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LncRNA H19 Genotypes and Haplotypes in Ovarian Cancer Patients

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ABSTRACT

Background & Objective: Functional long non-coding RNAs (lncRNAs) have a significant role in crucial pathways associated with cancer progression. The overexpression of the H19 gene has been documented in various types of cancer. The present study was conducted with aim to assess the correlations between two specific variations of the lncRNA H19 gene (rs217727C>T and rs2839698C>T) with the likelihood of developing ovarian cancer.

Materials & Methods: This case-control analysis was conducted on a sample of three hundred individuals, comprising 150 cases diagnosed with ovarian cancer and 200 control subjects. The tetra-primer ARMS-PCR method was used to assess the correlation between two single nucleotide polymorphisms (SNPs) of lncRNA H19 and the susceptibility to ovarian cancer.

Results: There was a strong association between the rs2839698 variant and the risk of ovarian cancer. The Co-dominant model of rs2839698 (CT vs. CC) demonstrated a statistically significant risk reduction, with an odds ratio (OR) of 0.45 (CI 0.26-0.78, P = 0.003). Similarly, the dominant model of rs2839698 (CT+TT vs. CC) exhibited a significant decrease in risk, with an OR of 0.54 (CI 0.32-0.91; P = 0.01). Lastly, the Over-dominant model of rs2839698 (CT vs. CC+TT) demonstrated a significant risk reduction, with an OR of 0.50 (CI 0.32-0.77; P = 0.01).

Conclusion: The results of the present study indicate that the rs2839698 variant in lncRNA H19 may influence the vulnerability to ovarian cancer in Iranian population.

Keywords: Carcinoma, Long non-coding RNA, Polymorphism, rs2839698

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1. Introduction

Ovarian cancer is a prevalent gynecological malignancy associated with a significant mortality rate, and its underlying molecular mechanisms remain mostly unknown **(1)**. The incidence of ovarian cancer accounted for approximately 4% of tumor cases in women, typically exhibiting no symptoms during the early stages **(2)**. Thus far, there has been a dispute regarding the efficacy of radiotherapy and chemotherapy in cancer treatment; however, the 5-year survival rate has consistently remained at a low level **(3)**. Ovarian cancer is associated with several risk variables, including but not limited to age, obesity, family history, and other candidate genes **(4)**. Approximately, 10% of invasive ovarian cancer cases are attributed to hereditary predispositions **(5)**. One of these predisposing genetic biomarkers suggested the long non-coding RNAs (lncRNAs). Molecular profiling studies have shown that the gene expression levels of lncRNAs can be either reduced or elevated in several types of human malignancies **(6)**. The initial survey indicated that lncRNA genes have the potential to function as either oncogenes or tumor suppressor genes, exerting control over numerous molecular signaling pathways associated with malignancies **(7)**. This dubious knowledge gives rise to a wide range of studies focused on investigating the functioning of non-coding sites and their role in advancing cancer. These surveys have identified lncRNA molecules that contain SNPs associated with malignancy **(8)**. LncRNA H19, a crucial gene inherited from the mother, was first identified in the 1990s **(9)**. The H19 gene, which consists of five exons and four introns, is responsible for encoding a non-coding RNA that is 2.3-kb long, capped, spliced, and polyadenylated. It is known to have a crucial function in embryonic development and growth regulation **(10)**. H19 is involved in multiple cancer types, such as breast, lung, esophageal, gastric, pancreatic, colorectal, liver, bladder, and cervical cancer. H19 functions as an oncogene or a suppressor gene, potentially contributing to the diversity observed among many cancer types. Prior studies have demonstrated a significant correlation between H19 gene polymorphisms and malignancies **(10-12)**.

The present study was conducted with aim to assess the correlation between two SNPs in long non-coding RNA H19 (rs217727C>T) and (rs2839698C>T) and the susceptibility to ovarian cancer. Personalized medicine holds particular significance in enhancing cancer diagnosis and therapeutics, surpassing other domains.

2. Materials and Methods

This study was conducted on 150 patients diagnosed with ovarian cancer by independent pathologists at Shiraz Namazi Hospital, and 200 controls who had no prior history of cancer. The selection of patients was based on the criteria of matching age, ethnicity, and genetic background between the two groups. The study received permission from the Regional Ethics Committee of Islamic Azad University, Arsanjan Branch (ethical code: IR.IAU.A.REC.1401.019). Before the commencement of the trial, all participants were required to submit written informed consent. The inclusion criteria for the control group were healthy women age and ethnicity matched, without a family history of malignancies, with the same geographical region participating in the routine evaluation.

