; low-grade non-metastasis, high-grade non-metastasis, low-grade metastasis, and high-grade metastasis. It indicates that survivin plays a role in ovarian cancer tumorigenesis and can be used as a prognostic biomarker. This supports the role of survivin in the process of carcinogenesis as apoptotic inhibitors, mitotic regulators, angiogenesis, and cell movement [11]. Based on the study conducted by Kurosaki and his colleagues that the level of survivin expression is highly correlated with the clinical stage, tumor stage, and histopathological grading which shows superior accuracy for detecting ovarian cancer [12]. The degree of histopathology is influenced by cell proliferation and mitotic activity. There are several pathways for the role of survivin in their effects on the carcinogenesis process in serous carcinoma of ovary, in this case especially in the high-grade group. In high-grade, it has very fast growth, a very aggressive tumor and has a high-grade nucleus, which is associated with its mitotic figure. High-grade cancer has characteristics of TP53 and genetic mutations that are very unstable [13]. Survivin is also able to perform the role of metastasis because of the process of angiogenesis. Metastasis is influenced by the ability of cells to invade, enter the bloodstream, adhesion, extravasation, growth, and angiogenesis. Based on the study conducted by Lv and his colleagues 2010, survivin triggering angiogenesis seems to be related to its ability to maintain the integrity of the microtubule structure and inhibit apoptosis in endothelial cells needed for viability and cytoprotective

endothelial cells. Alternatively, in tumors with few endothelial cells, survivin triggers VEGF synthesis/release encouraging vasculogenic-mimicry [14,15]. For ovarian cancer, the most common site of metastasis is omentum [16]. Based on the study conducted by Altieri and his colleagues 2014, Jaiswal 2015, and Di Palma and his colleagues 2013, survivin mRNA expression is associated with prognostic factors, such as the clinical stage of the tumor, the degree of histopathology or lymph involvement and this correlation is associated with the development of ovarian cancer. The moreover survivin expression, the worse the prognosis is [17,18,19,20] . In the high-grade metastasis serous carcinoma of ovary group, there were very strong positive survivin expressions, and in the non-metastatic low grade and non-metastatic high-grade group, there were weak positive expressions. This shows a significant relationship between survivin expression with the degree of histopathology and angiogenesis that is associated with the grade of histopathology and the incidence of metastasis [21,22].

## 5. Consluison

From the results of this study, it can be concluded as follows; the higher the expression of survivin, the higher the histopathological grade of serous carcinoma of ovary, and the higher the incidence of metastasis to omentum. So that survivin can be used as a prognostic biomarker, especially for high-grade metastasis serous carcinoma of ovary, which shows a very strong survivin expression.

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