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Association of *BRCA1/2* Mutation and *TP53* Over-Expression with Sporadic vs. Hereditary Iraqi Ovarian Cancer Patients

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Author's contribution

This whole work was carried out by IKA.

Original Research Article

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ABSTRACT

Aims: In Iraq, ovarian cancer is the fifth most common cause of death, and the 6th in the list of most common cancers. While *TP53* is the most common tumor suppressor gene involved with human malignancies, ovarian cancer is already known to be linked to the variations in the breast cancer genes, *BRCA1* and *BRCA2*. Herein we aim to estimate the rate of *BRCA1, BRCA2* gene mutation and *TP53* immuno-expression in patients with hereditary vs. sporadic ovarian cancer and to show the correlation of these biomarkers to some clinicopathological parameters.
Study Design: This is a correlational case-control study design.
Place and Duration of Study: The present study was performed in the Department of microbiology; Genetic section, College of Medicine, Babylon University. Samples taken from patients referred to general teaching hospitals and some of the private laboratories in Al-Hilla and Al-Najaf governorate, in the middle of Iraq, over a period from January 2013 to November 2013.
Methodology: Fifty-eight patients with ovarian carcinoma (30 sporadic and 28 hereditary), their ages ranging between 28-77 years, and thirty healthy women as control were included in this study. Genetic study using PCR technique was employed for *BRCA1/2* gene mutation detection. Avidin-Biotin Complex (ABC) method was employed for immune-histochemical detection of *Tp53* gene over-expression.
Results: *BRCA1l2* gene mutation was found in 21 and 13 cases out of 28 hereditary and 30 sporadic ovarian cancer cases respectively. *TP53* over-expression was detected in 18 and 17 cases out of 28 hereditary and 30 sporadic ovarian cancer cases. *BRCA1l2*

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gene mutation and *TP53* over-expression was reported more frequently in higher stage of tumor (*P*=0.05). The large majority of cases were diagnosed in later ages, patients with sporadic cancer got the disease later than hereditary ones.
Conclusion: *BRCA1*/*2* and *Tp53* genes alterations appears to be more important in hereditary than in sporadic ovarian cancer pathogenesis and evolution, as they are frequently associated with biologically aggressive tumors (high stages). Furthermore, *TP53* gene over-expression was found to be more correlated with the cancer occurrence than *BRCA1*/*2* mutation.

Keywords: BRCA1l2 gene; TP53 gene; ovarian carcinoma; sporadic; hereditary; Iraqi patients.

1. INTRODUCTION

The past decade has witnessed a rapid growth of researches into the prevention and early detection of ovarian cancer [1]. While it is moderately getting frequent in the Middle-east [2]. In Iraq, according to recent cancer registries, ovarian cancer is the fifth most common cause of death in all cancer death, and the 6th in the list of most common cancers [3]. Although it is still to know that the role of molecular and biological factors in ovarian cancer is controversial. The exact cause of ovarian cancer is unknown [4].

The most significant risk factor for ovarian cancer is an inherited genetic mutation [5,6,7]. Several oncogenes and onco-supressor genes have been implicated in ovarian carcinogenesis [3]. *BRCA1, BRCA2* and *TP53* (genes map to chromosome 17 and 13) are known biomarkers of ovarian cancer [8]. *BRCA1* and *BRCA2* are human genes that belong to a class of genes known as tumor suppressors. In normal cells, *BRCA1* and *BRCA2* help ensure the stability of the cells genetic material (DNA) and help prevent uncontrolled cell growth. The frequency of *BRCA1* and *BRCA2 genes* mutations varied greatly among different countries and populations [5]. Mutation in these genes has been linked to the development of hereditary ovarian cancer [9]. The *TP53* tumor suppressor gene encodes a transcription factor that plays a critical role in the regulation of cell cycle progression, DNA repair, and cell death [10,11]. The guardian of the genome, *TP53* is the most common tumor suppressor gene involved with human malignancies [12,13]. Occurring together, mutation in *BRCA1* or *BRCA2* and over-expression of *TP53*, make ovarian cancer to exhibit relatively aggressive features [14,15].

Only few percentages of women with ovarian carcinoma are diagnosed at a localized stage mostly because symptoms are vague and a reliable screening method for early detection has not been established [16]. *TP53* mutations were significantly more frequent in ovarian carcinomas with advanced stages III and IV [11]. Most patients with ovarian cancer present with stage III or higher disease and require aggressive treatment [17].

In view of that, it seems feasible to estimate the rate of *BRCA1*/2 mutation and *TP53* immunoexpression in different histological stages of ovarian cancer and to assess whether these biomarkers are correlated to some common clinicopathological parameters such as tumor staging, and patients ages in comparison between groups of local Iraqi hereditary vs. sporadic ovarian cancer patients.

