Indonesian Journal of Biomedical Sciences Volume 7, Number 2, July-December 2013: 67-73
Print-ISSN: 2085-4773, E-ISSN: 2302-2906.

ASSOCIATION OF B-CELL LYMPHOMA PROTEIN-2 AND CASPASE-3 EXPRESSION IN OVARIAN CANCER

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ABSTRACT

Ovarian cancer remains a major problem of women's health in the world, including Indonesia, and is associated with high rates of incidence and mortality. There are many efforts in early diagnosis on ovarian cancer, but until now there have not been found any satisfactory method. On the other hand, knowledge and research in the field of molecular biology become more advance, one of them is a mechanism to control the growth of cells in ovarian cancer through a process of programmed cell death or apoptosis. B-cell lymphoma protein 2 (Bcl-2) and caspase-3 are proteins that play a role on the mechanism of apoptosis. The purpose of this study was to determine the expression of Bcl-2 and caspase-3 and their association with ovarian cancer. Materials and method: The design of this study was a cross-sectional study. Expression of Bcl-2 and caspase-3 examined by immunohistochemistry under light microscope with 400x light power field and expression as a negative when the protein expressed in 10% or less of cells and as a positive when the protein expressed in more than 10% of cells. A number of 45 subjects were recruited in this study. Thirthy one of 45 subjects showed the expression of Bcl-2 positive (68.9%), while the positive expression of caspase-3 present in 20 subjects (44.4%). There was a significant association 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 significant association between stage of disease with expression of Bcl-2 (p=0.002; lambda=0.3) dan expression of caspase-3 (p=0.001; lambda=0.3). Conclusion: It concluded that there is a significant association between the expression of Bcl-2 and the expression of caspase-3 in ovarian cancer.

Keywords: Ovarian, cancer, Bcl-2 expression, Caspase-3 expression

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 ovarian cancer. In the world, the incidence of ovarian cancer in 2008 was 9.4%.[1,2] The incidene 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.[1] Reports indicate that the incidence of ovarian cancer was varied. In 2008, the number of ovarian cancer cases in the United States is 21,650 cases[2] and in the UK is 6500 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 become 10 per 100,000 women.[6] In Indonesia, the incidence rate of ovarian cancer is uncertain. Based on 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% to 35%.[8,9]

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

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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 efforts in early diagnosis of ovarian cancer, but until now there have not been found a satisfactory methode. 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. Ovarian cancer treatment through understanding of the carcinogenic mechanisms was 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, i.e. Bcl-2 and caspase-3. Bcl-2 proteins work against the p53 tumor suppressors 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 expresion 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.

METHOD

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: (1) a block of paraffin was examined histopathologically, so it was definitely diagnosed as ovarian cancer, (2) the patient's medical records can be found, and the necessary data is complete. While the exclusion criteria were: (1) patients who had been given chemo/radiotherapy preoperatively, (2) the tissue was damaged due to various technical reasons, (3) the patient's medical records could not be found or the necessary data was not complete.

Ovarian cancer tissue that has been paraffined then processed for immunohistochemical examination of Bcl-2 and caspase-3 protein. Epidemiological data of patient is obtained secondary from patient's medical records from 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. Expresion was 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

The number of subjects which is examined the expression of Bcl-2 and caspase-3 and a complete of patient's medical record in the study as many as 45 subjects.

Characteristic of the subjects

Of the 45 subjects, the youngest age is 19 years old and the oldest was 70 years old. Median age is 43 years old with mean age of subjects was 41.3 ± 13.7 years as indicate in Table 1.

Table 1
Characteristic of the subjects

Characteristic	Total	%
Age (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
Histopatological type		
Musinous	3	6.7
Endometrioid	3	6.7
Serous	26	57.8
Seromucous	1	2.2
Clear cell	1	2.2
Germ cell	6	13.3
Teratoma immature	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

The number of subjects that have parity are in the range of 0 - 9, with an average parity 1.5 ± 1.9. Of the 45 subjects, the commonest histopathological type was serous (57.8%). On the other hand, the rare histopathological type is clear-cell type and seromucous 2.2%, respectively.

Ovarian cancer is kind of disease where the stage of disease is determined through surgery (surgical staging) according to the FIGO classification. From 45 samples of the study, disease stage varies as shown in Table 1. No subjects suffering from ovarian cancer stage IIA, IIB, and IIIA.

