Research Article

Expression of B-Cell Lymphoma Protein-2 (Bcl-2) and Caspase-3 are Related with Ovarian Cancer

Ekspresi B-Cell Lymphoma Protein-2 (Bcl-2) dan Caspase-3 Berhubungan dengan Kanker Ovarium

I Nyoman G Budiana

Department of Obstetrics and Gynecology
Faculty of Medicine University of Udayana/
Sanglah General Hospital
Denpasar

Abstract

Objective: To determine the expression of Bcl-2 and caspase-3 and their relationship with ovarian cancer.

Method: This study was a cross-sectional study. Speciment was tissue sample from ovarian cancer patients collected from parafin-block to determine the Bcl-2 and caspase-3 expression and demographic data obtained secondary from patients medical records. Expression of Bcl-2 and caspase-3 was examined by immunohistochemistry under light microscope with 400x light power field. The result was recorded as negative when the protein was expressed in 10% or less of cells and as a positive when the protein was expressed in more than 10% of cells.

Result: A total of 45 samples was included as research subjects. 31 of 45 subjects showed the expression of Bcl-2 positive (68.9%), while the positive expression of caspase-3 was presented in 20 subjects (44.4%). There was a relationship between the expression of Bcl-2 with the expression of caspase-3 in ovarian cancer patients (p=0.002; Lambda=0.4). There was also a negative relationship, where the subjects with positive expression of Bcl-2 showed negative expression of caspase-3. In contrast, subjects with negative expression of Bcl-2 showed positive expression of caspase-3.

Conclusion: There was a significance relationship between the expression of Bcl-2 with the expression of caspase-3 in ovarian cancer patients.

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Keywords: Bcl-2 expression, caspase-3 expression, ovarian cancer

Abstrak

***Tujuan**: Untuk mengetahui ekspresi Bcl-2 dan caspase-3 serta hubungan di antara keduanya pada kanker ovarium.*

***Metode**: Rancangan penelitian adalah potong lintang. Preparat dari parafin blok pasien-pasien kanker ovarium diperiksa untuk mengetahui ekspresi Bcl-2 dan caspase-3 serta data-data demografi diperoleh dari catatan medik pasien. Ekspresi Bcl-2 dan caspase-3 diperiksa secara imunohistokimia dengan mikroskop cahaya pada pembesaran 400x. Ekspresi negatif bila jumlah sel yang tercatat sebanyak 10% atau kurang. Ekspresi positif bila jumlah sel yang tercatat lebih dari 10%.*

***Hasil**: Sebanyak 45 subjek diikutsertakan dalam penelitian. Tiga puluh satu dari 45 subjek memperlihatkan ekspresi Bcl-2 positif (68,9%) dan 20 subjek memperlihatkan ekspresi caspase-3 positif (44,4%). Terdapat hubungan antara ekspresi Bcl-2 dengan ekspresi caspase-3 pada kanker ovarium (p=0,002; Lambda=0,4). Juga terdapat hubungan negatif, di mana subjek dengan ekspresi Bcl-2 positif memperlihatkan ekspresi caspase-3 negatif, sebaliknya subjek dengan ekspresi Bcl-2 negatif memperlihatkan ekspresi caspase-3 positif.*

***Kesimpulan**: Terdapat hubungan yang bermakna antara ekspresi Bcl-2 dengan ekspresi caspase-3 pada kanker ovarium.*

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***Kata kunci**: ekspresi Bcl-2, ekspresi caspase-3, kanker ovarium*

Correspondence: I Nyoman Gede Budiana. Department of Obstetrics and Gynecology, Sanglah General Hospital/Faculty of Medicine University of Udayana, Denpasar. Telephone: (0361) 223868, Email: b_d_na@yahoo.com

INTRODUCTION

Over the past three decades, ovarian cancer remains a major problem of women's health in the world, including Indonesia. It is associated with high morbidity and mortality that is caused by cancer. In the world, the incidence of ovarian cancer in 2008 was 9.4%.[1,2] The incidence rate ranks 7th among cancers in women after breast cancer, colorectal, cervical, lung, stomach, and corpus uteri. Incidence rate of ovarian cancer ranks 3rd among gynecologic cancers after breast and cervical cancer. In some countries, reported that the incidence of ovarian cancer varied. In 2008, the number of ovarian cancer cases in the United States is 21,650 cases[2] and in the UK is 6,500 cases.[3] While in Europe varies between 12 per 100,000 women in Southern Europe and 19 per 100,000 women in Northern Europe in 2008.[4]