2. MATERIALS AND METHODS

After Institutional Ethics Committee approval, and the acquisition of a written signed informed consent from all the participants, fifty eight blood and tissue samples taken from female patients with ovarian cancer were included in this study. The present study was designed as a correlational case-control study, carried out in the Department of microbiology; Genetic section, College of Medicine, Babylon University. All cases and controls were collected from general teaching hospitals and some of the private laboratories in Al-Hilla and Al-Najaf governorate, in the middle of Iraq, over a period from January 2013 to November 2013. Patients' ages ranged from 28 to 77 years, with a mean age of 56 years. Most cancers are considered sporadic. In people who have sporadic cancer, they did not inherit cancer-causing mutations from their parents. Instead, certain cells in their body developed mutations that led to cancer [1]. In contrary, only approximately 10% of women with ovarian cancer are carriers of a breast/ovarian cancer susceptibility gene, people with hereditary cancer inherit a mutated gene from their parents. Every cell in the person's body contains the mutation. They can pass that altered gene along to children [6].

The inclusion criteria for hereditary cancer cases was based upon that, beside a documented medical diagnosis of having ovarian cancer, a positive family history of previously diagnosed cases of breast/ovarian cancer in the first or second degree relatives is needed to be engaged in the study, whereas sporadic cancer patients should have no such positive family history. The control groups were those patients who had negative family history and were referred to the hospital as having a suspected clinical ovarian cancer, and by histopathological tests they were revealed as having normal ovarian tissues.

Confirmation of histopathological diagnosis and grading of tumors were carried out after reviewing all slides before proceeding further to the genetic and immunohistochemical analysis.

For *BRCA* gene mutation analysis, duplicate blood samples were collected in EDTA tubes and were transferred to the laboratory as soon as possible, for DNA extraction [using a Promega DNA purification kit (Promega corporation, www.promega.com , catalogue no. LA1620) and in accordance with the manufacturer's protocols, genomic DNA was extracted from peripheral blood lymphocytes)]. Blood samples were subjected to screening for the presence of mutation in *BRCA*1 exon2, *BRCA1* exon20, and *BRCA*2 exon11, mutation by mean of PCR technique. To detect *BRCA*1 and *BRCA*2 mutations, DNA was amplified by polymerase chain reaction (PCR), exon 2 and 20 of *BRCA*1 and exon 11 of *BRCA2* genes, using the primer pairs and PCR conditions supplied by Lahad *et al*. (2007)[18]. PCR products for exon 2 and 20 of *BRCA*1 and exon 11 of *BRCA2* genes were sized 275, 425, and 534 base-pair respectively, as illustrated in Fig.1.

Avidin-Biotin Complex (ABC) method was employed for immunohistochemical detection of p53 protien. Tissue sections of 4.μ were taken from the formalin-fixed, paraffin embedded blocks for immunohistochemistry. The initial tissue section, stained with H&E, was used to confirm that ovarian tumor histopathological type was present in the frozen specimen. This method was employed for immunohistochemical detection of p53 using Monoclonal Mouse Anti-Human p53 Protein, 1 ml DAKO, Clone DO-7, Code N7001. Faint staining pattern, whether cytoplasmic or nuclear, that could only be detected by using higher magnification (objective 40). While strong staining pattern, easily seen by low magnification (objective 4). The criterion for positive immunoreaction is dark brown precipitate (nuclear for p53). While the intensity of the staining was assessed by counting the percentage of positive cells in 100

malignant cells at objective 40 total magnification. The immunostaining was calculated as the percentage of immunoreactive cells per total number of malignant cells. Each sample was scanned for at least five fields with a high power magnification [3,8].

The intensity of p53 nuclear stain (scoring system) was classified into:

Score 0: Negative, none or <5% of the cells revealed positivity for the marker.
Score +1: Weak or mild staining, (5-10%) positive of tumor cells.
Score +2: Moderate staining, less than 25% of tumor cells are stained positive.
Score +3: Strong staining, (25-50%) of tumor cells are stained positive.
Score +4: Highly strong staining, over 50% of tumor cells are stained positive.

A control group of 12 samples with normal ovarian tissues were involved in this study.

2.1 Statistical Analysis

Statistical analysis of all results were preceded by the help of SPSS version 15 software statistical package using P value at level of significance less than 0.05.

