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ASSOCIATION OF FAS-670 GENE POLYMORPHISM WITH RISK OF OVARIAN CANCER IN NORTH INDIAN POPULATION

Gynecology

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ABSTRACT

Ovarian cancer is the second most common gynaecological cancer. Apoptosis is a normal component of the development and health of multicellular organisms. Abnormal regulation of apoptosis is probably to contribute to pathogenesis of cancer. Fas, is a transmembrane receptor that plays a central role in apoptotic signaling. SNPs at -670 of Fas gene alters the function or structure of the encoded proteins, and is sufficient for its decreased expression and reducing the cell apoptosis leading to malignant transformation.

METHODS: A single-nucleotide polymorphism at -670 of Fas gene promoter (A/G) was examined in a total of 75 blood samples from (25 normal healthy and 50 ovarian cancer), using PCR-RFLP technique.

RESULTS: There was no significant difference found in the genotype prevalence between control and ovarian tumor patients. The Fas -670 GG genotype was not associated with an increased risk for the development of ovarian tumor (OR=1.0108 95% CI=0.274-3.7201) compared with the AA genotype.

KEYWORDS

Apoptosis, Fas, Single-nucleotide Polymorphism.

Introduction

Ovarian cancer is a disease in which normal ovarian cells begin to grow in an uncontrolled, abnormal manner and produce tumors in one or both ovaries.

Ovarian cancer is the sixth most common cancer and the second most common gynaecological cancer and the most common cause of death in malignancy among women as most ovarian carcinoma are diagnosed in late stage of disease.

Most ovarian cancers have several acquired gene mutations. It is now well established that ovarian follicles undergo atresia via apoptosis.

Apoptosis, or programmed cell death, is a normal component of the development and health of multicellular organisms. Cells die in response to a variety of stimuli and during apoptosis they do so in a controlled, regulated fashion. Apoptosis is a physiologic process for the elimination of specific types of cells, occurring extensively in embryonic development, metamorphosis, and differentiation and plays an important role in maintaining homeostasis, Abnormal regulation of apoptosis is probably to contribute to pathogenesis of cancer[4].

Fas, also known as CD95 or APO-1, is a transmembrane receptor that plays a central role in apoptotic signaling in many cell types[1]. The Fas receptor binds the Fas ligand (FasL), a transmembrane protein part of the TNF family[2]. And initiate the death signal cascade, which results in apoptotic cell death[1,3].

Accumulating evidence has showed that decreased expression of FAS favour malignant progression by reducing the tumor cell apoptosis.

FAS gene is highly polymorphic. In the promoter region of the FAS gene, however, the most extensively studied single nucleotide polymorphism is A to G substitution at position−670[5]. SNPs in the coding regions of Fas genes that alter the function or structure of the encoded proteins is sufficient for its decreased expression[6] and reducing the cell apoptosis leading to malignant transformation.

Recent studies have demonstrated that the A/G SNP at Fas promoter -670 is closely associated with the pathogenesis of autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus[7,8].

However, the correlation between this SNP and cancer susceptibility including the risk of gynaecological malignancies has not been extensively studied, and previous experimental results are controversial.

In this study 50 ovarian tumor patients and 25 control were analysed for SNP of A / G substitution at the position -670 of FAS gene to reevaluate the possible association between this SNP and the risk of ovarian cancer in a north indian population.

MATERIALS AND METHODS-

BLOOD SAMPLE-

We conducted genotype analysis of Fas gene promoter -670 using blood samples from 50 ovarian cancer patients, attending our out patient department and admitted for laparotomy and 25 normal healthy women. All patients were of north Indian population. This study was approved by our institutional review board, and all samples were obtained with consent.

DNA EXTRACTION-

Genomic DNA was extracted from peripheral blood leukocytes. 5 ml blood was mixed with 0.85% NaCl and centrifuged at 7000 RPM for 7 min. at 25° celcius. 15 ml of solution A(Sucrose, $MgCl_2$, Tritron X and dH_2O) was added to pellet and again centrifuged, and pellet was resuspended in 2 ml solution B (Tris-cl, EDTA, NaCl, SDS, MQ H_2O). 0.5 ml Solution C (Sodium perchlorate, MQ H_2O)was then added followed by addition of 2 ml ice chilled chloroform, mixture was then centrifugated at 7000 RPM for 7 minutes at 25°celcius. The aqueous layer then was carefully taken out in separate test tube and approximately equal amount of isopropanol is added to precipitate out the DNA. It was then washed with 70% alcohol and then followed by centrifugation at 10000 RPM at 4°celcius for 10 minutes. Finally DNA was dissolved in TE according to the amount of DNA precipated.