In Asia, the incidence of ovarian cancer is generally lower than the population in Europe and

North America. In Japan, the incidence of ovarian cancer is increased since 1970, but still lower than western countries.[5] Ushijima (2009) reported the incidence of ovarian cancer in Japan increased after the age of 60 and became 10 per 100,000 women.[6] In Indonesia, the incidence rate of ovarian cancer is uncertain. According to the report of Cancer Register Board Indonesian Ministry of Health that is obtained from 13 Central Pathology Laboratory in Indonesia showed that the proportion of ovarian cancer is 4.9%.[7] Reports from some teaching hospital in Indonesia showed that the proportion of ovarian cancer ranged from 32.5%[8] to 35%[9].

In addition to the high incidence rate, mortality rate of ovarian cancer is also high among gynecologic cancers. In the world, the rate of mortality of ovarian cancer in 2008 is 5.1%.[2] The most important factor that influence the high mortality rate of ovarian cancer is 70-75% of cases diagnosed at an advanced stage even terminal where 5-year survival rate is 20 - of 30%. However, when found in stage I, the 5-year survival rate reach 90-95%.[10] Thus, although the incidence of ovarian cancer was on the 3rd place, but the cancer is the number one cause of death among gynecological cancers.

The difficulty of finding ovarian cancer at an early stage related to the difficulty of finding the accurate screening and early detection methods. There are many effort in early diagnosis of ovarian cancer, but until now there have not been found a satisfactory method. Screening modality such as ultrasound, tumor markers CA-125, α-fetoprotein, and other efforts have not been able to reduce the incidence and mortality rate of ovarian cancer. Similarly, several attempts therapy such as surgery, chemotherapy, and radiation, as monotherapy or combination has not been given satisfactory results. On the other hand, knowledge and research in the field of molecular biology is become more advanced. Treatment of ovarian cancer through the understanding of the carcinogenic mechanisms become more promising in the future.

One mechanism to control cell growth is a process of programmed cell death or apoptosis. This mechanism is a complex process and involves a variety of proteins, one of them is the protein Bcl-2 and caspase-3. Bcl-2 proteins work against the p53 tumor suppressor protein that disrupt cell cycle regulation. The cells will undergo proliferation and resistance to stimulation that would normally lead to cells death.[11] Several studies have reported that expression of Bcl-2 in ovarian cancer was significantly higher than in benign tumor.[12] Meanwhile, caspase-3 protein is one of the 14 caspase that is known by human.[13] Caspase-3 acts as an apoptosis executor and cell death due to specific stimuli. In addition, caspase-3 plays an important role in the changes in cell morphology and biochemical events associated with the implementation and complete the process of apoptosis.[14] There is a significant difference in the expression of caspase-3 in epithelial ovarian cancer, borderline ovarian tumors, benign ovarian tumors, and normal ovarian tissue. Caspase-3 is also a factor of poor prognosis in epithelial ovarian cancer.[15]

Studies of oncogenes, tumor suppressor genes and proteins involved in the process of apoptosis in ovarian cancer have been carried out. However, most research only focused on a single gene, and in high-risk families so that the results are less representative in extrapolation. Protein expression of Bcl-2 and caspase-3 are known to differ in malignant ovarian tumors, boderline, benign, and normal ovarian cells. However, no one has studied the relationship between the expression of these proteins in ovarian cancer. The relationship between protein Bcl-2 and caspase-3 is involved in apoptosis process will contribute to the role of both proteins in ovarian cancer carcinogenesis. In the future, a growing number of proteins are known to play a role in the carcinogenesis of ovarian cancer, there are more effort in screening method, early diagnosis, and genetic therapies that can be applied to reduce the incidence and mortality rate of ovarian cancer.

