Prevalence of BRCA1 Expression in Epithelial Ovarian Cancer: Immunohistochemical Study

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The 99 formalin fixed paraffin-embedded ovarian tissue specimens of Epithelial Ovarian Cancer(EOC) patients treated at the Gynecologic Oncology Unit, King Chulalongkorn Memorial Hospital between January 1, 1996 and December 31, 1999, were immuno-stained with BRCA1 antibody using the immunohistochemical method. According to the criteria for BRCA1 immunohistochemical evaluation (neoplastic nuclear staining more than 10%), 12 (12.1%) of the 99 specimens showed positive BRCA1 expression. No associated statistical significance between clinicopathological variables and BRCA1 expression was detected. Survival analysis was performed in 87 patients who were followed-up for more than 6 months and recent status were available. During a median follow-up of 43 months, median survival time was 46 months (range 6-84 months). No association between BRCA1 expression and survival outcomes was found (Disease free survival and overall survival) in the presented patients.

Keywords: *BRCA1, Ovarian cancer, Immunohistochemistry*

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BRCA1 gene is located on chromosome 17q21 and encodes a protein of 220 kilodaltons consisting of 1,863 amino acid[1]. Germline mutations in this gene are thought to be the most common cause of hereditary ovarian cancer[2]. Although loss of heterozygosity at this locus frequently occurs in sporadic ovarian cancer and allelic deletion at the BRCA1 locus in tumors invariably involves the wild type chromosome suggesting that BRCA1 are tumor suppressor genes, it remains uncertain whether somatic mutations in BRCA1 play a role in their development[3-5]. Berchuck et al found that both germline and somatic BRCA1 mutations are accompanied by loss of heterozygosity, suggest that loss of this tumor suppressor gene is a critical event in the development of these cancers[6]. Many studies reported prognostic significance of BRCA1 mutation but are still controversial[7-9]. Johansson et al suggested that the survival of carriers of the BRCA1 mutation would be similar or worse than that of patients of ovarian cancer in general[10]. Rubin et al reported an apparently significantly prolonged survival for ovarian cancer patients with BRCA1 mutation[11].

In the past, almost all studies used the DNA analysis techniques to identify BRCA1 mutation. Byrne et al reported that immunohistochemical analysis could identify BRCA1 mutation and promise as a rapid and inexpensive method[12]. In the present study, the authors attempted to assess the prevalence of BRCA1 expression in EOC by immunohistochemical analysis.

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Material and Method

Patients

The tumor registry of the EOC patients treated at the Gynecologic Oncology Unit, King Chulalongkorn Memorial Hospital between 1 January 1996 - 31 December 1999 were reviewed. The formalin fixed paraffin-embedded ovarian tissues were obtained. The patients were excluded if the specimen or clinical data were not available. All of the enrolled patients were re-evaluated FIGO stage according to the surgical staging criteria classified by FIGO in 1985[13]. Histopathological type and grade of ovarian specimens were

reviewed by a gynecologic pathologist. All patients were followed up until 31 December 2003 or death. The schedule of follow-up was every month in the first year, every 3 months in another 2 years, and then every 6 months. Patients who developed recurrent disease were treated with a variation of chemotherapy, surgery, radiotherapy, or hormonal treatment according to the committee of Gynecologic Oncology Board. Disease free survival (DFS) was determined as time from primary surgery to the development of recurrent disease by physical examination and imaging or tumor markers. Overall survival (OS) was started at the time primary surgery was performed until the patients died or were lost to follow-up. Patients, who were lost to follow-up without evidence of the disease, were called to interview their recent status by phone.

Immunohistochemistry of BRCA1

Tissue sections from the paraffin-embedded ovarian tissue block, selected by gynecologic pathologist, were subjected to immunohistochemical stained. Section (3-micrometer-thick) were cut and mounted on slides pretreated with 3-aminopropyltriethoxylane. Sections were deparaffinized, hydrated, and then treated with 1% hydrogen peroxide in methanol for 35 minutes to block endogenous peroxidase activity then washed in phosphate-buffered saline solution (PBS). Each sample slide was placed in 10 mM citrate-phosphate buffer solution and heated in microwave oven. Samples were then incubated with normal horse serum and stained with BRCA1antibody (Ab No.345P; BioGenex Inc, San Ramon, CA94583, USA) All sections were evaluated with light microscope by one gynecologic pathologist (S.T.).The regions of greatest immunostaining were selected for cell count. The percentage of BRCA1 immuno-staining was scoring. Specimens were considered as positive BRCA1 expression when neoplastic nuclear staining > 10%[14].

