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BRCA1 Gene Expression is Down Regulated in Both Familial and Sporadic Breast Cancer Cases in Baghdad- Iraq

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Abstract

Breast cancer is the commonest cancer and the leading cause of malignancies-related mortality in women worldwide. Understanding the underlying biology of the disease could improve patients' stratification and may offer novel therapeutic targets and strategies. This study was set to investigate the association between *BRCA1* gene expression and some of the clinical features of breast cancer patients in Baghdad-Iraq. Eighty peripheral blood samples were collected from sixty patients diagnosed with breast cancer and twenty healthy age-matched controls for *BRCA1* qPCR gene expression analysis.

The results showed a significant reduction in *BRCA1* gene expression in all of the breast cancer patients with the vast majority of them (75%) having *BRCA1*expression below 25%. The down regulation of *BRCA1* expression also showed consistency in breast cancer patients of both sporadic (n=45) and family history (n=15) cases,with expression averages of 18% and 20.19%, respectively. Moreover, the redcuation in *BRCA1* expression was negatively associated with the disease's grades,asbreast cancer patients with the advanced stage III (n=19) showed the lowest expression average of *BRCA1* (13.8%) as compared to those in stages II (n=29) and I (n=12) of the disease (17.7% and 19.8%, respectively).

Overall, the study highlights the key role of *BRCA1*gene expression in the development of breast cancer and suggests its potential utility in the diagnosis strategies and preventing the progression of the disease, especially the sporadic type.

Keywords: *BRCA1* expression, qPCR, Breast cancer.

انخفاض التعبير الجيني لـ *BRCA1* في كلاً من مريضات سرطان الثدي المتوارث وغير المتوارث في بغداد – العراق

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الخلاصة

سرطان الثدي هو أكثر أنواع السرطان شيوعًا والسبب الرئيسي للوفيات المرتبطة بالأورام الخبيثة لدى النساء في جميع أنحاء العالم. إن فهم الأساس البيولوجي للمرض يمكن أن يسهم في تقسيم المرضى استنادا

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للمُعلمات البيولوجية التي يظهرها المرض والتي قد تستخدم كأهداف واستراتيجيات علاجية جديدة. هدفت الدراسة الى استقصاءالعلاقة بين التعبير الجيني لجين*BRCA1*وبعض السمات السريرية لمرض سرطان الثدي في بغداد – العراق.

اشتملت الدراسة على جمع ثمانين عينة دممنستين مريضة تم تشخيصهم بسرطان الثدي وعشرين عينة دم من نساء غير مصابات بالمرض(مجموعة السيطرة)لتحليل التعبيرالجيني لجين *BRCA1*بأستخدام تقنية qPCR.

أظهرت النتائج انخفاضًا كبيرًافي تعبير جين*BRCA1*في جميع مريضات سرطان الثدي حيث ان الغالبية العظمى منهن (75٪) لديهن تعبير الـ *BRCA1*أقل من 25٪. وأظهرت النتائج انخفاض التعبير الجيني لل*BRCA1*في كل من حالات سرطان الثدي غير المتوارثة (Sporadic) (n= 45) وكذلك المريضات اللأتي لديهن تاريخ عائلي للمرض (n = 15) بمتوسط تعبيرجيني تراوح بين 18 ٪ و 20.19 ٪ ،على التوالي. علاوةعلى ذلك،اظهرت النتائج أن الأنخفاض في تعبير*BRCA1*مرتبط عكسيامع درجات تطورالمرض،حيث أظهرت مريضات سرطان الثدي في المرحلة المتقدمة (المرحلةالثالثة، n = 19)أدنى معدل تعبيرل*BRCA1* (13.8٪) مقارنة بتلك الموجودة في المرحلة الثانية (n = 29) والمرحلة الأولى (n = 12) من المرض (17.7 ٪ و 19.8 ٪ ،على التوالي).

بشكل عام،الدراسةاكدت الدورالمهم لل*BRCA1*في تطورسرطان الثدي وتقترح احتمالية الأفادة من استخدام مستويات تعبيره الجين يفياس ستراتيجيات تشخيص ومنع تطور سرطان الثدي،وخاصةالنوع غير المتوارث من المرض.