METHODS

This study is an observational study with cross-sectional design. Subjects were patients with ovarian cancer tissues obtained from laparotomy surgery in Sanglah Hospital in Denpasar, which is based on histopathologic examination that is known as malignant ovarian tumors (ovarian cancer). Ovarian cancer classification refers to the classification of WHO in 2003.[16] Ovarian tumors tissue that have parrafined and stored in Laboratory of Pathology Anatomi, Faculty of Medicine, Udayana University/Sanglah Hospital in Denpasar, which was diagnosed with ovarian cancer based on histopathological examination, was selected as research subjects. Inclusion criteria were: a block of paraffin was examined histopathologically, so it

was definitely diagnosed as ovarian cancer, the patient's medical records can be found, and the necessary data is complete. While the exclusion criteria were: patients who had been given chemo/radiotherapy preoperatively, the tissue was damaged due to various technical reasons, the patient's medical records could not be found or the necessary data was not complete.

Ovarian cancer tissue that has been prepared into paraffin block then processed for immunohistochemical examination of Bcl-2 and caspase-3 protein. Epidemiological data of patient is obtained secondary from patients medical records that is recorded on the data collection sheet. Examination of protein expression of Bcl-2 and caspase-3 was performed by immunohistochemistry. Bcl-2 protein expression examined using monoclonal antibody staining methode specific primary monoclonal mouse anti-human Bcl-2 protein clone 124, while caspase-3 protein expression examined using biotin-avidin indirect staining of primary mouse monoclonal antibody (Triton, Alameda, CA). Protein expression of Bcl-2 and caspase-3 was assessed semiquantitatively under light microscope with 400x light power light. Expression positive if protein was expressed in more than 10% of cells, whereas expression negative if protein was expressed in 10% or less of cells.

RESULTS

We examined the expression of Bcl-2 and caspase-3 and the complete of patient's medical record in 45 subjects. Of the 45 subjects, the youngest age was 19 years old, the oldest was 70 years old, the median age was 43 years old with mean age of subjects was 41.3 ± 13.7 years. The most common age group patients with ovarian cancer is over 50 years old (24.4%).

Table 1. Characteristics of Subjects.

Characteristics	Total (%)
Age group (years)	
• ≤ 20	1 (2.2)
• 21 - 25	7 (15.6)
• 26 - 30	4 (8.8)
• 31 - 35	7 (15.6)
• 36 - 40	1 (2.2)
• 41 - 45	7 (15.6)
• 46 - 50	7 (15.6)
• > 50	11 (24.4)
Parity	
• 0	22 (48.9)
• 1	5 (11.1)
• 2	8 (17.8)
• 3	4 (8.9)
• 4	2 (4.4)
• ≥ 4	4 (8.9)
Histopathological type	
• Musinus	3 (6.7)
• Endometrioid	3 (6.7)
• Serus	26 (57.8)
• Seromukus	1 (2.2)
• Clear-cell	1 (2.2)
• Germ-cell	6 (13.3)
• Teratomaimmature	2 (4.4)
• Granulosa cell tumor	3 (6.7)
Stage of disease	
• IA	4 (8.9)
• IB	1 (2.2)
• IC	14 (31.2)
• IIC	1 (2.2)
• IIIB	9 (20.0)
• IIIC	10 (22.2)
• IV	6 (13.3)
Bcl-2 expression	
• Positive	31 (68.9)
• Negative	14 (31.1)
Caspase-3 expression	
• Positive	20 (44.4)
• Negative	25 (55.6)
Total	45 (100)

Relationship between Expression of Bcl-2 with Expression of Caspase-3

Table 2 shows the relationship between the expression of Bcl-2 with the expression of caspase-3 (p=0.002; Lambda=0.4). There are negative relationship also, where the subjects with positive expression of Bcl-2 showed negative expression of caspase-3. In contrast, subjects with negative expression of Bcl-2 showed positive expression of caspase-3. Based on stage of disease, the expression of Bcl-2 positive is increasing along with the stage of disease (p=0.002; Lambda=0.1). Meanwhile, subject with positive expression of caspase-3 is getting higher on early-stage disease, in contrast

to subject with advanced stage of disease showing negative expression of caspase-3 (p=0.000; Lambda=0.3).

Table 2. Relationship between Expression of Bcl-2 with the Expression of Caspase-3.

