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The association between KRAS gene expression and breast tumors in a sample of Iraqi women

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Abstract

In Iraq, breast cancer is considered one of the most widespread cancer types causing death in women due to changes in the genes that control cell growth and proliferation. KRAS gene plays a role in 30% of human cancers. This study aims to evaluate the expression of the KRAS gene and its role in women with breast tumours. A total of 120 women (60 women with breast tumours and 60 samples from apparently healthy women) were enrolled in this study to determine the expression of the KRAS gene. The RT-qPCR was used for this purpose. Fibroadenoma (FA) was the most common tumours, accounting for 53% of cases, followed by fibrocystic change (26%). Other benign tumours accounted for 20% of the cases; however, IDC is the most common type of invasive breast cancer, accounting for 70% of all breast cancers. Moreover, KRAS gene expression in women with breast tumours (benign and malignant) was not- significantly different from that seen in normal healthy women.

Keywords: breast tumors, KRAS, gene expression, Iraqi women

1. Introduction

Breast cancer (BC) is one of the most common cancers that cause death in women, and its prevalence is increasing in Asia. Invasive BCs are a diverse group of tumours that vary in clinical presentation, behavior, and morphology (1, 2). In Iraq, from 2000 to 2019, BC accounted for roughly one-third of all cancer cases recorded in the country (3).

In the progression of breast cancer, multiple genetic events can activate dominant-acting oncogenes and disrupt the function of specific tumour suppressor genes (4). In addition, the *KRAS* gene is a proto-oncogene located on chromosome 12p12.1 that codes for a protein termed small GTPase transductor (5, 6). KRAS protein plays an important role in the epidermal growth factor receptor (EGFR) signalling pathway, and oncogenic mutations in protein can drive downstream activation of this pathway. *KRAS* 3'-untranslated regions (UTR) of humans contain several putative tumour suppressor lethal-7 (*let-7*) complementary sites (*LCS*) (7). *KRAS* gene is controlled at the post-transcriptional level by a highly complicated interplay of *cis-acting* elements inside its 3′ UTR due to its infrequently long 3′ UTR length (8). Therefore, genetic variants of *KRAS* 3'-UTR may prevent *let-7* miRNA from binding to *KRAS* and control the activity of *KRAS* to regulate the expression of its protein. A number of studies have found that the SNPs in the *KRAS* 3'-UTR might cause high levels of the KRAS oncogenic protein and lower levels of the *let-7* miRNA (7, 9,10).

Moreover, MicroRNAs (MiRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level by binding to complementary sites in the 3′ UTR of target messenger RNAs (mRNAs) (11,12). This case-control study aims to determine the role of *KRAS* gene expression and its association with the incidence of breast tumours.

2. Materials and Methods

2.1. Subjects

A total of 120 women participated in this study. Sixty women (who attended the Oncology Teaching Hospital in Baghdad) were diagnosed with breast tumours (both malignant and benign), with 60 appearing to be healthy controls. Details of clinical data and demographic characteristics for patients were collected from medical reports. The women with breast tumours were early-diagnosed cases before surgery or treatment.

2.2. RNA Isolation

RNA was extracted from whole blood samples using the Trizol TM Reagent (Promega, USA) in accordance with the manufacturer's protocol. The RNA concentration was determined using a Quantus fluorometer (Promega, USA). Aliquots of 199µl of diluted QuantiFluor Dye were mixed with 1 µl of RNA after 5 minutes of incubation at room temperature, RNA concentration was measured and stored in an -80 °C deep freeze until use.

2.3. Real-time PCR and cDNA synthesis

RNA was reversed to cDNA using one-step *RT-qPCR* (Promega, USA). The expression level of *KRAS* and *GAPDH* as reference genes was also detected by using the *RT-qPCR* SYBR Green assay. Primer sequences for KRAS gene Forward

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(5′- TGAGGACTGGGGAGGGCTTT 3′) Reverse (5′- AGGCATCATCAACACCCTGTCT 3′) (13) and for *GAPDH* Forward (5′- AGAAGGCTG GGGCTCATT TG 3′) Reverse (5′- AGG GGCCAT CCACAG TCTTC 3′). The mRNA expression level was normalized by using Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as a housekeeping gene (HKG). *RT-qPCR* was performed using a total of 10 µl PCR reaction mixture that contains 1 µl of RNA, 0.25 µl of reverse transcriptase, 0.5 µl of primers for both forward and reverse primers, 0.25 µl of MgCl2, 2.5 µl of nuclease-free water, and 5 µl of *GoTaq 1-Step RT-qPCR* Master Mix (Promega, USA). *RT-qPCR* program was carried out under thermal-cycling conditions involving reverse transcriptase enzyme activation step at 37 °C for 15 min (one cycle), initial denaturation step at 95 °C for 10 min, followed by 40 cycles for 95 °C for 15s as denaturation, annealing step at 57 °C for 20 s, extension step at 72 °C for 7 min.

