

RESEARCH ARTICLE

No Association between BRCA1 Immunohistochemical Expression and Tumor Grade, Stage or Overall Survival in Platinum-Treated Epithelial Ovarian Cancer Patients

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Abstract

Background: The aim of this work is to assess the frequency of BRCA1 protein immunohistochemical (IHC) expression in epithelial ovarian cancer (EOC) and to evaluate the association of BRCA1 expression with clinical and pathological characteristics and the overall survival (OS) of patients treated with postoperative platinum-based chemotherapeutic agents. **Materials and Methods:** This retrospective study was conducted on 35 cases of epithelial ovarian cancer selected from the files of the Pathology Department, Faculty of Medicine, Mansoura University, Egypt. Immunohistochemistry (IHC) was performed for BRCA1 gene protein. BRCA1 expression was compared to patient's age, tumor histology, grade, stage and OS time. Statistical analysis was carried out with the SPSS version 16.0 to assess significant associations. **Results:** BRCA1 nuclear expression was detected in 40% of EOC, in which a mild increase in the percentage of positive cases was observed with serous histology, stage IV, and grade 3 carcinomas. There was a significant statistical difference in BRCA1 expression with regard to histological subtypes of EOC ($p=0.048$), but not grade or stage. Mean OS and survival rate were slightly better for BRCA1 expressing group, but there was no statistically significant difference ($p=0.528$). **Conclusions:** No association between BRCA1 immunohistochemical expression and tumor grade, stage or overall survival was noted in platinum-treated epithelial ovarian cancer patients.

Keywords: BRCA1 - ovarian carcinoma - immunohistochemistry - histological subtype - overall survival

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Introduction

Although ovarian cancer is the second most common gynecological malignancy, it accounts for the highest mortality rate among gynecological cancers (Altekruse et al., 2010). Epithelial ovarian cancer (EOC) has a 5-year survival rate of less than 25% and a 10-year survival rate approaching zero. More than 60% of women diagnosed with this cancer have reached stage III or stage IV when the cancer has already spread beyond the ovaries and the poor survival of patients developing this disease is, in part, attributable to the difficulties in diagnosis at an early stage and frequent metastasis to remote organs, but also to the lack of effective therapy for ovarian carcinoma (Sowter and Ashworth, 2005; Altekruse et al., 2010; Ji et al., 2014).

In the present, the BRCA1 mutation has become an interesting issue in breast and ovarian cancers. It also increases susceptibility to pancreatic cancer and other cancers in females (Sirisabya et al., 2007; Kooshyar et al., 2013; Mahdi et al., 2013). It is believed that 24-40% of ovarian cancers have dysfunction in the BRCA1 or BRCA2 (BRCAness) genes (Swisher, 2003; Skytte et al., 2011), due to either inherited (germ-line) or somatic

mutations or due to epigenetic BRCA1 silencing caused by DNA hypermethylation (Quinn et al., 2009; Skytte et al., 2011; Lan et al., 2013). Germ-line mutations in the BRCA1 gene predispose for approximately 6–15% of invasive EOC in the general population. Also, sporadic tumors exhibiting BRCAness behavior may exhibit deregulation of molecular pathways similar to those occurring in tumors with inherited BRCA gene mutations (Bolton et al., 2012; Wysham et al., 2012), and BRCA1 hypermethylation have been identified among 18.6% of EOC (Lan et al., 2013).

BRCA1 gene positioned on human 17q21 chromosome encodes a protein of 220 kilodaltons consisting of 1,863 amino acid. It contains 24 exons of which 22 ones have the coding function (Han et al., 2013). This gene functions as a tumor suppressor, with loss of function of both alleles required for tumorigenic progression. It performs many vital cellular functions, including recognition and repair of double stranded DNA breaks in cell cycles, cell-cycle checkpoint control, chromatin remodeling, transcriptional regulation of gene expression and mitosis (Gowen et al., 1998; Boyd et al., 2000; Swisher, 2003; Sowter and Ashworth, 2005; O'Donovan and Livingston,

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2010). It especially participates in nucleotide excision repair and homologous recombination repair (Han et al., 2013). Those cells with alterations in homologous recombination pathway genes are unable to repair DNA double-strand breaks, resulting in genomic instability and a predisposition to malignant transformation (Tutt and Ashworth, 2002; Mahdi et al., 2013). Conversely, homologous recombination pathway deficiencies can also impair tumor cells' ability to repair DNA cross-links introduced by chemotherapeutic agents such as Cisplatin (Venkitaraman, 2008).