DNA extraction and Tetra–ARMS PCR

The DNA extraction kit (Favorgen, Taiwan) was utilized to acquire DNA from the entire blood samples of the participants. In this study, the determination of DNA purity was conducted using spectrophotometry with an Eppendorf Biophotometer manufactured in Germany. The genomic sequences of lncRNA H19 were acquired from the NCBI website (http://www.ncbi.nlm.nih.gov). The design of all primers tailored explicitly for tetra-primer ARMS-PCR. The tetra-primer ARMS-PCR procedures are capable of precisely and reliably genotyping single-nucleotide polymorphisms, and they are user-friendly. The method entails conducting a polymerase chain reaction (PCR) in a vial containing two sets of primers, followed by electrophoresis on an agarose gel **(13-15)**.

The locations of the SNPs and tetra-primer ARMS-PCR primers are presented in Table 1. The 20-µL polymerase chain reaction (PCR) procedures consisted of genomic DNA (50 ng), 0.5 µM of each primer, 1 U of DNA polymerase Taq, 250 µM of dNTPs, and 1.5 mM of MgCl2. The cycling conditions of the polymerase chain reaction (PCR) involved an initial denaturation step lasting 5 minutes at a temperature of 95 °C. This was followed by 30 cycles of 30 seconds at 95 °C for rs217727 and rs2839698. Annealing was performed at 60 °C for rs217727 and 61.5 °C for rs2839698. Finally, an extension step was carried out at a temperature of 72 °C for 30 seconds, with a final extension step of 72 °C for 5 minutes. The PCR results were verified using 2% agarose gel. Approximately, 20% of the random samples were subjected to sequencing in order to ensure the quality of genotyping. No case of genotyping errors was detected.

Statistical analysis

The mean ages are typically represented as the mean ± standard deviation (SD). The odds ratio (OR) and its accompanying 95% confidence interval (CI) were utilized to demonstrate the correlation between the risk of ovarian cancer and H19 polymorphisms. The frequencies of H19 haplotypes in patients with ovarian cancer and patients with linkage disequilibrium (LD) were determined using the SHEsis algorithm (http://analysis.biox.cn/myAnalysis.php). LD was obtained by calculating the squared correlation (r2) and D'. The value of D' was determined by dividing the unstandardized value of D by its maximum or minimum value. All analyses were conducted with a significance level of $p<0.05$.

Table 1. The primers used for detection of rs217727 and rs2839698 polymorphisms in lncRNA H19.

SNP	Primer name	Primer Sequence 5'-3'	Length	PCR product size
rs217727	FI	ACATCTTCATCGCCACCCCCTGCTGT	26	205 bp (T allele)
	RI	TGTGATGGCTGGTGGTCAACCGTTCG	26	248 bp (C allele)
	FO	GACTAAGGAATCGGCTCTGGAAGGTGAG	28	399 bp
	RO	GATGGAGGAAACAGAGTCGTGGAGGCTT	28	399 bp
rs2839698	FI	CTGATGTCAGTGAGGAGTGTGGAGTATGC	29	187 bp (C allele)
	RI	GCCCTGTCTACACGATGCCTGGACA	25	273 bp (T allele)
	FO	GAAAAAGACCTGGCTAGGACCGAGGAG	27	404 bp
	RO	ATCAAACCCTGCCCACCAGCTCCCCTC	27	404 bp

3. Results

Demographic characteristics of the studied population

A total of 200 healthy women and 150 patients diagnosed with ovarian cancer were included in this research. The mean age of the participants was 48.76 ± 13.10 years for women diagnosed with cancer (ages ranged from 19 to 100 years) and 48.37 ± 13.98 years for healthy women without cancer (ages ranged from 19 to 85 years). Table 2 provides a comprehensive overview of the demographic features of the participants involved in the study. There was no significant difference between the case and control groups regarding to age, age at Menarche and age at menopause. The BMI was markedly lower in women with ovarian cancer (P<0.001).

Genotyping of lncRNA H19 rs217727and rs2839698 polymorphisms

The tetra-primer-ARMS-PCR method was employed to genotype the lncRNA H19 rs217727 and rs2839698. The analysis of the genotypes of the rs217727 polymorphism revealed that the patient group exhibited a frequency of 78 (52.00%) for the CC genotype, 11 (7.33%) for the TT genotype, 61 (40.66%) for the CT genotype, and in the control group, 90 (45.00%) for the CC genotype, 9 (4.50%) for the TT genotype, and 101 (50.50%) for the CT genotype. About rs2839698, the case group exhibited frequencies of 41 (27.33%), 42 (28.00%), and 67 (44.66%) for the CC, TT, and CT genotypes, respectively. In the control group, the frequencies were 34 (17.00%), 43 (21.50%), and 123 (61.50%) for the CC, TT, and CT genotypes, respectively.

