Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
2020, 17(2):426-430 **E-ISSN: 2411-7986**

DOI: http://dx.doi.org/10.21123/bsj.2020.17.2.0426

Lack of Association between *LCS6* Variant in *KRAS* Gene with the Occurrence of Breast Tumors in Iraqi Women

***Roya H. Al-Haddad*[1*]** ***Hameed M. Jasim*[1]** ***Wieeam Abdulfattah Saleh*[2]**

Received 22/6/2019, Accepted 27/10/2019, Published 1/6/2020

Abstract:

Breast cancer is the most commonly diagnosed cancer and remains one of the main reasons of cancer-related mortality in women worldwide. *KRAS* variant rs61764370 (T>G) is associated with an increased risk of occurrence of many cancers, Here The case-control study was accomplished on 135 women including 45 women with breast cancer patients, 45 women with benign breast lesions and 45 healthy women to analyze the association of *KRAS* variant rs (61764370 T>G) with breast cancer. *LCS 6* variant in *KRAS* gene was amplified by using specific primers, then genotype was detected after sequencing the PCR products. The results showed that the genotype and allele frequency of TT and GT allele of *KRAS* gene were statistically non-significant ($p< 0.01$) among breast cancer patients and breast benign lesions compared with healthy controls in Iraqi women.

Key words: Breast benign lesions, Breast cancer, *KRAS*, rs 61764370.

Introduction:

Breast cancer is the main lethal cancer in females all over the world, most of the cases occur in postmenopausal women, but a great number of younger women are afflicted (1). The genetic type of cancer development is based on that the transformation of a normal breast cell into a tumor depends on the change of genes found in the normal cell (2). *KRAS* is one of the most important oncogenes tumorigenesis. It encodes protein and the product can bind both guanosine triphosphate (GTP) and guanosine diphosphate (GDP) and plays an important role in the regulation of signal transduction (3). Latest evidence has displayed that single nucleotide polymorphisms (SNPs) which reside in *KRAS* 3'-untranslated regions (UTR) of human contains multiple supposed tumor suppressor lethal-7 (*let-7*) complementary sites (LCS). These SNPs can regulate the activity of *KRAS* and prevent the *let-7* miRNA from binding to *KRAS* in order to adjust the expression of its protein (4).

A number of studies have analyzed the influence of *KRAS* rs61764370 T>G polymorphism on the risk of various cancers. Among these are, Christensen *et al*., who showed that patients with head and neck squamous cell carcinoma, who carry the *KRAS-LCS6* variant allele had a reduced survival time compared to individuals with wild type (5). Ratner *et al*., collected DNA using standard isolation methods from tissue, blood, buccal cell samples, or saliva, to demonstrate that *KRAS* variant is associated with the risk of developing epithelial ovarian cancer (6), Kjersem *et al.,* found out that the *LCS6* variant allele does not occur to be a risk factor for occurrence of colorectal polyps or colorectal cancer (7). In another study conducted by Cerne *et al.,* it was shown that extracted DNA from whole blood in familial breast cancer cases and controls, had no significant correlation between the *KRAS* variant and risk of (sporadic and familial) breast cancer (8). Also another study by Uvirovaa *et al.,* mentioned that *KRAS-LCS 6* (rs61764370) was non-significantly associated with breast cancer (9). Dai *et al*., proposed that G allele of rs61764370 has a shorter life and a higher risk of reappearance or metastasis of colorectal cancer (10). Zhang and Shi showed that genotype GG, GT of (rs61764370) was not a factor for estimating cancer risk in Caucasian people (11).

This study was carried out in Iraq to determine the influence of *KRAS* gene polymorphism (rs61764370 T>G) on breast cancer and breast benign lesions compared to healthy women.

