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Original Article

Evaluation of the Role of the KRAS Gene Polymorphism LCS6 (rs61764370) in Iraqi Women with Ovarian Cancer

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Abstract

In carcinogenesis, KRAS is an essential oncogene that plays a key function. The polymorphism of rs61764370 is a candidate for cancer susceptibility in KRAS3' untranslated region. The current study aimed to determine the role and impact of the KRAS gene polymorphism (rs61764370 T>G) on the risk of ovarian cancer development in the Iraqi population. In total, 84 ovarian cancer patients and 28 ovarian benign tumors were involved in a case-control study. DNA extraction from the formalin-fixed/paraffin-embedded tissues, followed by the sequencing of PCR products was carried out in genetic analysis for the detection of the KRAS polymorphism (rs61764370 T>G). The results showed that the frequencies of the KRAS gene polymorphism (rs61764370) in ovarian cancer patients were 78 (92.85%) and 6 (7.15%) with genotypes homozygote TT and heterozygote TG, respectively. These corresponding values in patients with benign ovarian tumors were 25 (89.3%) and 3 (10.7%) with homozygote TT and heterozygote TG, respectively. None of the patients either with malignant or benign tumors have been detected with homozygote genotype GG. Genotype frequency of the TT and TG showed that the heterozygote TT genotype vs. TG and T allele vs. G allele were more frequent in malignant and benign tumors ($P \leq 0.01$). Statistically, there was no association between the KRAS polymorphism and the clinical characteristics of ovarian cancer patients, such as age, family history, menopause, histological type, tumor size, or tumor stage. In conclusion, a significant association was found between rs61764370 and the risk of ovarian cancer in the Iraqi population, particularly those with genotypes homozygote TT. On the other hand, genotypes had no relationship with any of the clinical characteristics of ovarian cancer patients. Additional well-designed studies with larger sample sizes are recommended to validate the precise role of KRAS LCS6 variations in ovarian cancer risk.

Keywords: KRAS, LCS6 Polymorphism, Ovarian cancer, rs61764370 T>G

1. Introduction

Many candidate gene studies have found putative common alleles of the sensitivity to ovarian cancer; however, most probably reflect erroneous associations because none of the hypotheses with low previous association probabilities were reported at statistical importance levels required for testing (1). Numerous gene studies have found putative common alleles of ovarian cancer sensitivity; however, these are almost certainly false associations since none of the hypotheses with low prior association probabilities were reported at the statistical significance levels required for testing (2). One of the most important oncogenes involved in cancer incidence and progression is KRAS, a member of the *Ras* gene family. *Ras* genes are a group of genes that encode GDP/GTP-binding proteins, which are involved in carcinogenesis (3). A single-nucleotide polymorphism (SNP) located in the 30-UTR of the KRAS oncogene, known as rs61764370 or LCS6 polymorphism, was discovered in 2010 and linked to the risk of unselected epithelial ovarian cancer. This variation and BRCA1

mutations have been linked in a significant way. Women with these mutations have a higher risk of cancer according to this link. This variation was also associated with a family history of the disease and shorter progression-free survival in women even in the absence of BRCA gene mutations (4). The variant of alterations of allele T to allele G located in LCS6 from KRAS 3'-UTR is rs61764370. It can diminish the mature Let-7's binding capacity to target KRAS mRNA, which results in increased KRAS activity.

In addition, in tumors of patients holding the G-allele in comparison with T-allele, a considerably lower expression of Let-7 was found. Those findings have demonstrated a link between rs61764370 and aberrant KRAS expression with Let-7 level. This locus could therefore be associated with cancer and considered a cancer-susceptible candidate locus (5). Numerous studies have investigated the influence on the risk of different cancers of KRAS rs61764370 T/G polymorphism. Non-small cell lung cancer (6), premenopausal triple-negative breast cancer (7), and ovarian cancer have all been linked to the LCS6 variant allele (4). This study aimed to determine if there is a link between polymorphism and ovarian cancer risk in Iraqi women, given the importance of the KRAS gene and the LCS6 variant in cancer progression.