The *KRAS* gene expression was determined by the ΔCt method, the 2^−ΔΔCt equation (14), and the relative quantitative method using the comparative Ct formula:

Folding = 2^−ΔΔCT where:
ΔCT = CT (target gene) − CT (reference gene).
ΔΔCT = ΔCT (Patients) − ΔCT (Control).

2.4. Statistical analysis

Data were represented as mean ± S.D. SPSS software (Armonk, NY: IBM Corp) was used for the statistical analysis. The least significant difference –the LSD test (ANOVA) was used to compare between means. The value of $P<0.05$ was considered to be statistically significant.

3. Results

The age of benign breast tumour cases varies from 17-53 years old, with a mean age and median of 32±2.3 and 34, respectively. Results showed that fibroadenoma was the most prevalent type of benign breast tumour, representing 53.3% of the total samples of benign breast tumours; fibrocystic changes were 26.6%, breast cyst was 11%, and intra-ductal papilloma was 9% as shown in Fig. 1.

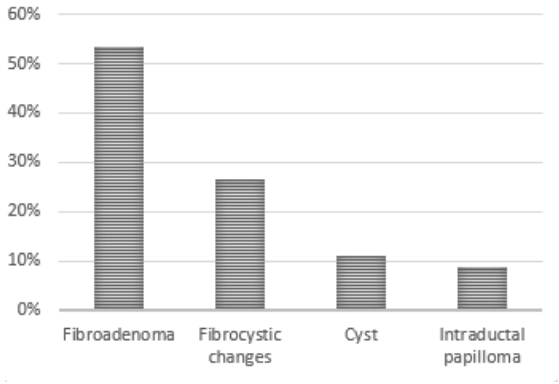

Fig. 1. Frequencies of histopathological for breast benign tumors in Iraqi women

While the age of Iraqi women with malignant breast tumours varied from 35-70 years old, with a mean age of 51±1.2 years and a median of 52. There are three Histopathological types characterized in this study. The first was invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), and ductal carcinoma in situ (DCIS), with frequencies of 78%, 15%, and 7%, respectively, as shown in Fig. 2.

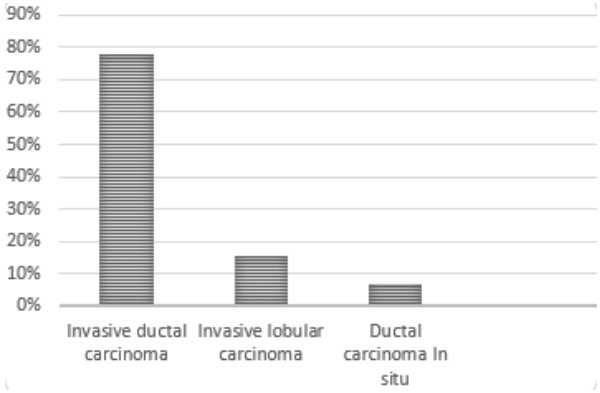

Fig. 2. Frequencies of histopathological for malignant breast tumours in Iraqi women.

QRT-PCR was used to determine the level of *KRAS* mRNA in the blood sample of case-control subjects (women with malignant breast tumours, women with benign breast tumours and healthy control women).

Results showed that the fluorescence measured during each PCR cycle was proportional to the number of PCR products. The actual C_t value was inversely proportional to the total RNA concentration in blood samples.

The melting curve revealed the specificity of the primers designed for *KRAS* and *GAPDH* indicated by a specific single peak in the melting curve graph. No primer dimer was seen in PCR amplification (Fig. 3 and 4).

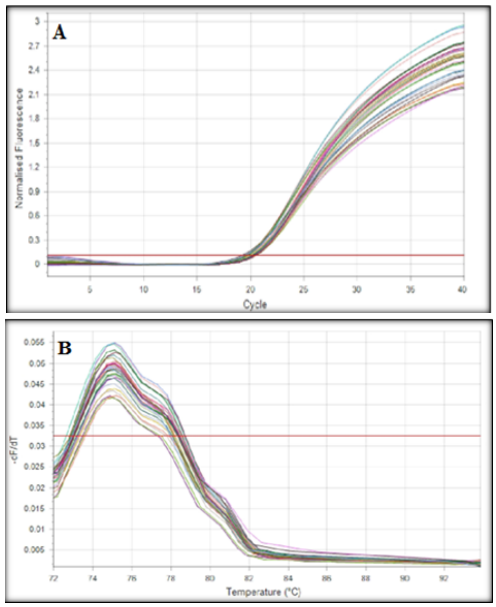

Fig. 3. Melting curve analysis of the real-time polymerase chain reaction (RT-PCR) for *KRAS* expression. A: Ct values the photograph was taken directly from Mic-qPCR. B: The dissociation stage was performed.

