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# ABCG2 (BCRP) m RNA expression level by using real- time PCR and immunohistochemistry associated with clinicopathological features in Iraqi women with stage II-III breast cancer.

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## Abstract:

**Background:** Breast cancer is the most frequent cancer and cause of death among women worldwide. ABCG2 (ATP–binding cassette sub-family G member 2) is an ABC transporter superfamily and endogenous expression of ABCG2 in different certain cancer reflect intrinsic drug resistance. It is also a molecular determinant of pharmacokinetic properties of many drugs in humans. In this study, we determined the expression levels of ABCG2 in breast tissues of Iraqi women with stage II and III breast cancer. The correlation between the expression levels of ABCG2 and clinicopathological features was analyzed.
**Methods:** The expression levels of ABCG2 in the breast was determined by using real- time PCR and immunohistochemistry in 64 patients with stage II and III samples and in 21 benign tumors from Iraqi women.
**Results:** We found that the expression level of ABCG2 mRNA were significantly increased in breast cancer stage II-III tissues than those in benign tumor tissues. There was a significant variation between the mRNA levels of ABCG2 in stage II and stage III at $P< 0.05$. Immunohistochemistry revealed that the protein expression levels of ABCG2, was also increased in 83% of patients with stage II and III breast cancer as compared to 17% in benign tumor. The increased expression levels of ABCG2, in stage II-III breast cancer were correlated to tumor stages ($P=0.03$), tumor grades ($P=0.01$), tumor types ($P=0.01$) and lymph node metastasis ($p=0.0001$), respectively.
**Conclusion:** ABCG2 expression level in Iraqi women with stage II and III breast cancer were highly correlated with tumor stages, grades, types and metastasis and they could be used a potential markers which can prediction tumor behavior, progression and prognosis. Over expression of ABCG2 protein lead to treatment failure, tumor relapse and tumor metastasis by induction cancer cells against cytotoxic drugs.

***Key words:*** *ABCG2, Breast cancer, real-time PCR, Iraqi women.*

## Introduction:

Breast cancer is one of the most frequent malignant tumors among women, with an estimate of more than 1.4 million new breast cancer cases diagnosed worldwide each year and accounting for 23% of all new neoplasm cases (1). Approximately half a million women die from breast cancer (2).

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Breast cancer resistance protein (BCRP) or ATP-binding cassette (ABC), subfamily G, member 2 (ABCG2) belong to a large superfamilly of membrane transporter which catalyze the ATP dependent transport of different xenobiotics and endogenous compounds out of the cells (3). In normal human tissues; BCRP is highly expressed in the small intestine, brain endothelium and placenta (3).

BCRP (ABCG2) was discovered initially in the multidrug resistant breast cancer cell, as showing that it confers resistance to the chemotherapeutic agents like Mitoxantrone, topotecane and methotrexate, So ABCG2 has the capacity to efflux (ex-

truding) these various chemotherapy drugs out of the cells and may contribute to drug resistance of cancer cells (3,4).

Studies into the role of transporters in cancer cytotoxic drug resistance and its expansion as a therapeutic target has been recently revealed (5). Recurrent metastatic breast carcinoma may be due to the presence of drug resistant adult stem cells called side population cells, which are ABCG2 phenotype expression (6).

The information about the participation of ABCG2 in breast cancer progression, metastasis and resistance to chemotherapy in Iraqi women with breast cancer are rare. In this study, the expression levels of ABCG2 in Iraqi women with stage II-III breast cancer as well as in benign breast tumors were examined by real-time PCR and immunohistochemistry. In addition, correlations between the expression levels of ABCG2 and patient's clinicopathological features were investigated.

## Patients and Methods:

### Patients and tumor characteristics

This study involved 64 breast cancers as well as 21 benign tumors surgically harvested from women admitted to Alkarama hospital in Baghdad between June 2013 and April 2014. All patients with breast cancer recruited in this study were diagnosed as stages II-III of where (60.9 %) of patients were stage II and (39.1%) were stage III breast cancer (Table 1). The mean age of patients was 64 years (range was 36 to 77 years). Final needle aspiration (FNA) technique, mammogram and histophathological examination were used for the diagnosis of all cases. All the patients enrolled in this study have not received chemo- or radiation therapy. Fresh breast cancer tissue, as well as benign tissue samples were collected and divided into two parts: one part was fixed in 10% formalin and embedded in paraffin to be used in immunohistochemisty staining and pathological examination for the determination of tumor type, grade, lymph node metastasis and ER, PR and HER2/Neu hormones receptor status. Tumor grades and tumor stage were evaluated according to modified bloom-Richardson grading system and (TNM) staging system (7, 8). The other part of fresh samples was stored in (-80°C) for subsequent use for RNA extraction.