2. Materials and Methods

The current study used tissue sections from 84 patients with primary and advanced stages of ovarian cancer, as well as different tumor types histologically. Numerous Iraqi hospitals provided these sections after patients underwent surgeries, such as total abdominal hysterectomy, subtotal abdominal hysterectomy, vaginal hysterectomy, bilateral salpingo-oophorectomy, and endometrial biopsy. A total of 28 tissue sections from benign ovarian tumors were used as a control group. Patients' demographic and clinical data (age, menopausal status, tumor size, tumor subtype, tumor stage, and family history) were obtained from their medical records. For further molecular analysis, the formalin-fixed/paraffin-embedded tissues were sectioned into 10µm sections and placed in DNase-RNase tubes.

2.1. Genomic DNA Isolation

DNA extraction and purification from tissue sections were carried out in accordance with the manufacturer's instructions (QIAamp DNA FFPE Tissue Mini Kit, Qiagen/USA).

2.2. DNA Amplification and Genotyping

The primers for KRAS rs61764370 T/G polymorphism were LCS6 forward: GCGTGTGCCACTACACTCAA and LCS6 reverse: GGTGACTGGCATCTGGTAGG. The PCR reaction was started in a volume of 20 µl. The following substances were included in the reaction tube: 0.5 µl of primers mix, 2 µl of extracted DNA, 7.5 of free water, 10 µl of reaction buffer. The reaction conditions were as follows: an initial 10 min step at 95°C for enzyme activation, followed by 40 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec for denaturation, annealing, and extension, respectively, followed by a final 5-min extension at 72°C, and then incubation at 4°C to time end. The resulting DNA fragments were separated on a 2% agarose gel electrophoresis and then visualized under UV light after ethidium staining to clarify DNA bands. The PCR products were purified using the Charge Switch PCR Clean-up kit according to the manufacturer's instructions before sequencing. ABI Big Dye terminator ready reactions Kit (Applied Biosystems, USA) and ABI Automated DNA Sequencer 3730 were used for the sequencing. The Mutation Surveyor Software of sequencing reading (version 3.24) was used to analyze the data.

2.3. Statistical Analysis

The Statistical Analysis System program (SAS, 2012) was employed to determine the impact on the study parameters of various variables. The least significant difference (ANOVA variation analysis) or t-test has been used in this study in order to compare the means.

3. Results

A total of 84 ovarian cancer samples were examined for the expression and identification of the LCS6 SNP

in the KRAS 3-UTR. This study included 28 samples with benign ovarian tumors as a control group. The pathological and clinical features of ovarian cancer patients are shown in table 1. The patients' ages ranged from 14 to 70 years, with a median of 47 years. According to the family history, all ovarian cancer samples tested negative for family history. In terms of menopause state, the premenopausal and postmenopausal states were determined at 40 (47.6%), and 44 (52.4%), respectively. The majority of the samples (n=63; 75%) were in stage I, while the remaining (n=21, 25%) were in stage III considering the International Federation of Gynecology and Obstetrics surgical staging system. According to tumor histology types, the samples were divided into five clinical groups of serous epithelial tumors (n=34; 40.47%), mucinous epithelial tumors (n=20; 23.8%), endometrioid tumors (n=21; 25%), germ cell tumors (n=6; 7.14%), and (n=3; 3.57%) Burner tumors. Results of genotyping in both benign and malignant ovarian tumors are listed in table 2. In the Iraqi population, the frequencies of the KRAS gene polymorphism (rs61764370) in ovarian cancer patients were found to be 78 (92.85%) and 6 (7.15%) with genotypes homozygote TT and heterozygote TG, respectively. Patients with benign ovarian tumors were homozygote TT in 25 (89.3%) cases and heterozygote TG in 3 (10.7%) cases. There was no homozygote genotype GG found in any of the patients, whether they had malignant or benign tumors. Individuals with homozygote TT had a significantly higher risk of ovarian cancer than those with the TG genotype (P=0.0001). The homozygote TT genotype was more common in both malignant and benign tumors ($P \leq 0.01$) than the TG genotype (Figure 1). There were no significant differences between malignant and benign tumors in the distribution of genotypes. The frequency of T vs. G alleles, on the other hand, was significantly higher in both study groups ($P \leq 0.01$) (0.96% and 0.95% in the malignant and benign ovarian tumors, respectively). However, no statistically significant relationship was found between genotype distribution and clinical characteristics of ovarian cancer patients, including age, family history, menopause, histological type, tumor size, and tumor stage.

