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HLA-G5 and G7 Isoforms in Pregnant Women

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ABSTRACT

Human leukocyte antigen-G which is an immune tolerance effecter molecule has an important role in the maintenance of fetus during pregnancy. Abortion is one of the complications of pregnancy period. In this research, we have studied levels of HLA-G 5, HLA-G7 isoforms in the abortion-threatened pregnant women in comparison with controls.

In a case-control study, 101 abortion-threatened women and 101 healthy pregnant women (healthy controls) with age range 21-32 years were studied. Gene expression of HLA-5 and HLA-7 isoforms was analyzed by real-time polymerase chain reaction after mRNA extraction and cDNA synthesis.

The results indicated that HLA-G5 was significantly lower in abortion-threatened women in comparison with the control group whereas HLA-G7 was not significantly differentbetween the 2 groups.

HLA-G is a vital molecule during pregnancy that can be a key factor in prevention of abortion. It is concluded that determination of HLA-G5 can be of value in pregnancy.

Keywords: Abortion; HLA-G; Pregnancy

INTRODUCTION

Abortion is an important complication during pregnancy. Annually, 20% of pregnancies fail because of unknown factors.[1,2]

During pregnancy, immune system plays a key role in maintenance of fetus. Human leukocyte antigen-G (HLA-G) has 7 isoforms including: HLA-G1, G2, G3, G4, G5, G6, and G7.[3] HLA-G1, G2, G3 and G4 are surface molecules and HLA-G5, G6, G7 are soluble molecules.[4] It has been demonstrated that HLA-G increases in cancer, transplantation, and infectious diseases.[5] This molecule has an interesting role in pregnancy.[6] HLA-G is expressed on the trophoblastic cells in placenta and reacts with inhibitory receptors on natural killer cells (NK) and T cells.[7] Therefore, it prevents maternal uterine natural killer cell attack against fetus. Up to now, many studies reported the role of HLA-G molecules in some diseases.[4,8,9] It has been previously reported that HLA-G was up-regulated after Interferon Beta(IFN- β) therapy and prevented unwanted

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reaction in diabetes type 1.[10] In addition, HLA-G is an important molecule in immune tolerance between mother and fetus which has a basic role to preventfetal loss. Furthermore, it has a key role in the blastocysts development and induction of ovum replacement.[11] The role of HLA-G different isoforms is less known in pregnancy.

Thus, we evaluated HLA-G5 and G7 isoforms in abortion-threatened women in comparison with healthy pregnant women.

MATERIALS AND METHODS

In a case-control study, 101 abortion-threaened women and 101 healthy pregnant women (control) with age range of 21-32 years were included**.** Informed consent was obtained and the study was approved by Mazandaran University Ethical Committee. The participants referred to obstetrics clinic in Mazandaran province of Iran before twenty weeks of pregnancy (Table1)**.**

Isolation of Human Peripheral Blood Mononuclear Cells (PBMCs)

5 ml peripheral blood after dilution was added gently to 2.5 ml Ficoll histopaque 1.077 (Lymphoprep, Norway) solution in a falcon tube and centrifuged at 3000 rpm for 20 minutes. A ring including mononuclear cells collected and was washed by RPMI-1640 medium (Sigma, USA) and centrifuged at 3000 rpm for 5 minutes. Then, cell counting was done with a hemocytometer and trypan blue exclusion test was determined for cell viability.

Total RNA Isolation, mRNA Extraction and cDNA Synthesis

Gene expression of HLA-G5 and -G7 isoforms was analyzed by Real time polymerase chain reaction (RT-PCR) after mRNA extraction and cDNA production from PBMCs.

Total RNA extraction was performed using standard kit in accordance to manufacturer's protocol (Bioneer, South Korea). A DNase treatment was included in the procedure for elimination of any genomic DNA (Qiagen, Hilden, Germany).The cDNA production kit (Fermentas Germany) was used for cDNA synthesis.

RT-PCR

Quantitative RT-PCR was performed using specific primers for HLA-G5 and -G7 and hypoxanthine-guanine phosphoribosyl transferase (was chosen as a reference gene) genes (Table 2) with the QuantiFast SYBR Green PCR Master Mix.

Quantitative RT-PCR reactions were performed in a 20 µL volume containing 1× QuantiTect SYBR Green PCR master mix (Qiagen, Hilden, Germany), 1 µM of forward and reverse primers and 1 µL of first strand cDNA according to manufacturer's procedure. After an

Table 1. Demographic characteristics and some clinical parameters of case and control groups

	Abortion-threatened women (No=101)	Healthy pregnant women (No=101)	*P* value
Age (y) (mean±SD)	25.43±3.23	24.01±2.43	0.373
Recurrent abortion (mean±SD)	1.4±0.57	-	-
Weight (mean±SD)	66.80±6.50	66.02±7.66	0.799
Blood sugar (mean±SD)	87.73±8.3	88.5±9.04	0.124
Hemoglobin (mean±SD)	11.87±0.6	12.07±0.6	0.667
Hematocrit (mean±SD)	36.33±1.97	35.7 ± 1.810	0.412
Blood urea nitrogen (mean±SD)	25.43±3.23	24.01±2.43	0.373
Creatinin (mean±SD)	0.75±0.23	0.68±0.14	0.657
T4	7.32±2.52	8.09±2.43	0.257
TSH (mean±SD)	2.04±1.47	2.66±1.29	0.077
VDRL	Negative	Negative	-
Hepatitie B surface antigen	Negative	Negative	-
Immunosuppressive Drug Consumption	Negative	Negative	-
Familial disorders	Negative	Negative	-