**Table 1: Clincopathological features of patients with stage II-III breast cancer.**

| Parameter | Patients (n) | Percentage (%) |
|---|---|---|
| **Age of patients** | | |
| ≤50 years | 17 | 26.6 (%) |
| ≥50 years | 47 | 73.4 (%) |
| **Tumor Stages** | | |
| Stage II | 39 | 60.9 (%) |
| Stage III | 25 | 39.1 (%) |
| **Tumor Grades** | | |
| Grade I | 8 | 12.5 (%) |
| Grade II | 36 | 56.3 (%) |
| Grade III | 20 | 31.2 (%) |
| **Tumor Types** | | |
| IDC | 48 | 75 (%) |
| ILC | 10 | 15.6 (%) |
| Other | 6 | 9.4 (%) |
| **Lymph Node Metastasis** | | |
| Positive | 29 | 45.3 (%) |
| Negative | 35 | 54.7 (%) |
| **ER Status** | | |
| Positive | 47 | 73.4 (%) |
| Negative | 17 | 26.6 (%) |
| **PR Status** | | |
| Positive | 44 | 68.8 (%) |
| Negative | 20 | 31.3 (%) |
| **HER2/new Receptor Status** | | |
| Positive | 21 | 32.8 (%) |
| Negative | 43 | 67.2 (%) |

**IDC: Invasive Ductal Carcinoma, ILC: Invasive lobular carcinoma, ER: Estrogen Receptor, PR: Progesterone Receptor, HER2/New: Epidermal growth Factor Receptor.**

**RNA extraction and cDNA preparation**

To determine the mRNA levels of ABCG2, total RNA was extracted from frozen tissues using RNA miniprep kit (Agilent biotechnology Inc., USA) according to manufacture instructions. Pure extracted RNA was used for the synthesis of cDNA using Revert Aid First Strand cDNA synthesis kit (Thermo Fisher Scientific Inc., USA) according to instructions.

**Real time-PCR**

The mRNA levels of ABCG2 in breast tissues from breast cancer and benign tumors were determined by real-time PCR. The mRNA accession numbers of all genes included in this study were obtained from NCBI (National Center for Biotechnology Information) database. All mRNA sequences obtained are curated sequences. Primer-Blast tool at NCBI was used to design all genes including in this study (Table 2).

**Table 2: The sequences of specific primers used for determination of ABCG2 and β-actin by real-time PCR.**

| | PCR product (bp) | Primer pair sequences (5' to 3') | Accession No. |
|---|---|---|---|
| ABCG2 | 131 | F:GTGTGTCTGGAGGAGAAAGAAA<br>R:CTTGAGTCTAAGCCAGTTGTAGG | NM-001257386 |
| B-actin | 78 | F: CCGCAAATGCTTCTAGGCG<br>R: TGTTTTCTGCGCAAGTTAGGT | NM-001101.3 |

The PCR amplification reaction was carried out using AccuPower® green StarTM qPCR PreMix (BioNeer, k-6210, Korea) using Stratagene Mx 3005P (Agilent technology, LA, USA) system. The reaction was performed in a 20 µl volume using the following thermal profile: 5 minutes at 95° C for 1 cycle, followed by 40 cycles at 94° C for 15 seconds, 55° C for 30 seconds and 72° C for 30 seconds. The relative amount of gene expression was calculated using the equation $=2^{-\Delta\Delta ct}$, which was then expressed by Mann-Whitney U test to compared the significant difference among medians of all markers.

**Immunohistochemistry staining**

Protein expression of ABCG2 was evaluated immunohistochemically. Four mm of paraffin embedded section was cut and mounted on positive charge glass slides and deparaffinized in xylene and rehydrated by series of ethanol solution. The slides were incubated with primary antibodies: anti-ABCG2 (B2738A US-bio, USA), overnight at 4°C. Slides were incubated with horseradish Peroxidase conjugate detection reagent (DAKO biotechnology) and with complex avidin-biotin for 30 minutes at room temperature for each. The slides were visualized using diaminobenzidine (DAB) and countered with hematoxiline, dehydrated with graduated ethanol and then with zylene. Finally the slides were mounted with water-free mounting medium (DPX) and then analyzed by light microscope at (400x). Placenta tissue was used as a positive control. Negative control was treated with all above steps except the incubation with primary antibody.

