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Crescent Journal of Medical and Biological Sciences
Vol. 5, No. 3, July 2018, 194–197
eISSN 2148-9696

The Relationship of Maternal *KIR* and Parental *HLA-C* Genes With Risk of Recurrent Spontaneous Abortion: A Regional Study in Lorestan Province, Iran

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Abstract

Objectives: Natural killer cells (NKs) are one of the most important cells which play a key role in fetomaternal immune tolerance. This immune tolerance is induced by the interaction of fetal human leucocyte antigens (HLAs) and maternal killer-cell immunoglobulin-like receptors (KIRs). Hence, we intended to investigate the relationship of maternal *KIR*, parental *HLA-C*, and maternal-parental *KIR+HLA-C* with the risk of recurrent spontaneous abortion (RSA).

Materials and Methods: The present regional study in Lorestan province of Iran was conducted as a case-control study on 200 couples. Polymerase chain reaction with sequence-specific primers (PCR-SSP) was used in order to detect genes.

Results: A significant correlation was found for maternal *KIR2DS1* in combination with paternal *HLA-C2* (P=0.0089; OR=2.25). Likewise, a significant relation was found for maternal C1C2 in combination with paternal C1 or C2 (P=0.0289; OR=2.25). No significant relation was found for *KIR* genes alone.

Conclusions: Our study showed a significant relation for maternal *KIR2DS1* in combination with paternal *HLA-C2* as a risk factor in our region. Investigations on this combination for increasing the success rate of assisted reproduction, for first trimester abortions occurring after implantation and early placentation, for stillbirth groups, and for successful and unsuccessful pregnancies with malformed embryos and fetuses are suggested.

Keywords: Recurrent spontaneous abortion, KIR, HLA-C

Introduction

A healthy immune system does not normally reject the semi-allograft fetus. The immune system has 2 roles in implantation and pregnancy; impeding the formation of abnormal embryos, and protecting the fetomaternal interaction by releasing angiogenic factors, cytokines and adhesive molecules. These 2 roles are not mutually exclusive, because it can be justified through immune tolerance (1-3).

Natural killer cells (NKs) are one of the most important lymphocytes in immune tolerance. They identify self-cells through their killer-cell immunoglobulin-like receptors (KIRs) expressed on their surface. The KIRs interact with their ligands, the human leukocyte antigens (HLAs) being the identification cards of self-cells. These interactions usually result in immune tolerance in normal conditions. Both *KIR* and *HLA* have loci in human genome and are inherited as haplotypes. In addition, each gene of their loci is polymorphic. Thus interactions of different KIRs with different HLAs result in different outcomes including inhibitory and activating responses. *KIR* gene cluster is located on chromosome 19. This cluster has 2 types of genes including 8 inhibitory (*2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5*, *3DL1*, *3DL2* and *3DL3*) and 6 activating (*2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5* and *3DS1*) genes, and 2 pseudogenes. Some of these genes like *KIR2DL4* exist in all individuals (4,5). In addition, HLA has 2 classes of I and II and the class I, in turn, is divided into classical and non-classical HLAs (3,6,7).

Since involving NKs in implantation are maternal and half part of the involving HLAs of blastocyst are paternal, in the present study we attempted to evaluate maternal *KIR*, parental *HLA-C* and maternal-parental *KIR+HLA-C* interaction in both recurrent spontaneous abortion (RSA) group and healthy controls.

Materials and Methods

Subjects

For the current case-control study, 100 couples participated in each group. The criterion for the RSA group was the history of at least three times of unexplained RSA. The criteria for the healthy controls were history of

Received 8 July 2017, **Accepted** 13 December 2017, **Available online** 23 December 2017

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two successful delivery and absence of any pregnancy complications such as preeclampsia. The patients were included in the study through convenient sampling across those who had referred to Asalian hospital of obstetrics and gynecology, Khorramabad, west of Iran, for fertility consultation.

Genetic Assay

In order to extract genomic DNA, 2 mL of peripheral blood was obtained and then DNA was extracted from their leukocytes using the EXTRA GENE I kit (BAG, Lich, Germany). Polymerase chain reaction with sequence-specific primers (PCR-SSP) was used for DNA genotyping. For the presence or absence of the *KIR* genes, we used KIR TYPE kit (BAG, Lich, Germany) and for genotyping their HLA-C ligands (HLA-C1, C2), we used EPI-TOP TYPE kit (BAG, Lich, Germany). The frequencies of *HLA* and *KIR* genes were calculated through direct counting.

Statistical Analysis

The significance of associations was determined using χ^2 test with Yates' correction (or Fisher's exact test if necessary) in 2 by 2 tables for each gene. Significance level and confidence intervals (CI) were considered as 0.05 and 95%, respectively. If significant, a suitable multiple test correction would be carried out.

Results

For the maternal *KIR* genes and genotypes, no significant difference was observed between the RSA cases and the controls, although the frequency of *KIR2DS1* was higher in the RSA group (Table 1); hence no multiple test correction was needed. In addition, no significant difference was observed for maternal *HLA-C* genes (Table 2), as well as maternal *KIR+HLA-C* combinations (Table 3). No significant difference was observed for paternal *HLA-C* genes (Table 4). A significant correlation was found for maternal *KIR2DS1* in combination with paternal *HLA-C2* (48% vs. 29%, $P=0.0089$, CI = 1.27-3.974, OR = 2.25) (Table 5). After applying Bonferroni correction (n = 4 tests in Table 5), the *P* value would be <0.05 again. Moreover, a significant correlation was found for maternal "C1 and C2" in combination with paternal "C1 or C2" *HLA-C* genotypes (30% vs. 16%, $P=0.0289$, CI = 1.138-4.437, OR = 2.25) (Table 6). Of course after applying Bonferroni correction (n = 4 tests in Table 6), the relation would not be remained significant.

