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Correlation of Maternal KIR and Parental HLA-C Genes Diversity With Risk of Preeclampsia in Lorestan Province of Iran

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Abstract

Objectives: Fetomaternal immune tolerance induced by natural killer cells (NKs) is a necessary phenomenon associated with maternal killer-cell immunoglobulin-like receptors (KIRs) and fetal human leukocyte antigens (HLAs). We aimed to investigate maternal KIR, parental HLA-C, and maternal-parental KIR-HLA combination in 2 preeclampsia and control groups.

Materials and Methods: A total of 200 couples participated in this case-control study. DNA samples were assayed through polymerase chain reaction with sequence specific primers (PCR-SSP).

Results: No significant difference was observed between the cases and the controls regarding the maternal *KIR* genes and genotypes and paternal *HLA-C* genes. A significant relation was found for maternal *KIR* and paternal *HLA-C* combination. The relation was for the inhibitory combination *KIR2DL1+HLA-C2* in the preeclampsia group ($P<0.05$; odds ratio [OR] = 2.02; sensitivity = 79%). In addition, maternal *AA* genotype of *KIR* in combination with paternal *HLA-C1C2* was a risk factor ($P<00.05$; OR = 3.24; specificity = 92%).

Conclusions: The inhibitory maternal-paternal combinations *KIR2DL1+HLA-C2* and *AA+HLA-C1C2* seem to be more associated with risk of preeclampsia. Prediction of the risk of preeclampsia with the help of maternal *KIR* typing and paternal *HLA-C* typing can be possible in future.

Keywords: HLA-C, KIR, NK cells, Preeclampsia

Introduction

Preeclampsia is a pregnancy specific syndrome occurring in 3%-14% of all pregnancies worldwide based on recent reports (1). There are a variety of approaches to its pathogenesis. Among these approaches, immune system and its involved molecules are notable (2). The fascinating feature of immune system is that it does not normally reject the semi-allograft fetus. Two roles can be considered for immune system in implantation and pregnancy; the first one is impeding the formation of abnormal embryos, and the second one is maintenance of the fetomaternal interaction through immune tolerance signals.

Natural killer-cells (NKs) are the most important cells in the immune tolerance. The NKs identify self-cells through their killer-cell immunoglobulin-like receptors (KIRs) expressed on their surface. These KIRs interact with the human leukocyte antigens (HLAs) expressed on surface of nuclear self-cells. KIR has 8 inhibitory (*2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5*, *3DL1*, *3DL2* and *3DL3*) and 6 activating (*2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5* and *3DS1*) genes. In human genome, both *HLA* and *KIR* have loci (not locus) and therefore they are inherited as haplotypes. As well, each gene of their loci can be polymorphic. Hence interactions of different KIRs with different HLAs lead to different outcomes. Therefore from an anthropological point of view, people of ethnicities have different KIR-HLA interactions (3-7). There are 2 classes of HLA, I and II, and class I can be classical or non-classical. HLA-G is a non-classical HLA expressed on the semi-allograft embryonic cells. This HLA interacts with KIR2DL4 molecules and triggers the immune tolerance (8-13). NKs might have (or not) the marker CD16 which is a weapon for antibody-dependent cell-mediated cytotoxicity (ADCC). Usually $CD56^{dim}$ NKs are $CD16^{+}$; therefor $CD16^{+}CD56^{dim}$ NKs are called cytotoxic NKs. On the other hand, $CD16^{-}CD56^{bright}$ NKs are called immune-regulatory NKs (2, 14-16). About 90% of uterine NKs (UNKs) are immune-regulatory. Hence UNKs are not usually cytotoxic for embryo (2, 15).

The fascinating point is that how the immune system is both killing and protective. In other words, this system is a bodyguard to protect the self and kill the non-self. Pregnancy is a semi-allograft transplantation. Thus there

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is a question that "what is the action of immune system in such conditions; protection or rejection of the graft". The best answer for the above question is immune tolerance (2, 17, 18). Of course immune tolerance is not the only reason for acceptance of this graft, because adhesive molecules like selectins (2) and integrins (19) are also important in early implantation. Such molecules and cytokines are produced by immune cells.

Since the involved NKs in placenta are maternal and on the other hand half part of the involved fetal HLAs are paternally originated, in the current study we intended to investigate maternal *KIR* genes and genotypes, maternal and paternal *HLA-C*, and maternal-paternal *KIR+HLA-C* interactions in both preeclampsia and control groups. As a hypothesis, the inhibitory interactions can be more associated with preeclampsia, because of lower activity of the NKs having a high capacity of cytokines, angiogentic and adhesive molecules.