Statistical analysis

The association between BRCA1 expression and clinical variables were analyzed by standard Chi-square tests, or, when appropriate, Fisher's exact test. Kaplan-Meier and log rank survival analyses were used to compare negative and positive BRCA1 expression patients. A p-value of less than 0.05 was considered statistically significant.

Results

Two hundred and thirty-five patients with diagnosis of EOC, clinical data and ovarian tissue specimens were available in 108 cases. Nine cases were excluded (5 cases of neo-adjuvant chemotherapy used, and 4 cases of low malignant potential ovarian cancer). Ninety-nine cases were enrolled in the present study. The median age at diagnosis for the 99 subjects was 47 years (range 24-74 years). Fifty-five patients (51.5%) had early stage, and 48 patients (48.5%) had advanced stage. Histopathological diagnosis, 28 (28.3%) were endometrioid carcinoma, 23 (23.2%) clear cell carcinoma, 22 (22.2%) serous cystadenocarcinoma, 19 (19.2%) mucinous cystadenocarcinoma, six (6.1%) mixed type, and one (1%) undifferentiated adenocarcinoma. From the operative records, ascitic fluid more than 100 ml was presented in 54 cases (54.5%), Complete surgical staging was performed in 54 cases (54.5%), optimal cytoreductive surgery(residual tumor < 2 cm) in 64 cases (64.6%) and suboptimal cytoreductive surgery (residual tumor > 2 cm) in 35 cases (35.4%).

According to the criteria for BRCA1 immunohistochemical evaluation (neoplastic nuclear staining more than 10%), 12 (12.1%) of the 99 specimens showed positive BRCA1 expression. The authors also compared the expression of BRCA1 with the clinicopathological variables of the 99 patients with EOC. As shown in

Table 1. Association of BRCA1 expression and variables of epithelial ovarian cancer patients

		NBRCA1		p-value
		Negative	Positive	
Total		87 (87.9%)	12 (12.1%)	
Age	< 60	75	12	NS
	≥ 60	12	0	
Stage	I	32	7	NS
	II	10	2	
	III	40	3	
	IV	5	0	
Grade	I	27	3	NS
	II	28	4	
	III	32	5	
Histology	Serous	20	2	NS
	Mucinous	17	2	
	Endometrioid	26	2	
	Clear cell	19	4	
	Mixed	4	2	
	Undefined	1	0	
Ascites	Negative	40	5	NS
	Positive	47	7	
Residual	No	39	7	NS
	< 2 cm	15	3	
	≥ 2 cm	33	2	

Table1, there was no associated statistical significance between the BRCA1 expression and the clinicopathological variables (Table 1).

The survival analysis was performed in 87 patients with follow-up of more than 6 months and available recent status. The median survival time was 46 months (range 6-84 months) (Fig. 1), median disease free survival was 37 months (range2-84 months) (Fig. 2). There was no statistical significant difference in survival and disease-free survival between positive and

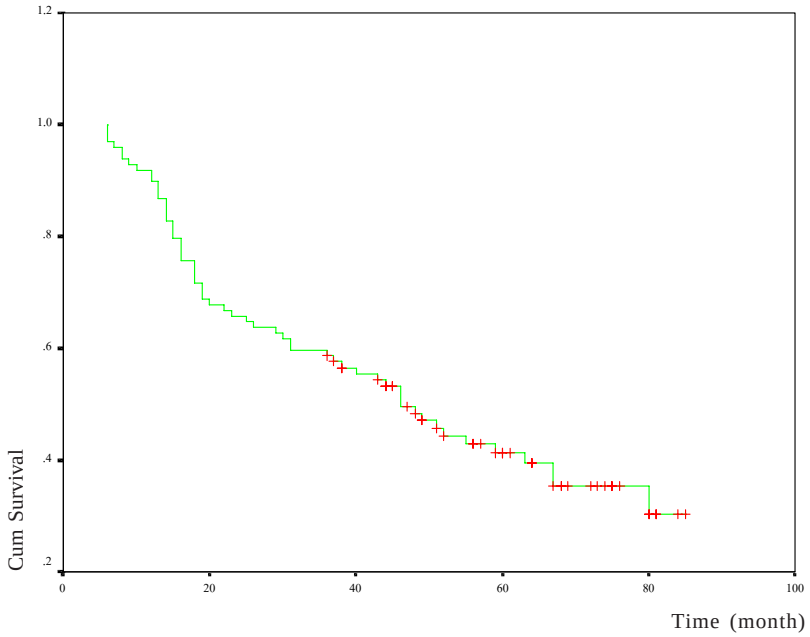

Fig. 1 Survival of patients with epithelial ovarian cancer (n = 287)