Discussion

In this case-control study, we found that maternal *KIR2DS1* is a risk factor for RSA just in combination with paternal *HLA-C2*. This finding was similar to some previous studies, while it disagreed with some other studies.

This objective in reproductive immunology goes back to 2004. Witt et al found no significant association for

Table 1. Distribution of Maternal *KIR* Genes and Genotypes in the RSA and Control Groups

Maternal KIR Genes and Genotypes	Couples With Recurrent Miscarriage (n=100) No. (%)	Healthy Couples (n=100) No. (%)
KIR genes		
Inhibitory		
2DL1	93	95
2DL2/3	100	100
2DL4	100	100
2DL5	58	60
3DL1	93	95
3DL2	100	100
3DL3	100	100
Activating		
2DS1	49	40
2DS2	59	54
2DS3	38	34
2DS4	95	95
2DS5	35	34
3DS1	41	40
Pseudogenes		
2DP1	98	98
3DP1	100	100
KIR genotypes		
AA	27	30
Bx	73	70

Table 2. Distribution of Maternal *HLA-C* Genes in the RSA and Control Groups

Maternal HLA Ligand	Couples With Recurrent Miscarriage (n=100) No. (%)	Healthy Couples (n=100) No. (%)
C1	78	80
C2	65	74

Table 3. Distribution of Maternal *KIR+HLA* Combinations in the RSA and Control Groups

Maternal *KIR+HLA* Combinations	Couples with Recurrent Miscarriage (n=100) No. (%)	Healthy Couples (n=100) No. (%)
Inhibitory combinations		
2DL2/3+C1	78	80
2DL1+C2	64	70
Activating combinations		
2DS2+C1	45	39
2DS1+C2	33	28

Table 4. Distribution of Paternal *HLA-C* Genes in the RSA and Control Groups

Paternal HLA Ligand	Couples With Recurrent Miscarriage (n=100) No. (%)	Healthy Couples (n=100) No. (%)
C1	77	77
C2	72	73

Table 5. Distribution of Maternal *KIR*+ Paternal *HLA* Combinations in the RSA and Control Groups

Maternal *KIR*+ Paternal *HLA* Combinations	Couples with Recurrent Miscarriage (n=100) No. (%)	Healthy Couples (n=100) No. (%)
Inhibitory combinations		
2DL2/3+C1	77	77
2DL1+C2	66	65
Activating combinations		
2DS2+C1	45	42
2DS1+C2	48[a]	29

[a] Significant differences (48% vs. 29%, $P=0.009$, CI=1.27-3.974, OR=2.25).

Table 6. Distribution of Maternal and Paternal HLA-C Genotypes in the RSA and Control Groups

Maternal HLA Ligand Genotypes	Paternal HLA Ligand Genotypes	Couples With Recurrent Miscarriage (n=100) No. (%)	Healthy Couples (n=100) No. (%)
C1 or C2	C1 or C2	20	28
C1 or C2	C1 and C2	25	28
C1 and C2	C1 or C2	30[a]	16
C1 and C2	C1 and C2	25	28

[a] Significant differences (30% vs. 16%, $P=0.029$, CI=1.138-4.437, OR=2.25).

maternal *KIR* genes with the risk of RSA in a Brazilian population (8). Yamada et al evaluated different immune markers such as CD94, CD158 (the very KIR) and CD161 through flow cytometry in 20 RSA women and 15 fertile controls. They found a lower level of CD158a (the very KIR2DL1) in the RSA group (9). Varla-Leftherioti et al evaluated only *KIR2DL1*, *2DL2* and *2DL3* among the *KIR* genes in a low sample size (10). Wang et al found a risk association for *KIR2DS1* in a Chinese population. They evaluated *HLA-C* in the couples like what we did (11). Contrary to our results and those of some previous studies, Hiby et al found a strongly protecting association for *KIR2DS1* in a Caucasian population (12). Vargas et al found a risk association for a number of maternal activating *KIR* genes (13). Faridi et al found that RSA was more associated with activating and more protected with inhibitory *KIR* genes (14). Nowak et al found that RSA can be associated with *KIR* genotypes. Despite some other studies, they found that RSA was more frequent in the patients who had got genotypes with 6 inhibitory genes (15). Nowak et alfound that female heterozygote of *HLA-C* in combination with *AA* genotype of *KIR* (a genotype containing inhibitory genes of *KIR* plus the activating gene *2DS4*) could be a protecting factor for RSA (16). Khosravifar et alinvestigated the role of maternal *KIR* and paternal *HLA-C* in an Iranian population. They found that RSA was associated with maternal *HLA-C2* (17). Ozturk et alfound a protecting role for *AA* genotype (18).

We had some limitations in this study. The major limitation of our study was that we could not identify abortions related with genetic abnormality. Likewise, some abortions might be due to anti-phospholipid syndrome (APS). As far as we know, this point was not mentioned in the methods of any of the above-cited studies.

Conclusions

Our case-control investigation showed a significant relation for maternal *KIR2DS1* in combination with paternal *HLA-C2* as a risk factor in our region. In order to clarify this role, future researches are suggested on this combination for increasing the success rate of assisted reproduction, for first trimester abortions occurring after implantation and early placentation, for stillbirth groups, and for successful and unsuccessful pregnancies with malformed embryos and fetuses.

Conflict of Interests

The authors declare no conflict of interests.

Ethical Issues

We received informed consent from the participants. This study was approved by the Ethics Committee of Lorestan University of Medical Sciences (Ethics No. lums.rec.1394,10).

Financial Support

This study was financially supported by Lorestan University of Medical Sciences, Khorramabad, Iran.

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