Introduction

Breast cancer is the most common cancer affecting women with more than two million diagnosed cases and 626,679 deaths worldwide in 2018. Recently, a statistical cancer report showed that breast cancer is on the top of the list of cancer related death causes across the world. Globally, this disease represents approximately a quarter of cancers among women[1], while in Iraq one-third of all the registered women's malignancies is breast cancer [2]. Similar to the other types of cancer, hereditary factors, including germ-line mutations in *BRCA1* genes and the familial history of other malignancies, account for only 5 -10 % of breast cancer cases. Other non-inherited factors are thought to be the major drivers for the international spread and differences in the disease incidence[3]. It is believed that the prolonged exposure to exogenous/ endogenous hormones also contributes to raising the risk of breast cancer [4, 5].

However, a very recent finding demonstrated that heritable epigenetic aberrations associated with the risk of breast cancer development in women did not carry known germ-line mutations of the disease[6]. This finding supports the significant impact of epigenetic modifications in the initiation and progression of breast cancer through the modulation of the transcription activity of key genes involved in cellular transformation. *BRCA1* is a well-established breast cancer susceptibility gene; its germ line mutations account for 40–50% of familial breast cancer cases and reported to increase the life-long risk to 50–80% [7]. *BRCA1* is a tumour suppressor gene that has a significant role in regulating both the signalling of DNA damage and also in DNA repair. This gene has been frequently reported to be mutated in hereditary breast and ovarian cancers. Although somatic mutations have not been well characterized, loss of heterozygosity, decreased expression levels of *BRCA1* mRNA and protein expression, and hypermethylation of the *BRCA1* promoter region have been shown in breast carcinoma by a number of studies [8-10]. This indicates the significant involvement of *BRCA1* expression in the development of both familial and sporadic breast cancer cases [11].

The crucial role of *BRCA1* gene in breast cancer could be attributed to its influence on the chromatin modulation, thus connecting*BRCA1*dysreguation to both epigenetic and genetic instability. Indeed, a recent study has identified that aberrant DNA methylation signature, as epigenetic marks, was able to detect breast cancer up to one year earlier than mammography could do[12]. *BRCA1* has been linked to regulate the inactivation of epigenetically silenced heterochromatin of X chromosome (the normally inactive X chromosome in females, Barr body); the loss of inactive X chromosome is frequently reported in breast and ovarian malignancies [13-15]. It is believed that heterochromatin disruption is a cancer's common event leading to the extensive genomic dysregulation and the development of some cancers [16, 17].Here the expression levels of *BRCA1gene*were investigated for their association with some of the clinical features in a set of breast cancer patients in Iraq.

Subjects and Methods

Blood samples

For RNA extraction and purification, peripheral blood (PBL) samples were collected from eighty participants in this study during the period of November 2017 till July 2018. The blood samples were obtained from 60 patients (age mean 47.08 years, range30-69years) diagnosed with breast cancer and attending the Oncology Hospital in the Medical City- Baghdad, Iraq, along with20 samples of healthy women as controls. Blood samples were collected according to the ethical considerations, the hospital ethical committee, and verbal patients consent. Information regarding the disease diagnosis, patient's age, family history of the disease and other clinical features used in this study were acquired from the medical record of each patient. All of the study design items and procedures were approved by the Researches Development Unit in the Medical City–Baghdad, Ministry of Health and Environment, Iraq.

RNA extraction and *BRCA1*gene expression using real time-PCR

For *BRCA1*gene expression levels measurement, RNA was extracted from the peripheral blood samples (250µl) of breast cancer patients and healthy controls. The RNA extraction was performed using AccuZolTM extraction kit following the protocol provided by Bioneer Company. The extracted RNA samples were nanodropped to check their purity and concentrations. Thereafter, cDNA was synthesized from the extracted RNA samples, using AccuPowerRRocketScriptTM RT PreMix kit (Bioneer) according to the manufacturer's instruction. *BRCA1* gene expression was quantified by real time PCR technique using the following primer set: *BRCA1*- forward: CAT GCT ACT TCT CAA CCA GAA, and *BRCA1*- reverse: RTGT AGG CTC CTT TTG GTT ATA TTC. GAPDH was used as a reference gene with the following primers sequence: GAPDH-forward: TGCACCACCAACTGCTTAGC and *GAPDH*-reverse GGCATGGACTGTGGTCATGAG. All qPCR amplifications were performed in triplicate, with each one having a final volume of 10µl. These amplifications included 20 ng of cDNA, 300 ng of primer mix. 5 µl of Syber Green and 4.25 µl of distilled water. The results were presented and analyzed using Excel data analysis software.