Bcl-2 and caspase-3 expression

Distribution of subject based on Bcl-2 and caspase-3 expression was presented in Table 2. From this table it can be seen that 68.9 % was positively expressed Bcl-2 and 31.1 % was negatively expressed Bcl-2.

Table 2
Distribution of subjects by Bcl-2 and caspase-3 expression

Characteristic	Total	%
Bcl-2 expression		
Positive	31	68.9
Negative	14	31.1
Caspase-3 expression		
Positive	20	44.4
Negative	25	55.6

Association between expression of Bcl-2 with expression of caspase-3 was presented in Table 3.

Table 3
Association between expresion of Bcl-2 with the expression of caspase-3

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

Lambda=0.4

Table 3 shows association between expression of Bcl-2 to expression of caspase-3 (p=0.002; Lambda=0.4).

Association between expression of Bcl-2 to stage of disease

Table 4 shows the expression of Bcl-2 positive is increasing along with the stage of disease (p=0.002; Lambda=0.1).

Tabel 4
Distribution of subjects by stage of disease and Bcl-2 expression

Stage	Bcl-2 expresion		*p*
	positive	negative	
IA	1 (3.2)	3 (21.5)	0.002
IB	0	1 (7.1)	
IC	5 (16.1)	9 (64.3)	
IIC	0	1 (7.1)	
IIIB	9 (29.0)	0	
IIIC	10 (32.3)	0	
IV	6 (19.4)	0	
Total	31 (100)	14 (100)	

Lambda=0.3

Association between expresion of caspase-3 with stage of disease

Table 5 shows that the number of 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 5
Distribution of subjects by stage of disease and caspase-3 expression

Stage	Caspase-3 expression (%)		*p*
	Positive	Negative	
IA	4 (20.0)	0	0.0001
IB	1 (5.0)	0	
IC	13 (65.0)	1 (4.0)	
IIC	0	1 (4.0)	
IIIB	0	9 (36.0)	
IIIC	2 (10.0)	8 (32.0)	
IV	0	6 (24.0)	
Total	20 (100)	25 (100)	

Lambda=0.3

DISCUSSION

Age

In this study we found the proportion of ovarian cancer on age 20 years old for 2.2%, with the largest proportion in the age group over 50 years old. These results are similar with the data from SEER cancer statistic review that reported between the years of 2005-2009 the proportion of ovarian cancer at the age under 20 years old is 1.3%. While the largest proportion occurred in the age group of 55-64 years old 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 rare 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

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] Another 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 associated with a reduced risk of ovarian cancer, where multiparity has relative risk of ovarian cancer 0.6-0.8.[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] Risk of ovarian cancer is associated to 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, also occurred invagination of epithelial surface into the stroma and form inclusion cyst.[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]

Histopathological Type

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

Research by Rivas-Corchado LM, et al. suggested the commonest histopathological type of ovarian cancer is serous that accounted for 25%, followed by endometrioid and mucinous types, 20% for each type and granulosa cell types 7%.[20]

While the granulose type is obtained with almost the same results, as many as 6.7 %. In other literature ovarian cancer serous type remains the commonest, accounted for 75%, followed by musinous (20%) and endometrioid (2%). While the type of clear-cell, Brenner, and undifferentiated carcinoma are 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 1C has the largestproportion as many as 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) as many as 44.5%.[20]

Report from ACOG Committee Opinion, 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 reaching 90-95%.[10]

Association between Bcl-2 and Caspase-3 expression

In this research, positive expression of Bcl-2 in ovarian cancer is 68.9%, while the negative expression of Bcl-2 31.1% (Table 3). 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% (Table 2). 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 association between the expression of Bcl-2 with the expression of caspase-3 in ovarian cancer (p=0.002; lambda=0,4). Table 3 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 corelation 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.

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 avtivate CAD endonuklease.[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.

Association of Bcl-2 and caspase-3 expression with Stage of Disease

When associated with stage of disease, there is a significant association between the expression of Bcl-2 to stage of disease (p=0.002; lambda=0.3). Table 4 showes 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 association between the expression of Bcl-2 with stage of disease, the present study also found a significant association between the expression of caspase-3 with stage of disease (p=0.000). Table 5 showed that the positive expression of caspase-3 is higher on the early-stage disease, in contrast to the advance stage that showed negative expression of caspase-3 (p=0.000; Lambda=0.3). Other 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.

CONCLUSION

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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