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Caspase-3 expression correlation with cell differentiation grade, stage, and residual tumor size in epithelial ovarian cancer

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ABSTRACT

Background: Ovarian cancer has a high incidence and a high mortality rate, and often found in terminal stage. In ovarian malignancy, one of the important proteins presents in the apoptosis process is Caspase-3. Caspase-3 has an important role in modifying the cell morphologic and several biochemistry condition of the apoptosis process.

Objectives: Our study aims to analyze the correlation between Caspase-3 expression and the cell differentiation grade, the stage of the disease, and the residual tumor size after primary surgery in epithelial ovarian cancer.

Method: We conducted a cross-sectional study. The sample was recruited in Dr. Soetomo Hospital Surabaya. They were ovarian cancer patients with complete medical records, which ovarian tumor specimens were deparaffinized in the pathology anatomy laboratory, Faculty of Medicine, Airlangga University, Surabaya, Indonesia. Patients were excluded if they received a chemotherapy or a radiotherapy before the surgery, or their paraffin wax embedded tissue were damaged. The Caspase-3 expression was examined immunohistochemically. The correlation among the variables were analyzed using the Spearman and Phi correlation test.

Results: From 42 patients, Caspase-3 expression was positive in the 47.6%, while the 52.4% was negative. It was found that most samples with a good grade of cell differentiation showed a positive Caspase-3 expression ($r=-0.215$, $p<0.05$). Moreover, in most samples with the early stage of the disease had a positive Caspase-3 expression ($\varphi=-0.583$, $p<0.05$). In addition, most samples with residual tumor less than 1 cm in diameter, also showed a positive Caspase-3 expression ($\varphi=-0.336$, $p=0.029$).

Conclusion: A statistically significant negative correlation was present between Caspase-3 expression and the cell differentiation grade, the stage of the disease, and the residual tumor after the primary surgery.

Keywords: Caspase-3, Grade Of Cell Differentiation, Stage Of Disease, Residual Tumor

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INTRODUCTION

Ovarian cancer remains as a big health problem in the world and in Indonesia. It has a high incidence and a high mortality rate. In 2008, the global prevalence of ovarian cancer was 9.4%.[1,2] Based on Cancer Registry Agency of the Indonesian Ministry of Health record, which data were collated from 13 centers of pathology anatomy laboratories, the incidence of ovarian cancer was 4.9%.[3]

Several medical center in Indonesia reported the incidence as high as 19.6% to 35%.[3,4] In addition to the high incidence, ovarian cancer has the highest mortality rate among other gynecology malignancies. Although the prevalence of ovarian cancer is the third highest, which comes after cervical and breast cancer, this malignancy is the most prevalent cause of death in gynecology malignancy cases.

There are many prognostic factors influencing the outcome of the ovarian cancer patient, i.e. the stage, the degree of cell differentiation and the residual tumor size after a primary operation. As much as 70-75% of cases diagnosed in the terminal stage, contributing to the high mortality rate. The 5-year survival rate is 20-30%. But, when diagnosed in the early stage (first stage), the 5-year survival rate is 90-95%.[5] The degree of cell differentiation is evaluated using a microscope to determine the morphology characteristic, the aggressivity or the biologic behavior of the cancer cell. The residual tumor size is related to the clinical response to chemotherapy and the survival rate.[6]

Numerous research was conducted around the molecular biology aspects of ovarian cancer, especially about the apoptosis process. One of the proteins performing a major role in apoptosis process is Caspase-3. It presence in an apoptosis process is activated by an extrinsic or an intrinsic pathway. Caspase-3 plays a role in the cell morphologic change and in various biochemistry related to apoptosis process.[7] This research aimed to discover the correlation between the Caspase-3 expression

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and the degree of cell differentiation, the stage and the tumor residue in epithelial ovarian cancer.

METHODS

We conducted a cross-sectional study in August 2012 to March 2013. The sample was the ovarian cancer patients whose ovarian tumor specimen was deparaffinized in the pathology anatomy laboratory of Dr. Soetomo Hospital in Surabaya, East Java, Indonesia. The inclusion criteria were: (1) the patient's histopathology examination (paraffin specimen) showed an epithelial ovarian cancer, (2) the patient has a complete medical record. The exclusion criteria were: (3) received a chemotherapy or a radiotherapy before a specimen was taken, (2) the paraffin specimen was found damaged.