Results

BRCA1 gene expression is down regulated in all of the studied breast cancer patients

All of the studied breast cancer patients showed down regulation of *BRCA1* gene expression, confirming the suggested crucial role for this gene in the disease initiation and progression. Of interest, 75% of the patients had *BRCA1*gene expression level below 25% and more than one third (36.6%) had *BRCA1* expression level below 9% (Figure-1).

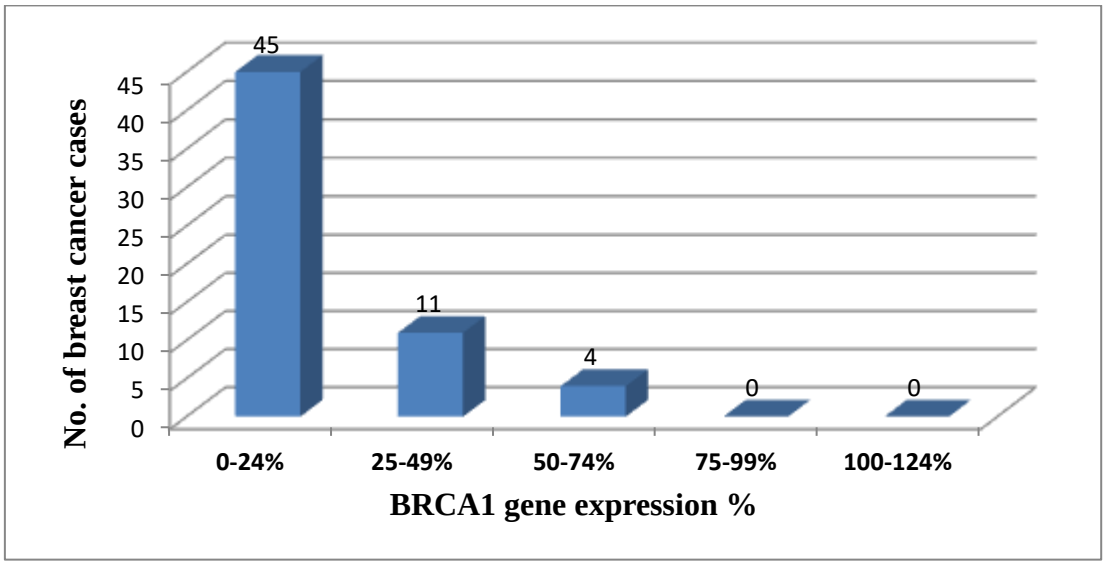

Figure 1-BRCA1 gene expression in the studied breast cancer patients. All of the studied breast cancer patients showed down regulation of *BRCA1*gene expression.

*BRCA1*gene expression levels were also shownto be down regulated for both sporadic (n=45) and family history (n=15) breast cancer patients, with expression averagesof 18% and 20.19%, respectively (Figure-2). The differences were not significant between the sporadic and patients with family historyof the disease (T-test, P=0.681).The reduced *BRCA1*gene expression in both sporadic and inherited breast cancer casesconfirms the important role for this gene in both types of this disease. The downregulation of *BRCA1* in the sporadic type of breastcancer could be due to either epigenetic or genetic alterations/ somatic mutations.

