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Association between KRAS rs 712 G>T Polymorphism with the Incidence of Breast tumors in Iraqi Women

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Abstract: Recently, Single nucleotide polymorphisms (SNPs) located in the 3'-UTR of the *KRAS (KRAS* rs712 (G>T)) which interrupts let-7 miRNA binding site was stated to be associated with the occurrence of many human cancers. This study aimed to assess the association of *KRAS* rs712 polymorphism with the occurrence of breast cancer and breast benign tumor in Iraqi women. rs712 in 3' UTR of the *KRAS* gene was amplified by using specific primers, then genotype was detected after sequencing of the amplified products. Results of genotyping showed that mutant type TT was significantly associated in women with breast cancer versus women breast benign tumors and in women with breast benign tumors versus healthy women. However, the results showed there is no significant association in women with breast cancer versus healthy women. Based on the results, rs712 polymorphism in the 3' UTR of the *KRAS* gene was considered as a risk factor with the occurrence of breast cancer and breast benign tumor in Iraqi women compared with healthy women.

Keywords: Breast cancer, Breast benign tumor, rs712, KRAS.

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Introduction

In Iraq, breast cancer has become the main risk to women's health and it is the main cause of death following cardiovascular diseases, together with a cancer-related death rate of 23 % (1). Many studies were carried out in Iraq to investigate the genetic abnormalities association with breast cancer (2,3,4,5). KRAS gene is a cellular oncogene, which belongs to the ras family located on 12p12.1 chromosome and includes 6 exons and 5 introns. This gene encodes for GDP/GTP-binding protein that acts as a signal transducer and this protein belongs to the small GTPase superfamily (6). The latest study showed that single nucleotide polymorphisms (SNPs) residing in human *KRAS* 3'-UTR contains numerous suspected tumor suppressors, like lethal-7 (let-7) supplementary sites (LCS). These SNPs regulate the activity of *KRAS* and block let-7 miRNA from binding to *KRAS* in order to change the expression of its protein (7).

Several researchers have reported the association of *KRAS* rs712 which is located in *LCS1* with various cancers, among them, Li *et al*., (8) who indicated that the T allele of *KRAS* rs712 was significantly related with increased risk of gastric cancer. Zhao *et al.* (9) proposed that *KRAS* rs712 could enhance susceptibility to develop cancer in the Chinese population. On the other hand, Liang *et al*., (10) and Jiang *et al.*, (11)

found that rs712 may induce the etiology of Cervical Squamous Cell Carcinoma and the occurrence of colorectal cancer in a Chinese population. Furthermore, it was found that *KRAS* rs712 polymorphism was correlated with increased casual of Hepatocellular carcinoma (12). Based on the literature review, this presented study was the first carried out in Iraq to determine the correlation between the *KRAS* (rs712 G>T) with the occurrence of breast cancer and breast benign tumor in Iraqi women, which can provide new prospects for cancer treatment and prevention.

Subjects, Material, and methods

Subjects

In this study, a total of one hundred thirty-five women had been enrolled. Forty-five of them with early diagnosed with breast cancer, forty-five of them with early diagnosed with breast benign tumor (all these women attend to the oncology teaching hospital in Baghdad from different governorates of Iraq), in addition to forty-five normal healthy women. Women with recurrent breast cancer were excluded from this study. Details of clinical data and demographic characteristics of the patients with breast cancer only, were collected by medical record review according to the American Joint Committee on Cancer (AJCC) (13), which includes: age, tumor site and size, grade, recurrence/metastasis status, estrogen and progesterone receptor and *HER2 new* status.

Genomic DNA isolation

Genomic DNA was extracted from the EDTA-coated venous blood samples using ReliaPrep™ Blood gDNA Miniprep System (Promega/ USA) as stated by the manufacturer's instructions. The concentration of DNA was assessed by Quantus™ Fluorometer (Promega/ USA) for further analysis.

Amplification of rs712 in *KRAS* gene

KRAS variant (rs 712) was amplified using Polymerase chain reaction (PCR) via specific primers mentioned by Sanaei *et al*., (14) nucleotide sequence for the forward primer: 5`-AAGGCATACTAGTACAAGTGGTA A- 3` and reverse primer 5`-TGTGTTCCCTCAATGTTTCAGT -3`, the mixture of reaction (25µl) was prepared by adding 12.5 µl of 2X Go*Taq* Green master mix, 8.5 µl of Nuclease-Free Water, 1 µl of each primer and 2 µl of DNA template, the PCR condition was: 30 cycle of 95 °C for 30s, 56 °C for 30S, 72 °C for 30s followed by a final extension step for 7min at 72 °C. After amplification, agarose gel electrophoresis was adopted to approve the occurrence of amplification.