rs217727 and rs2839698 variants and the risk of ovarian cancer

The potential correlation between rs217727 and rs2839698 and the risk of ovarian cancer was evaluated by employing four separate genetic models, including codominant, dominant, recessive, and over-dominant. The multivariate logistic regression analysis conducted on the rs217727 instance indicated that there was no significant association between any of the genotypes and the risk of cancer. Regarding rs2839698, the multivariate logistic regression analysis showed that patients with CT genotype in the co-dominant model had a considerably lower risk of ovarian cancer, with a decrease of 0.45 times compared to those with CC genotype (OR = 0.45, 95% CI = 0.26-0.78, P = 0.003). In the dominant model, the risk of ovarian cancer decreased by a factor of 0.54 in patients with CT+TT genotypes compared to those with CC genotypes (OR = 2.14, 95% CI = 1.27-3.6, P = 0.01). Similarly, in the over-dominant model, the risk decreased by 0.50 in patients with CT genotype compared to those with CC+TT genotypes (OR = 0.50, 95% CI = 0.32-0.77, P = 0.001) (Table 3).

Distribution of haplotypes among case and control groups

The frequency distribution of haplotypes is presented in Table 4. None of the haplotypes (rs217727 and rs2839698) in the various haplotype models were shown to be linked to the risk of ovarian cancer. The association disequilibrium patterns of the two H19 single nucleotide polymorphisms (rs217727 and rs2839698) were examined by the computation of D' and r2 values. The coefficient of determination (D′) was 0.343, and r2 was 0.046 in the ovarian cancer group, while in the control group, D′ was 0.121 and r2 was 0.007. The total population values were D′=0.235 and r2=0.024.

Table 2. The demographic and clinical characteristics of women with ovarian cancer and controls

Characteristics	Patients Mean± SD	Controls Mean± SD	P-value
Age, year	48.76±13.10	48.37±13.98	0.77
Age at Menarche	12.62±1.48	12.42±1.37	0.16
Age at Menopause	48.42±4.46	49.26±4.47	0.07
BMI (kg/m^2)	25.45±4.94	27.89±4.63	<0.001
Tumor Type	**Number (%)**		
Serous carcinoma	100 (66.66%)		
Sex cord tumor	8 (5.33%)		
Mucinous carcinoma	22 (14.66%)		

Characteristics	Patients Mean± SD	Controls Mean± SD	P-value
Squamous cell carcinoma	4 (2.66%)		
Endometroid carcinoma	5 (3.33%)		
Borderline serous adenocarcinoma	9 (6.00%)		
Germ cell tumor	1 (0.66 %)		
Adenocarcinoma of mulerian origin	1 (0.66%)		

Table 3. Associations between rs217727 and rs2839698 SNPs and risk of ovarian cancer by Logistic regression analysis

SNP	Genotype	Case (%)	Control (%)	OR (95% CI)	P-value
rs217727 Co-dominant	CC	78 (52.00%)	90 (45.00%)	1	
	TT	11 (7.33%)	9 (4.50%)	1.40 (0.54-3.69)	0.46
	CT	61 (40.66%)	101 (50.50%)	0.69 (0.44-1.08)	0.10
Dominant	CC	78 (52.00%)	90 (45.00%)	1	
	CT+TT	72 (48.00%)	110 (55.00%)	0.75 (0.49-1.15)	0.19
Recessive	CT+CC	139 (92.66%)	191 (95.50%)	1	
	TT	11 (7.33%)	9 (4.50%)	1.67 (0.66-4.30)	0.25
Over-dominant	CC+TT	89 (59.33%)	99 (49.50%)	1	
	CT	61 (40.66%)	101 (50.50%)	0.67 (0.43-1.03)	0.06
	T allele	83 (27.66%)	119 (29.75%)	1.10 (0.79-1.54)	0.54
	C allele	217 (72.33%)	281 (70.25%)		
rs2839698 Co-dominant	CC	41 (27.33%)	34 (17.00%)	1	
	TT	42 (28.00%)	43 (21.50%)	0.81 (0.43-1.51)	0.50
	CT	67 (44.66%)	123 (61.50%)	0.45 (0.26-0.78)	0.003
Dominant	CC	41 (27.33%)	34 (17.00%)	1	
	CT+TT	109 (72.66%)	166 (83.00%)	0.54 (0.32-0.91)	0.01
Recessive	CT+CC	108 (72.00%)	157 (78.50%)	1	
	TT	42 (28.00%)	43 (21.50%)	1.41 (0.86-2.32)	0.16
Over-dominant	CC+TT	83(55.33%)	77 (38.50%)	1	
	CT	67 (44.66%)	123 (61.50%)	0.50 (0.32-0.77)	0.001
	C allele	149 (49.66%)	191 (47.75%)	1	
	T allele	151 (50.33%)	209 (52.25%)	0.92 (0.68-1.25)	0.61

Table 4. Analysis for the Haplotype frequencies of *H19* between ovarian cancer cases and controls