The Caspase-3 expression was examined using an immunochemistry technique by utilizing *Novocastra*™ Lyophilized Mouse Monoclonal Antibody CPP32 (Caspase-3) staining produced by *Leica Biosystem* Newcastle Ltd.-United Kingdom. The Caspase-3 expression interpretation was done semi-quantitatively by counting the percentage of the positive malignant cells in 200 malignant cells, using an Olympus binocular light microscope in 400x magnification. The staining was positive if the nucleus and or the cytoplasm becomes brown. The scoring was based on the percentage of the stained nucleus and or the cytoplasm. The scoring criteria were: 0 if there was no stained nucleus and or cytoplasm, +1 if 1 to 10% of the nucleus or the cytoplasm was stained, +3 if there was more than 10% of the nucleus or the cytoplasm stained.[8] The Caspase-3 expression was categorized into negative for the 0 and the +1, and claimed as positive if it scored +2 or +3.[8]

The ovarian cancer degree of differentiation was classified according to Shimizu-Silverberg grading system. The scoring is based on the cell component (glandular, papillary, or solid), the degree of atypical nucleus (mild, moderate, severe), and the mitotic index per 10 HPF (0-9, 10-24, and ≥25). The score from each component was then summarized as a total score to determine the degree of differentiation.[9] The grading system consists of 3 degrees of differentiation: 1st degree or a good differentiation if the score 3-5, 2nd degree or a moderate differentiation if the score 6-7, and 3rd degree or a poor differentiation if the score 8-9.[10-12]

The primary surgery result and the metastatic finding in the patient medical record used the ovarian cancer staging based on the International Federation of Gynecology and Obstetrics (FIGO) 2008 classification. The stages are: early stage for stage I and II, and advance stage for stage III and IV. The residual tumor size is the largest diameter of the tumor left after the primary surgery.

The criteria of the tumor residue size are: optimal if the residual tumor diameter is less or the same as 1 cm, and suboptimal if the residual tumor so more than 1 cm.[13] The Spearman correlation test and Phi association test was conducted to examine the statistical correlation between the variables.

RESULTS

There were 42 patients included in our study. The youngest was 25 and the oldest was 70, with average age of 47.5±9.6 years.

In 47.6% of the sample, the Caspase-3 expression was positive, while the rest was negative (52.4%).

Table 1 Characteristics Based on Age, Parity, Histopathology, Degree of Differentiation, Disease Stage, Tumor Residual Size

Characteristics	f	%
Age (in year)		
21 – 30	4	9.5
31 – 40	4	9.5
41 – 50	15	35.7
51 – 60	17	40.5
> 60	2	4.8
Parity		
0	14	33.3
1	11	26.2
2	10	23.8
3	5	11.9
4	1	2.4
>4	1	2.4
Histopathology		
Endometrioid	14	33.3
Serous	18	42.9
Mucinous	8	19.1
Clear-cell	2	4.7
Degree of Differentiation		
Good	13	30.9
Moderate	7	16.7
Poor	22	52.4
Ovarian cancer stage		
Early	15	35.7
Advance	27	64.3
Tumor residual		
≤ 1 cm	22	52.4
> 1 cm	20	47.6

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Table 2 Distribution Based on Caspase-3 Expression and The Cell Differentiation

Cell degree of differentiation	Caspase-3 expression		Total f (%)
	Negative f (%)	Positive f (%)	
1	4 (9.5)	9 (21.4)	13 (30.9)
2	5 (11.9)	2 (4.8)	7 (16.7)
3	13 (30.9)	9 (21.4)	22 (52.4)
Total f (%)	22 (52.4)	20 (47.6)	42 (100)

Spearman correlation test; r=-0.215; p=0.017

Table 3 Distribution Based on Caspase-3 Expression and Staging

Stage	Caspase-3 expression		Total f (%)
	Negative f (%)	Positive f (%)	
Early	2 (4.8)	13 (31.0)	15 (35.7)
Advance	20 (47.6)	7 (16.7)	27 (64.3)
Total (%)	22 (52.4)	20 (47.6)	42 (100)

Phi association test; φ=-0.583; p<0.001

Table 4 Distribution Based on Caspase-3 Expression and Residual Tumor Size

Residual Tumor Size	Caspase-3 Expression		Total f (%)
	Negative f (%)	Positive f (%)	
≤ 1 cm	8 (19.0)	14 (33.3)	22 (52.4)
> 1 cm	14 (33.3)	6 (14.3)	20 (47.6)
Total (%)	22 (52.4)	20 (47.6)	42 (100)