[1] College of Biotechnology, Al-Nahrain University, Baghdad, Iraq.
[2] Oncology Teaching Hospital, Ministry of Health, Baghdad, Iraq.
[*]Corresponding author: royaalhadad84@gmail.com
[*]ORCID ID: https://orcid.org/0000-0001-5862-0278

Baghdad Science Journal
P-ISSN: 2078-8665
E-ISSN: 2411-7986

Materials and methods

Subjects:

A total of 135 women were enrolled in this study, forty five of them were had a breast cancer with mean age of 51 years, forty five of them with breast benign lesion with mean age of 32 (all these women attended oncology teaching hospital in Baghdad from different governorates of Iraq) in addition to forty five healthy control women with mean age 50 years. Details of clinical data and demographic characteristics for the patients with breast cancer only were collected by medical record review according to American joint committee on Cancer (AJCC) (12), which includes: the age, smoking status, tumor site and size, grade, recurrence/metastasis status, estrogen, progesterone receptor, *HER2 neu* status and family history.

Genomic DNA isolation

Genomic DNA was isolated from the EDTA-coated venous blood samples using ReliaPrep™ Blood gDNA Miniprep System (Promega/ USA) according to the manufacturer's instructions. The DNA concentration was measured by Quantus™ Fluorometer (Promega/ USA).

Amplification of *LCS6* variant in *KRAS* gene

Polymerase chain reaction (PCR) was used for the identification of *LCS6* variant in 3'UTR of *KRAS* gene by using specific primers mentioned by Sanaei *et al.*, (13) (rs 61764370) the forward primer: 5`- GTGTCAGAGTCTCGCTCTTGTC -3` and reverse primer: 5`-AGACCACACTAGCACTACCTAAGGA-3, the reaction mixture (25 µl) was prepared by adding 12.5 µl of 2X Go*Taq* Green master mix, 1 µl of each primer, 8.5 µl of Nuclease-Free Water and 2 µl of DNA template. The PCR condition was: 30 cycle of 95 °C for 30s, 57 °C for 30S, 72 °C for 30s followed by a final extension step for 7 min at 72 °C. After amplification, agarose gel electrophoresis was used to approve the presence of amplification products.

Sequencing of the amplified products

PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequencer used by Macrogen Corporation – Korea. Results of sequencing were analyzed by using Geneious software.

Statistical analysis

SPSS (statistical package for social sciences) version 24 was used for the statistical analysis (14), to determine the significant difference ($P<0.01$) between odd ratio and allele frequencies of T>G polymorphism in *KRAS* gene among each case study.

Results and Discussion:

Patients with invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) accounted 75.5 % and 15.5 % respectively. It was found that the breast cancer patients who expressed the estrogen receptor (ER) (82.2%) and the progesterone receptor (PR) were (75.5%). According to the tumor-node-metastasis (TNM) status, it was found that 51.1% of the breast cancer patients were at stage II; other patients (48.9%) were at other stages (I, III, IV). The medical files showed that 60 % of breast cancer patients were with lymph node metastasis as shown in Table (1).

Table 1. Clinical and histological characteristics of Iraqi women having breast cancer

Characteristics	Cases (45) No (%)
Histological type	
IDC	34 (75.5)
ILC	7 (15.5)
Others	4 (8.9)
Grade	
I	6 (13.3)
II	26 (57.8)
III	9 (20)
IV	4 (8.9)
Tumor size	
Stage I	13 (28.9)
Stage II	23 (51.1)
Stage III	6 (13.3)
Stage IV	3 (6.7)
Lymph node	
(+)	27 (60)
(-)	18 (40)
ER status	
(+)	37 (82.2)
(-)	8 (17.7)
PR status	
(+)	34 (75.5)
(-)	11 (24.4)
HER2 neu status	
(+)	12 (26.6)
(-)	33 (73.3)

To ascertain if the *KRAS* single nucleotide polymorphisms (rs61764370 T>G) was associated with the incidence breast cancer we first amplify the *Let-7* complementary site 6 of *KRAS* gene in each case study by using specific primers with product size of 385bp obtained and visualized after Agarose gel electrophoresis by UV light, then size of fragment was calculated by using gel documentation system as shown in Fig. (1).