Evaluation of immunohistochemical staining was carried out blind to the patient's data and pathological features. The percentage and intensity of the staining were considered in this study. Normal cells that present in the whole tissue were scored as negative 0 percentage (no positive staining), score 1: (1-10% positive tumor cells), score2: (11-50% positive tumor cells) and score 3 (51-100 % positive tumor cells). The staining intensity was considered as 0 (no staining), 1(+, weak positive staining = yellow), 2 (++, moderate positive staining= yellow brown) and 3 (+++, strong positive staining = brown) (9). Both 0 and score 1 were considered as low expression and score 2 and score 3 were considered as high expression. Expression of each gene over 10 % was considered positive.

**Statistical Analysis**

The comparison between breast carcinoma stage II, stage III and benign tumors was performed by using prism pad graph version 6 (Graphic pad Soft ware Inc, San Diego CA, USA). Comparison of expression values were performed by the non-parametric Mann-Whitney U test. A chi-square ($\chi 2$) statistic was used to investigate whether expression values differed between breast cancer tissues stage II-III and benign tumors. $P< 0.05$ value was considered as statistically significant.

## Results:

**Histopathological examination**

This study examined the expression levels of ABCG2 in sixty four patients with stage II and III breast cancer and 21 benign tumors using Real time-PCR and immunohistochemisty. Stage II patients represented 60.9 % while stage III represented 39.1 %. Histophathological examination showed that 12.5 % of breast cancer cases are grade I (well differentiated), 56.3% with grade II (moderate differentiated) and 31.2 % of patients were grade III (poor differentiated). The majority of cancers were ductal carcinoma, where 75% of the cases were pure invasive ductal carcinoma, 15.6% were invasive lobular carcinoma and 9.4% were other cancers (invasive medullary carcinoma and mixed infiltrative ductal carcinoma and lobular carcinoma). Twenty nine patients (45.4%) had lymph node metastasis. The hormone receptor status was positive for ER,

PR and HER2/neu in stage II-III breast cancer are (73.4%, 68.8% and 32.8%, respectively) (Table 1)

**ABCG2 gene expression in breast cancer and benign tumors.**

Amplification blot and melting curve of ABCG2 performed by real time-PCR are illustrated in Figure (1) and Figure (2). Figure (3) showed the expression levels of ABCG2 in breast tissues from stage II-III breast carcinoma and benign tumors as determined by real-time PCR. The mRNA expression level of ABCG2 was highly increased in breast cancer tissues stage III (median:17.48 , p= 0.0001) and in stage II ( median: 6.618, p=0.008) as compared to that in benign tumors tissues (median: 2.189) with around 8 times in stage III and 3 times in stage II the median expression of benign tumor tissues (Figure 3)

