Significant role of Loss or Reduced BRCA1 gene expression in clinical implication of ovarian cancer

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ABSTRACT

Background: BRCA1 immunohistochemistry (IHC) provides a rapid initial screen to detect BRCA1 dysfunction in ovarian cancer that resulting from genetic alterations.

Aim: To assess the expression of BRCA1 protein by IHC analysis among a group of Iraqi ovarian cancer patients to evaluate the patterns of expression and its correlation with the clinicopathological parameters in attempting to evaluate a significance role of BRCA1 gene implication in ovarian cancer.

Methods: Forty three paraffin embedded samples of ovarian cancer cases were analyzed for BRCA1dysfunction by IHC analysis. The semi-quantitative approach using modified histochemical score (H-score) was achieved to assess the patterns of BRCA1 gene expression.

Results: Complete loss of BRCA1 nuclear expression was detected in 30.2% of the cases while, reduced expression occurred in 46.5% of cases, giving rise to 76.7% of all cases detected with altered BRCA1 nuclear expression. Altered BRCA1 expression was found to be higher in age group ≤ 45 years (78.3%) in comparison with those of ages >45 years. Altered BRCA1 expression was significantly correlated with the high grade and with the unilateral tumor site when compared with the low grade and bilateral tumor site ($P \leq 0.05$), and was insignificantly correlated with the high stage ovarian tumors, 11.6% of cases were detected by cytoplasmic BRCA1 expression and no association was found between cytoplasmic expression and tumor grade, stage and tumor site.

Conclusion: Altered BRCA1 expression may play a significant role in the progression of ovarian cancer.

Recommendation: BRCA1 IHC is a clinically useful approach to detect the BRCA1 dysfunction and the H-score assessment reflects good estimation for BRCA1expression patterns.

Introduction

Ovarian cancer is regarded as the sixth most common cancer type in women [1]. It is responsible for most of the death in the gynecological field [2]. It is considered as the seventh cause of death in women worldwide and seen in all ages and ethnic groups [1]. It represents 3% of all female cancers [3]. According to Iraqi cancer registry, ovarian cancer is the fifth most common cause of death, and the sixth in the list of most common cancers in Iraq, which represents about (3.8%)[4,5]. Ovarian cancer is a heterogeneous disease that requires an understanding of its biology, detection of its growth determinants and assessment of specific prognostic factors to predict the outcome of the disease and to manipulate the therapeutic strategies [6].

BRCA1 gene is found to have genetic alterations in a variety of neoplasm, including breast, ovarian and fallopian tube tumors [7, 8]. It is frequently inactivated in 5-10% of inherited ovarian cancer cases and about 60% of the sporadic cases [9]. BRCA1gene is a tumor suppressor gene that is located on chromosome 17q21, it consists of 24 exons, that encodes 1863 amino acids with a molecular weight of 220 kD. Its phosphoprotein plays a major role in controlling multiple cellular processes including the DNA repair, cell cycle checkpoint control, transcriptional regulator and maintainance of the genomic integrity [10].

Germline mutations of the BRCA1 gene was presented in 28% of a hereditary breast and ovarian cancer syndrome (HBOC), which is characterized by an increased risk for breast, ovarian, prostate and pancreatic cancer[11]. Somatic mutation in BRCA1 was found in a very low frequency of sporadic ovarian cancer, but it is not reported in sporadic breast cancer. Epigenetic alterations in the BRCA1 gene and other DNA repairing genes may play an important role in sporadic ovarian cancer [12]. Loss of heterozygosity at BRCA1 region was seen in 40-80% and 30-60% of sporadic breast and ovarian cancers respectively. Promoter hypermethylation is regarded as an alternative mechanism to the intragenic mutations which are responsible for the loss of the activity of the tumor suppressor genes and it occurs in 5-20% of sporadic ovarian cancer [13].