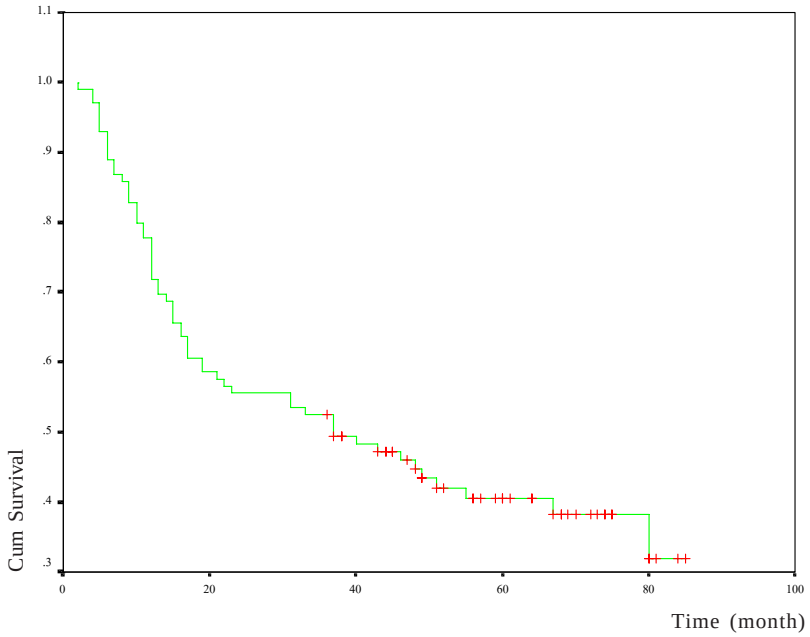

Fig. 2 Disease free survival of patients with epithelial ovarian cancer (n = 287)