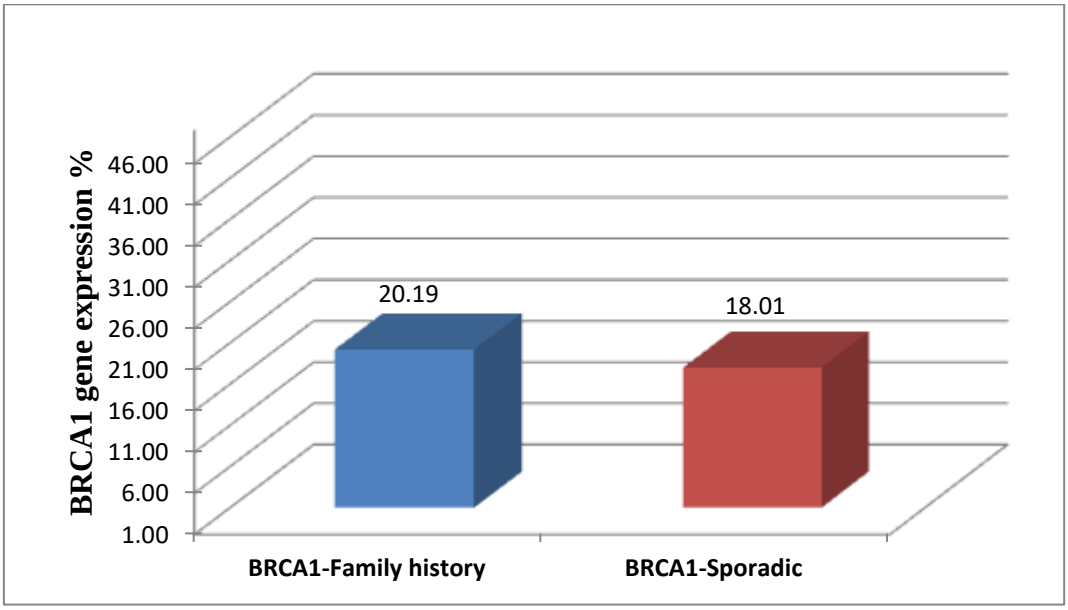

Figure 2-BRCA1 gene expression in breast cancer patients of inherited and sporadic breast cancer.

Although all of breast cancer patients showed down regulation of *BRCA1* gene expression, the comparison between the different disease grades resulted in significant differences ($P < 0.05\%$). Breast cancer patients with advanced disease grades showed higher levels of *BRCA1*gene expression in comparison to those with lower grades. The mean expression of *BRCA1* was 29.28%, 16.51% and 13% for breast cancer patients in grade III (n=10), grade II (n=40) and grade I (n=10), respectively.

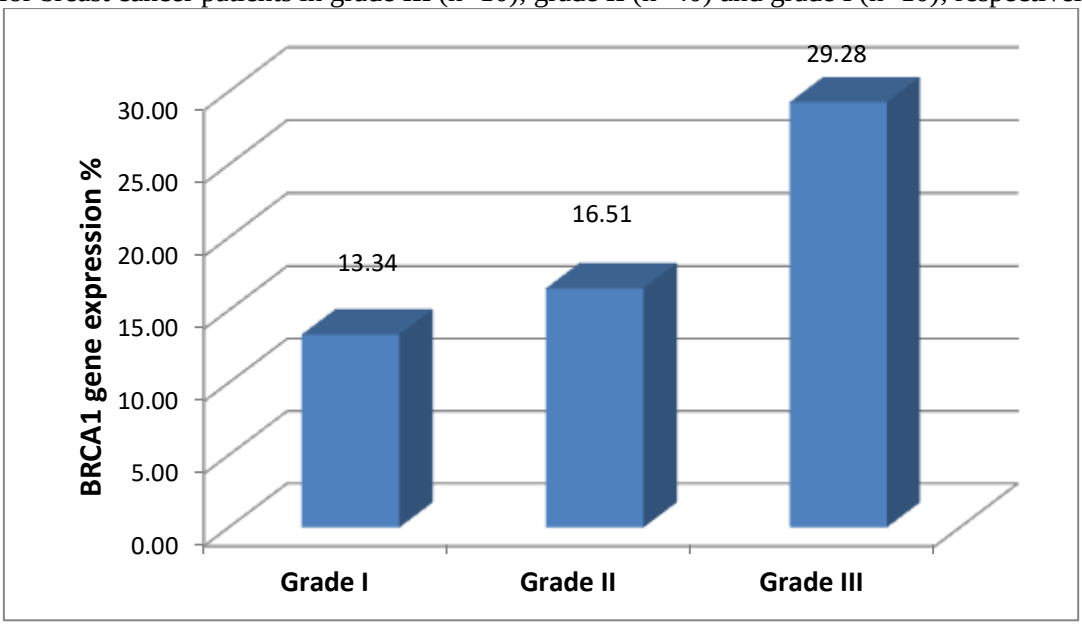

Figure 3-*BRCA1* gene expression in breast cancer patients by disease grade. The mean expression of *BRCA1* was 29.28%, 16.51% and 13% for breast cancer patients in grade III, grade II and grade I, respectively.