BRCA1 immunohistochemistry (IHC) is regarded as an inexpensive and rapid initial screen to detect BRCA1 dysfunction in ovarian cancer which has the ability to predict patients having BRCA1 germline mutation and also has the ability to detect other BRCA1 genetic defect such as somatic

mutation and promoter methylation[14,16] Therefore, IHC analysis was used in the present study to assess the expression of BRCA1 protein among a group of Iraqi ovarian cancer patients to evaluate the patterns of expression and its correlation with the clinicopathological parameters in attempting to evaluate a significant role of BRCA1 gene implication in ovarian cancer.

Methods

Forty three formalin-fixed, paraffin-embedded ovarian cancer tissue samples were collected between2014-2015. Cases were collected from the laboratory unit of Al-Sadder Medical City and from the private laboratories in AL-Najaf and Wassit governorates. The clinicopathological parameters such as the age, site, stage and grade of the patients from whom, tumors were obtained were available. The study was carried out in the Medical Genetics Laboratory of Euphrates Unit for Cancer Research, College of Medicine, Kufa University. Ethical approval was obtained from the local Medical Ethics Committee, Faculty of Medicine, and University of Kufa.

The patient's ages ranged from 10-80 y with a mean age of 43 y. The biopsy specimens were taken from the patients as pre-chemotherapy surgeries, either as a part of total abdominal hysterectomy (TAH) with bilateral salpingo-oophorectomy (BSO), oophorectomy alone, as ovarian cystectomy or as Tru-cut biopsy. Cases with recurrence or post-chemotherapy were excluded from this study. The diagnosis was confirmed by the reviewing of slides stained with Hematoxylin and Eosin by a certified pathologist for histological assessment of type, grade and FIGO stage [17] of tumors.

The estimation of BRCA1 protein expression was done by an immunohistochemical technique. Monoclonal Mouse Anti-Human BRCA1 protein (1 ml, Clone GLK-2, Code M3606) and Labeled Streptavidin-Biotin LSAB+ System-Horseradish Peroxidase (Code K0679) (DakoCytomation/Denmark) were used in the current immunohistochemical analysis. Tissue sections (5μm) from the paraffin-embedded tumor blocks were placed on positively charged slides (Fisher scientific Co., Pittsburgh, PA). The sections were deparaffinized with xylene followed by rehydration in serial alcohol solutions, and then pre-treated with antigen retrieval solution (0.01M, citrate buffer, pH 9.0, DakoCytomation/Denmark) in water-bath at 95ºC for 30 min. The tissue sections were incubated in 0.3% hydrogen peroxide for 10 min to block the endogenous peroxidase

activity. The slides then incubated with monoclonal primary antibody with a dilution of 1:50 for 30 min in a humidified chamber at 37 º C. The slides was subsequently incubated with a biotinylated universal secondary antibody (15 min) and with streptavidin-Biotin horseradish peroxidase (30 min). Then, slides were incubated with 3, 3'-diaminobenzidine (DAB) substrate chromogen solution and counterstained with hematoxylin. Tissue sections of ovarian cancer samples well known to be positive for BRCA1 were used as a positive control for each set of immuostaining, while negative control slides were incubated with phosphate buffered saline (PBS) instead of the primary antibody. Infiltrating lymphocytes were used as an internal control for BRCA1 immunoexpression.

The semiquantitative approach was applied to assess the BRCA1 immuno-histochemical reactivity. The modified histochemical score (H-score) was used at which the intensity of the staining and the percentage of the stained cells were calculated [6, 18]. The score index of staining intensity consists of 0 to 3 (0: negative, 1: weak, 2: moderate and 3: strong) levels. The distribution represented the calculated percentage of stained tumor cells at each intensity [19], 5% or more of the positively stained tumor cells was regarded as a positive expression of BRCA1. A final score of 0-300 is the result of multiplying the intensity of the stained cells by the percentage of positive cells. Patterns of BRCA1 expression were nuclear, cytoplasmic, or both nuclear and cytoplasmic. A further classification of a positive nuclear expression of BRCA1 was performed based on the cut-off value, median of expression, (H-score =47) in two groups: above and below the median corresponding to strong and reduced expression, respectively. Also, the assessment of cytoplasmic expression (sub-cellular localization) was conducted.