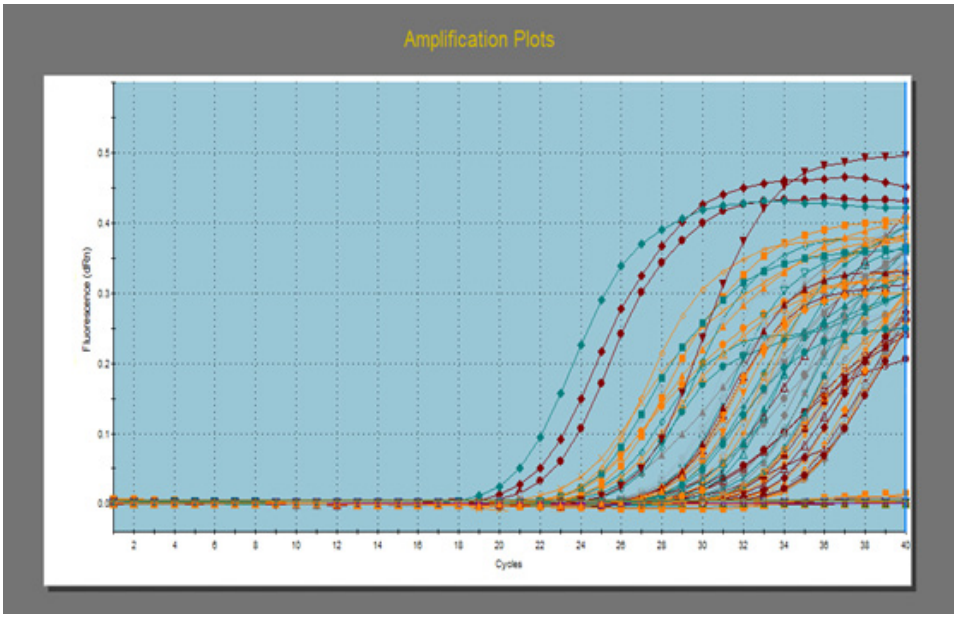


**Figure (1): Amplification blot of ABCG2 as determined by real time PCR**

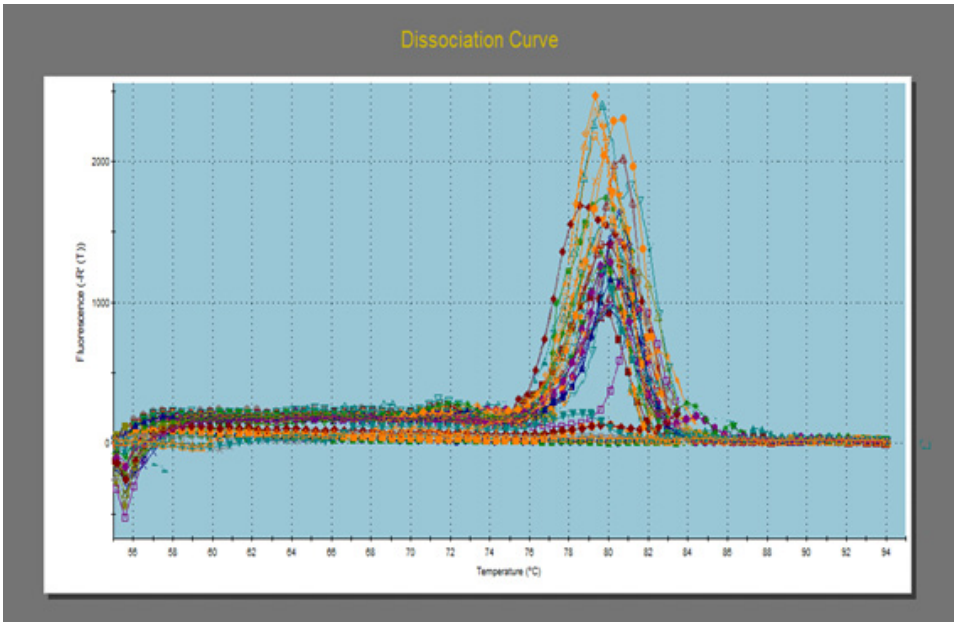


**Figure (2): ABCG2 melting curve as determined by real time-PCR.**

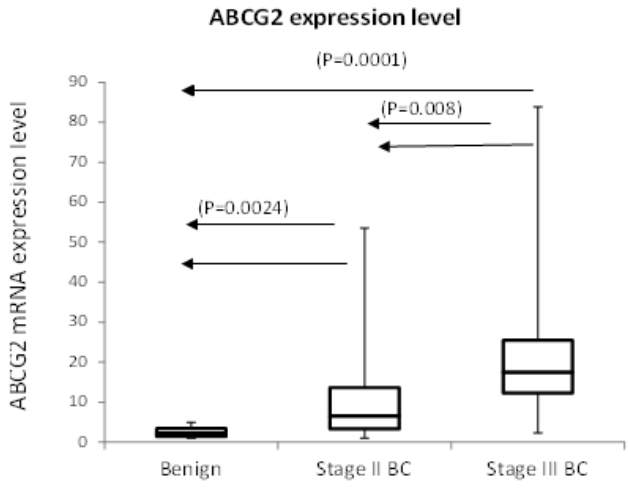


**Figure (3): Box plot to show the expression levels of ABCG2 in benign breast tumors and stage II–III breast cancer in Iraqi women, as determined by real-time PCR. The box indicate the significant variation in mRNA levels of ABCG2 in benign tumors (n=17), stage II (n=32) and stage III breast cancer (n=18). The horizontal line within a box marked the median, and the lines extending from a box reach to maximum and minimum data values. The expression level of each gene was normalized to corresponding expression of β-actin. The figure illustrated significant variation in ABCG2 expression level in stage II-III breast cancer tissues when compared with benign tissues. On the other hand, ABCG2 expression shows significantly variation at mRNA level between stage II and stage III breast cancer tissues.**

**Protein expression levels of ABCG2 as determined by immunohistochemisty**

Immunohistochemistry was carryout to determine the expression patterns of ABCG2 in 64 breast cancer tissues stage II-III breast cancer as well as in 21 benign tumor tissues. Placenta tissue was used as positive control for the expression levels of ABCG2 marker. As shown in Figure (4), ABCG2 was mainly expressed in the plasma membrane and the cytoplasm of cells. Negative control showed no positive expression and thus confirmed the specificity of ABCG2 antibody. Immunostaining showed that 53 out of 64 breast cancer tissues showed high positive expression of ABCG2 (P=0.0001). The overall positive ratio was (82.2%). Approximately 18% of breast cancer tissues showed low ABCG2 expression (Table 4).