In contrast to the association with the disease grade, *BRCA1* gene expression levels showed negative association with the breast cancer stage. Patients in the advanced breast cancer stage (stage III) had the lowest expression average of *BRCA1* (13.8%) as compared to those in stage II (n=29) and

I (n=12) of the disease (17.7% and 19.8% respectively, Figures-3, 4). However, the differences were not significant among the compared breast cancer stages in the studied groups of patients (T-test, P= 0.420).

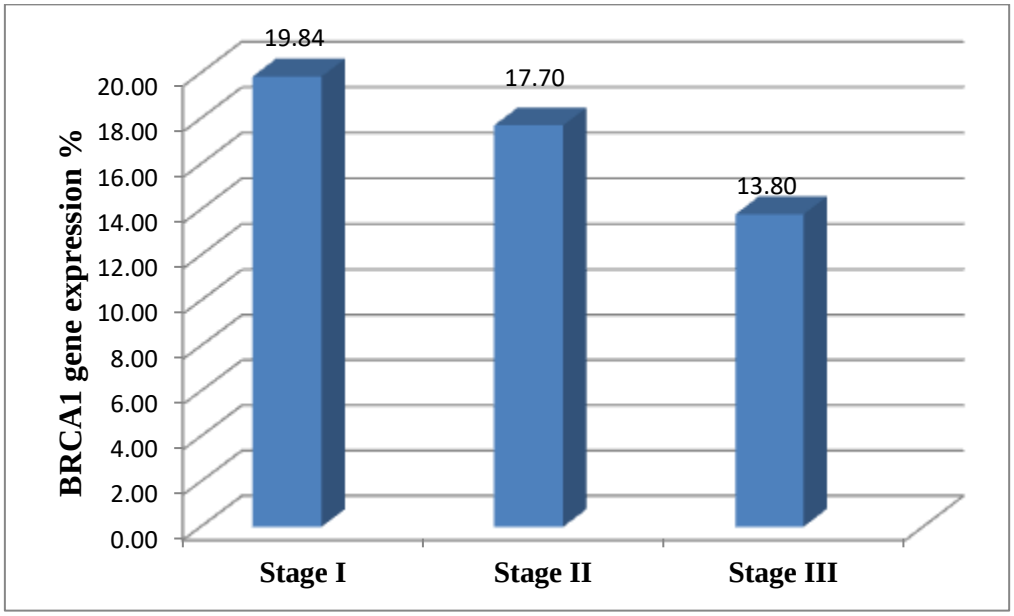

Figure 3-*BRCA1* gene expression in breast cancer patients by disease stage.*BRCA1* expression levels are shown to be negatively correlated with the disease stages.

***BRCA1* gene expression variations corresponding to different ages and menopausal status of the studied breast cancer patients**

Even though *BCRA1* gene expression was down regulated in all of the studied breast cancer patients, the results showed variations in the average of its expression among the different age groups. The *BRCA1* expression in the breast cancer age group of 30-39 years was 10.92, followed by 12.07% for the 50-69 age groups, while the highest *BRCA1* gene expression (29.07%) was for the 40-49 years age. Significant differences were obtained when the *BRCA1* expression was compared between the following age-groups: 40-49 and 50-69 years old groups (T-test, P=0.0004); 30-39 and 50-69 years old groups (T-test, P=0.013).

