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Mutation of BRAF V600E in Iraqi Female Patients Diagnosed With Breast Cancer

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Abstract:

This study aimed to investigate the possible presence of BRAF V600E mutation in the Iraqi female patients who diagnosed with breast cancer in different ages, in which Blood and tissue samples were collected from 46 female patients with age (46.73±3.54);Those were divided into two groups; who took chemotherapy (31 persons) as treated group and without chemotherapy as an untreated group (15 persons) and from (23) healthy person with age(47.93±3.05). Polymerase chain reaction (PCR) were done with newly designed primers. The results revealed no correlation between breast cancer occurrence and BRAF V600E mutation in the Iraqi patients enrolled in the current study.

Keywords: BRAF gene, Breast cancer, Iraq, Mutation, PCR

الخلاصة

هدفت هذه الدراسة الى التحري عن وجود طفرة BRAF V600E من عدمها في الاناث التي تم تشخيصهن بالاصابة بسرطان الثدي وفي اعمار مختلفة، حيث تم جمع عينات الدم والانسجة من ٤٦ مريضة وباعمار(٤٦ ±٣,٥٤)، حيث تم تقسيمها اللى مجموعتين، المجموعة التي تحت العلاج الكيمياوي وعددها ٣١ مصابة والمجموعة الاخرى بدون علاج كيمياوي وعددها ١٥ مصابة كما تم مقارنة النتائج مع مجموعة الاصحاء كمجموعة سيطرة وعددهم ٢٣ شخصا وباعمار(٤٧,٩٣±٣,٠٥) سنة.تم اجراء تفاعل البلمرة المتسلسل (PCR) وباستخدام بوادئ تم تصميمها ضمن الدراسة الحالية على جميع العينات. اظهرت النتائج عدم وجود علاقة مابين الاصابة بسرطان الثدي وطفرة BRAF V600E في المرضى العراقين المشمولين بالدراسة الحالية.

الكلمات المفتاحية: سرطان الثدي ، طفرة ، العراق

Introduction

Breast cancer is the mainly diagnosed malignant tumor of women in a lot of countries (Zekri, Bahnassy *et al.*, 2012).In Iraq and other Arab countries, breast cancer comes first among other malignancies (Najjar and Easson 2010; Al-Hashimi and Wang 2014). Breast cancer incidence represented about 34 % of the total cancer cases among females from (2000-2009) registered in Iraq (Al-Hashimi and Wang,2014).

The gene BRAF is a "serine/threonine protein kinase, encoded on chromosome 7q34" that initiates the MAP kinase/ERK-signaling pathway(Cui *et al.*,2010). Raf kinases (A-Raf, B-Raf, and C-Raf) arouse the mitogen-activated protein kinase (MAPK) cascade, which composed of mitogen-activated protein/extracellular signal–regulated kinase (MEK) and extracellular-signal regulated kinase (ERK).As the three Raf kinase members, B-Raf is the most strong MEK activator with major roles in cell development, cell cycle progression, and survival(Manousaridis and Mavridou *et al.*,2013; Favre,2014) and is frequently deregulated in cancer(Wong,2009). Activation of Ras/Raf/MEK/ERK pathway is found in more than 25% human cancers(Zhang *et al.*,2016).

The BRAF gene is regularly active in the mitogen-activated protein kinase/ extracellular signal-regulated kinase signaling pathway which has an effect on cell growth and differentiation(Wang *et al.*,2015).Malignant tumors arise through the

gathering of epigenetic and genetic alternations (Kikuchi *et al.*,2013). V600E mutation of BRAF that alters the encoded amino acid from valine to glutamic acid at the position 600 (Janku *et al.*, 2012),Association of BRAF mutation with many kinds of cancer have been studied (Millington,2013) neck cancer (Kikuchi *et al.*, 2013), thyroid cancer (Kikuchi *et al.*, 2013),lung cancer (Goldman and Gray,2015) and breast cancer (Kim *et al.*,2013).