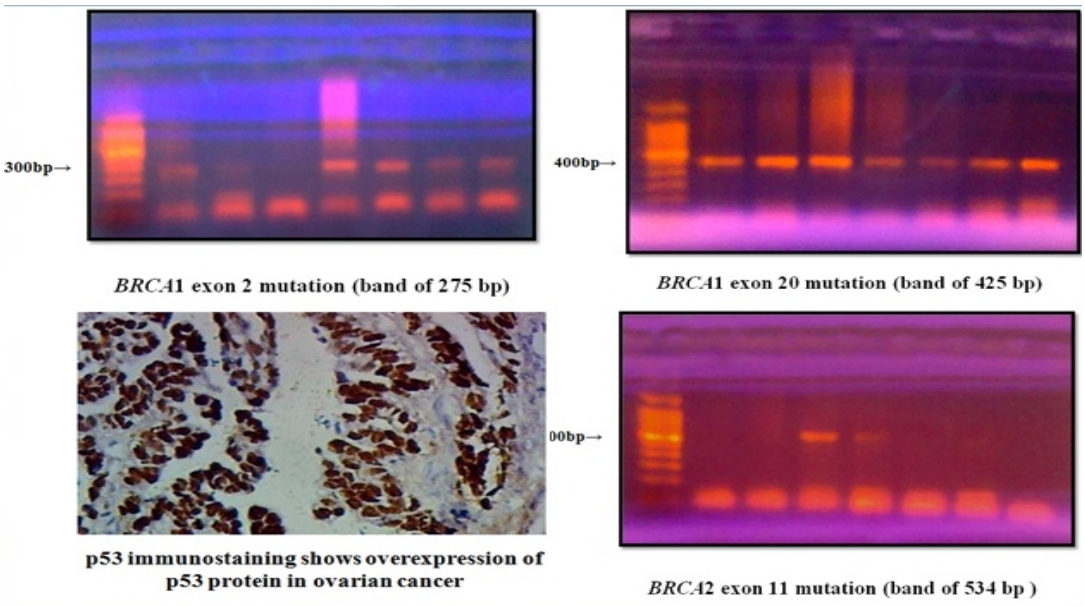

Fig. 1. Electrophoretic imaging of *BRCA*1/2 gene mutation (upper two and lower right) and p53 immunostaining (lower left)

3. RESULTS AND DISCUSSION

3.1 Results

Genetic analysis of the present work found that, there was a strong positive correlation between the detection of *BRCA* and *TP53* genes alterations among hereditary, and to lesser extent sporadic ovarian cancer patients (*P*=0.05).

We attempted to assess the frequency of genetic alterations behind the increased incidence of ovarian cancer in comparison to studies done in other countries (Czech Republic [1], Iran [5], United Kingdom and the United States [6], Turkey [15], Israel [18,19], England [20],and Russia [21]). In order to estimate the rate of *BRCA1, BRCA2* gene mutation and *TP53* immuno-expression in patients with hereditary and sporadic ovarian cancer, a genetic analysis using PCR technique was performed for the detection of *BRCA* gene mutation, while Avidin-Biotin Complex (ABC) method was employed for immunohistochemical detection of p53 protein Fig. 1.

Outcome of the genetic analysis demonstrated that there was a high frequency of *BRCA* gene mutation among patients with hereditary, and to lesser extent sporadic ovarian cancer, while these mutations hardly ever appeared in the control group participants. Similarly, results about *TP53* gene over-expression were more frequently associated with the cancer group patients but barely in the control participants Table 1. The statistical analysis of this study acknowledged that, there was a strong positive association between a mutated *BRCA1* gene and the occurrence of ovarian cancer for both hereditary and sporadic group patients (OR: 12.13, C.I. 95%:2.41-61.03; and OR: 5.09, C.I. 95%:0.98-26.43 respectively, *P*=0.000105). Similarly, concerning *BRCA2* gene mutation analysis results illustrated that, once more there was a strong positive association between a mutated *BRCA2* gene and the occurrence of ovarian cancer for both hereditary and sporadic group patients (OR:11.60, C.I. 95%:1.34-100.41 ; and OR:5.80, C.I. 95%:0.63-53.01 respectively, *P*=0.000007). Further statistical analysis was applied on whether there was any positive correlation between the over-expressed p53 protein and ovarian cancer development, results revealed that there was a very strong association between this genetic alteration and both hereditary and sporadic ovarian cancer development (OR:118.79, C.I. 95%:47.45-297.38; and OR:79.07, C.I. 95%:13.77-196.79 respectively, *P*=0.000001). Furthermore, the combined *BRCA* gene mutation and *TP53* gene alteration was studied concomitantly, showing more frequent occurrence among hereditary than sporadic cancer patients (57.1% in hereditary cancer patients and 30% in sporadic cancer patients, *P*=0.001).