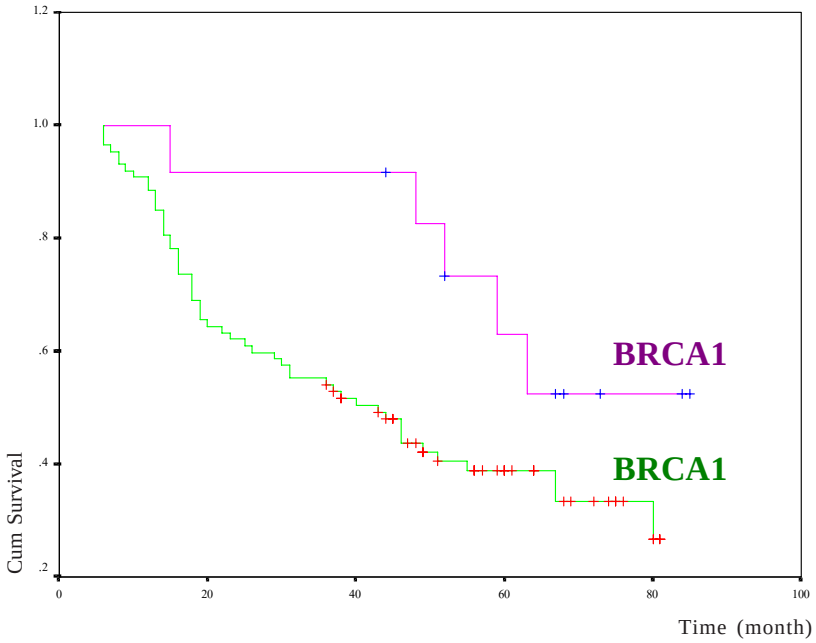

Fig. 3 Survival of patients with epithelial ovarian cancer by BRCA1 expression

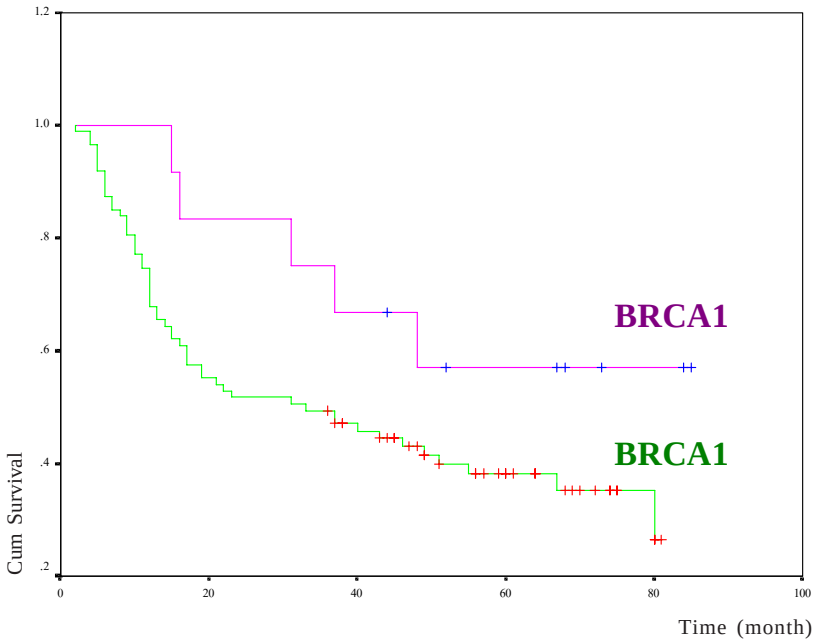

Fig. 4 Disease free survival of patients with epithelial ovarian cancer by BRCA1 expression

negative BRCA1 expression groups (Fig. 3, 4).

Discussion

In the past, the link between the BRCA1 gene and hereditary breast and ovarian carcinoma was established[15]. In the present, the BRCA1 mutation has become an interesting issue in sporadic breast and ovarian cancers because the BRCA1 region also

displays loss of heterozygosity (LOH) in 30-70% of the sporadic group. These observations support that BRCA1 is a tumor suppressor gene that has a role in both inherited and sporadic breast and ovarian cancers[16,17]. David et al reported the prevalence of BRCA1 mutation analyzed by DNA analysis was 19.4% in EOC[18]. Although the method of localization the BRCA1 mutation is controversial, Byrne et al reported that immunohistochemical analysis could identify BRCA1 mutation and promise as a rapid and inexpensive method[12]. In the present study, which is the largest and the first immunohistochemical study of BRCA1 in EOC in Thailand, the prevalence was 12.1%. The prevalence from the present study was lower than David's study, may be from fewer specimens, different patient groups and different methods of analysis. Comparison of BRCA1 expression and clinicalpathological variables were performed, all of positive BRCA1 expression specimens were found in patients with age < 60 group but associated no statistical significance between the BRCA1 expression and the clinicopathological variables, which may be from not enough patients to show the difference between groups.

Many studies reported better survival of the BRCA1 carrier group[11,18,19]. Boyd et al suggested two possible mechanisms for better survival in this group: a potential, indolent clinical behavior through a slower rate of cell division or alternatively, a more favorable response to chemotherapy[19]. Pierce LG et al and Sharan SK et al suggested that the BRCA1 genes are involved in cellular response to DNA damage[20,21]. Thangaraju et al has shown that BRCA1 facilitates stress-induced apoptosis in breast and ovarian cancer cell lines[22]. It is possible that reduced BRCA1 protein may up regulate the threshold for drug-induced apoptosis; therefore, the patients with reduced BRCA1 expression have a worse prognosis[14]. In the present study, the survival analysis was performed and the authors also found better survival in positive BRCA1 expression group but no statistical significant difference in survival between positive and negative BRCA1 expression groups was found. However, more patients are needed to answer the difference.

Conclusion

The prevalence of BRCA1 expression in EOC in Thailand, using immunohistochemical analysis, was 12.1%. There was no associated statistical significance between the BRCA1 expression and clinicopathological variables and no statistical significant difference in survival between positive and negative BRCA1 expression groups.

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การตรวจพบโปรตีน BRCA1 ในมะเร็งรังไข่ชนิด epithelial carcinoma

นครินทร์ ศิริทรัพย์, ธาริณี แม่นชนะ, วิชัย เติมรุ่งเรืองเลิศ, สุรางค์ ตรีรัตนชาติ, นวพันธ์ จารุรักษ์, ดำรง ตรีสุโกศล

ได้ทำการศึกษาการตรวจพบโปรตีน BRCA1 โดยการตรวจด้วยวิธี immunohistochemical ในชิ้นเนื้อ formalin-fixed ของผู้ป่วยมะเร็งรังไข่ชนิด epithelial carcinoma ที่เข้ารับการรักษาในโรงพยาบาลจุฬาลงกรณ์ระหว่าง 1 มกราคม พ.ศ. 2539 - 31 ธันวาคม พ.ศ. 2542 ที่สามารถจัดหาชิ้นเนื้อและประวัติได้รวม 99 ราย พบอุบัติการณ์การตรวจพบโปรตีน BRCA1 เท่ากับ 12.1% พบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติในแต่ละตัวแปรทางคลินิก และได้ทำการศึกษาผลต่อระยะการมีชีวิตรอดพบว่า การตรวจพบโปรตีน BRCA1 ไม่มีผลต่อระยะการมีชีวิตรอดอย่างมีนัยสำคัญทางสถิติ