Sequencing of the amplified products

PCR products sent for Sanger sequencing using ABI3730XL, an automated DNA sequencer used by Macrogen Corporation – Korea. The alignment of the Results was executed utilizing Geneious software.

Statistical analysis

Statistical analysis in this work was made using statistical package for social sciences (SPSS). This analysis includes odds ratio (OR) and confidence interval

(CI) 95%, which performed to investigate the association between polymorphism and breast tumors. χ^2-test performed to study the rs712 genotype frequency differences between each two groups. *P*-value was considered to be statistically significant (P<0.01). Hardy-Weinberg equilibrium (HWE) was calculated for each case study.

cancer who expressed the estrogen receptor (ER), progesterone receptor (PR) were 82.2%, 75.5% respectively. Besides, women with invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC) were accounted 15.5% and 75.5% respectively. According to the TNM, it was found that 51.1% of the

Results and Discussion

Histological biomarkers had been utilized to provide information for the diagnosis of the disease and its consequences. The age of breast cancer patients under study was (20-60) year, benign breast tumor was (20-45) years and healthy women were (30-50) years. It was noticed that women with breast breast cancer patients were at stage II of tumor; other patients were at Stage I, stage III and stage IV stages with 28.8%, 13.3%, and 6.6% respectively. The medical records showed that 60% of breast cancer patients with metastasis lymph node as shown in Table (1).

Table (1): Clinical and histological characteristics for Iraqi women with 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 (28.8)
Stage II	23 (51.1)
Stage III	6 (13.3)
Stage IV	3 (6.6)
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)

rs 712 of the *KRAS* gene in each case study was amplified using specific primers. Results of amplification showed that an amplicon product of 426bp was obtained and visualized after Agarose gel electrophoresis by UV light, then the size of the fragment was calculated by using the gel documentation system as shown in Figure (1).

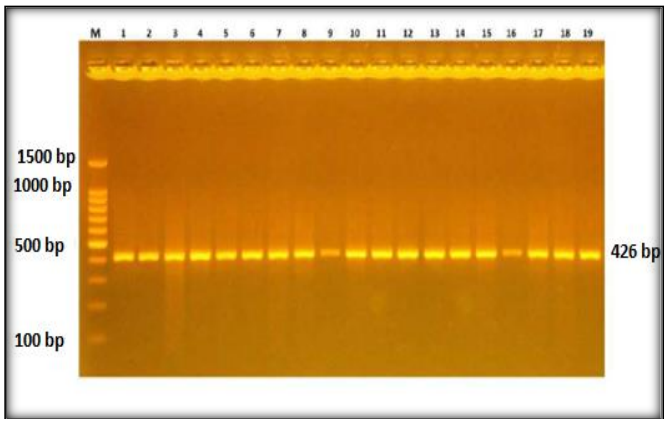

Figure (1): PCR products for rs712 (G>T) of *KRAS* gene for human samples were fractionated on 1.5 % agarose gel electrophoresis (100 volts for 75 minutes) Lane (1): 1000 bp DNA marker lane (2-8) women with breast cancer, lane (9-14) women with a breast benign tumor, and lane (15-19) healthy women.

PCR product was genotyped in women with breast cancer, women with benign tumor and healthy women, then sequenced and analyzed to assess the allele frequency of this polymorphism in patients and control groups by Geneious software, as shown in Figure (2).

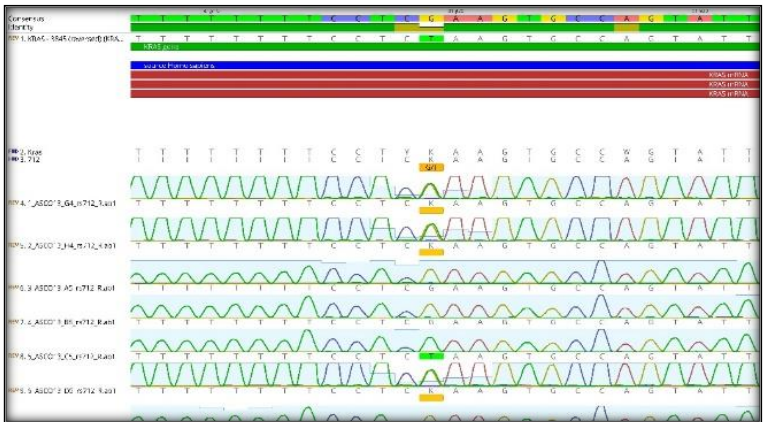

Figure (2): The sequence of the *KRAS* (rs712) gene when alignment with reference sequencing shows the substitution of nucleotide GG to (GT and TT).

Results displayed that allele frequency of G and T in breast cancer women was 0.63 % and 0.37% respectively. Also, allele frequency for G and T alleles for women with benign tumor were 0.40% and 0.60%

respectively. Allele frequency for the G and T alleles in healthy women was 0.57% and 0.43% respectively.