POLYMORPHISM ANALYSIS OF THE −670 (A/G) FAS GENE-

Polymorphism chain reaction followed by digestion with restriction enzyme MvaI was used to detect the A to G transition polymorphism at position −670 of FAS gene. Two sequence-specific oligonucleotide primers were used for the polymerase chain reaction (PCR): the forward primer(5′-CTACCTAAGAGCTATCTACCGTTC-3′) was used in combination with the reverse primer (5′-GGCTGTCCATGTTGTGGCTGC-3′). PCR was performed by using 100 ng genomic DNA as template,(figure 1) 200 µM dNTPs, 1X reaction buffer, 5pM/µl of each primer and 0.5 U of Taq DNA polymerase, in a total 20 µL reaction volume.

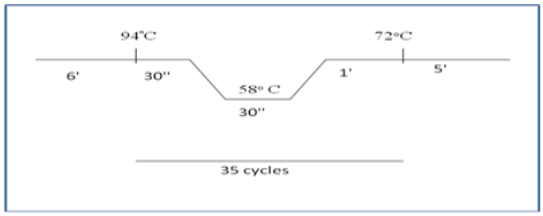

Figure 1: PCR condition for FAS -670 A/G polymorphism

The 331-bp PCR products were digested with restriction enzyme MvaI and analysed by agarose-gel electrophoresis.

RESULTS-

After digestion with MvaI enzyme, two fragments, 233 and 98 bp, were produced if A nucleotide was present at -670 position. In the presence of G substitution at this position, the resultant 233-bp fragment was further cleaved into two fragments, 189 and 44 bp.

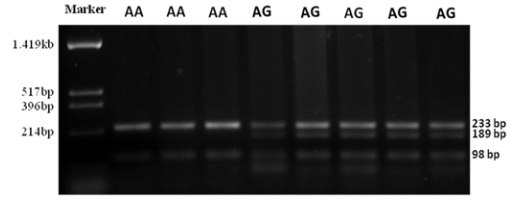

Figure 2: A-670G SNP mutation detection of FAS gene by PCR-RFLP.

Table 1:- Genotypic frequencies of Fas promoter-2670 in control subjects and ovarian tumor patients-

Sample	AA	AG	GG
Control	7 (28%)	12 (48%)	6 (24%)
Ovarian tumor cases	15 (30%)	22 (44%)	13 (26%)

χ^2=0.108 p=0.947.

Table 2: Risk of ovarian cancer associated with Fas promoter -670 genotypes

Genotype	Cases (n=50)		Control(n=25)		OR (95%CI)
	No.	%	No.	%	
AA	15	30	7	28	1.00 (referent)
AG	22	44	12	48	0.858 (0.795-2.638)
GG	13	26	6	24	1.0108 (0.274-3.7201)
Total	50	100	25	100	

Table 1, shows Genotype frequencies for FAS gene in the control and ovarian cancer patients, The homozygous AA and GG genotypes were observed in 28% and 24%, respectively, of the normal population, whereas the heterozygous AG genotypes were observed in 48%.whereas in ovarian tumor cases the homozygous AA and GG genotypes were observed in 30% and 26%, respectively, and heterozygous AG genotype was observed in 44% cases. There was no significant difference found in the genotype prevalence between control subjects and ovarian tumor patients. As shown in Table 2, the Fas -670 GG genotype was not associated with an increased risk for the development of ovarian tumor (OR=1.0108 95% CI=0.274-3.7201) compared with the AA genotype.

DISCUSSION-

The FAS receptor-ligand system is a key regulator of apoptotic cell death. Several previous studies addressed the association of this SNP with autoimmune diseases[5,7,8,9]. Lai et al[10]. conducted Fas promoter -670 polymorphism analysis and reported that the frequency of AA genotype and A allele increased in accordance with the multistep carcinogenesis from cervical intraepithelial lesion to invasive squamous cell cancer. Tamandani, DMK et al[11]have found Association of Fas-670 gene polymorphism with risk of cervical cancer in North Indian population. but the correlation between this SNP and cancer susceptibility including the risk of gynaecological malignancies specially with ovarian cancer has not been extensively studied. H Das et al[12] study results indicated that down-regulation of Fas expression is a common abnormality in gynaecological cancers including ovarian cancer. M. UEDA et al[13] analysed SNP at -670 of Fas gene promoter (A/G) in a total of 354 blood samples from normal healthy women and gynecological cancer patients. And found that Fas -670 GG genotype was associated with an increased risk for the development of cervical cancer but no significant difference in the genotype or allele prevalence between control subjects and ovarian cancer patients, in Japanese population. Here in our current study we found that the frequency of GG genotype was not found statistically higher in cases in correspond to control group (^{2}=0.108 p=0.947) and demonstrated that the germ-line polymorphism of Fas gene promoter -670 is not associated with the risk of ovarian cancer with OR-1.0108 (CI-0.274-3.7201) in north Indian population.

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