Statistical Package of Social Science software (SPSS, version 20) was used to calculate Fisher's exact and Chi square probability, and Odds ratios (ORs). The Fisher's exact and Chi square probability are considered statistically significant at P-value ≤ 0.05, while the strength of associations was measured by calculating Odds ratios (ORs>1 indicates positive association and a value of <1 indicates negative association).

Results

Forty three primary ovarian cancer cases were enrolled, the ages of patients ranged between10-80 y with a mean of 43 y, 53.5% of cases did not exceed 45 y and 46.5% of cases aged more than 45 y.

The clinicopathological characteristics: Serous type of ovarian cancer was found to be the most common histological type in 28 (65.2%) cases in comparison to 7 cases of mucinous cystadenocarcinoma, 5 cases of granulosa cell tumor, 1 case of yolk sac tumor, 1 case of sertoli-leydig cell tumor and 1 case of dysgerminoma (Table 1, Fig 1). Grading of the cancer cases revealed that 23.2% of cases were of grade I, 16.3% of grade II and 60.5% of grade III (Table 1, Fig 1). Staging of cancer cases pointed out 32.5% of cases have stage I, 16.3% of stage II, 37.2% and 14% of stages III and IV respectively. The site of cancer was found to be unilateral in 55.8% and bilateral in 44.2% of cases (Table 1).

BRCA1 protein expression analysis: Immunohistochemical analysis indicated that BRCA1 staining was mainly localized to the nuclei as well as to the cytoplasm in some cases, showing nuclear, cytoplasmic, or both nuclear and cytoplasmic expression Patterns. The cut-off value of positive nuclear BRCA1 immunostaining was 47 and the H-score distribution (0-300) showed values range from 5 to 170 of nuclear BRCA1 expression. Thirteen out of the 43 cases (30.2%) were observed to loss BRCA1 nuclear expression. H-score determination demonstrated reduced immunostaining in 46.5% of cases, whereas strong expression was evident in 23.3% of the malignant tumors. The internal control (Infiltrating lymphocytes) showed positively nuclear BRCA1staining, consistent with wild type expression of BRCA1 gene. The cytoplasmic expression was clear in 37.2% of cases, 25.6% of them were associated with the nuclear expression while 11.6% of cases were found to have only cytoplasmic expression (Table 2, Fig 2).

Altered BRCA1 expression (complete loss or reduced expression) was observed in 33 cases (76.7%) out of 43 studied cases, it was higher in age group $\leq$ 45 y (78.3%). However, strong nuclear BRCA1 expression was illustrated in age group > 45 y (25%) (OR, 2.75; CI, 0.63-11.97) (Table 2).

Altered BRCA1 expression was higher in serous cystadenocarcinoma (82.1%) and granulosa cell tumors (80%) and less frequent in mucinous cystadenocarcinoma (57.1%). Moreover, the only 1 case of dysgerminoma and yolk sac tumor showed altered expression (Fig 3). Altered nuclear BRCA1 expression was highlighted to be significantly correlated with the grade of ovarian tumors (OR, 5.3; CI, 1.14-25.1) ($P<0.05$), and with the unilateral tumor site (OR=5.00; CI, 1.04-24.03) ($P<0.05$), but not with the high stage of ovarian tumors (Table 2).

Table 1:

Clinicopathological characteristics of the presented ovarian tumor patients.

Parameter		Total patients	percentage %
		43	
Age	≤45	23	53.5%
	>45	20	46.5%
Histological types	serous	28	65.1%
	Mucinous	7	16.3%
	others	8	18.6%
Tumor grade			
	I	10	23.2%
	II	7	16.3%
	III	26	60.5%
FIGO stage	I	14	32.5%
	II	7	16.3%
	III	16	37.2%
	IV	6	14%
Site of tumor	unilateral	24	55.8%
	Bilateral	19	44.2%

Table 2:

Nuclear BRCA1 expression in correlation to the clinicopathological features in malignant ovarian tumors.