Baghdad Science Journal
P-ISSN: 2078-8665
E-ISSN: 2411-7986

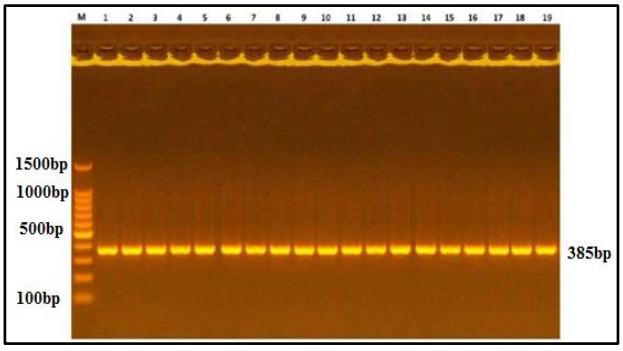

Figure 1. PCR products for rs61764370 (T>G) of *KRAS* gene for human samples was fractionated on 1.5 % agarose gel electrophoresis (100 volt for 75 minutes) Lane (1):1000 bp DNA marker lane (2-8) patients having breast cancer, lane (9-14) patients having breast benign lesions, and lane (15-19) control.

The PCR products were sequenced and analyzed to evaluate the frequency of this polymorphisms in patients with breast cancer as shown in Fig. (2).

The genotype and allele frequency of TT, GT are indicate in the Table-2. Results showed that the allele frequency of T and G in women with breast cancer was 0.93%, 0.07% respectively, while the allele frequency for T and G alleles for women with benign lesion was 0.94%, 0.06% respectively and the allele frequency for the same alleles in healthy women was 0.97%, 0.03% respectively. The genotype frequency of the TT, TG show no significant (NS) association in the three groups (malignant, benign and control) among Iraqi population.

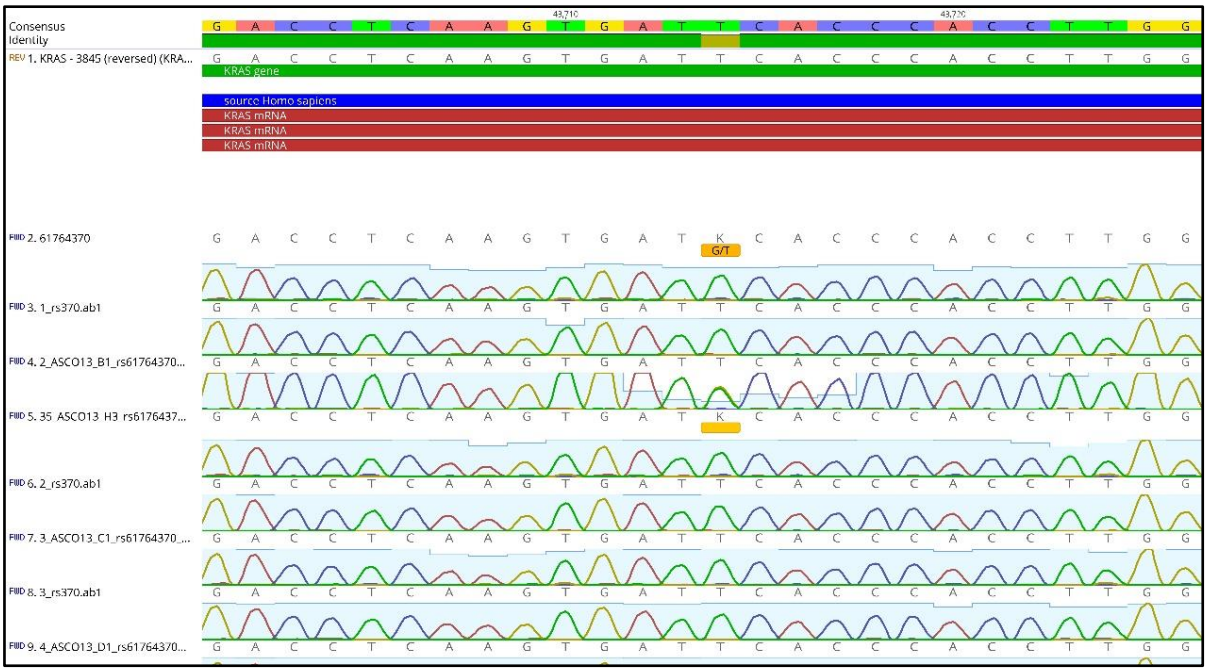

Figure 2. The sequence of *KRAS* (rs61764370 T>G) gene when alignment with reference sequence (showed substitute nucleotide from TT to TG).