**Table (4-8): ABCG2 expression levels in breast carcinoma tissues (n=64) and benign tumors (n=21).**

| ABCG2 | High expression n(%) | Low expression n(%) | P value |
|---|---|---|---|
| Cancer tissues | 53(82.8%) | 11(17.2%) | 0.0001 |
| Benign tissues | 4(19%) | 17(81%) | |

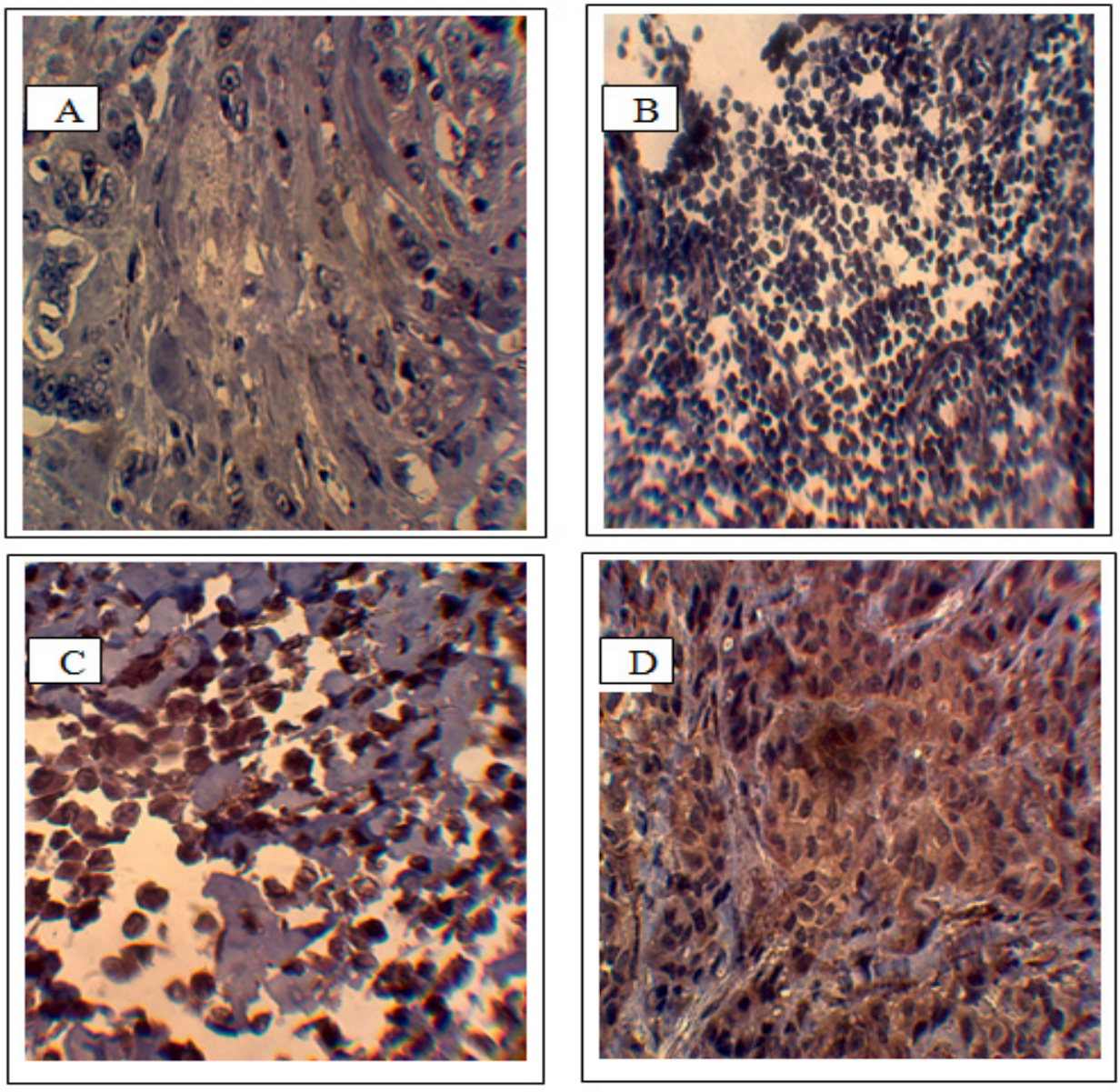


**Figure (4): Immunohistochemistry staining of ABCG2 in benign tumor tissues, stage II and stage III breast cancer tissues (400X). (A) Negative control. (B) Benign tumor section with slightly positive expression. (C): Stage II breast section showing positive ABCG2 in cytoplasm and cytomembran of breast cancer cells. (D) Representative image showing High ABCG2 expression in poorly differentiated breast cancer tissues.**

**Relation between ABCG2 expression and clincopathological data**

The expression level of ABCG2 correlated with clinicopathological feature was summarized in (Table 5). ABCG2 was increased in stage III as compared with stage II (P= 0.03) (Table 5). ABCG2 expression levels were significantly correlated with tumor grades (P= 0.01), and with tumor types (P= 0.01) (Table 5). ABCG2 expression levels showed also significantly elevated in patients with lymph node metastasis when compared to the negative metastatic group (P= 0.0001) (Table 5). As shown in (Table 5), there was no significant variation between the expression levels of ABCG2 with age and ER, PR and Her2/neu hormone receptor status.

**Table (5): Relationship between the ABCG2 expression levels and pathological data of patients**

| Pathological Data | No. of patients | ABCG2 | | P value |
|---|---|---|---|---|
| | | High expression | Low expression | |
| **Age of Patients** | | | | |
| ≥ 50 years | 47 | 17(40%) | 28(60%) | **0.37** |
| ≤ 50 years | 17 | 9(53%) | 8(47%) | |
| **Tumor staging** | | | | |
| Stage II | 39 | 14(36%) | 25(64%) | *0.03 |
| Stage III | **25** | 3(12%) | 22(88%) | |
| **Tumor grading** | | | | |
| Grade I | 8 | 5(62%) | 3(38%) | **0.01 |
| Grade II | 36 | 19(53% | 17(37%) | |
| Grade III | 20 | 16(80%) | 4(20%) | |