Parameter	Total Patients	BRCA1 expression in malignant ovarian tumors (N=43)				odd ratio 95%CI	P-value
		altered expression (N=33) (76.7%)			Strong expression (N=10) (23.3%)		
		Negative (N=13) (30.2%)	Reduced (N=20) (46.5%)	Total			
	43						
Age of patients						2.75 (0.63-11.97)	0.3
≤45	23	9 (39.1%)	9 (39.1%)	18	5 (21.8%)		
>45	20	4 (20%)	11 (55%)	15	5 (25%)		
Tumor grade							0.03
Low (I&II)	17	3 (17.6%)	7 (41.2%)	10	7 (41.2%)	5.3 (1.14-25.1)	
High (III)	26	10 (38.5%)	13 (50%)	23	3 (11.5%)		
FIGO stage						1.1 (0.25-4.37)	0.6
Low (I&II)	21	8 (38.1%)	8(38.1%)	16	5(23.8%)		
High (III&IV)	22	5(22.7%)	12(54.6%)	17	5(22.7%)		
Tumor site							
Unilateral	24	10 (41.7%)	8 (33.3%)	18	6 (25%)	5.00 (1.04- 24.03)	0.03
Bilateral	19	3 (15.8%)	12 (63.1%)	15	4 (21.1%)		

