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Research Article

Investigation of BRAF V600E Mutation in Breast Cancer Patients

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Abstract

The B-Raf is the essential protein in signal pathways inside cells which is affected by cell growth direction. The B-Raf protein encoded by the BRAF gene that is located at chromosome 7, BRAF gene is also pointed out as proto-oncogene. This study aimed to detect the substitution at codon 600 causing a change of valine to glutamic acid (V600E) mutation in Iraqi females to assist its role in initiating breast cancer. Sixty biopsies tissue from breast cancer Iraqi women and 20 women with benign lesions were enrolled in this study. DNA was extracted from breast cancer biopsies samples. PCR and DNA Sequencing techniques were used to screen the BRAF V600E gene mutation as it is an essential event in the initiation of cancer. The results revealed that none Iraqi breast cancer women had BRAF V600E mutation, The annotated BRAF gene has been deposited in DDBJ/GenBank under the accession number LC547435. In conclusion: The present data indicate no BRAF V600E mutation in Iraqi breast cancer females and may not possess a role in breast cancer initiation. The current results may be refer to ineffectiveness of Vemurafenib and Encorafenib therapies that specific for patients with the BRAF V600 mutation. Other studies with large numbers of patients are needed to confirm the result of this study, as the high prevalence of breast cancer among Iraqi women.

Keywords

BRAF V600E, Breast, Cancer, BRAF Gene, Iraq

1. Introduction

Breast cancer considers an important malignant disease among females worldwide, about 24% of new cancer cases and more than 12% of cancer deaths in 2018 [1]. Breast cancer is a neoplasm resulting from genetic mutations and heterogeneity [2].

Many genes are important in the initiation, development, and progression of breast cancer. The *BRCA1* (BReast CAncer 1) gene is one of the important genes in breast cancer; a significant reduction in the expression of the BRCA1 gene in Iraqi breast cancer patients suggests its potential role in breast cancer development [3]. Also, high expression of the human epidermal growth factor receptor-2 (HER2) was found in 30% of breast cancer patients expressed gene plays a critical role in breast cancer development and progression. [4].

BRAF is a family of Raf kinase that contains many proteins, including BRAF, the vital member in the activation of MEK

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kinase in the Ras-Raf-MEK-ERK pathway [5], thus regulates many biological functions such as cell proliferation, cell differentiation, and programmed death (apoptosis) in response to several extracellular stimuli factors such as, hormones, growth factors, cytokines and environmental stress in addition to activate of signaling pathway (Raf-MEK-ERK) which also known to stimulate the expression of many target genes [6, 7].

About 90% of BRAF mutations was a missense mutation that occurs at 1796 of BRAF gene results in substitution at codon 600 causing a change of valine to glutamic acid (V600E). BRAF V600E mutation is reported to be found in various cancers such as malignant melanoma, colorectal carcinoma thyroid papillary carcinoma, glioma, ovary serous carcinoma, and others cancer. [8]. A series of articles suggested a promising treatment option for patients with BRAF mutated cancer and provide insights into the future use of checkpoint inhibitors in patients with BRAF mutant colon cancer [9, 10].

This study aims to investigate the BRAF V600E role as biomarker detection of breast cancer by screening of its mutation status using Automating sequences technique in Iraqi breast cancer females and its relation with the clinicopathological parameter.

2. Material and Methods

2.1. Tissue Samples of Breast Cancer

Biopsies from sixty Iraqi females with breast cancer, in addition to 20 women with benign lesions were enrolled in this study; ages of all participated women were between (20-72) years with an average age of 46 year, attended to the Oncology Teaching Hospital of the Medical City in Baghdad from July 2021 to April 2022. Biopsy samples were taken with ethical permission from the hospital and all participants. Patients selection and diagnosis were carried out by the consultant medical staff and pathologist committee at the Hospital. All patients were diagnosed at an early stage, and no chemotherapy or radiotherapy was administered Hospital.

2.2. PCR and BRAF Gene Sequencing

Extraction of genomic DNA from biopsy tissue using the QIAamp DNA Mini kit (Qiagen) in Hilden, Germany. Fragment DNA of the *BRAF* gene (exon fifteen) was selected to amplify using the following sequences (5'- ATGCTTGCTCTGATAGGAAAATGA and, 5'- AGCAGCATCTCAGGGCCA) for forward and reveres primers respectively as described by [11]. The volume of PCR mixture reaction (25µl) of for *BRAF* gene amplification consist of 5.5 µl of extracted genomic DNA, 16.5 µl of master mix and 1.5 µM of each primer PCR; amplifications were carried out in an Applied Biosystem 96 thermocycler. Amplifications reaction was achieved using a 15-minute at 95 ℃ as initial denaturation, followed by 35 cycles of 30 seconds at 94 ℃, 30 seconds at 59 ℃, and 30 seconds at 72 ℃, final extension for 10 min at 72 ℃. After staining with ethidium bromide, the bands of PCR products were running in 1.5% agarose gel along with a molecular marker (Kapa universal ladder) in a separate well. Automating sequences technique was used for BRAF gene analysis.

3. Results

3.1. Characterization of Patients

In this study, sixty women diagnosed with breast cancer participated, in addition to 20 females with benign lesions. The patients had a mean age of 46 years, ranging from 20-72 years old. The tumor severity was classified as moderately differentiated adenocarcinoma in 65% of cases (39 out 60) of patients, well-differentiated adenocarcinoma in 11.66% (7 out 60) pateints, and poorly differentiated adenocarcinoma in 23.33% (14 out 60) patients. The study also found that 45% (27 out 60) of patients had tumors on the right side, while 23.4% (14 out 60) of patents had tumors on the left side of the breast (Table 1).