Table 1. Clinical and pathological features of ovarian cancer patients

Age groups	
Children (0-14 years)	9 (10.7%)
Teenagers and young adults (15-24 years)	3 (3.57%)
Adults (25-49 years)	33 (39.28%)
Old age (50-74 years)	39 (46.42%)
Menopausal state	
Premenopausal	40 (47.6%)
Postmenopausal	44 (52.4%)
Family history	
Positive	0
Negative	84 (100%)
International federation of gynecology and obstetrics tumor stage	
Stage I	63 (75%)
Stage III	21 (25%)
Tumor histopathological types	
Serous tumors	34 (40.47%)
Mucinous tumors	20 (23.8%)
Endometriod tumors	21 (25%)
Germ cell tumors	6 (7.14%)
Burner tumors	3 (3,57%

Table 2. Genotypes and allele frequency of KRAS LCS6 polymorphisms in the study groups

Genotypes	Malignant ovarian tumor	Benign ovarian tumor	OR (95%Cl)	*P*-value
TT	78 (92.85%)	25 (89.3%)	1.00	0.437 NS
TG	6 (7.15%)	3 (10.7%)	1.56	0.437 NS
GG	0 (0.00%)	0 (0.00%)	0	1.00 NS
P-value	0.0001 **	0.0001 **	---	---
Alleles				
T	0.96	0.95	--	--
G	0.04	0.05	--	--

** ($P \leq 0.01$)

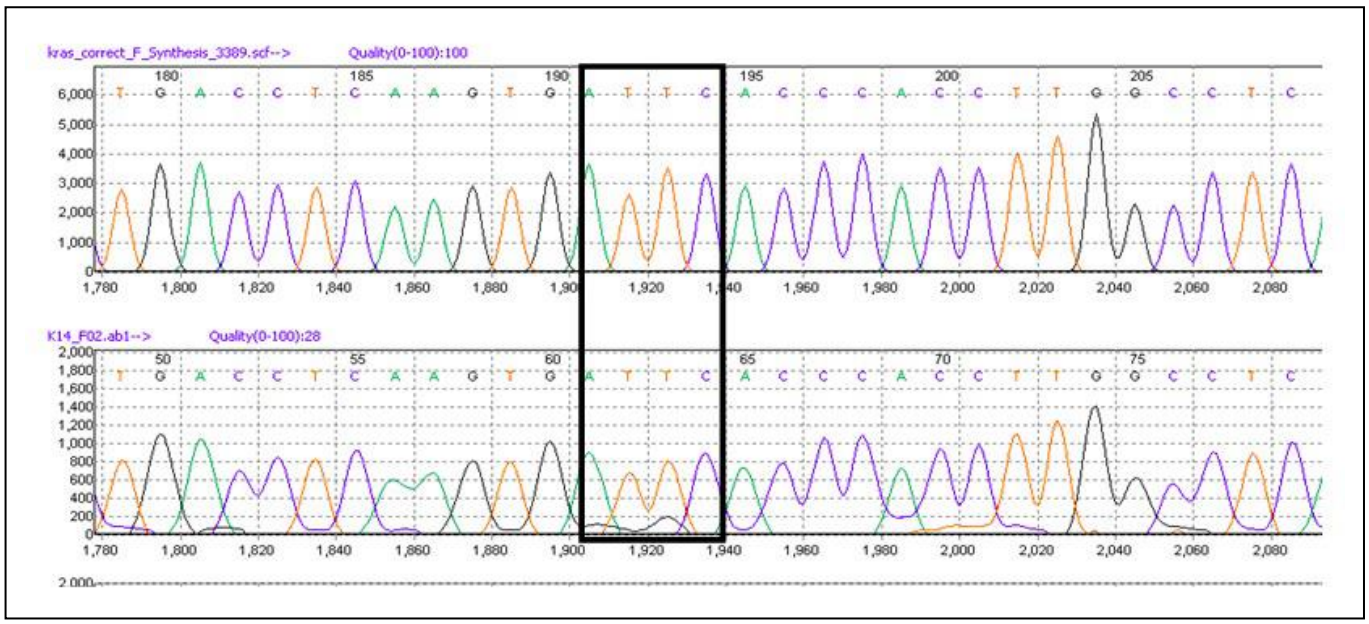

Figure 1. Sequencing results revealing TT genotype (Up) and TG genotype (Down)