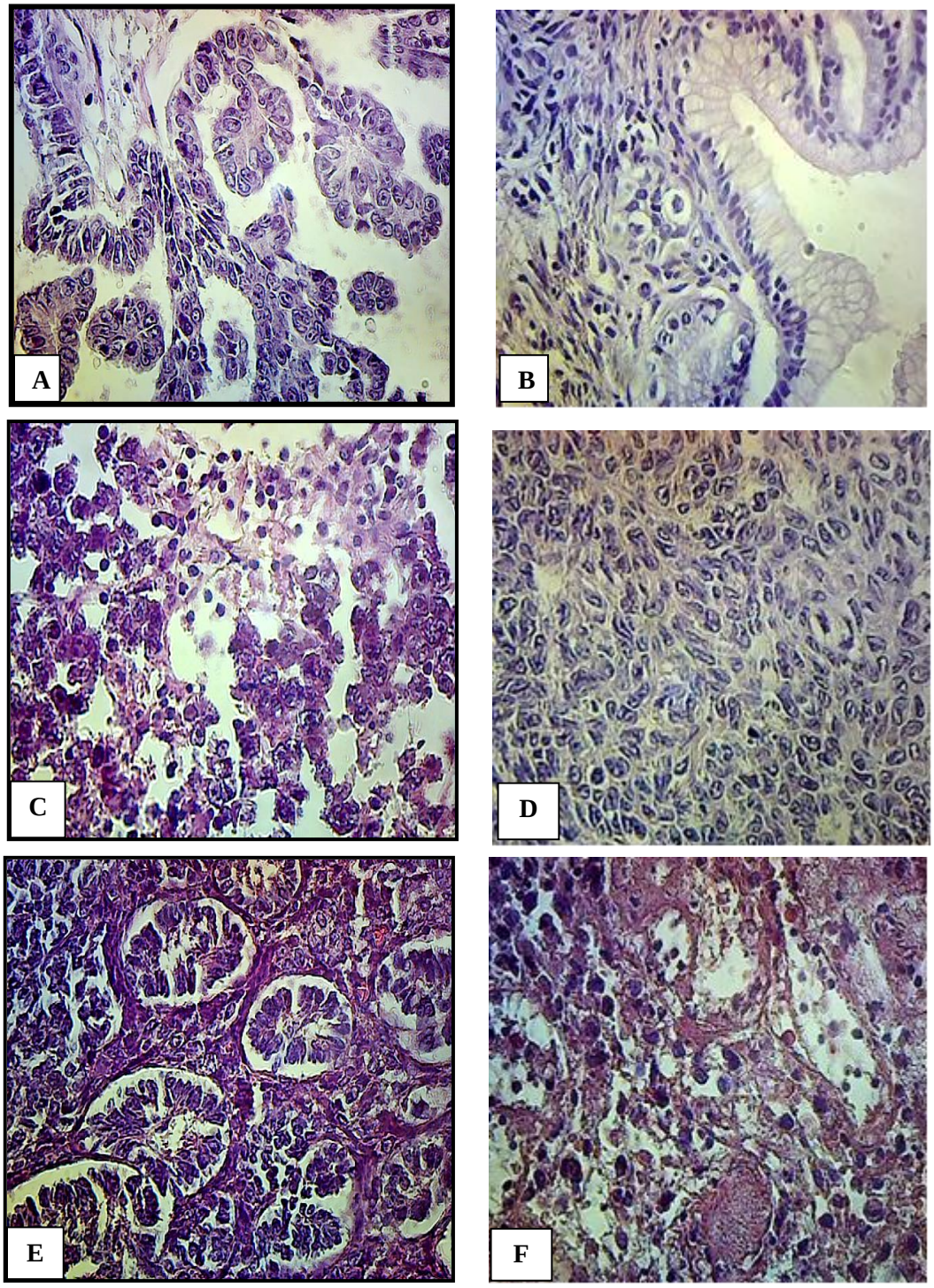

Figure.1 Histological types of the studied ovarian cancer: (A) Serous cystadenocarcinoma, well Differentiated (grade I) (B) Mucinous cystadenocarcinoma, well Differentiated (grade I) (C) Dysgerminoma, Poorly Differentiated (grade III) (D) Granulosa cell tumor, Poorly Differentiated (grade III) (E) Sertoli-leydig cell tumor, Poorly Differentiated (grade III) and (F) Yolk-sac tumor, Poorly Differentiated (grade III) [Hematoxylin and Eosin (H&E),40X].