Phi association test; φ=-0.336; p=0.029

DISCUSSION

The Characteristics

The most frequent age in our study was 51 to 60 years old (40.5%). The result is similar to the data presented by the Surveillance, Epidemiology, and End Results (SEER) of the U.S. National Cancer Institute (NCI), which stated that the most ovarian cancer proportion (23.4%) in 2005-2009 was at the 55 to 64 years old.[2] Moreover, a research in Thailand showed a peak incidence at 45-60 years old.[14] The average age in this study was 47.5±9.6 years old. Our study confirmed many literatures, showing the increase of ovarian cancer incidence parallel to the increase of age. Ovarian cancer rarely occurs in patients under 40.[15] The peak incidence of ovarian cancer is at 50 years old, and increases towards the 70. But, it decreases towards 80 years old.[16]

The parity in this study were 0-5. Most proportion of ovarian cancer with nulliparity was 33,3%. There was a tendency showing that a higher parity will impact to low risk incidence ovarian cancer. In this study, the lowest proportion at parity 4 and more than 4, respectively 2.4%. Study by Rivas-Corchado LM, et al found 25% ovarian cancer case from 40 patients in their study were 0 parity.[17] Parity is one of risk factors that increase the incidence of pregnancy. Ovarian cancer incidence decrease parallel to the increase of parity.[15] Multiparity related to decrease of risk of ovarian cancer, whereas have relative risk about 0.6-0.8.[18]

From 42 samples, the most frequent type was serous carcinoma (42.9%), followed by endometrioid (33.3%), mucinous (19.1%), and the fewest was clear cell (4.7%). Studies found the most histopathology type was serous at about 25%, followed by endometrioid and mucinous around 20%.[17] Other study also found serous ovarian cancer as the most frequent type. The clear cell, Brenner, and undifferentiated carcinoma was found in less than 1%.[19]

Based on the degree of cell differentiation, the most frequent was the poor differentiation, about 52.4%. This result is similar to a study by Hoskin WJ, et al.[20] While other study found the differentiation from the most frequent to the least: moderate, poor, and good differentiation.[21]

Our study shows 64.3% was in advance stage and 35.7% was an early stage ovarian cancer. This result is similar to many research, that found advance stage ovarian cancer in stage III 57.28%, and stage IV 56.2%.[22] ACOG Committee Opinion, in 2002, reported 70-75% ovarian cancer diagnosed in the advance stage. When found in the advance stage, the five years' survival rate was 20-30%. But, if the case found in the early stage, the five years' survival rate was 90-95%.[23]

Based on the size of the tumor residual after the primary operation, our study had 52.4% with residual ≤ 1cm and 47.6% with residual >1cm. A prospective and multicenter study involved 226 patients' ovarian cancer epithelial type found minimal residual tumor for about 87.6%.[24]

Correlation between Caspase-3 expression and degree of cell differentiation

Our study found negative relation between Caspase-3 expression and ovarian cancer degree of cell differentiation (r=-0.215, p<0.05). Duo and Tong reported a similar result, where Caspase-3 expression correlated with cancer cell differentiation and could be used to determine the cancer prognosis.[25] Caspase-3 is the main Caspase executioner among other Caspase executioner, like Caspase-6 and Caspase-7. Caspase-3 activated many nucleus substrates, such as Lamin A, Actin, Gas2, and α-fodrin, which cause a cell shrinkage and an irregularity of the cell membrane. Caspase-3 activated CAD which is cause DNA fragmentation.[26] These protein activities cause morphologic

and biochemistry change in the cell apoptotic process, i.e. the cell shrunk, condensed protein, DNA chromosome fragmented, nuclei degraded, which includes cytoskeleton protein and cell disintegration become apoptosis bodies.[27]

Cells show apoptotic process better if they give positive Caspase-3 expression than cells that do not express Caspase-3. An uncontrolled proliferating cell caused by gene abnormality will be apoptotic if the cell repair process ineffective. A good apoptotic process expresses a positive Caspase-3, and it will degrade the abnormal cell as an effort to maintain homeostasis. But, if the apoptotic mechanism is ineffective, which Capcase-3 expression is negative, the abnormal cell will proliferate uncontrollably. A histology examination will find atypical cells and many mitotic, as a result of poor cell differentiation.[28]

Correlation between Caspase-3 expression and ovarian cancer stage

The study found negative association between Caspase-3 expression and ovarian cancer stage (φ=-0.583; p<0.05). Other studies also shown similar result, whereas Caspase-3 expression significance related to ovarian cancer stage.[26] As Caspase-3 in an executioner in the apoptotic process, positive Caspase-3 expression show apoptotic process in good function, and it related to early stage. Nonetheless, if negative Caspase-3 expression, the apoptotic process is disrupted. The disruption of apoptotic protein, like Caspase- 3, will disrupt homeostatic mechanism and induced uncontrolled proliferation process, leading to advance stage of disease.[28]

