Original Article

BRCA1 Protein Expression in Ovarian Serous Carcinoma in Southern Thailand

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Background: Ovarian serous carcinoma is the most common type of ovarian cancer. Abnormal protein expression in this type of cancer cells may identify prognosis of patient.

Objective: To determine the breast cancer type 1 (BRCA1) status by using immunostaining in ovarian serous carcinoma patients and compare with clinicopathological characteristics and survival outcome.

Materials and Methods: Serous ovarian cancer specimens and clinicopathological data were collected from Songkhlanagarind hospital between 2008 and 2017. There were 166 cases during that time. The BRCA1 protein expression was evaluated by tissue microarray and immunohistochemical staining of BRCA1 (clone MS110).

Results: The results showed loss of BRCA1 expression in 44 cases or 25.4%. In seven patients who had history of breast cancer, four cases or 57.0% lost BRCA1 protein expression. The median overall survival was 44.3 months, 32.5 months in loss of the expression group and 50.4 months in the expression group. The BRCA1 expression was not associated with survival outcome (p=0.90). Tumor staging was an independence prognostic factor.

Conclusion: The percentage of BRCA1 loss of expression in ovarian serous cancer was slightly high in Southern Thailand, but BRCA1 loss of expression did not statically associated with survival outcome.

Keywords: BRCA1; Immunohistochemistry; Ovarian cancer; Serous carcinoma; Survival

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Ovarian cancer is a common malignancy in women worldwide and the fourth most common tumor of female in Southern Thailand[1]. The most common type of ovarian cancer in Thailand is serous carcinoma[2]. Serous carcinoma is categorized into two subtypes, high-grade and low-grade cancer. The high-grade cancer has more cellular atypia with poorer prognosis than the low-grade cancer.

Breast cancer type 1 (BRCA1) and breast cancer type 2 (BRCA2) gene are tumor suppressor genes located on chromosomes 17q21 and 13q12.3. The function of these genes is DNA homologous recombination repair. The causes of BRCA1 gene defect are gene mutation, epigenetic BRCA1 silencing, and dysfunction of other genes and proteins involving in DNA repair[3-6]. An abnormal function of BRCA gene can cause cancer[7,8]. BRCA gene abnormality with co-existed with cellular tumor antigen p53 (p53) gene mutation is found in breast and ovarian cancer[9]. BRCA1 or 2 (BRCA1/2) mutations are found up to 18.0% of ovarian cancers and found in other cancer[10-15]. Women who inherited BRCA1/2 mutations have significantly increased risk of breast and ovarian cancers, varying depending on type and location of mutations[16-18].

Ovarian cancers with BRCA 1/2 germline mutations can present with more aggressive and high-grade histology but are frequently responsive to platinum chemotherapy, which improved the five years survival[19-21]. BRCA mutation-positives cancer patient can use anti-BRCA drug to improve survival outcome[22,23]. Therefore, the BRCA status has potential in predicting survival outcome and sensitivity to chemotherapy and targeted therapy in breast and ovarian cancers[24-26]. Immunohistochemistry is

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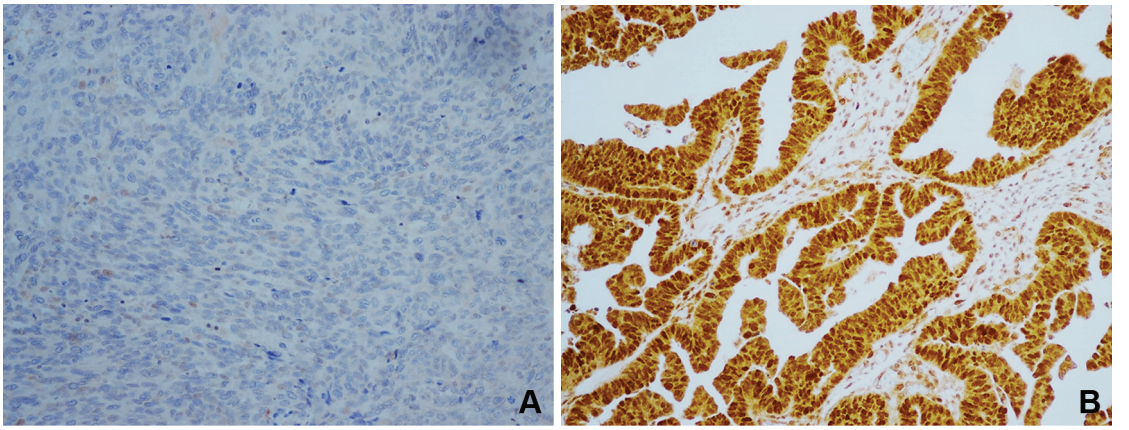