| **Tumor types** | | | | |
| IDC | 48 | 39(81%) | 9(19%) | **0.01 |
| ILC | 10 | 6(60%) | 4(40%) | |
| Other | 6 | 3(50%) | 3(50%) | |
| **Lymph node metastasis** | | | | |
| Positive | 27 | 3 (11%) | 24(89%) | †0.0001 |
| Negative | 27 | 27(73%) | 10(27%) | |
| | 37 | | | |
| **ER status** | | | | |
| Positive | 49 | 28(58%) | 21(42%) | **0.47** |
| Negative | **15** | 7 (47%) | 8(53%) | |
| **PR status** | | | | |
| Positive | 47 | 23(49%) | 18(38%) | **0.14** |
| Negative | 47 | 7(42%) | 10(58%) | |
| | 17 | | | |
| **Her2/neu Stats** | | | | |
| Positive | 24 | 8(34%) | 16(66%) | **0.26** |
| Negative | 24 | 19(68%) | 21(32%) | |
| | **40** | | | |

**IDC: Invasive Ductal Carcinoma, ILC: Invasive lobular carcinoma, ER: Estrogen Receptor, PR: Progesterone Receptor, Her2/Neu: Epidermal growth Factor Receptor. **P≥0.01 and †P≥0.001.**

## Discussion:

ABCG2 or breast cancer resistance protein (BCRP) belongs to large family of ABC transporters consist of at least 48 members. Large numbers of human ABC protein are efflux transporters but just three of them including P-glycoprotein (P-gp), multidrug resistance protein (MDR) and breast cancer resistance protein (BCRP) are the major efflux transporters which have ability to efflux different cytotoxic drugs and may contributes in chemoresistance of cancer cells (10).

In this study, we evaluated the expression levels of ABCG2 in breast cancer tissues as well as in benign tumor tissues in Iraqi women. To our knowledge, this is the first study evaluate the correlation between the expression level of ABCG2 and

clinicopathological parameters of Iraqi women with stage II-III breast cancer at gene and protein levels.

Since it's discovered in 1998, ABCG2 was found to be a xenobiotics transporter which affect the duration of drug presence in the body, so that, ABCG2 play an important role in multidrug resistance in human breast cancer (11). Over expression of ABCG2 protein lead to treatment failure, tumor relapse and tumor metastasis by induction cancer cells against cytotoxic drugs (12).