In last years , the study of the molecular pathways of breast cancer has shown that the BRAF gene mutation is an important event in the process of this disease(Wang *et al.*, 2015). The V600E mutation of the BRAF gene was noticed in lung cancer(Ueda *et al.*, 2008; Ohba *et al.*,2015),central nervous system neoplasms (Behling *et al.*,2016), papillary thyroid carcinoma (Kim *et al.*, 2016), colorectal cancer (Sueda *et al.*,2016) .in breast cancer in which some studies showed the absence the mutations (Barras *et al.*,2016) while the other proved the presence of the mutation (Davies, *et al.* 2002; Jung *et al.,* 2016).So it was questionable issue in the field of molecular biology of cancer.

Materials and methods

Blood samples were collected from (23) healthy person with age (47.93±3.05). Blood and tissue samples were collected from 46 female patients with age (46.73±3.54). Those also divided into two groups those were taken therapy (31 persons) as treated group and those without therapy as an untreated group (15 persons) .The patients and healthy groups are matched in age. Five milliliters of venous blood samples were collected in Na-EDTA tubes. The serum and whole blood kept frozen at (-20°C) until further application in different assays and DNA extraction was done by genomic DNA extraction blood kit from (Favorgen, South Korea). The extracted DNA was stored at -20 □ C. mutant primers were designed as shown in table (1) , these Oligonucleotides used in this study were designed based on BRAF V600E sequence in NCBI database and these primers have been synthesized by (Bioneer, South Korea). Polymerase chain reactions (PCR) were conducted using the AccuPower PCR premix with the thermal cycler LABNET (USA), the reaction conditions were as follows: initial denaturation step at 94°C/5 min followed by 40 cycles; consisting of denaturation step at 94 °C/30 s, annealing step at 60 °C/60 s, and extension step at 72 °C/ 50 s with a final extension step at 72 °C/5 min. The PCR products were analyzed by 1.5 % agarose gel electrophoresis containing 0.5 µg/ml Ethidium bromide and visualized under ultraviolet light for detection of amplified DAN bands.

Table(1): PCR Oligonucleotides used to detect BRAF V600E mutation

Primers	Sequence	Amplicon length
BRAF Forward	5'TCCTAACACATTTCAAGCCCCA3'	312 bp
BRAF Reverse (Mutant)	5'GACCCACTCCATCGAGATTTCT 3'	

Results

BRAF is the family member most strongly activated by Ras (Ascierto *et al.*, 2012). BRAF is a serine/threonine protein kinase(Wong,2009). The correlation between the BRAF V600E mutation and breast cancer is unresolved, according to previous studies; some researchers showed that there may be a possible role of that mutation in the breast cancer, while the others found no clear correlation.