Correlation between Caspase-3 and tumor residual

This study found negative association which statistically significance between Caspase-3 expression and tumor residual (φ=-0.336; p=0.029). We have not found another study in Caspase-3 and tumor residual correlation to the epithelial ovarian cancer. The residual tumor diameter determines the response of the disease to adjuvant therapy and survival rate.[7] Each 10% increase of cito reduction related to 5.5% increase of median survival. It is important to achieve an optimal cito reduction to about 20-90%.[29] The success rate of the debulking procedure depends on many factors, such as the operation technique, anesthesiology technique, post operation treatment, chemotherapy regiment and patient education level.[30] Beside those factors, mainly maximal cito reduction procedure and tumor biological factors play important role to predict the size of the residual tumor.[31]

Caspase-3 plays an important role in the ovarian cancer carcinogenesis. Caspase-3 is a Caspase executioner the Caspase pathway to maintain the apoptotic process. Inactive Caspase-3 in the form of negative Caspase-3 expression causes a gene abnormality and a failure to repair, which continues to an uncontrolled proliferation process.[32] Caspase-3 expressions is related to the degree of cell differentiation and the stage of ovarian cancer. Samples with negative Caspase-3 expression had poor cell differentiation and advance stage ovarian cancer. It showed cancer with negative Caspase-3 expression had a high aggressive behavior. A poorer degree of cell differentiation results in an advance stage of ovarian cancer and commonly left a larger tumor residual after the debulking procedure.

CONCLUSION

There was statistically negative correlation between Caspase-3 expression and the degree of cell differentiation, the stage and size of the residual tumor after the primary operation of epithelial ovarian cancer.

REFERENCES

1. Ferlay, J., Shin, H., R., Bray, F., Forman, D., Mathers, C., Parkin, D., M. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer* 2010; 127:2893-917.
2. Jemal, A., Bray, F., Center, M., M., Ferlay, J., Ward, E., Forman, D. Global cancer statistics. CA *Cancer J Clin* 2011;61(2):69-90.
3. Aziz, M., F. Gynecological cancer in Indonesia. *J Gynecol Oncol* 2009;20(1-8):8-10.
4. Karyana, K. Profil penderita kanker ovarium di RS Sanglah Denpasar [tesis]. Denpasar: Bag/SMF Obstetri dan Ginekologi FK UNUD/RS Sanglah Denpasar; 2005.
5. The American College of Obstetricians and Gynecologist. The role of the Obstetrician-Gynecologist in the early detection of epithelial ovarian cancer. Committee Opinion number 477. March 2011:1-5.
6. Diaz-Montes, T., P., Bristow, R. Clinical predictors of outcome in epithelial ovarian carcinoma. Dalam: Levenback, C., F., Sood, A., K., Lu, K., H., Coleman, R., L., penyunting. Prognostic and predictive factors in gynecologic cancers. United Kingdom: Informa Healthcare; 2007. p.3-11.
7. Vranic, A. Caspase-3 and surviving expression in primary atypical and malignant meningiomas. ISRN Neuroscience 2013; 2013:1-5.
8. Yang, C., Ionescu-Tiba, V., Burns, K., dkk. The role of the cyclin D1-dependent kinases in ErbB2-mediated breast cancer. American Journal of Pathology 2004;164(3):1031-8.
9. Fan, T., J., Han, L., H., Ceng, R., S., Liang, J. Caspase family proteases and apoptosis. Acta Biochimica et Biophysica Sinica 2005;37(11):719-27.
10. Vang, R., Shih, I., M., Kurman, R., J. Ovarian low-grade and high-grade serous carcinoma: pathogenesis, clinicopathologic, and molecular biologic features and diagnostic problems. Adv Anat Pathol 2009; 16:267-82.
11. Tavassoli, F., A., Devilee, P. Tumours of the Breast and Female Genital Organs. World Health Organization Classification of Tumors. Lyon, France: IARC Press; 2003. p.114.
12. Shimizu, Y., Kamoi, S., Amada, S., Akiyama, F., Silverberg, S., G. Toward the development of a universal grading system for ovarian epithelial carcinoma: testing of a proposed in a series of 461 patients with uniform treatment and follow-up. Cancer 1998; 82:893-901.