In human, ABCG2 gene was located in chromosome 4(q21-q22). ABCG2 was expressed in the cytoplasm and cell membrane of epithelial and stromal cells in mammary gland. Previous study demonstrated that ABCG2 was expressed in solid tumors with recurrent expression in melanoma, lung, endometrium and digestive tract tumors (13).

In breast cancer, different studies reports low ABCG2 expression level and didn't revealed significant correlation between ABCG2 over expression and patients clinicopathological features (14, 15). In contrast to these studies, the current study showed that the expression level of ABCG2 was significantly increased in stage II-III breast cancer tissues (83%) as compared with those in benign tumor tissues. On the other hand, the expression level of ABCG2 was positively correlated with tumor grades (P=0.04), tumor types (P=0.004) and with positive lymph node metastasis (P=0.009).

These results suggested that high ABCG2 expression level may have some correlation with worse biological behavior and clinical aggressiveness. Beside to that, the results of the current study showed a positive correlation between ABCG2 expression level and different breast cancer stages (p=0.05) suggested that the patients with high expression level of ABCG2 may have a worse prognosis than those with low expression level of ABCG2.

The current study revealed negative correlation between the expression level of ABCG2 and other clincopathological data such as age and ER, PR and her2/neu hormon receptor status. Previous study demonstrated that estradiol (E2) and peroxisome proliferator-activated receptor g (PPARg) control ABCG2 expression. These results showed that the effect of E2 on ABCG2 is not constant that result from the differences between ERα and ERβ. Each of these form effect on ABCG2 expression by different way and this explain why there is no significant variation between the high ABCG2 expression level and ER receptor. However, further study to estimate by which mechanisms involved ABCG2 that cause the changes in biological behavior of breast cancer in Iraqi women was required.

In conclusion, our results showed that the expression level of ABCG2 was increased in stage II-III breast cancer in Iraqi women. ABCG2 can be used as worthy markers for detection of breast cancer stage, grade and type as well as for the detection of breast cancer metastasis.

## References:


1. Jemal A, Bray F, Center MM, Ferlay J, Ward E (2011). Global cancer statistics. CA Cancer J Clin 61(2): 69–90.
2. DeSantis C, Howlader N, Cronin K (2011). Breast cancer incidence rates in US women are no longer declining.Ca. Epidemimol. Biomarkers Prev., 20:733-739.
3. Ni Z., Bikadi Z, Rosenberg MF, Mao Q (2010). Structure and function of the human breast cancer resistance protein (BCRP/ABCG2). Curr Drug Metab.; 11 (7): 603-17.
4. Zhou S, Morris J J, Barnes Y, Lan L, Schuetz JD, Sorrentino BP (2002). Bcrp1 gene expression is required for normal numbers of side population stem cells in mice, and confers relative protection to mitoxantrone in hematopoietic cells in vivo. Proceedings of the National Academy of Sciences of the United States of America 99 (19): 12339–12344.
5. Lei X, Peng S, Shujun X, Zhiyan L, Yan W, Peng G, Genyin Z (2011). ABCG2 is associated with HER-2 expression, lymph node metastasis and clinical stage in breast invasive ductal carcinoma. Diagnosis Pathology 6: 90-97.
6. Britton KM, Eyre R. Harvey IJ Stemke-Hale K, Browel D, Lennard TW, Meeson AP (2012). Breast cancer, side population cells & ABCG2 expression. Cancer Lett. ; 323 (1): 97-105.
7. National Comprehensive Cancer Network (NCCN) guidelines, Breast Cancer" (Version 2.2011).
8. Singletary S., Connolly J. (2006). Breast cancer staging: work with the sixth edition of the AJCC Cancer Staging Manual. CA Cancer J. Clin.; 56:37-47.
9. Su P, Zhang Q, Yang Q. (2010). Research immunohistochemical analysis of metadherin in proliferative and cancerous breast tissue. Diagnostic Pathology; 5:38.
10. Qingchng M, Jashvant DU (2015). Role of the breast cancer resistance protein (BCRP/ABCG2) in drug transport. APPS journal 17 (1): 65-82.
11. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi A K, Ross DD (1998). A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc. Nat. Acad. Sci. 95: 15665-15670.
12. Dean M, Fojo T, Bates S (2005). Tumor stem cells and drug resistance. Nat Rev Cancer 5:275-84.
13. Diestra JE, Scheffer GL, Catala I, Maliepaard M, Schellens JH, Scheper RJ, Germa-Lluch JR, Izquierdo MA (2002). Frequent expression of the multi-drug resistance associated protein CRP/MXR/ABCP/ABCG2 in human tumours detected by the BXP-
14. monoclonal antibody in paraffin embedded material. J Pathol. 198:213-219.
15. Kanzaki A, Toi M, Nakayama K, Bando H, Mutoh M, Uchida T, Fukumoto M, Takebayashi Y: Expression of multidrug resistance-related transporters in human breast carcinoma. Japanese Journal of Cancer Research 2011, 92:452-458
16. Faneyte IF, Kristel PM, Maliepaard M, Scheffer GL, Scheper RJ, Schellens JH, van De Vijver MJ (2012). Expression of the breast cancer resistance protein in breast cancer. Clinical Cancer Research 8:1068-1074.