Table 2. Primer sequences used in this research

HLA	Sequence	Exon	bp
HLA-G5	5-CGGAGTATTGGGAAGAGGAGA-3	Exon3	388
	5-TGGTACCCGCGCGCTGCAG-3	Exon4-Intron4	
HLA-G7	5-GGGCTACGGAATGAAGTTCTC-3	Exon1-2	169
	5-GCACTCACCCGCCCAGGTC-3	Exon2-Intron2	
HGPRT	5-CTAATTATGGACAGGACTGAACG-3	Exon12-3	211
	5-TTGACTGGTCATTACAATAGCTC-3	Exon4-3	

Table 3. HLA-G expression in the PBMCs of abortion-threatened women (cases) in comparison with healthy pregnant women (controls) by RT-PCR. The results are mean ± SD. Statistically significant differences between groups were tested using students T test.

HLA	Fold change		*P* value
	Control Group	Case Group	
HLA-G7 (Mean±SD)	1.12±0.41	1.03±0.47	0.241
HLA-G5 (Mean±SD)	1.64±0.55	0.4±0.16	0.0001

initial 5-min at 95°C as activation step, 40 cycles consisting of denaturation at 95°C, 10 s; annealing and extension at 60°C, 30 s were performed. In each PCR run, preparation of standard curve was carried out by serial dilution of cDNA from samples. Expression was assayed by comparison with the standard curve of the specific target and reference gene in each PCR run.

Statistical Analysis

For statistical analysis, we used paired *t*-tests. The *p* values were determined in all cases and were considered to be significant at $p<0.05$). Data are presented as mean ± SD.

RESULTS

General Characteristics

In this case–control study, a total of 202 individuals were included.

HLA-G Isoforms Expression on PBMC

There was a significant decrease in HLA-G5 level in abortion-threatened pregnant women in comparison with healthy pregnant women (Table 3; $p=0.0001$). HLA-G7 expression in patients (threatened-abortion) was less than control group. In control group, HLA-G5 and G7 showed an increase in comparison with threatened-abortion women.

DISCUSSION

In the present study, we evaluated two immune-tolerance molecules (HLA-G5 and G7) in PBMC of healthy pregnant women as control in comparison with abortion-threatened women before twenty weeks of pregnancy. The studies have demonstrated that mononuclear cell-bearing HLA-G acquires tolerogenic potential to down regulate various immune activities.[6,10,12,13] Our results showed that HLA-G5 and G7 isoforms were down regulated in the abortion-threatened women (Figure1). It means that these molecules are transiently up-regulated after pregnancy. Many studies demonstrated that women's age is one of the important factors in abortion.[14,15] Sipak et al (2007) showed that the level of soluble HLA-G in some isoforms is different from the others.[16] In this research, HLA-G5 expression level was higher than HLA-G7 in healthy pregnant women and HLA-G5 level was lower than HLA-G7 in abortion-threatened women.

Thus, it is recommended that determination of HLA-G5 as a soluble isoform may be a marker in pregnancy. Probably, expression of HLA-G5 isoform is controlled by genetic factors. Furthermore, HLA-G5 may be a major isoform in tolerance between mother and fetus that its determination may be a key factor in prevention of fetal loss.

HLA-G5 in comparison with the other isoforms was up regulated in the healthy pregnant women. HLA-G5 is an inhibitory constituent and may exert a key effect

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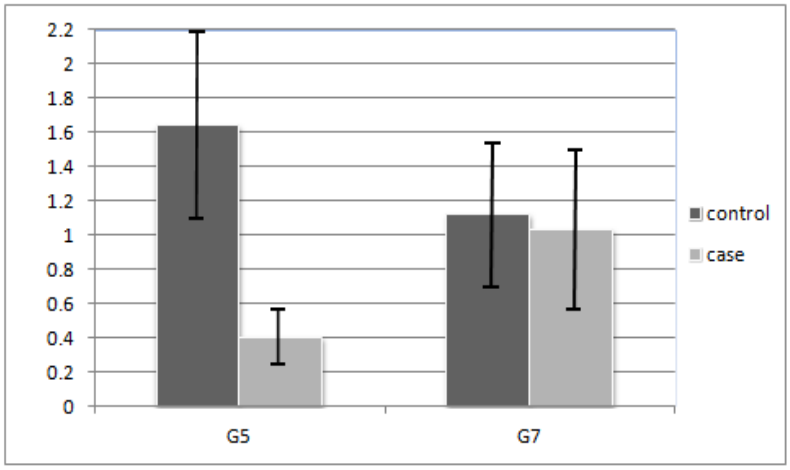

Figure1. The comparison of relative expression of two isoforms of HLA-G (HLA-G7 and G5) in abortion-threatened women and the control group.

on T cell proliferative response. Also, it is possible that the decrease of soluble HLA-G5 contribute to allogenic $CD4^+$ T cell proliferative responses in semiallogenic fetus. On the other hand, HLA-G5 may be an important molecule for stimulating of T regulatory cells in the healthy pregnant women. In addition, HLA-G has an important role in secretion of IL-10 and TGF-β by T cells.[10] Thus, one of the abortion factors in abortion-threatened women could be down-regulation of inhibitory cytokines by cell-bearing HLA-G5. It is concluded that further studies are needed on the role of HLA-G5 in pregnancy.

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