Table 2. Genotype and allele frequency of (rs61764370 T >G) polymorphisms in breast cancer patients, breast benign lesions patients and control

Polymorphism(rs61764370)	Malignant(G1)	Benign(G2)	Control(G3)
	Genotype frequency, No (%)		
TT	39(86.67%)	40(88.89%)	42(93.33%)
TG	6(13.33%)	5(11.11%)	3(6.67%)
GG	0(0.00%)	0(0.00%)	0(0.00%)
	Malignant vs benign	OR = 1.2 95%CI(0.34 - 4.36)	0.7
	Benign vs control	OR = 1.8 95% CI (0.4-7.8)	0.5
TT vs TG	Malignant vs control	OR = 2.2 95% CI (0.5-9.2)	0.3
	Allele frequency (%)		
T	0.93	0.94	0.97
G	0.07	0.06	0.03

In this case-control study it was found that there are no significant differences in allele and genotype frequency of rs61764370 T>G in breast cancer patients compared with benign lesions and healthy controls, similar results reported that the occurrence of the *KRAS-LCS6* (rs61764370) TG genotype was associated with non-significantly with the risk of breast cancer by Cerne *et al.,* (8) and Uvirovaa *et al.,* (9). Contrary, Hollestelle *et al*., stated that *KRAS* variant (rs61764370 T>G) frequencies might be increased among *BRCA1* carriers but not *BRCA2* or non *BRCA1/BRCA2* families among controls (15). On the other hand, Paranjape *et al*., (16) and Ustinova *et al*., (17) found that, the *KRAS* variant was significantly associated with breast cancer at menopausal age. Another study by Pilarski, *et al*., (18), found that *KRAS* variant is considered as a genetic marker for hereditary breast ovarian cancer families. In this study the homozygous genotype GG was not found in patients with breast cancer, patients with benign breast disease and controls in Iraqi women.

Chin *et al*., (19) found that *LCS6* variant in the *KRAS* 3'-UTR varied across geographic populations, it was infrequently in Asian (like Iraq in this research) and Native American populations while in African populations were less frequently, however, it occurs most frequently in European populations.

Conclusion:

This work indicates that the genotype frequency of GT (heterozygous) and TT (wild type) in single nucleotide polymorphism (rs61764370 T<G) of *KRAS* gene is not associated with the incidence of breast cancer and breast benign lesions as a risk factor compared with normal Iraqi women.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in Al-Nahrain University.

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فقدان العلاقه بين التباين الوراثي *LCS6* في جين *KRAS* مع حدوث الاصابة باورام الثدي في النساء العراقيات

رؤيا هادي الحداد[1] **حميد مجيد جاسم[1]** **وئام عبد الفتاح صالح[2]**

[1] كليه التقنيات الاحيائية، جامعه النهرين، بغداد، العراق.
[2] مستشفى الاورام التعليمي، وزاره االصحة، بغداد، العراق.

الخلاصة:

سرطان الثدي هو أكثر أنواع السرطان شيوعًا ولا يزال أحد الأسباب الرئيسية للوفيات المرتبطة بالسرطان لدى النساء في جميع أنحاء العالم. يرتبط التباين الوراثي (T<G rs61764370) بزياده خطر الاصابه بالعديد من انواع السرطانات . أجريت هذه الدراسه على135 امراه حيث تضمنت 45 عينه من النساء العراقيات مصابات بسرطان الثدي و45 مصابه باورام الثدي الحميده و45 من النساء الاصحاء لتحليل العلاقه بين التغايرات الوراثيه احاديه النيوكليوتيده (rs61764370) مع الاصابه بسرطان الثدي تم تضخيم LCS 6 variant لجين *KRAS* باستخدام بادئات محددة ، ثم تم الكشف عن النمط وراثيّ بعد استخدام التعاقب النيوكليوتي لنواتج تفاعل السلسله المتبلمره . ولقد أظهرت النتائج أن النمط الوراثي وتكرار الأليل من TT و GT أليل الجين *KRAS* كانت غير ذات دلالة إحصائية (P <0.01) بين مرضى سرطان الثدي واورام الثدي الحميده مقارنة مع عينات النساء العراقيات الاصحاء.

الكلمات المفتاحية: اورام الثدي الحميدة، سرطان الثدي، *KRAS* ، rs 61764370.