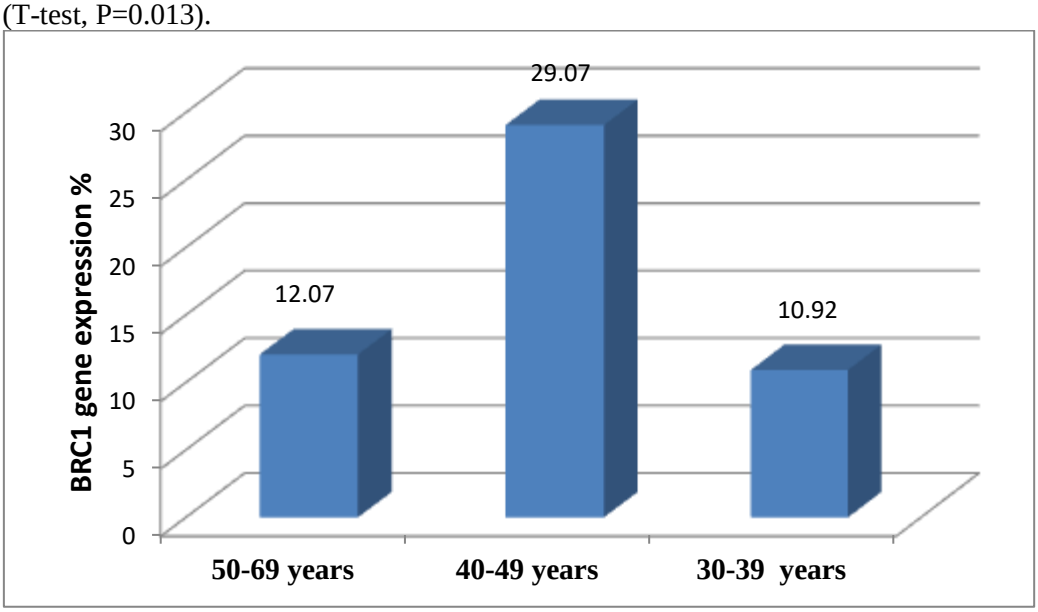

Figure 3-*BRCA1* gene expression in breast cancer cases by age of patients.

BRCA1 expression seemed much more down-regulated (expression average of 14.32%) in post-menopause breast cancer patients (n=28) in comparison to those in the pre-menopause patients (n=32,

expression average of 21.87%). However, these differences were not significant when T-test was applied (P=0.085).

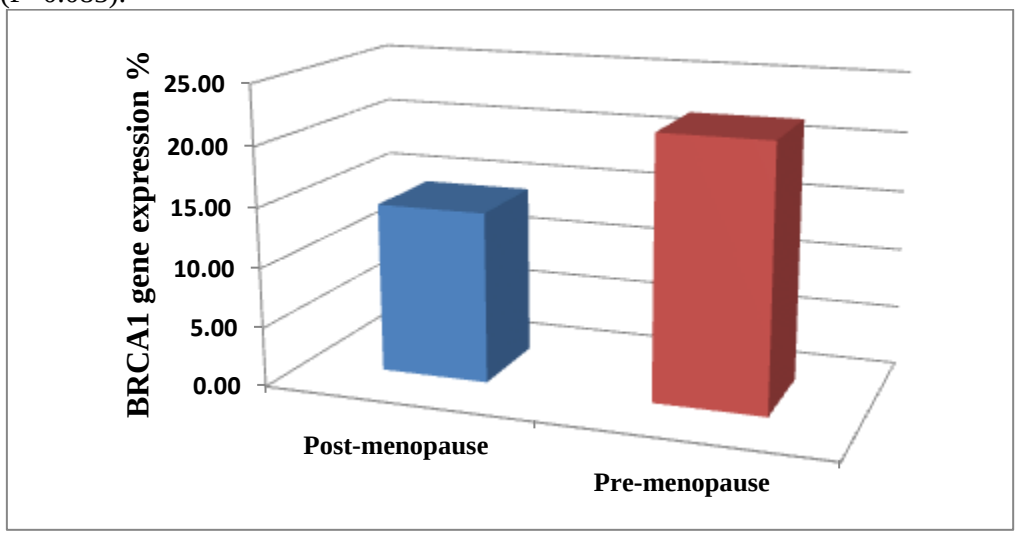

Figure 4-*BRCA1* gene expression in breast cancer patients by pre- versus post menopause stages

Discussion

Globally, breast cancer is the commonest women's cancer with an increased risk that is expected to affect one of every eight women throughout lifespan [18]. This disease also represents the leading cause of cancer-related mortality in women worldwide[19]. Understanding the underlying biology of the disease could improve patients' stratification and may offer novel therapeutic targets and strategies. This study was set to investigate the association between *BRCA1* gene expression and some of the clinical features of breast cancer patients in Baghdad-Iraq.