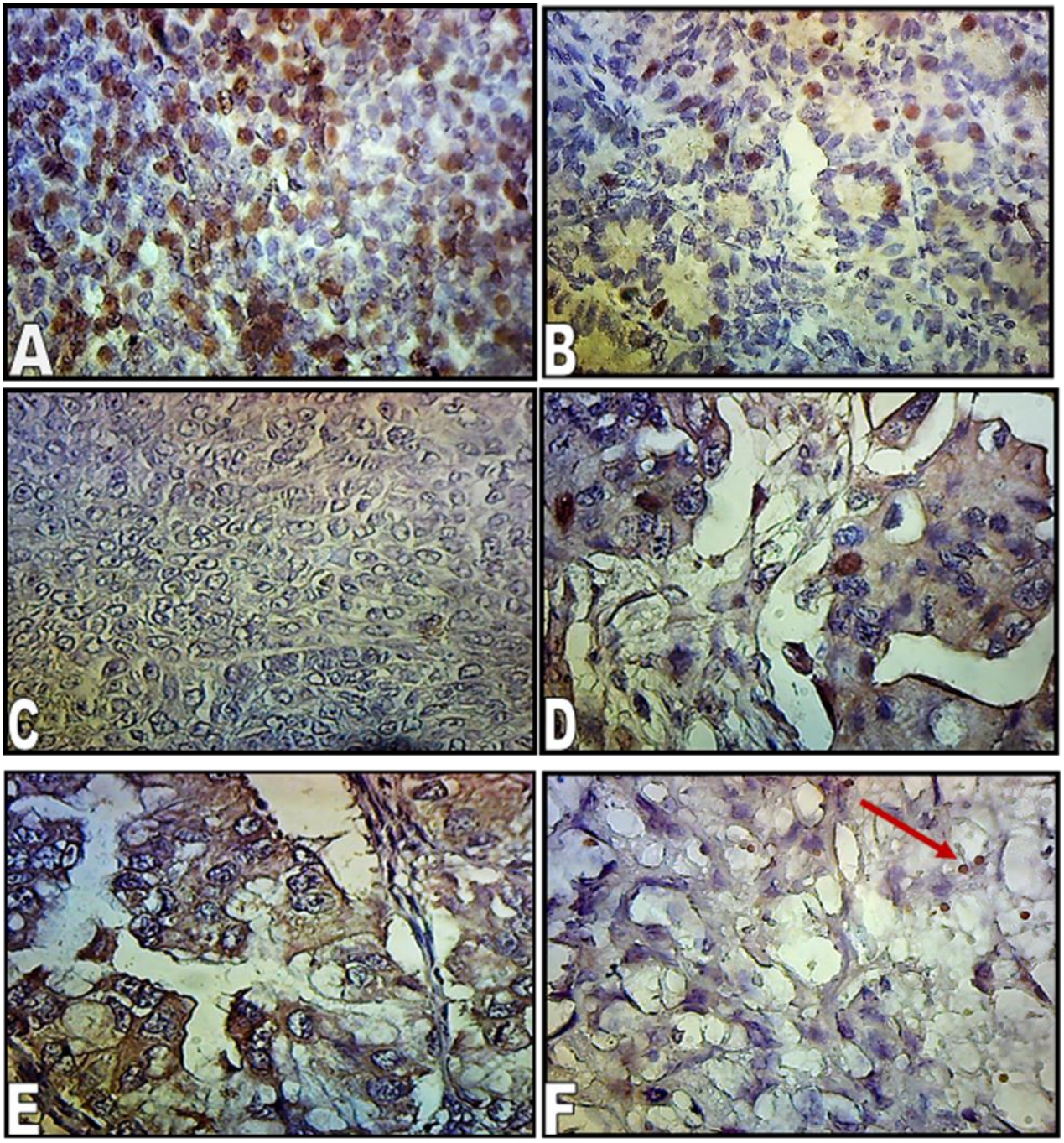

Figure. 2 BRCA1 expression: (A) a case of serous cystadenocarcinoma showing strong BRCA1 nuclear expression, (B) a case of granulosa cell tumor showing reduced BRCA1 nuclear expression, (C)) a case of serous cystadenocarcinoma showing loss of BRCA1 immunostaining (D) a case of serous cystadenocarcinoma showing nuclear and cytoplasmic immunostaining, (E) a case of serous cystadenocarcinoma showing cytoplasmic expression of BRCA1 protein, (F) infiltrating lymphocytes showing strong immunostaining (arrow) (40X).

Discussion

The complex genomic organization of BRCA1 gene and the wide range of mutations occurred and the heterogeneity of ovarian cancer suggesting that rapid screening technique represents a major technical diagnostic step in ovarian tumors [6]. Detection of BRCA dysfunction in ovarian carcinomas has prognostic and therapeutic significance. BRCA1 immunohistochemistry provides effective, an inexpensive and rapid initial screening method to detect BRCA1 dysfunction in ovarian cancer that resulting from genetic alterations such as BRCA1 germline mutation, somatic mutation and promoter methylation [14, 16].

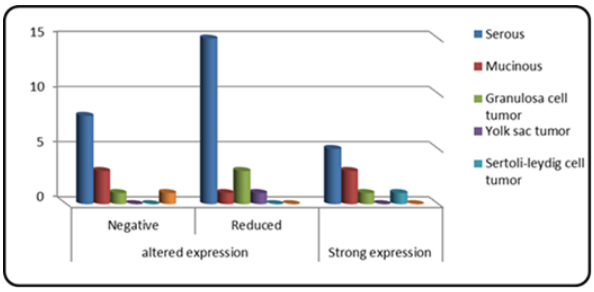

Figure.3 Distribution of nuclear BRCA1 expression among histological types of ovarian cancer.