Results also showed that the G allele is a major in both groups under study (malignant and control) respectively. whereas, the T allele is more common in women with breast benign tumor (0.60) which was significantly different (P<0.01) than healthy women or women with breast cancer (0.37, 0.43) with as indicated in the Table (2).

Table (2): Allele and genotype frequency of rs712 (G > T) for women with breast cancer, women with breast benign tumor, and healthy women control.

Polymorphism	Malignant group	Benign group	Control group
Genotype frequency, No (%)			
GG	18 (40.00)	12 (26.67)	12 (26.67)
GT	21(46.67)	12 (26.67)	27 (60.00)
TT	6 (13.33)	21 (46.67)	6 (13.33)
Dominant GG vs GT + TT	Malignant vs benign	O.R. = 0.55 95% CI (0.22-1.32)	*P=0.2*
	Benign vs control	O.R.=1.00 95% CI (0.4-2.55)	*P= 1.00*
	Malignant vs control	O.R. =0.55 95% CI (0.22 -1.32)	*P = 0.2*
Recessive TT vs GG+GT	Malignant vs benign	O.R.= 0.2 95% CI (0.06- 0.49)	*P = 0.001*
	Benign vs control	O.R.= 5.68 95% CI (2.01 - 16.09)	*P = 0.001*
	Malignant vs control	O.R.= 1.00 95% CI (0.3- 3.4)	*P=1.00*
Allele frequency, No (%)			
G	(28) 0.63	(18) 0.40	(26) 0.57
T	(17) 0.37	(27) 0.60	(19) 0.43
P- values	0.9	0.003	0.1

Genotype frequency of GG, GT, and TT for women with breast cancer were 40%, 46.67%, and 13.33% respectively. Moreover, the genotype frequency of GG, GT, TT in the second group of women with benign tumor were 26.67%، 26.67% and 46.67% respectively, while the genotype frequency of GG, GT, and TT for healthy women were 26.67%, 60.00%, and 13.33% respectively. The odd ratio for recessive mode TT vs GG+GT was 0.2, confidence interval (CI) (95%CI) =0.06- 0.49, P = 0.001 in breast cancer women versus women with breast benign tumor. The odd ratio for the TT vs GG+GT was 1.00, 95% CI= 0.3-3.4, P=1.00 in women with breast cancer versus healthy women and the odd ratio for TT vs GG+GT was 5.68, 95% CI =2.01 -16.09, P =0.001 in women with breast benign tumor versus healthy women.

Lifestyle- associated agents can't causing cancer, but are other threats correlated to the genesis of cancer, as in uncontrolled exposure to many carcinogens in the environment, including radiations and microorganisms (15). However, the correlation of rs712 polymorphism with cancer may be

related to environmental causes that can induce tumoral epigenetic changes (16).

Results of this work showed that, homozygous TT (mutant type) in rs712 polymorphism was related with incidence of breast tumors compared with healthy women, and that is in agreement with other studies which showed a relationship between polymorphism and various types of cancer including gastric cancer (8), colorectal cancer (11) and hepatocellular carcinoma (12). Furthermore, Kim *et al*., (17) revealed that sequencing of the complete 3'-UTR region of KRAS in epithelial ovarian cancers and non-small cell lung cancers that rs712 may have a functional role in the control of the *KRAS* gene by interrupting let-7 sites. On the other hand, Sanaei *et al*., (14) exhibited that *KRAS* rs712 TT vs GG+GT genotype in a recessive model was correlated with a diminished risk of breast cancer. In contrast, other studies reported that there is no significant association between *KRAS* rs712 with papillary thyroid cancer (18), nasopharyngeal carcinoma (19) and breast cancer (20).

Conclusion

It had been found that single nucleotide polymorphisms (SNPs) in 3′ UTR of the *KRAS* gene have the capability to be such genetic biomarkers. To evaluate the influence of the *KRAS* variant (rs 712) on breast cancer and benign breast tumor among healthy women, this study showed a significant association in the recessive model TT vs GT+GG in women with breast tumors among healthy women in Iraqi population.