BRCA1 mutation associated ovarian carcinomas were described to have distinct molecular genetic and clinicopathological features compared with sporadic ovarian cancer groups. They tend to be predominantly serous adenocarcinomas, with mucinous carcinomas and borderline tumors under-represented. They are diagnosed at a younger age and are almost of high-grade and advanced-stage (Boyd et al., 2000; Werness et al., 2004; Prat et al., 2005; Sowter and Ashworth, 2005). Nevertheless, the prognostic significance of BRCA1 mutation is still a matter of controversy, especially regarding survival (Boyd et al., 2000; Sowter and Ashworth, 2005; Sirisabya et al., 2007; Bolton et al., 2012). Ovarian cancers associated with germline BRCA1 mutations were initially shown to have a more favorable clinical course and prolonged overall survival than matched sporadic cancers (Rubin et al., 1996). This was confirmed (Aida et al., 1996; Boyd et al., 2000) or contradicted (Johannsson et al., 1998; Pharoah et al., 1999) by subsequent studies. However, recent retrospective studies confirmed that absent/low BRCA1 protein expression is a favorable prognostic indicator in epithelial ovarian cancer patients that predicts for an improved clinical response to Platinum-based chemotherapy and for likely higher survival rates (Venkitaraman, 2008; Carser et al., 2011; Joo et al., 2011; Lesnock et al., 2013).

Currently, there are no differences between the treatments provided for sporadic and hereditary ovarian cancer. However, a large proportion of patients treated with Platinum-based chemotherapy fail to benefit from it. Therefore, predictive biomarkers are needed for personalized medicine which identifies subpopulations of patients who most likely respond to a given therapy and those who not and there are indications that targeted therapy is effective in women with BRCA-associated tumors (Joo et al., 2011; Lesnock et al., 2013; Li et al., 2013). Thus the aim of this work is to assess the frequency of BRCA1 protein immunohistochemical (IHC) expression in epithelial ovarian cancer (EOC) and to evaluate the association of BRCA1 expression with the clinical and pathological characteristics and the overall survival (OS) of patients treated with postoperative Platinum-based chemotherapeutic agents.

Materials and Methods

Patient selection and clinicopathological criteria

This retrospective study was conducted on 35 cases of epithelial ovarian malignant tumors (6 serous borderline tumors, 14 serous carcinomas, 4 mucinous borderline

tumors, 6 mucinous carcinomas and 5 endometrioid carcinomas). Cases were selected from the files of the Pathology Department, Faculty of Medicine, Mansoura University, Egypt, during the period between January 2006 to December 2007, according to the availability of tumor-representative paraffin tissue blocks and the clinicopathological data. All patients received postoperative Platinum-based therapy at Radiotherapy and Nuclear medicine Department of the same University. Overall survival (OS) starting from the time of primary surgery was calculated until the patients died or was lost to follow-up.

Haematoxyline and eosin (H&E) slides were reviewed to re-evaluate histopathological type according to the latest World Health Organization (WHO) classification and grade the tumors according to Gynecologic Oncology Group (GOG) grading system. Staging was reviewed according to International Federation of Gynecology and Obstetrics (FIGO) surgical staging criteria.

Immunohistochemistry (IHC)

BRCA1 gene protein immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissues -sectioned at 4-5 μ m- using the standard avidin-biotin-peroxidase technique (Anti-BRCA1; Ab-1423; rabbit polyclonal antibody that detects endogenous levels of total BRCA1 protein; Mybiosource corporation product Catalog # MBS132398). Positive controls prepared from human breast carcinoma tissue as well as negative control slides were processed with the tumor tissue slides. All IHC sections were examined for BRCA1 expression with light microscope by two pathologists at least blinded to clinical outcome in archival tumor specimens. The regions of greatest immunostaining were selected for evaluation. Specimens were considered as positive (aberrant) for BRCA1 expression when neoplastic cell nuclear staining scored more than 10% (Sirisabya et al., 2007; Lesnock et al., 2013).

Statistical analysis

Statistical analysis was carried out with the SPSS version 16.0 (Chicago, USA). The association of BRCA1 protein expression with ovarian carcinoma patients' clinicopathologic variables including: histopathological type, GOG grade, FIGO stage and patient OS time, was assessed by the Pearson chi-Square test (χ^2) test. Survival curves were plotted by Kaplan-Meier method and compared by the log-rank test. $P \leq .05$ was considered as statistically significant.

Results

According to the criteria for BRCA1 immunohistochemical evaluation, 14 (40%) of the 35 studied EOC expressed BRCA1 gene protein. The mean age at diagnosis was slightly lower for the BRCA1-positive cases being 42 years (± 13 SD) compared to 45 years (± 12 SD) for BRCA1-negative cases.