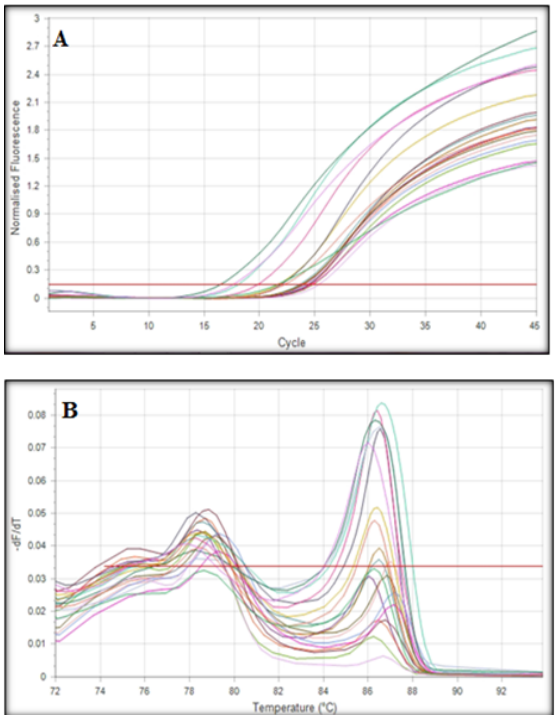

Fig. 4. Melting curve analysis of the real-time polymerase chain reaction (RT-PCR) for glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). A: Ct values ranged from 17 to 24. B: The dissociation stage was performed

The *KRAS* gene expression in women with breast tumours (benign and malignant) was non-significantly different from that seen in apparently normal healthy women (Fig. 5)

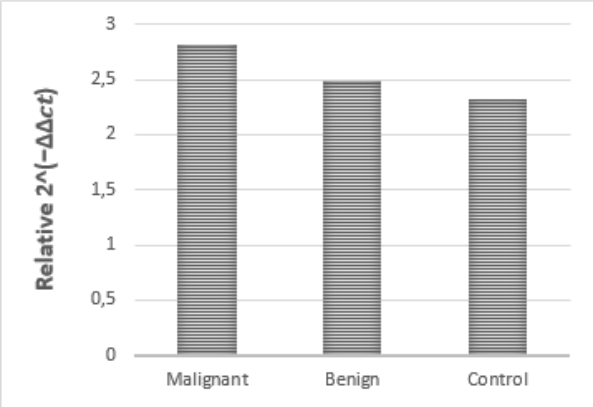

Fig. 5. Expression of *KRAS* mRNA in women with breast tumors (malignant and benign) compared to apparently healthy women

4. Discussion

In humans, *KRAS* proto-oncogene contains six exons, and it encodes guanosine triphosphatase (GTPase) KRAS isoform, a single amino acid substitution in *KRAS* is responsible for an activating mutation. In addition, the *KRAS* gene is known to be associated with human malignancies (15). SNPs in 3′-UTRs alter microRNA target recognition by disrupting sequence complementarity; some polymorphisms interfere with miRNA function and affect the expression of miRNA targets (16). According to the results of chin et al. (17), the prognostic role of LCS6 variants in KRAS 3'-UTR causes an increase in the expression of KRAS. In addition, (18) stated that LCS6 variants of the KRAS gene were statistically non-significant in women with breast tumours compared with healthy control. Therefore, there are no comparatively different levels of KRAS mRNA, which may also be due to the human mRNA being mostly targeted by several miRNAs at different target sites, which may enable recompensing of the loss of a single binding site (19).

Furthermore, miRNAs have been involved in regulating various cellular processes, including tumour development and progression, indicating that they can act as either oncogenes or tumour suppressors. The irregular expression of miRNAs plays a significant role in the progression of cancer; therefore, reducing the expression of these miRNAs may cause negative regulation of *KRAS*. In addition, the molecular mechanism of miRNA mediated in the control of KRAS would be useful for developing therapy (20, 21). Several miRNAs were acting as indirect targets to inhibit or increase KRAS-driven tumorigenesis; for instance: in NSCLC, miR-21 promotes *KRAS*-driven tumorigenesis by affecting negative regulators of the Ras/MAPK pathway (22). While miR-96, miR-30c and miR-181a, miR-143, and miR-145 have been shown to control KRAS in several cancers (23, 24). Also, another study found that miR-16 inhibits the expression and tumorigenesis of KRAS, which directly targets the 3′-UTR of KRAS mRNA (25). This paper shown that fibroadenoma occurs at an early age; however, invasive ductal carcinoma is the most prevalent type of malignant breast tumour. The results of this study indicate that the expression of the *KRAS* gene, which is conducted by *RT-qPCR*, has no correlation with breast tumours compared to healthy women, so the *KRAS* gene has not been represented as a risk factor in breast tumours.

Ethical statement

The study was ethically approved by the Iraqi Ministry of Health (decree order 18815 on 23/5/2018) and the scientific committee of Al-Nahrain University's College of Biotechnology.

Conflict of interest

The authors have no conflict of interest

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Authors' contributions

Concept: A.H.R., J.M.H., Design: A.H.R., J.M.H., Data Collection or Processing: A.H.R., J.M.H., Analysis or Interpretation: A.H.R., J.M.H., Literature Search: A.H.R., J.M.H., Writing: A.H.R., J.M.H.

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