Figure 1. Example pictures of negative immunostaining (A) and positive staining (B) of BRCA1 protein.

used to detect abnormal expression of BRCA gene because this test has high specificity compared to molecular study[27,28]. Immunohistochemistry is also a non-complicated test and available in pathological laboratories appropriated for using as a screening test of BRCA gene expression. Studies showed correlation between BRCA gene mutation and high-grade serous ovarian carcinoma[29,30]. A study in Thai patients revealed BRCA germline mutation in 11.4% of non-mucinous ovarian cancer[31]. However, there was no study about BRCA1 gene mutation and expression in the Southern Thailand that may be different from other regions. Therefore, knowing of incidence of BRCA1 gene mutation in Southern Thailand might suggest the plan of screening test and plan of treatment.

The objective of the present study was to determine the prevalence of abnormal BRCA1 immunostaining in ovarian serous carcinoma patients and to compare BRCA1 status with clinicopathological characteristics and survival outcome.

Materials and Methods

Study population

The inclusion criteria of study population were patients diagnosed as primary ovarian serous carcinoma at Songklanagarind hospital between January 2008 and June 2017. All cases were performed ovarian resection and had tissue-embedded paraffin blocks. The exclusion criteria were cases missing survival data or inadequate paraffin block for immunohistochemistry. One hundred sixty-six cases were studied. Clinical data including age, family history, follow-up information, tumor size, staging, metastatic status, and tumor grade information were collected. The present study was approved by the Ethical Committee of Faculty of Medicine, Prince of Songkhla University (REC 61-031-5-1).

Immunohistochemistry

All hematoxylin and eosin (H&E) stained slides of all cases meeting the criteria were reviewed to confirm the diagnosis. Using a tissue microarray (TMA), the two areas of tumor in each donor block were core with a 2-mm diameter needle and transferred to a recipient paraffin block. Immunohistochemical staining was done by a mouse monoclonal antibody of BRCA1 (clone MS110: Abcam). The immunostaining was performed by using Leica BOND-MAX automated immunostainer. All immunostaining slides had positive control section for quality control.

All sections were examined by one general pathologist and one pathologist resident. The results of the immunostaining were evaluated by the percentage of positive tumor cells of any intensity. The positive staining indicated the normal expression of BRCA1 gene. The positive result was nuclear staining more than 10.0%, and tumor positivity l0% or less was classify as abnormal BRCA1 expression or negative result[28]. The example picture of BRCA1 immunostaining result is showed in Figure 1.

Statistical analysis

The clinicopathological characteristics of the patients were presented in percent, mean, and median and compared using Wilcoxon rank-sum test, chi-square tests, or Fisher's exact tests. The Kaplan-Meier method was used to estimate the overall survival (OS) outcome, and the log-rank test was performed to compare the survival difference in each group. Univariate analysis and multivariate-adjusted Cox regression models were used to evaluate independent prognostic factors. Difference was considered

Table 1. Comparison between BRCA expression and clinicopathological characteristics