Materials and Methods

Subjects

For the present case-control study, a number of 100 couples were included in each group (totally 400 individuals). The inclusion criterion for the case group was having history of idiopathic preeclampsia (blood pressure >140/90 mm Hg after 20 weeks of gestation and proteinuria >300 mg/24 h (20)), and the exclusion criterion was history of pregnancy complications other than preeclampsia, or history of any hormonal or genetic problems. The criteria for the control group were history of 2 successful deliveries and absence of any pregnancy complication. The patients were included in the study through convenient sampling across those who were referred to Asalian hospital of Obstetrics and Gynecology, Khorramabad, west of Iran, for fertility consult. The ethnicities of the patients were Lur (21) and Lak (22) from Lorestan province, Iran.

Genetic Assay

Genomic DNA was extracted from peripheral blood leukocytes (2 mL) using the EXTRA GENE I kit (BAG, Lich, Germany). DNA samples were genotyped using polymerase chain reaction with sequence specific primers (PCR-SSP) (23). For the presence or absence of *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). These kits have been previously controlled and evaluated by their companies and also by some researchers (24). The frequencies of *HLA* and *KIR* genes were calculated through direct counting. Assessment of other types of non-classical HLAs like HLA-G was of the limitations of our study.

Statistical Analysis

The significance of associations was determined using the χ^2 test with Yate correction and degree of freedom (*df*) = 1. Significance level and CI were considered as 0.05 and 95%, respectively. Bonferroni's correction was used for multiple comparisons. For each significant relation, the sensitivities, specificities and positive predicting values (PPVs) were calculated for medical diagnosis and prediction aims. Since the study was not cohort, conventional calculation of PPV would not be valid. Hence we used the corrected formula based on disease prevalence (25). In the case of ours, the prevalence of preeclampsia was 5% based on a meta-analysis (26). The CI of the medical diagnosis accuracy amounts were calculated through the formula $[1.96 \times \sqrt{}[P(1-P)/n]$. For CI calculation of PPV, P = 5% was considered based on the real prevalence of preeclampsia in Iran, and for CI calculation of sensitivity and specificity P = 50% was considered.

Results

Among maternal *KIR* genes, no significant difference was observed between the cases and the controls after adjusting Bonferroni correction (Table 1). In addition, there was observed no significant relationship for maternal *KIR* genotypes (Figure 1). Many of these genotypes have been previously reported in Iranian and Lur populations (21). Among maternal *HLA-C* genes and genotypes,

Table 1. Distribution of Maternal KIR Genes and Genotypes in the Couples With Preeclampsia and Healthy Couples

Maternal KIR Genes and Genotypes	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)
KIR genes		
Inhibitory		
2DL1	96*	85
2DL2	51	59
2DL3	86	77
2DL4	100	100
2DL5A	41	39
2DL5B	40	37
3DL1	95	94
3DL2	100	100
3DL3	100	100
Activating		
2DS1	40	42
2DS2	54	57
2DS3	35	34
2DS4	95	94
2DS4-full	34	34
2DS4-del	85	83
2DS5	36	36
3DS1	43	37
Pseudogenes		
2DP1	96*	85
3DP1-full	34	32
3DP1-del	96	96
KIR genotypes		
AA	32	28
Bx (AB+BB)	68	72

* Significant at *P*<0.05; however Bonferroni adjusted *P* value was not significant.