Haplotype		Case (frequency)	Control (frequency)	χ^2	p-value	OR (95% CI)
rs217727	rs2839698					
C	C	0.311	0.317	0.022	0.883	0.976 (0.707-1.348)
C	T	0.412	0.386	0.486	0.485	1.115 (0.821-1.513)
T	C	0.185	0.161	0.718	0.396	1.186 (0.799-1.760)
T	T	0.091	0.137	3.386	0.065	0.636 (0.391-1.033)

4. Discussion

The purpose of the present study was to evaluate the impact of two genetic polymorphisms (rs217727 and rs2839698) in the lncRNA-H19 gene on the risk of ovarian cancer. This study is the inaugural investigation into the association between H19 polymorphisms and the susceptibility to ovarian cancer within the Iranian population. There was no association between H19 rs217727 polymorphism and ovarian cancer. However, a noteworthy correlation was identified between the H19 rs2839698 polymorphism and the risk of ovarian cancer in the Co-dominant, Dominant, and Over-dominant models.

The findings indicate that lncRNAs are a sort of RNA subtype that plays a role in various biological pathways, including cell proliferation and the advancement of cancer cells **(16)**. While the detailed characterization of lncRNAs is lacking, it is evident that they play a crucial role in regulating gene expression during developmental and physiological processes. In addition to its well-documented involvement in facilitating cellular development, proliferation, invasion, migration, epithelial-mesenchymal transition (EMT), and metastasis, new findings have revealed that H19 also has inhibitory effects on tumor cell apoptosis. H19 exhibits increased expression in various cancer types and could be regarded as a potential biomarker for the diagnosis, treatment, and risk of the cancer **(17)**. Two studies have been conducted to evaluate the impact of nucleotide alterations in the H19 gene on susceptibility to ovarian cancer **(18)**. The findings regarding the association between SNPs and cancer risk have been inconclusive and contradictory **(10, 18)**. In a study conducted by Song, Ramus **(19)**, the individuals carrying the minor allele of rs2107425 had a reduced susceptibility to both overall ovarian cancer and serous-type ovarian cancer. Zhang et al. assessed the correlation between four SNPs (rs2525885, rs2839698, rs3741206, rs3741219) and the risk of ovarian cancer **(20)**. The genotype TC+CC was found to be significantly related to a decrease in the risk of ovarian cancer, with the TT genotype serving as the reference. Conversely, in the recessive model, the genotype AA was associated with a 59% decrease in ovarian cancer risk. Also, they found that, the allele C of rs2525885 and allele A of rs2839698 was associated with elevated ovarian cancer genetic susceptibility under both dominant and recessive models **(20)**. Consistent with our results, the genotype frequencies of the H19 gene in the present study indicated a substantial association between rs2839698 and susceptibility to ovarian cancer in the Iranian population. A significant reduction in the risk of ovarian cancer was observed in individuals with the genotype CT in both co-dominant and over-dominant genetic models, as well as those with the genotype CT+TT in a dominant genetic model. The present study is an initial observational survey that provides novel insights into the impact of the H19 (rs2839698) gene variant on the development of ovarian cancer in Iranian patients. Other studies have been conducted to evaluate the correlations between the rs217727 polymorphism and the susceptibility to various malignancies **(10, 21)**. No correlation was found between H19 rs217727 and ovarian cancer.

The current investigation was subject to various constraints about its analytical capability, sample composition, and many failure factors associated with specific variables. It is essential to acknowledge that integrating H19 variants in gene expression change and activity is not feasible. Therefore, further studies are necessary to confirm if H19 polymorphisms have an impact on the carcinogenesis of ovarian cancer. Using PCR-based approaches significantly reduces the cost and time needed to genotype a large number of tests to identify genetic indicators associated with the risk of ovarian cancer. Nevertheless, compared to alternative methods like Sanger sequencing or Next-generation sequencing (NGS) technologies employed in the present study, the genotyping accuracy is comparatively lower. This poses an issue that needs to be addressed. However, based on our theory, identifying H19 polymorphism in regular diagnostic and prognostic tests might be viewed not only as complementary biomarkers but also as independent tests in personalized medicine. This implies that every ovarian cancer patient can be monitored according to their H19 SNP profile.

5. Conclusion

The findings of the present study indicate a significant correlation between the rs2839698 variant, which may have significant implications for the development of ovarian cancer. Consequently, these

variations could be regarded as potential predictive biomarkers for individuals with ovarian cancer.

6. Declarations

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Ethical Considerations

The study was approved by the Regional Ethics Committee of Islamic Azad University, Arsanjan Branch (ethical code: IR.IAU.A.REC.1401.019).

Authors' Contributions

M.J.M. designed this study. M.J.M. wrote the main manuscript text. F.S. and M.F. collected all the clinical data. M.J.M. and F.S. performed the statistical analysis. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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