Characteristic	BRCA1 negative	BRCA1 positive	Total	p-value
Age; median (IQR)	52 (47, 61)	58 (50, 64)	57 (49, 63)	0.03
Grade (n=79); n (%)				0.24
High	17 (81.0)	37 (63.8)	54 (68.4)	
Low	4 (19.0)	21 (36.2)	25 (31.6)	
Size; median (IQR)	9 (7, 11)	7 (5, 11)	7.5 (5, 11)	0.17
History of breast cancer (n=155); n (%)				0.38
No	37 (92.5)	111 (96.5)	148 (95.5)	
Yes	3 (7.5)	4 (3.5)	7 (4.5)	
Family history of breast/ovarian cancer (n=114); n (%)				0.39
No	24 (88.9)	82 (94.3)	106 (93.0)	
Yes	3 (11.1)	5 (5.7)	8 (7.0)	
Stage (n=150); n (%)				0.84
1	5 (12.8)	17 (15.3)	22 (14.7)	
2	5 (12.8)	20 (18.0)	25 (16.7)	
3	23 (59.0)	59 (53.2)	82 (54.7)	
4	6 (15.4)	15 (13.5)	21 (14.0)	
Platinum-based chemotherapy (n=160); n (%)				0.39
No	22 (53.7)	68 (57.1)	90 (56.3)	
Yes	19 (46.3)	51 (42.9)	70 (43.7)	

BRCA1=breast cancer type 1; IQR=interquartile range

significant when the p-value was less than 0.05. All statistical analyses were calculated by R program studio 3.3.1.

Results

Immunohistochemical findings

The demographic data and immunostaining results of all cases are shown in Table 1. The results showed loss of BRCA1 expression in 44 cases or 25.4%. Median age of BRCA1 expression group was higher than negative (p=0.03). Most cases were high grade serous ovarian cancer at 68.4%. The negative expression group had higher proportion of high-grade tumors at 81.0% than the positive group at 63.8% but did not reach statistical significance. The results show no association between BRCA1 expression and platinum-based chemotherapy.

Seven cases had a history of breast cancer with three cases or 7.5% in the negative group and four cases or 3.5% in the positive group. The family history of breast or ovarian cancer was found in eight cases including three cases in the negative and five cases in the positive BRCA1 group. Most cases were in stage 3 at 54.7%.

Survival analysis

The survival between stages and both expression groups were shown by Kaplan-Meier curves (Figure 2). The median survival of all cases was 44.3 months, 32.5 months in abnormal expression group, and 50.4 months in normal expression group. The survival outcome was not associated with BRCA1 expression (p=0.90). The overall 3-year survival was 55.9% (95% CI 48.6 to 64.4). There was a statistical difference of survival time between early and advanced stages of serous carcinoma by Log-Rank test (p<0.01) (Figure 3). The subgroup survival analysis of variables, including age, tumor grade and platinum chemotherapy showed no prognostic significance (p=0.93, 0.20, and 0.90, respectively).

Univariate and multivariate Cox's regression analysis of survival time showed no survival difference between both groups of BRCA1 staining. The only independent prognostic factor was tumor staging. The summary of Cox's regression analysis is showed in Table 2.

Discussion

In the present study, loss of BRCA1 expression was found in 25.4%. This percentage is similar to the other studies, which ranged between 8% to 20.0%[10-13]. Abnormal BRCA1 expression is significantly associated with younger ages. This supports the previous studies that some BRCA1 defect is hereditary. The results also showed higher percentage of negative staining in groups with

Table 2. Univariate and multivariate Cox's regression analysis of survival time

Characteristic	Univariate analysis; hazard ratio (95% CI)	p-value	Multivariate analysis; hazard ratio (95% CI)	p-value
Age	1.01 (0.98 to 1.02)	0.53	1.00 (0.96 to 1.03)	0.87
Grade		0.15		0.37
High	Ref.=1		Ref.=1	
Low	0.6 (0.30 to 1.23)		0.63 (0.23 to 1.77)	
Size	0.93 (0.88 to 0.98)	0.04	0.97 (0.87 to 1.07)	0.52
BRCA status		0.68		0.74
Negative	Ref.=1		Ref.=1	
Positive	0.91 (0.88 to 0.98)		1.15 (0.49 to 2.70)	
Stage		<0.01		0.03
1	Ref.=1		Ref.=1	
2	1.46 (0.48 to 4.45)		1.29 (0.10 to 16.07)	
3	5.48 (2.19 to 13.7)		6.10 (0.74 to 50.06)	
4	5.45 (2.01 to 14.77)		10.14 (1.22 to 84.33)	