4. Discussion

KRAS LCS6 polymorphisms are important molecular markers in medicine since they are involved in the cancer development pathway and influence drug response, making them potential therapeutic targets (8). Genetic variations in the binding site of Let-7 microRNA within the KRAS gene have been linked to ovarian cancer predisposition, and it has also been detected as a marker for ovarian cancer detection (4). The current study has found that homozygote TT is the most common genotype in both study groups (92.85% and 89.3% in malignant and benign ovarian tumors, respectively), followed by heterozygote TG (7.15% and 10.7%), while the homozygote GG was not detected. There were no statistically significant differences between malignant and benign ovarian tumors in genotype distribution. Furthermore, no statistically significant relationship was found between genotype distribution and any of the clinical features of ovarian cancer patients studied. The findings of the current study revealed that the TT vs. TG genotype and T vs. G allele were significantly associated with ovarian cancer. Pharoah, Palmieri (9) confirmed our findings and hypothesized that rs61764370 was associated significantly with ovarian cancer risk, as well as familial ovarian cancer.

A study on a Caucasian population found no link between the rs61764370 genotype GT/GG and the risk of developing the cancers studied, such as head and neck carcinoma, cancers of breast, ovaries, lung, and colorectal cancer (10). On the other hand, Caiola, Rulli (11) confirmed our findings stating that the KRAS-LCS6 polymorphism had no bearing on ovarian cancer. Several studies, including those conducted by D'Hooghe, Grechukhina (12), as well as Farahani, Shahbazi (13), have confirmed the lack of association of this polymorphism with endometriosis. KRAS-LCS6 polymorphism, on the other hand, has been proposed as a genetic diagnostic marker for breast and ovarian cancer detection in families with a hereditary predisposition (14).

RAS proteins are involved in a variety of biological processes, including cell proliferation regulation, tumor cell replication and differentiation, as well as tumor growth and progression (15). In the current study, no differences were found in the analyzed group of patients in the distribution of polymorphism and clinical features, such as family history, menopausal state, and tumor stage. Ganzinelli, Rulli (16) discovered that the KRAS-LCS6 polymorphism had no effect on survival rate or tumor progression in patients with non-small cell lung cancer, and the polymorphism had no association with any of the patients' baseline characteristics.

KRAS LCS6 polymorphisms were recently discovered to be a key player in increasing the risk of carcinogenesis and survival in metastatic colorectal cancer patients, particularly those who received anti-epidermal growth factor receptor therapy (17). The present study showed that the frequency of T vs. G alleles was significantly higher in both study groups. These findings are similar to the results reported by Al-Haddad (18) who found that the frequencies of the T allele were 0.93% and 0.94% in breast cancer patients and cases with benign lesions, respectively. Similarly, Farokhzad, Hosseini (19) found that the T allele was frequent in 84% of the lung cancer patients and 96% of healthy control. The same results were obtained by Sanaei, Hashemi (20) who recorded that the T allele was frequent in 56% of breast cancer patients and 63% of control. In the same line, Mohthash, Shah (21) found the same results as they detected T allele in 65% of breast cancer patients and 82% of healthy control.

Regarding the limitations of this study, one can refer to the relatively small sample size, insufficient data, and lack of such studies of ovarian cancer in the geographical region, which made comparing the findings of the current study difficult. To our knowledge, no research has been conducted on the relationship between this polymorphism and the risk of ovarian cancer in Iraqi or neighboring populations.

5. Conclusion

In conclusion, the results confirmed a significant association between rs61764370 and ovarian cancer risk. However, no statistically significant differences were found between study groups in terms of allele frequency distribution with a predominance of wild-type TT. Furthermore, genotypes showed no association with any of the clinical features of ovarian cancer patients. Given the current study's limitations, additional well-designed studies with larger sample sizes are strongly recommended to validate the exact role of KRAS LCS6 variations in ovarian cancer risk.

Authors' Contribution

Study concept and design: M. G. J.
Acquisition of data: M. G. J.
Analysis and interpretation of data: M. G. J.
Drafting of the manuscript: M. G. J.
Critical revision of the manuscript for important intellectual content: M. G. J.
Statistical analysis: M. G. J.
Administrative, technical, and material support: M. G. J.

Ethics

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Baghdad University, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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