		Expression of caspase-3		Total	
		Negative	Positive		
Expression of Bcl-2	Positive	22	9	31	p=0.002
	Negative	3	11	14	Lambda=0.4
Total		25	20	45	

DISCUSSION

In this study we found the proportion of ovarian cancer on patients aged 20 years old was 2.2%, with the largest proportion were in the age group over 50 years old. These results are similar with the data from SEER cancer statistic review reporting that the proportion of ovarian cancer at the age under 20 years old was 1.3%. While the largest proportion occurred in the age group of 55-64 years old were as many as 23.4%.[17]

These results are consistent with the reports in the published literature that stated the incidence of ovarian cancer is increased along with the age. Ovarian cancer is very rarely occurred under 40 years old.[18] Peak incidence of ovarian cancer occurs at age 50 years old, and increasing gradually until 70 years old, then decline after 80 years old.[19]

Parity in this study ranged from 0-9. The largest proportion of patients with parity 0 is 48.9%. There is a trend of the larger parity proportion, the lower incidence of ovarian cancer, where parity that is more that 4 is as many as 8.9%.

Research by Rivas-Corchado LM et al found that from 40 ovarian cancer patients, 25% occurred in patients with parity 0.[20] Several studies have found the risk of epithelial ovarian cancer is higher in women with higher social economy status. This is related with only a few of those women have childrends.[21] Parity is a factor that increases the risk of ovarian cancer. The risk of ovarian cancer is decreased along with the increasing number of pregnancy.[18] Multiparity is associated with a reduced risk of ovarian cancer.[22]

Protective effect against the development of ovarian cancer such as multiparity, supports the concept of incessant ovulation which is a contributing factor in the development of ovarian cancer. This concept was first proposed by Fathalla.[23] The risk of ovarian cancer is associated with the number of ovulatory cycles. When ovulation occurs, the epithelium surface was damaged. The epithelial damage stimulates epithelial cells to undergo proliferation as an attempt to repair. At the time of ovulation, invagination of epithelial surface into the stroma and form inclusion cyst also occured.[18] Subsequent researchers found that the process that involved in the repairment of damaged ovarian epithelial surface that caused by traumatic ovulation particularly epithelial that cover inclusion cyst under the influence of oncogenic factors somehow will turn to malignancy. The larger number of lifetime ovulatory cycles in women, the higher her risk of developing epithelial ovarian cancer.[24,25]

From 45 subjects, the commonest histopathologic type was serous (57.8%). While the rare of histopathologic type is clear-cell type and seromucous 2.2%, respectively.

Research by Rivas-Corchado LM, et al suggested that the commonest histopathological type of ovarian cancer is serous, accounted for 25%, followed by endometrioid and mucinous types (20% for each type) and granulosa cell types (7%).[20] In other literature ovarian cancer serous type remains the commonest, accounted for 75%, followed by mucinous (20%) and endometrioid (2%). While the clear-cell, Brenner, and undifferentiated carcinoma are found to be less than 1%, respectively.[16]

Stage of Disease

Based on the stage of disease, from 45 subjects, there are no subjects with stage IIA, IIB, and IIIA. Stage IC has the largest proportion (31.2%). In contrast with the results of study by Rivas-Corchado LM, et al that found most cases in stage IA (32%). But cumulatively, the number of cases with advanced stage (stage III and IV) remains higher (55.5%) compared with early stage (stage I and II), which is only 44.5%.[20]

Report from ACOG Committee Opinion in 2002 found that as many as 70-75% of ovarian cancer cases are diagnosed at advanced stage.[10] This is an important factor that affect the high mortality rate

of ovarian cancer, where the 5-year survival rate at advanced stage is 20 - of 30%. However, when found in stage I, the 5-year survival rate reach 90-95%.[10]

Relationship between Bcl-2 and Caspase-3 Expression

In this research, the rate of positive expression of Bcl-2 in ovarian cancer was 68.9%, while the rate of negative expression of Bcl-2 was 31.1%. The similar results also obtained in a cohort study with 95 advanced ovarian cancer patients (stage IIIC-IV) that found the results of immunohistochemical examination toward positive protein Bcl-2 rate of 69.5%.[26] While other studies that involving 71 ovarian tumors serous, including 29 benign ovarian tumors, 14 borderline ovarian tumors, and 28 malignant ovarian tumors found the positive expression of Bcl-2 17.2%, 35.7%, and 25%, respectively.[27]