Table 1. Genetic analysis among ovarian cancer and controls

Genetic analysis	Hereditary ovarian cancer (28)	Sporadic ovarian cancer (30)	Control group (30)
BRCA1 mutation	13 (46.4%)	8 (26.6%)	2 (6.6%)
BRCA2 mutation	8 (28.5%)	5 (16.6%)	1 (3.3%)
TP53 over-expression	18 (64%)	17 (56.6%)	0 (0%)

***** *Among the hereditary ovarian cancer patients who got a mutated BRCA genes, 16 were also having combined p53 over expression. While only 9 of sporadic cancer patients got the combined mutation genotype.*

The risk of ovarian cancer goes up with age [22]. In the present study it was instituted that, while some women in their 20's and 30's get ovarian cancer, the large majority of cases were diagnosed in later ages. The mean age of patients with hereditary ovarian cancer was 52 years, while sporadic cancer patients get ovarian cancer older with mean age of 63 years. Table 2.

Regarding the supposed correlation between genetic alterations and cancer staging, results established that the studied genetic alterations were more oftenly reported in higher stages(more in stage III than stage IV) with significant level of expression (*P*=0.05) Table 3.

Table 2. Age distribution among hereditary vs. sporadic ovarian cancer patients

Age intervals	Hereditary ovarian cancer	Sporadic ovarian cancer
20-29	1	1
30-39	3	2
40-49	7	3
50-59	12	6
60-69	4	13
70-79	1	5
Total no.	28	30
Mean age / year	52	63

Table 3. Distribution of patients according to the stage of ovarian cancer

Stages of ovarian cancer	Hereditary ovarian cancer (28)	Sporadic ovarian cancer (30)
Stage: I	1 (3.6%)	1 (3.3%)
Stage: II	3 (10.7%)	2 (6.7%)
Stage: III	17 (60.7%)	19 (63.4%)
Stage: IV	7 (25%)	8 (26.6%)

3.2 Discussion

According to the health profile ranking of Iraq, there is an increase in cancer incidence to more than three folds from the older Iraqi cancer registries in the past two decades [2,3]. In the current study, it was affirmed that there was a statistically significant high frequency of genetic alterations base behind the increased incidence of ovarian cancer in comparison to studies done in other countries, may be due to some factors specially that our society was exposed to illegal weapons of carcinogenic materials during the Gulf Wars specially in areas in the middle of Iraq, where the study done [16].

Ovarian cancer is already known to be linked to the variations in the breast cancer genes, *BRCA1* and *BRCA2* [1,3,6,8,9,15,18,19,22]. In the present work we were 95% confident that subjects who have a hereditary family tendency to get ovarian cancer and have a mutated *BRCA1* gene are 12.13 times more risky than others to develop ovarian cancer. Similarly, subjects who do not have such predisposition to get ovarian cancer but have a mutated *BRCA1* gene are 5.09 times more susceptible to get ovarian cancer than others, both these results were statistically very significant (P=0.000105). Likewise, *BRCA2* gene mutation is associated with increased cancer risk for both hereditary (OR:11.60) and sporadic(OR:5.80) group patients (P=0.000007). This pronouncement illustrates the role of a mutated *BRCA* gene to play in ovarian cancer pathogenesis, especially for those with positive family history of the disease. Many authors confirm us about the frequently observed mutation of *BRCA1* and *BRCA2* in ovarian cancer [8,19,20]. They observe that the presence of mutations of such a high frequency permits the study of their clinical expression and penetrance [19]. In a study done in Israel about the penetrance and distribution of *BRCA* gene mutation among Ashkenazi women with ovarian cancer it was found that there was a surprisingly high frequency (45%) of germ-line *BRCA1/BRCA2* mutations in a series of Iraqi/Iranian origin Jewish women with ovarian cancer who were unselected for family history[18]. Some mutations in *BRCA* gene was thought to be specific for defined populations (ex.: Ashkenazi, Russian and Turkish populations), presence of these founder mutations in other populations

suggesting a certain level of admixture between individuals in spite of religious and cultural barriers separating these diverse populations[5].

The strongest known risk factor in patients with ovarian cancer is a family history of the disease [6]. In hereditary ovarian cancer the role of *BRCA* gene alteration is well established, although its frequency is variable, it still within a defined range [23]. As our data and those from literature cited here suggest, *BRCA* gene mutation (or their defects) appears to be important in approximately half of hereditary and one-quarter of sporadic ovarian cancer cases [1].