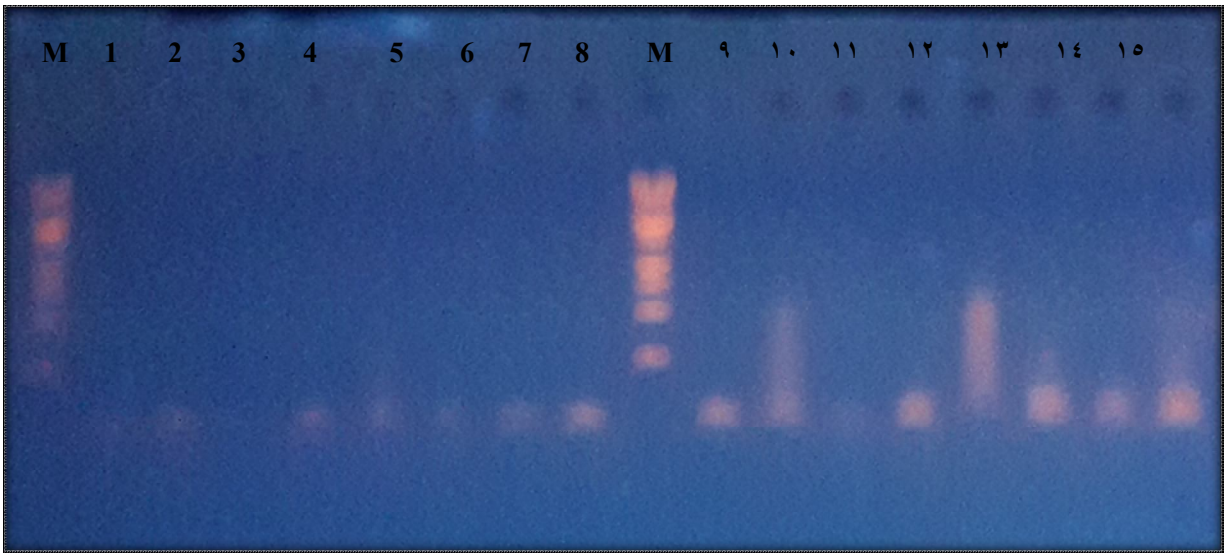

Figure (1): Electrophoresis pattern of PCR for detection of BRAF mutation, lanes No 1-8) refer to breast cancer patients samples, lanes(9-16) refer to healthy individuals sample. M: Refer to (100bp DNA Ladder). Agarose 1.5% with 5v/Cm.

Blood samples were collected from forty-six female patients age (46.73±3.54, mean ± SD) and Twenty-three healthy persons age(47.93±3.05, mean ± SD) and PCR assays were conducted using PCR reaction in which it was repeated three times to confirm the final results, the results revealed no BRAF V600E mutations were detected in the examined samples as shown in figure (1).

Discussion

In Melanoma, about 50% has BRAF mutation with 90% of them as V600E mutations(Ascierto *et al.*,2012).BRAF V600E mutation was recognized in 13.4% of colorectal cancer(Vandrovcova *et al.*,2006).

The correlation between BRAF V600E mutation and different types of cancer was controversial through previous studies; Zeng *et al.*(2016) showed that Hashimoto's thyroiditis was not clearly associated with BRAF(V600E) mutation I which Hashimoto's thyroiditis was negatively associated with BRAF(V600E) mutation. In differentiated thyroid cancer (DTC),BRAF(V600E) was not considerably associated with(CLNM) central lymph node metastases(Han *et al.*, 2016).

The results of the current study showed the lack of BRAF V600E mutation in the patients who diagnosed with breast cancer in Iraq , these findings are similar to results of Myers *et al.*(Myers *et al.*,2016) in which he indicated the rare presence of BRAF V600 mutation in the breast cancer patients which suggests other molecular causes behind the breast cancer occurance in pateints enroled in the current study, although The RAS–RAF–MEK–ERK kinase protein path play important roles in cellular responses to growth signals(Davies *et al.*,2002). Mutations in this signals chains are frequently noticed in altered cell lines and commonly linked with human cancers (Chang *et al.*,2003).RAS is mutated to an oncogenic form in more than 14% of human cancer (Davies *et al.*,2002). There are three Raf kinases-A-Raf, B-Raf, and C-Raf; only B-Raf is generally mutated in a many type of cancers in which it may bear a resemblance to the meiosis process in the

normal cells (Wong,2009). B-Raf is the most widespread mutation type which is based on a substitution of a Glutamic acid residue to a Valine residue at codon #600(Cui *et al.* 2010). Germ cells mutations in a number of members of RAS/RAF/MEK/ERK pathway cause genetic disorders (Tumurkhuu *et al.*,2010). In the direction of treatment of some types of cancer; the Ras/Raf/MEK/ERK pathway can be activated by chemotherapeutic drugs usually used in leukemia treatment through controlling the level of protein expression of that pathway(Steelman *et al.*,2011)and in treatment of Melanoma (Favre,2014).

The Ras/Raf/MEK/ERK pathway has an important role in osteosarcoma lung metastasis (Yu *et al.*,2011).

Conclusions

In this study, we concluded that there is no obvious correlation between breast cancer occurrence and BRAF V600E mutation in the Iraqi patients who participated in the current study.

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