Table 1. *The characteristic distribution of females with breast cancer.*

Characterization	Total no. (%)
Breast cancer females	60 (100)
Age years	
Average age	46
< 50	15 (25)
≥ 50	45 (75)
Gender	
Female	60 (100)
Site of tumor	
Right breast	27 (45)
Left breast	14 (23.4)
Bilateral breast	4 (6.6)
Axillary	15 (25)
Differentiation	
Moderately	39 (65)
Well	7 (11.66)
Poorly	14 (23.33)

3.2. PCR and BRAF Gene Sequence Analysis

Exon, fifteen of the *BRAF* gene, was amplified by PCR technique and screened for the presence of (V600E) mutations

in 80 women (60 breast cancer women and 20 females with benign lesions). The products size of PCR was more than 240 bp, automated direct sequencing was performed in all patient samples, and the data of sequence analysis were compared with the National Center for Biotechnology Information (NCBI) database (NCBI accession number HM459603). Our analyses revealed that none of the Iraqi breast cancer females had BRAF V600E mutation Figure 1. No association of *BRAF* gene mutation with clinical characteristics of breast cancer patients as no *BRAF* gene mutation was found in this study.

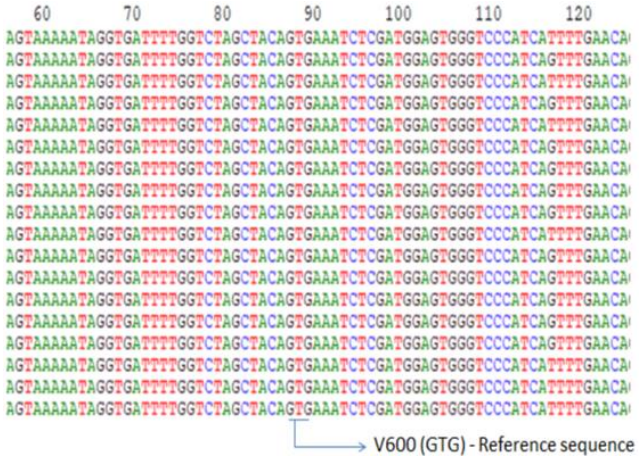

Figure 1. *A: Alignment of exon fifteen of BRAF gene sequences analysis breast samples comparable with Ref V600 GTG references sequences (NCBI accession number HM459603), B: Arrow refers to the representative analysis of V600 GTG position.*

4. Discussion

The Ras-Raf-MEK-ERK-activated signaling pathways are essential in the formation and development of tumors [12]. Mutations in related genes are well known to play a crucial role in the occurrence of various types of cancer in humans. The Raf kinase family has been the focus of several studies in different tumor types, including breast cancer in western populations. However, there have been limited reports on the frequency of BRAF mutations in Iraqi females with breast cancer. This study aimed to investigate the correlation between the occurrence of breast cancer in Iraqi females and the sequence analysis of the *BRAF* gene. We collected 60 breast tissue biopsies from breast cancer. In addition to 20 benign tumor samples, as it may be developed to breast cancer, all samples were screened for *BRAF* mutations V600E. The results revealed no mutation in the BRAF gene. These findings confirm the results of a previous Iraqi study conducted by Al-Askeri and Mutter [13], which also reported no V600E mutation in the *BRAF* gene in 46 females with breast cancer. In our previous study on Iraqi patients, it was found that no *BRAF* mutation in bowel inflammation and colorectal cancer samples. However, three of different heterogeneity mutations were reported in the exon fifteen of BRAF [14]. Our findings differ from other results, *BRAF* gene mutations has been stated in 30% of breast cancer type basal-like carcinomas [15, 16], also different from Zhu et al. [17] who suggested that the BRAF/MEK/ERK signaling pathway controls cell cycle progression in an ER status-dependent manner in breast cancer. On the other hand, Premalatha, with co-authors [18], reported that BRAF gene mutations may be have a role in the occurrence and development of breast cancer. Positive specific antibodies for BRAF gene mutation in triple-negative breast cancer was reported without of connection with clinicopathologic characteristics of breast cancer [19]. Another Chinese study reported that 16% of breast carcinoma women with BRAF harbored a mutation [20].While similarly to our results, a study by Kim and colleagues reported that BRAF mutations did not have any association with the initiation of breast cancer in the Korean population [21]. These differences could be due to genetic or environmental heterogeneity [22], the use of different samples and techniques for gene mutation detection. Variable cancer histologic subtypes and variations in DNA-reading thresholds may also influence prevalence rates and interpretation of positive results detected in forty-six breast cancer females. [23].

Annotation of the BRAF gene sequencing using the DDBJ Submission Tool. The annotated BRAF gene has been deposited in DDBJ/GenBank under the accession number LC547435.

5. Conclusions

Based on current data, there is no evidence of a BRAF mutation V600E in breast cancer patients. However, further studies with larger patient groups are necessary to obtain more accurate information about the prevalence of BRAF V600E in breast cancer, particularly among Iraqi women because may be refer to ineffectiveness of Vemurafenib and Encorafenib therapies that specific for patients with the BRAF V600 mutation.

Abbreviations

BRCA1	BReast CAncer Gene1
HER2	Human Epidermal Growth Factor Receptor-2
V600E	Valine (V) 600 Change to Glutamic Acid (E) in BRAF Gene
PCR	Polymerase Chain Reaction
NCBI	National Center for Biotechnology Information
DDBJ	DNA Data Bank of Japan

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Conflicts of Interest

The authors declare no conflicts of interest.

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