BRCA=breast cancer; CI=confidence interval

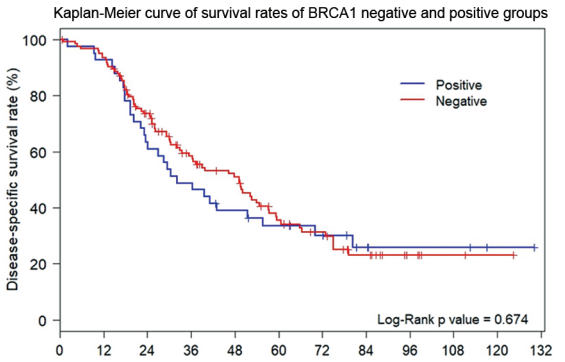

Figure 2. Survival rate of patient with BRCA1 expression in 166 patients of serous ovarian cancer.

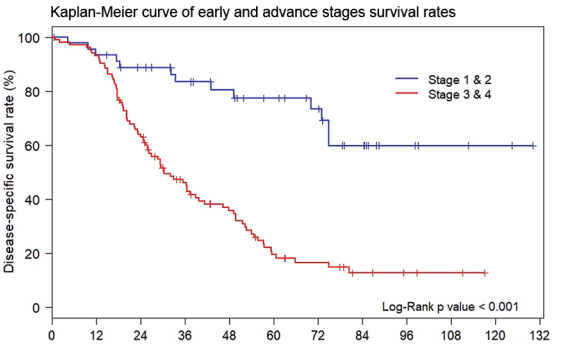

Figure 3. Survival rate of patient with early and advance stages in 166 patients of serous ovarian cancer.

breast cancer history at 57.1% or family history at 62.5%. These abnormalities might be associated with autosomal inheritance of BRCA1 gene defect of serous carcinomas and breast cancers. The present research also found the loss of expression group had shorter median survival time compared to the normal expression group. Loss of BRCA1 expression should inhibit the DNA repairing process causing high chance of mutation that led to high proliferation and aggression of tumor cells. The authors found the prognosis was associate with tumor staging, which is concordant with the previous study. The result of the present study showed no correlation between the BRCA1 negative patients and the OS, which was different from the previous meta-analysis study[32]. This difference might be caused by the limited number of negative BRCA1 patients in the present study. Therefore, further study should collect more cases.

Cancers, including serous carcinoma has been proved with loss of BRCA1 or BRCA2 from various mechanism such as germline mutation, sporadic mutation, or epigenetic mechanisms. Previous studies found that these tumors with BRCA1/2 function loss would respond better on platinum-based chemotherapy and the new targeted therapy, poly or adenosine diphosphate-ribose, polymerase (PARP) inhibitors. PARP inhibitor has been approved to treat patients with advanced stage ovarian serous carcinoma with BRCA gene mutation[33]. Therefore, patients have to go through genetic tests before they can be approved to use the drug. The immunohistochemistry was an effective test for detecting the BRCA loss expression. According to the present study, the loss of expression of BRCA1 in ovarian serous cancer in Southern Thailand was about 25% of all cases. Therefore, the PARP targeted therapy might have benefit to treat these patients. Using immunostaining for screening for BRCA1 is an applicable test. This study did not

include the immunostaining for BRCA2. Therefore, further study of screening BRCA1 and BRCA2 by immunostaining may have benefit for choosing the therapeutic drug.

Conclusion

The prevalence of BRCA1 loss of expression in ovarian serous cancer is slightly high in Southern Thailand. The BRCA1 expression was not associated with survival outcome.

What is already known on this topic?

The previous studies indicated that the germline mutation of BRCA1 in non-mucinous ovarian cancer is found about 11% in Thai people, but there is limited data of loss expression of BRCA1 protein in Southern Thailand.

What this study adds?

This study showed a high incidence of BRCA1 protein loss expression in serous ovarian cancer in Southern Thailand. The BRCA1 protein loss expression is not associated with survival outcome.

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Conflict of interest

No conflict of interest declared.

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