# دراسه التعبير الجيني لل ABCG2عند المريضات العراقيات المصابات بسرطان الثدي المرحله الثانيه والثالثه باستخدام طريقه الاستنساخ العكسي- تفاعل سلسلة البلمرة و باستخدام طريقة Immunohistochemistry .

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## الخلاصه:

هدفت هذه الدراسه الى دراسة التعبير الجيني للدلاله الخاصة للخلايا الجذعيه السرطانيه (بروتين المقاوم للعلاج في سرطان الثدي ABCG2) عند المريضات للعراقيات المصابات بسرطان الثدي في المرحلتين الثانية و الثالثة وذلك باستخدام طريقه الاستنساخ العكسي- تفاعل سلسلة البلمرة و باستخدام طريقة Im-munohistochemistry .

شملت الدراسة (64) مريضه مصابة بسرطان الثدي قسمت الى 39 مريضه مصابه بسرطان الثدي المرحلة الثانية (60.9%, و25 مريضة مصابة بسرطان الثدي من المرحلة الثالثة (39.1 ). وقد قورنت هذه النتائج مع 21 عينه لنساء مصابات باورام حميده في الثدي. جمعت العينات من المريضات في مستشفى الكرامه التعليمي - بغداد –من الفترة من حزيران 2013 الى نيسان 2014 وتم اخذ عينات نسيجيه من الاورام من المريضات وتم تقسيم هذه العينات الى جزئين : الجزء الاول تم حفظه في ماده الفورمالين لغرض التشخيص النسيجي و Immunohistochemistry و الجزء الثاني تم تبريده وحفظه في النايتروجين السائل لغرض دراسه التعبير الجيني للدلالات السرطانيه بواسطه طريقه الاستنساخ العكسي- تفاعل سلسلة البلمرة. بينت نتائج هذه الدراسه ان مستوى الدلاله لل ABCG2 قد ارتفعت بشكل معنوي P=0.008 في عينات المرحله الثانيه من السرطان و كانت الزياده معنويه ايضا في المرحله الثالثه من سرطان الثدي P=0.0045 وذلك عند المقارنه مع مستوى الدلاله في اورام الثدي الحميده. وعند استخدام طريقه Immunohistochemistry بينت هذه الدراسه وجود فروقا في تعبير هذه الدلاله ABCG2 في عينات مرضى السرطان المرحله الثانية والثالثة 82.8% مقارنه ب14.3%, في اورام الثدي الحميده. اوضحت هذه الدراسه وجود علاقة وثيقة بين زيادة التعبير لهذه الدلاله ABCG2 مع الحاله السريريه للمرضى, حيث ارتبط زياده التعبير مع نوع السرطان P= 0.01 و درجه السرطان P=0.01 ومع الانبثاث العقدي اللمفاوي P=0.0001 بينما ارتبط زياده التعبير لل ABCG2مع المرحله الثانيه و المرحلة الثالثة للسرطان P= 0.006. نستنتج من هذه الدراسه ان التعبير الجيني لدلاله الخلايا الجذعيه السرطانيهABCG2 قد ارتفع بشكل كبير عند المريضات العراقيات المصابات بسرطان الثدي من المرحلة الثانية و الثالثة وان هذه الزياده قد ارتبطت مع حاله المرضيات السريريه وان هذه الزيادة توضح زياده عدد الخلاي الجذعيه السرطانيه وقد تكون هذه الزياده احد الاسباب المهمه في زياده انتشار سرطان الثدي والانبثاث الى اعضاء اخرى داخل الجسم.