Coming in accordance with the reason for the selection of the recurrent *BRCA* gene mutations in this research, a study by Couch et al., (2007) [24], showed that the most commonly detected *BRCA*1 mutations are a deletion of adenine and guanine [*BRCA*1 185delAG (exon 2)], and insertion of cytosine [*BRCA*1 5382insC (exon 20)], and *BRCA*2 mutation is the deletion of thymine [*BRCA*2 6174delT (exon 11)], which have a high frequency in the general population. Results further solidified by another study [24] which investigated different mutations flanking both *BRCA1* and *BRCA2* genes and their individual risk on breast/ovarian cancer, illuminating that exon2 mutation in *BRCA1* gene was the most risky genetic alteration, followed by exon2, while in *BRCA2* gene exon 11 was the most commonly associated genetic alteration to ovarian cancer among carriers.

It is expected that ovarian cancer risk is due to a combination of several genes that individually carry a low to moderate risk of the disease [20]. Through this study it was clear that, *TP53* gene alterations was the most significant associate to ovarian cancer for both hereditary and sporadic groups of patients (OR:118.79, C.I. 95%:47.45-297.38; and OR:79.07, C.I. 95%:13.77-196.79 respectively, *P*=0.000001). Generally the present work confirms previous findings concerning the combined alteration in the expression of *BRCA* gene and *TP53* which are linked to accelerated tumor behavior and a poor prognosis in ovarian cancer. Wild-type p53 has been shown to play a role in many cellular functions including cell cycle regulation and apoptotic cell death [17]. Mutation of the *TP53* gene is the most common genetic alteration thus far in ovarian cancer [4], with mutations being present in approximately half of advanced stage ovarian carcinomas [10]. Beside *BRCA* gene mutations, in the present study, p53 immuno-expression was detected in 64% of hereditary ovarian cancer cases and in 56.6% of sporadic cases. These data are consistent with previous findings, signifying that *TP53* over-expression plays a significant role in ovarian cancer development [11].

The age distribution of hereditary and sporadic ovarian cancer among patients in our study was further supported by the fact that all women are at risk of developing ovarian cancer regardless of age; however, a woman's risk is highest during her 60s and increases with age through her late 70s [5]. The risk of developing ovarian cancer in a woman's lifetime is estimated to be 1 in 70. The incidence increases with age, reaching its peak in the eighth decade [4]. Furthermore, the later occurrence of ovarian cancer in sporadic cancer patient if compared to patients with hereditary cancer was explained as that, in women with positive family history of ovarian cancer the disease occurs at a younger age than sporadic cancer [25].

One of the most important clinicopathological parameters in studying a cancer is its staging. Here in, it was noticed that *BRCA* gene mutation and p53 immunoreactivity is significantly increased as the stage of tumor elevated. This finding may be justified by another author as that ovarian tumors with *BRCA* gene mutation and *TP53* over-expression are biologically

bearing more aggressive behavior [3]. As ovarian cancer usually presents with widespread intra-abdominal metastasis. Approximately 70% of women with ovarian cancer present with advanced stage disease with either regional or distant metastasis at the time of diagnosis. Researchers found that, *TP53* mutations were significantly more frequent in ovarian carcinomas with advanced stages III and IV [11]. It is possible that the correlation between *BRCA* gene mutation and p53 immunoreactivity and aggressive tumor behavior manifested by distant metastases may be a downstream of more fundamental molecular abnormalities, such as genomic instability, which is common in ovarian cancer, strongly correlates with the development of *BRCA and TP53* gene mutations. Saying that for some ovarian cancers, genomic instability might be the cause of the mutations rather than the result [17].

4. CONCLUSION

Research of *BRCA1*/*2* and *TP53* gene role in sporadic ovarian cancer is still beginning. As our data and those from literature cited here [1,8] suggested, these genes (or their defects) appear to be more important in hereditary than in sporadic ovarian cancer pathogenesis and evolution, as they are frequently associated with biologically aggressive tumors (high stages). Furthermore, *TP53* gene over-expression was found to be more correlated with the cancer occurrence than *BRCA1*/*2* mutation.

Consequently, further studies need to be focused on how these tumors differ in biological features or in response to therapy in correlation to the genetic base of the disease. And additional expanded genetic analysis of larger number of patients with ovarian cancer in a prospective studies with a longer duration of follow-up will provide a better insight in to the role of *BRCA1*/*2* and *TP53* gene alterations in sporadic vs. hereditary ovarian cancer.

CONSENT

This study was carried out after the acquisition of written signed informed consents from all the participants. Author declares that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

This study was carried out after Institutional Ethical Committee approval. Author hereby declares that this study has been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

The Author has declared that no competing interests exist.

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