Interestingly, all the studied breast cancer patients showed down regulation of *BRCA1* gene expression. The results of the present study confirm the crucial role for this gene in breast cancer development. Indeed, *BRCA1* expression was dramatically decreased in both sporadic and inherited breast cancer cases in the present study, which highlights the important role of this gene in both types of the disease. Down regulation of *BRCA1*gene expression in the sporadic type of breastcancer could be due to either epigenetic silencing or genetic alterations/ mutations. In this regard, Garcia's study showed that *BRCA1*gene expression was absent in 15 tumours (43%) and present in 20 (57%)[20].The decreased *BRCA1*expression might also be a secondary effect caused by changes in upstream regulatory pathways controlling *BRCA1*expression. In addition, environmental exposures to pollutants might alter *BRCA1*expression levels. Polycyclic aromatic hydrocarbons have been reported to be capable of reducing *BRCA1*mRNA expression inhuman breast carcinoma cells [21]. Loss of heterozygosity (LOH) at the *BRCA1* locus is also a common event that occurs in 46% of breast tumours [22], but only 20% of the tumours with LOH display inactivation of the remaining allele through promoter hypermethylation[23, 24]. Studies have highlighted a debated link between *BRCA1* and Xi (inactivated X-chromosome, Barr body) which might reflect a general relationship between *BRCA1* and heterochromatin. This could connect*BRCA1* to both epigenetic and genetic instability. It has been suggested that heterochromatic instability is a common but largely unexplored mechanism, leading to widespread genomic dysregulation and the progression of some cancers[25, 26].Felicio observed a higher proportion of breast cancer cases before the age of 50 in families with pathogenic *BRCA1* mutations [27]. *BRCA1* plays a crucial role in DNA repair and decreased*BRCA1* mRNA has been observed to influence both sporadic and hereditary breast cancer. *BRCA1* mRNA is reduced in sporadic breast cancer cells despite the absence of mutations. This reduction of *BRCA1* mRNA levels in sporadic breast cancer cases has been related to acquired DNA methylation of the *BRCA1* promoter and to abnormalities in the upstream pathways that regulate *BRCA1* expression. Aberrant methylation of *BRCA1* promoter is found in 10–15% of sporadic breast cancers, whereas it is not detectable in normal breast tissues [23]. Methylation in one *BRCA1* allele is often associated with the loss of the other allele at the same locus (loss of heterozygosity or allelic imbalance)and, thus, with the complete inactivation of the *BRCA1* gene, according to Knudson's "two hits" hypothesis of tumour suppressor

gene inactivation [28]. A fraction of sporadic breast cancers has a low *BRCA1* expression. *BRCA1* mutation carriers are more likely to achieve a pathological complete response with DNA damage-based chemotherapy compared to non-mutation carriers, while the presence of *BRCA1* increases sensitivity to anti-microtubule agents [29]. An increased risk (50%) of breast cancer development showed association with first degree relative carrying *BRCA1*or BRCA2 mutations [30].

Studies have also demonstrated that the decline of *BRCA1* expression depends on the grade of the tumour [31].In a recent study, most patients with mutation in *BRCA1* showed the histological grade III (61.0%) [27].The higher prevalence of triple negative cases among *BRCA1*mutated patients can be one of the factors responsible for the poor prognosis observed in these patients. Studies also pointed to the fact that tumors associated with the presence of *BRCA1* mutations often have higher histological grades, elevated mitotic counts, poor differentiation, and high frequency of necrotic areas and pleomorphism. These characteristics are commonly associated with a worse prognosis [32-34].

Several studies have reported an inverse correlation between *BRCA1* expression and advanced breast cancer stages [35-37]. All of the observations support the tumor suppressor role of *BRCA1* gene in breast cancer development. The relatively increased *BRCA1* expression in premenopausal patients in our study seems consistent with the findings of Kandula's and colleagues who showed an increase in *BRCA1* expression in premenopausal women with grades II and III of breast cancer [38].

Conclusion

Overall, the study identified a significant reduction in *BRCA1* gene expression in all of the studied breast cancer patients, including those with no family history of the disease. Considering the rarity of *BRCA1*mutations in the general population, *BRCA1* expression assessment could be considered to identify individuals with high risk of breast cancer, including those with family history of the disease who gave negative *BRAC1* mutations test.

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