Meanwhile, the positive expression of caspase-3 in this study is 44.4%, with the negative expression of caspase-3 is 55.6%. These results are similar with results of other studies, which examine the expression of caspase-3 by immunohistochemistry in 16 cases of benign ovarian tumors and 84 cases of ovarian cancer. Postive expression of caspase-3 is 93.4% in benign ovarian tumors and 48.8% in cancer ovarium.[28] Likewise, research on 112 cases of primary ovarian tumor, found positive caspase-3 expression in malignant ovarian tumors for 44, 4% which is significantly lower than the expression of caspase-3 in benign ovarian tumors that is 81.8% (p=0.01).[29] The expression of caspase-3 in ovarian cancer are consistently lower than the expression of caspase-3 in benign ovarian tumor and normal ovarian tissue.[30,31]

In this study we also found a significant correlation between the expression of Bcl-2 with the expression of caspase-3 in ovarian cancer (p=0.002). Table 2 showed that in the group with positive expression of Bcl-2, we also found cases with negative expression of caspase-3. And vice versa, when there are group of negative expression of the Bcl-2, there are also cases with positive expression of caspase-3. This suggests a negative relationship, cases with positive expression of Bcl-2 have negative expression of caspase-3, while the opposite cases with negative expression of Bcl-2 have a positive expression of caspase-3 (p=0.002; Lambda=0, 4).

On the mechanism of apoptosis, either through the extrinsic and intrinsic pathways, it is known that Bcl-2 acts as an anti apoptosis either through inhibit the release of cytochrome-c from mitochondria or by inhibiting the activity of caspase including caspase-3.[32] Caspase-3 is the most important executioner caspase among another executioner such as caspase-6 and caspase-7. Caspase-3 activity involved many substrates such as cytokeratin, PARP, fodrin cytoskeleton protein alpha plasma membrane, the protein core, and activate CAD endonuclease.[33] Activity of these proteins causes changes in morphology and biochemistry as seen in apoptotic cells include shrunken cells, protein condensation, chromosomal DNA fragmentation, degradation of the core including cytoskeleton proteins, and cells disintegration becoming apoptotic bodies.[14,34] In the mechanism of apoptosis, Bcl-2 activity is inversely proportional to caspase-3, where Bcl- 2 acts as an anti-apoptosis, while caspase-3 acts as executioner apoptosis.

Relationship of Bcl-2 and Caspase-3 Expression with Stage of Disease

When associated with stage of disease, there is a significant correlation between the expression of Bcl-2 with stage of disease (p=0.002). This study showed the positive expression of Bcl-2 is increasing along with the higher stage of disease (p=0.002; Lambda = 0.3). Another studies found that the expression of Bcl-2 positively related to unfavorable factors such as the degree of differentiation of the cells, advanced stage disease, and residual tumor.[35] Meanwhile, Lukyanova NY, et al found that the expression of Bcl-2 is decreased in patients with ovarian cancer with a good degree of cell differentiation and in patients with early stage ovarian cancer.[36] In contrast, the expression of Bcl-2 is increased along with the increasing degree of cell differentiation and stage of disease.[27] This finding is consistent with the role of Bcl-2 in apoptosis mechanism as an anti-apoptotic as described above.

Besides the relationship between the expression of Bcl-2 with stage of disease, the present study also found a significant correlation between the expression of caspase-3 with stage of disease (p=0.000). Subjects with positive expression of caspase-3 is higher on the early-stage disease, in contrast to the advanced stage that showed negative expression of caspase-3 (p=0.000; Lambda=0.3). Another studies have also found similar results, in

which the expression of caspase-3 in malignant ovarian tumors is significantly associated with the degree of cell differentiation and stage of disease.[29-31] In accordance with the role of caspase-3 in apoptosis mechanisms as an executioner caspase, then higher expression of caspase-3 means that the apoptotic mechanism went well. Conversely, if the expression of caspase-3 is low, apoptosis process will become impaired that will cause the stage of disease will be more advanced.

CONCLUSIONS

In this research, positive expression of Bcl-2 in ovarian cancer is 68.9%, while the expression of caspase-3 in ovarian cancer is 44.4%. Statistically, there is a significant association between the expression of Bcl-2 and the expression of caspase-3 in ovarian cancer. There was also a significant association between the expression of Bcl-2 and caspase-3 expression with stage ovarian cancer stage. The results of this study indicate a role of Bcl-2 and caspase-3 in ovarian cancer that can be used to develop strategies for diagnosis, treatment, and prognosis based on molecular mechanism. Further research is needed with better research methods such as case-control or cohort study.

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