As seen in Table 1, BRCA1 was more frequently expressed in tumors with serous histology (50%; Figure 1), followed by tumors with mucinous differentiation

Table 1. Association of BRCA1 Expression and Clinicopathological Variables and Overall Survival of Epithelial Ovarian Cancer Patients

	BRCA1 expression		p value of chi square test
	Positive	Negative	
Age			
< 60 y (no 31; 89%)	14 (45%)	17 (55%)	
≥ 60 y (no 4; 11%)	0 (0%)	4 (100%)	
Age range	21-57 y	24-62 y	
Mean age	42.4 y (±13 SD)	45 y (±12 SD)	
Histology			
Serous (no 20)	10 (50%)	10 (50%)	0.048*
Mucinous (no 10)	4 (40%)	6 (60%)	
Endometrioid (no 5)	0 (0%)	5 (100%)	
Stage			
I (no 20)	8 (40%)	12 (60%)	0.893
II (no 1)	0 (0%)	1 (100%)	
III (no 10)	4 (40%)	6 (60%)	
IV (no 4)	2 (50%)	2 (50%)	
Grade			
Borderline (no 10)	2 (20%)	8 (80%)	0.379
1 (no 6)	2 (33.3%)	4 (66.7%)	
2 (no 9)	4 (44.4%)	5 (55.6%)	
3 (no 10)	6 (60%)	4 (40%)	
Overall survival (OS)			
OS range	8- 56 m	10-60 m	0.528
Mean OS	44.5 m	42 m	
Total (no 35)	14 (40%)	21 (60%)	

*p value is significant if ≤0.05; no: number; y: years; SD: standard deviation; m: months

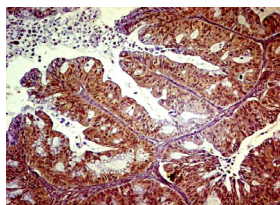


Figure 1. BRCA1 Nuclear Immunoreactivity in Papillary Serous Adenocarcinoma (Immunoperoxidase-DAB x100)

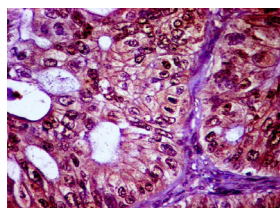


Figure 2. BRCA1 Nuclear Immunoreactivity in Mucinous Adenocarcinoma (Immunoperoxidase-DAB x400)

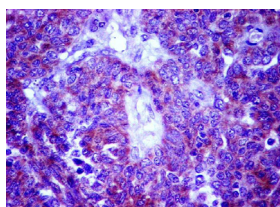


Figure 3. BRCA1-Negative Endometrioid Ovarian Carcinoma (Immunoperoxidase-DAB x400)

(40%; Figure 2), but endometrioid tumors were entirely BRCA1-negative (Figure 3). There was a significant statistical difference in BRCA1 expression with regards to histological subtypes of EOC ($p=0.048$). BRCA1 expression was evident in stage IV (50%) and grade 3

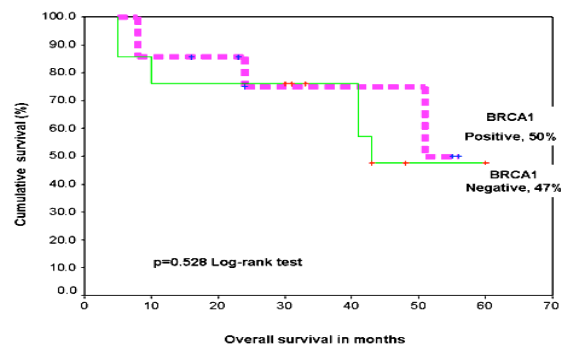


Figure 4. Kaplan-Meier Curves with Univariate Analyses (Log-rank, $p=0.528$) of Patients with Epithelial Ovarian Cancer, Subdivided according to BRCA1 Protein Immunohistochemical Expression. The Cumulative 5-year Survival rate was 50% in the BRCA1-Positive Group, (no=14), and it was 47% in the BRCA1-Negative Group (no=21)

carcinomas (60%) compared to other groups, however, there was no statistically significant association between BRCA1 expression and FIGO stage or tumor grade ($p=0.893$ and 0.379 respectively) among our patients.

The mean survival time for patients with BRCA1 expressing tumors was slightly longer than patients with BRCA1-negative tumors (44.5 and 42 months respectively) and the overall survival rate, assessed by the Kaplan-Meier method was 50% in BRCA1 expressing group, whereas it was 47% in the BRCA1-negative group (Figure 4), but there was no statistically significant difference in survival between both groups ($p=0.528$).

Discussion

In the current study, immunohistochemistry (IHC) was performed to detect the frequency of BRCA1 expression in 35 epithelial ovarian carcinomas (EOC). Almost all earlier studies used the DNA analysis techniques to identify BRCA1 mutation, however recent studies verified the feasibility of using immunohistochemistry as a promising, inexpensive, and rapid method for BRCA1 mutation detection (Carser et al., 2011; Skytte et al., 2011; Lesnock et al., 2013). The frequency of BRCA1 expression among our cases was 40%; a finding that matches with the previous studies (Carser et al., 2011; Skytte et al., 2011; Lesnock et al., 2013). On the contrary, Sirisabya et al. (2007) reported a markedly lower prevalence of 12%.