The present investigation revealed that the frequency of primary ovarian cancer cases was higher in age group <45 y than in age group >45 yrs (53.5% Vs 46.5%) (Table 1). This finding is in agreement with Deeba et al. [20] who found the mean age of cases was 40.6 y, and similar to the age range of 10-81 years published by Strigini et al.[21] while Ferrandina et al.[22] found the cases mean age was 58.5 with age range of 25-84 y. This difference in the age of presentation of ovarian cancer cases was supported by the fact that all women are at risk of ovarian cancer development regardless of age. However, the risk is highest at the postmenopausal period and increasing with age. Many factors may contribute to an early age ovarian cancer development like the genetic and environmental factors and the hazards of the wars [23]. Our study found that the serous type of ovarian cancer was the most common histological type (65.2%). This is consistent with findings of Bagnoli et al. [24] and Ferrandina et al. [22] who found most of cases (65% and 66.4%, respectively) were of serous cystadenocarcinoma. The current results may reflect the fact that the serous cystadeno-carcinoma represents the most common type of all surface epithelial tumors.

Most of cases in the present study were of high grade (grade III; 60.5%) and high (stage III; 37.2%) (Table 1), which are similar to the reported elsewhere. Ferrandina et al .[22] and Sabatier et al.[25] found that 67.3% and

59.2% of cases were of grade III, respectively. Others, found 50% of cases were of stage III, while Yoshikawa et al. [26] reported 45.7% of cases were of stage I. The possible explanation of such differences may reflect the absence of the early detection programs and the poor health educations, as well as the non-specific symptoms of ovarian cancer leading the patients to be presented with a high grade tumor [22].

The present results revealed a high percentage (76.7%) of malignant ovarian tumors having altered protein expression (complete loss or reduced expression) which falls in the range of 34-90% of aberrant expression in ovarian tumors reported previously[27,29]. Controversy, may be explained mainly due to the difference in the sample size, tumor staging, and condition of IHC assessment and interpretation of the BRCA1 expression.

Altered BRCA1 expression was found to be higher in samples of age group ≤45 y (78.3%) in comparison with those of age group >45 y which exhibited strong nuclear BRCA1 expression (25%) (OR, 2.75; CI, 0.63-11.97) (P>0.05) (Table 2). These results indicate that altered BRCA1 protein expression among the Iraqi ovarian cancer cases may be resulted from genetic alterations, at which environmental and war hazardous roles are involved. BRCA1 dysfunction can be primarily estimated by detection of altered BRCA1 protein expression [17]. Our findings also revealed that altered protein expression was observed more commonly in high grade and high stage ovarian tumors in comparison to the better differentiated tumors. These results are in consistence with previous findings of altered protein expression correlation with poor prognostic parameters [6]. In fact, the possible correlation between BRCA1 inactivation and the high stage tumors may be due to the molecular abnormalities resulted from the genomic instability, which is common in ovarian cancers. The present result showed that altered nuclear BRCA1 expression was significantly correlated with the unilateral tumor site (OR=5.00; CI 1.04-24.03) (P<0.05), indicate to BRCA1 dysfunction may be associated with development of ovarian cancer in one site.

The cytoplasmic expression was observed in 37.2% of studied malignant ovarian cases and no association was found between cytoplasmic expression and tumor grade, stage and tumor site. Previous studies conducted on sporadic breast cancer specimens showed similar findings. Rakha et al.[30] mentioned that BRCA1 cytoplasmic expression play an important role in breast

cancer. Kashima et al. [31] indicated that cytoplasmic expression detected in normal ovarian tissues represents the splice variant protein losing most of exon 11 (BRCA1-Δ exon 11) recognized by monoclonal antibody (GLK-2) used in the current study.

In conclusion, our data demonstrated that the altered BRCA1 expression may play a significant role in the progression of ovarian cancer. BRCA1 IHC is a clinically useful approach to detect the BRCA1 dysfunction and the H-score assessment is considered as a good estimation scoring to evaluate the altered BRCA expression. Large sample study is required to clarify the cellular localization of BRCA1 protein and potential significance of its abnormal cellular localization in ovarian cancer.

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