13. Berek, J.S., Natarajan, S. Ovarian and Fallopian Tube Cancer. In: Berek, J.S., editor. *Berek & Novak's Gynecology*. 14th. Ed. Philadhelpia: Lippincott William & Wilkins; 2007. p. 1457-1548.
14. Wilailak, S. Epidemiologic report of gynecological cancer in Thailand. *J Gynecol Oncol* 2009;20(2):80-3.
15. Whittemore, A., S., Harris, R., Itnyre, J. Collaborative ovarian cancer group: characteristics relating to ovarian cancer risk, collaborative analysis of 12 US case-control studies II invasive epithelial ovarian cancer in white women. *Am J Epidemiol* 1992;136(10):1212-20.
16. Yancik, R., Ries, L., G., Yates, J., W. Ovarian cancer in the elderly: an analysis of Surveillance, Epidemiology, and End Results Program data. *Am J Obstet Gynecol* 1986;154(3):639-47.
17. Rivas-Corchado, L., M., Gonzales-Geroniz, M., Hernandez-Herrera, R., J. Epidemiological profile of ovarian cancer. Ginecol Obstet Mex 2011;79(9):558-64.
18. Pelucchi, C., Galeone, C., Talamin, R., dkk. Lifetime ovulatory cycles and ovarian cancer risk in two Italian case-control studies. *Am J Obstet Gynecol* 2007;196(1):831-7.
19. Scully, R., E., Young, R., Clemet, P., B. Tumor of the ovary, maldeveloped gonad, and broad ligament. Dalam: Atlas of tumor pathology. 3rd series, fascicle 23. Washington DC: Armed Forces Institute of pathology; 1998. p.51-168.
20. Hoskin, W., J., McGuire, W., Brady, M., F., dkk. The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma. *Am J Obstet Gynecol* 1994; 170:974-80.
21. Sorbe, B., Frankendal, B., Veress, B. Importance of histologic grading in the prognosis of epithelial ovarian carcinoma. *Obstet Gynecol* 1982;59(5):576-82.
22. Zheng, Q-Q., Wong, P., Hui, R., Yao, A-M. Prognostic analysis of ovarian cancer patients using the Cox regression model. *Chinese Journal of Cancer* 2009;28(2):170-2.
23. The American College of Obstetricians and Gynecologist. The role of the generalist Obstetrician-Gynecologist in the early detection of ovarian cancer. Committee Opinion number 280. 2002:1-5.
24. Polterauer, S., Vergote, I., Concin, N., dkk. Prognostic value of residual tumor size in patients with epithelial ovarian cancer FIGO stages IIA-IV: analysis of the OVCAD data. Int *J Gynecol Cancer* 2012;22(3):380-5.
25. Duo, Y., Tong, L. Expression of Caspase-3 and Bcl-2 Protein in Epithelial Ovarian Tumor and Relation of the Expression with Cell Apoptosis and Proliferation. China Journal of Modern Medicine 2004; 8:8-15.
26. Porter, A., G., Janicke, R., U. Emerging roles of Caspase-3 in apoptosis. Cell Death and Differentiation 1999; 6:99-104.
27. Rastogi, R., P., Richa, Sinha, R., P. Apoptosis: Molecular mechanisms and pathogenicity. *EXCLI Journal* 2009; 8:155-81.
28. Wei, C., Ping, P. Expression and clinical significance of xiap and Caspase-3 protein in primary epithelia ovarian cancer. *Chinese Journal of Cellular and Molecular Immunology* 2010; 7:7-16.
29. Wakabayashi, M., T., Lin, P., S., Hakim, A., A. The role of cytoreductive/debulking surgery in ovarian cancer. J Natl Compr Canc Netw 2008;6(8):803-10.
30. Manipalviratn, S., Worasethsin, P., Triratanachat, S., Tresukosol, D. Impact of residual tumor on survival of patients with advanced stage common epithelial ovarian cancer at King Chulalongkorn Memorial Hospital from 1995 to 1999. *Thai Journal of Obstetrics and Gynaecology* 2002; 14:269-76.
31. Winter III, W., E., Maxwell, L., Tian, C., dkk. Tumor residual after surgical cytoreduction in prediction of clinical outcome in stage IV epithelial ovarian cancer: A Gynecologic Oncologic Group Study. *J Clin Oncol* 2008;26(1):83-9.
32. Fan, T., J., Han, L., H., Ceng, R., S., Liang, J. Caspase family proteases and apoptosis. Acta Biochimica et Biophysica Sinica 2005;37(11):719-27.