Reference

1. Alwan, N. (2016). Breast Cancer among Iraqi Women: Preliminary Findings from a Regional Comparative Breast Cancer Research Project. JGO, 2(5): 255-258.
2. Gaaib, J.N. and Abdul Hussein M. AL-Faisal (2019). Role of mir-let-7a Expression in Breast Cancer Diagnosis and Prognosis. Indian Journal of Natural Sciences, 9(53): 17177-17184.
3. Gaaib, J.N. AL-Faisal, A.H.M. and Ghanim, M.M. (2017). Evaluation of diagnostic and prognostic value of mucin (MUC 1) gene expression in breast cancer patients. Iraqi Journal of Biotechnology, 16(3): 177-185.
4. Gaaib, J.N.; AL-Faisal, A.H.M.; Tobal, K. and Al-Alwan, N. (2014). Evaluation the Diagnostic and Prognostic Value of Human Mammaglobin (MGB1) Gene Expression in Iraqi Breast Cancer Patients. Int.J.Advance Res.,2(4): 663-669.
5. Mahdi, G.M., Al-thwaini, A.N. and AlFaisal, A.M. (2009). Loss of heterozygosity in short arm of chromosome 8 in FFPT breast cancer tissues samples. Iraqi J.Biotechnol., 8(3):696-703.
6. Kranenburg, O. (2005). The KRAS oncogene : Past, present, and future. *Biochim Biophys Acta1*, 756: 81–82.
7. Roush, S. and Slack, F. J. (2018). The let-7 family of microRNAs. Trends Cell Biol., 18(10): 505–516.
8. Li, Z.; Pan, X.; Han, B. and Guo, X. (2013). A let-7 binding site polymorphism rs712 in the KRAS 3 ′ UTR is associated with an increased risk of gastric cancer. Tumor Biol., 34:3159–3163.
9. Zhao, W.H.; Qu, X.F.; Xing, Z.G.; Zhao, L.Q.; Qin, L. and Lv, C. (2015). Association of rs712 polymorphism in Kras gene 3′-untranslated region and cancer risk: a meta-analysis. J BUON., 20.:309–316.
10. Liang, Y.; Sun, R.; Li, L.; Yuan, F.; Liang, W.; Wang, L., *et al*. (2015). A Functional Polymorphism in the Promoter of MiR-143/145 Is Associated with the Risk of Cervical Squamous Cell Carcinoma in Chinese Women: A Case-Control Study. Medicine (Baltimore), 94: 1289.
11. Jiang, Q.H.; Peng, H.X.; Zhang, Y.; Tian,

P.; Xi, Z.L. and Chen, H. (2015). rs712 polymorphism within let-7 microRNA-binding sites might be involved in the initiation and progression of colorectal cancer in Chinese population. Onco Targets Ther., 8.: 3041–3045.

12. Xiong, D.; Song, Y.P.; Xiong, W. and Liang, Y.D. (2015). A let-7 *KRAS* rs712 polymorphism increases hepatocellular carcinoma risk. Genet. Mol. Res., 14:14050–14055.
13. Kim, I.; Choi, H. J.; Ryu, J. M.; Lee, S.K.; Han, Y.J.; Kim, S. W., *et al*. (2018). Breast Cancer Prognostic Validation of the American Joint Committee on Cancer 8th Staging System in 24,014 Korean Patients with Breast Cancer. J. Breast. Cancer. 21(2) :173-181.
14. Sanaei, S.; Hashemi, M.; Eskandari, E.; Hashemi, S.M. and Bahari, G. (2017). *KRAS* Gene Polymorphisms and their Impact on Breast Cancer Risk in an Iranian Population. Asian Paci. J. of Can. Prevention, 18 (5):1301-1305.
15. Irigaray, P.; Newby, J.A.; Clapp, R.; Hardell, L.; Howard, V.; Montagnier, L., *et al*. (2007). Lifestyle-related factors and environmental agents causing cancer : An overview, Biomed. Pharmacother, 61:640–658.
16. Schulte, P.A.; Whittaker, C. and Curran, C.P. (2015). Considerations for Using Genetic and Epigenetic Information in Occupational Health Risk Assessment and Standard Setting. J. Occup. Environ. Hyg., 12(1): S69–S81.
17. Kim, M.; Chen, X.; Chin, L.J.; Paranjape, T.; Speed, W.C.; *et al*. (2014). Extensive sequence variation in the 3' untranslated region of the KRAS gene in lung and ovarian cancer cases. Cell Cycle, 13(6): 1030-1040.
18. Jin, H.; Liang, Y.; Wang, X.; Zhu, J.; Sun, R.; Chen, P., *et al*. (2014). Association between a functional polymorphism rs712 within let-7-binding site and risk of papillary thyroid cancer. Med. Oncol., 31:221.
19. Pan, X.M.; Jia, J.; Guo, X.M.; Li, Z.H.; Zhang, Z.; Qin, H.J., *et al*. (2014). Lack of association between let-7 binding site polymorphism rs712 and risk of nasopharyngeal carcinoma. Fam. Cancer., 13:93–97.
20. Huang, X.; Yang, Y.; Guo, Y.; Cao, Z.L.; Cui, Z.W.; Hu, T.C. *et al*. (2015). Association of a let-7 KRAS rs712 polymorphism with the risk of breast cancer. Genet. Mol. Res., 14:16913–16920.