Attempts to define the prognostic significance of BRCA1 mutation status in ovarian cancer have produced conflicting results (Boyd et al., 2000; Bolton et al., 2012). Comparison of BRCA1 expression and the clinicopathological variables performed here, revealed a significant statistical difference in BRCA1 expression among different histological subtypes of EOC, but not among different stages or grades of EOC, although BRCA1 expression was more frequent in stage IV and grade 3 carcinomas. Our results were intermediate between Sirisabya et al. (2007), who found no correlation between the BRCA1 expression and any of the clinicopathological variables, and Werness et al. (2004), who detected fewer grade 1 and stage I cancers in BRCA1

positive patients than in BRCA1 negative patients. Similarly, polymorphisms of breast cancer susceptibility gene BRCA1 had no statistically significant correlation with clinicopathological characteristics of breast cancer in Saudi population (Hasan et al., 2013). In addition, Lan et al. (2013) observed no significant differences in the methylation frequencies of BRCA1 between stages and ages of ovarian cancer patients. On the other hand, Han et al. (2013) detected greater expression quantities of BRCA1 mRNA in stages II and III epithelial ovarian cancer than in phases I and IV.

Many investigators reported better survival of the BRCA1 mutation carrier patients compared with non-carriers (Rubin et al., 1996; Aida et al., 1998; Ben-David et al., 2002; Chetrit et al., 2008). For example, Rubin et al. (1996), found a median survival of 77 months in BRCA1 mutation carriers, compared to 29 months for age- and stage-matched controls, and Bolton et al. (2012) reported a 5-year overall survival of 44% for BRCA1 mutation-carriers and 36% for non-carriers. This may be explained by the slower rate of cell division and the improved response to chemotherapy via effects on DNA damage response (Boyd et al., 2000; Swisher, 2003; Li et al., 2013). We observed a slightly improved mean survival time and a higher cumulative survival rate for BRCA1 positive patients (mean 44.5 months and 50% respectively), compared to BRCA1 negative patients (mean 42 months and 47% respectively), but this difference was insignificant from the statistical point of view. This finding is in accordance with other previous studies which reported a similar or even more worse survival for BRCA1 mutation-carriers compared to the negative group (Johannsson et al., 1998; Pharoah et al., 1999; Sirisabya et al., 2007). In compatibility with these data, Carser et al. (2011) confirmed that patients with absent/low BRCA1 had a better clinical outcome compared to patients with high BRCA1 protein expression owing to the adverse histopathologic features observed in BRCA1 positive tumors. Also, Yang et al. (2012) reported that BRCA1 mutations were not significantly associated with beneficial OS; besides neither BRCA1 mutations nor BRCA1 methylation in ovarian cancer was associated with prognosis, improved survival or improved Platinum-based chemotherapy response in the later study. Moreover, recent studies (Radosa et al., 2011; Lesnock et al., 2013; Li et al., 2013; Lorusso et al., 2013), confirmed that EOC with negative BRCA1 protein expression shows a significantly better OS, prolonged treatment intervals and a tendency for an extended progression free time interval. In addition, they suggested that decreased BRCA1 expression predicts for improved sensitivity to Cisplatin-based chemotherapy.

Virtually, discrepancies in the results of published data about the survival in BRCA1 associated-EOC might make the comparison of results between studies problematic because BRCA1 plays a versatile role in tumor suppression through its ability to participate in DNA damage response, checkpoint control, mitotic spindle assembly, sister-chromatid decatenation, and centrosome duplication. The failure of one of these mechanisms could predispose BRCA1-mutated cells to tumorigenesis but not necessarily render the developed cancer cell sensitive

to DNA cross-link agents such as Cisplatin (Yang et al., 2012). Moreover, several factors may account for these divergent results between studies such as: small sample size resulting in imprecise survival estimates, different patient groups, stage of disease compared, the inclusion of various populations and several methods of analysis in different studies and the grouping of BRCA1 and BRCA2 carriers together for analysis, despite their potential prognostic differences (Sirisabya et al., 2007; Chetrit et al. 2008; Bolton et al., 2012).

In conclusion, in the current work, BRCA1 expression was detected in a substantial number of EOC, using immunohistochemical analysis. There was a trend BRCA1 expression to be associated with tumor histology, but not with grade or stage of the tumor in EOC. It seems that no remarkable difference exists in the impact of BRCA1 expression on the survival of BRCA1-positive and negative OEC patients treated with Platinum- based agents.

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