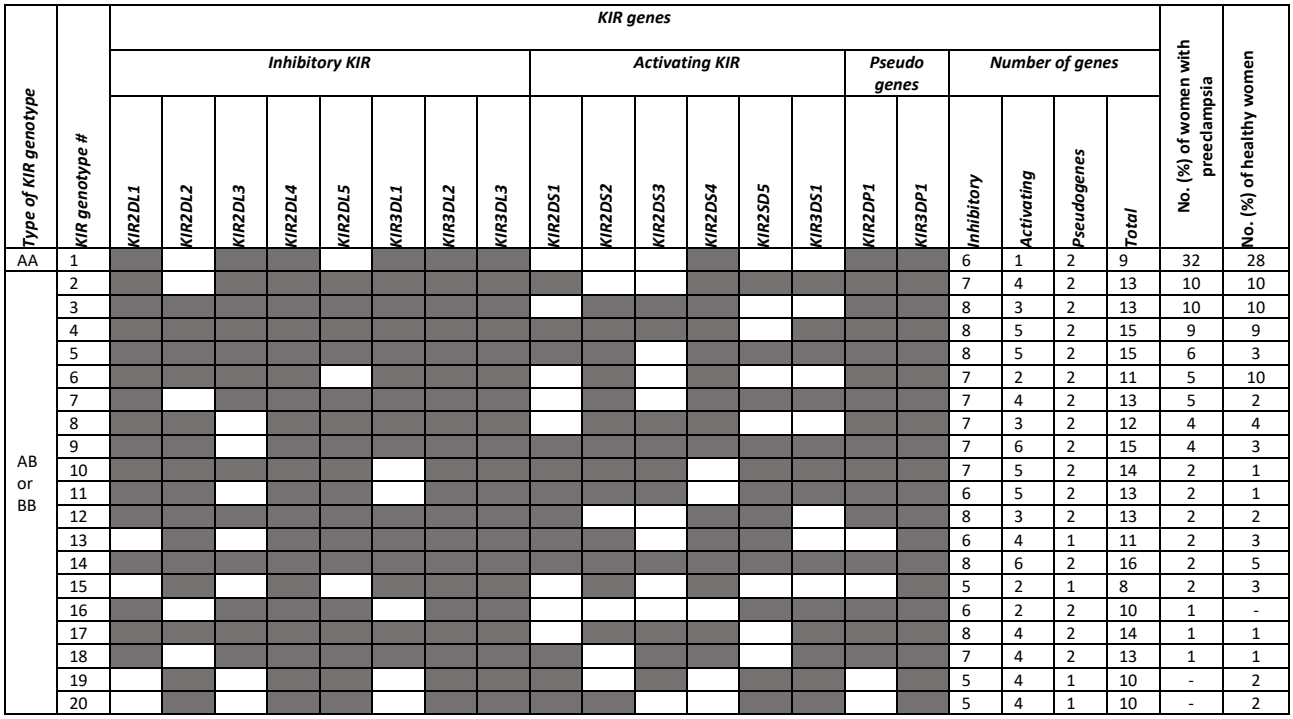

Figure 1. Distribution of *KIR* Genotypes in Women With Preeclampsia and Healthy Women. The grey rectangles indicate gene presence and the white rectangles indicate gene absence. Many of these genotypes have been previously reported in Iranian and Lur populations. No statistical relation was found.

HLA-C1 was a significant protecting factor ($P < 0.05$; odds ratio [OR] = 0.44) (Table 2). The same result was found for maternal *KIR2DL2/3+HLA-C1* combination, because all the patients had at least one of these 2 *KIRs* (Table 3). Paternal *HLA-C* genes and genotypes were not significantly different between the cases and the controls (Table 4).

As we had hypothesized, a significant relation was found for a maternal *KIR* and paternal *HLA-C* combination. The relation was for the inhibitory combination

Table 2. Distribution of Maternal HLA Ligand Genes in the Couples With Preeclampsia and Healthy Couples

Maternal HLA Ligand and Genotypes	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)
HLA Ligand genes				
C1	67	82	0.0231*	0.44 (0.23-0.86)
C2	78	68	NS[a]	NA[b]
HLA Ligand genotypes				
C1 or C2	55	50	NS	NA
C1 and C2	45	50	NS	NA

*Significant at $P<0.05$. [a] NS: non-significant. [b] NA: not applicable.

Table 3. Distribution of Maternal KIR+HLA Combinations in the Couples With Preeclampsia and Healthy Couples

Maternal HLA Ligand and Genotypes	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)
HLA Ligand genes				
2DL2/3+C1	67	82	0.0231*	0.44 (0.23-0.86)
2DL1+C2	74	64	NS[a]	NA[b]
Activating Combinations				
2DS2+C1	41	43	NS	NA
2DS1+C2	29	32	NS	NA

*Significant at $P<0.05$. [a] NS: non-significant. [b] NA: not applicable.

Table 4. Distribution of Paternal HLA Ligand Genes in the Couples With Preeclampsia and Healthy Couples

Paternal HLA Ligand and Genotypes	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)
HLA Ligand genes				
C1	74	72	NS[a]	NA[b]
C2	79	76	NS	NA
HLA Ligand genotypes				
C1 or C2	47	52	NS	NA
C1 and C2	53	48	NS	NA

[a] NS: non-significant. [b] NA: not applicable.

Table 5. Distribution of Maternal KIR + Paternal HLA Combinations in the Couples With Preeclampsia and Healthy Couples

Maternal KIR+Paternal HLA Combinations	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)
Inhibitory Combinations				
2DL2/3+C1	74	72	NS[a]	NA[b]
2DL1+C2	79	65	0.0406*	2.02 (1.07-3.81)
Activating Combinations				
2DS2+C1	42	47	NS	NA
2DS1+C2	28	34	NS	NA

*Significant at $P<0.05$. [a] NS: non-significant. [b] NA: not applicable.

KIR2DL1+HLA-C2 in the preeclampsia group ($P<0.05$; OR = 2.02) (Table 5). Another interesting finding of ours was that maternal homozygote genotypes of *HLA-C* in combination with paternal heterozygote genotype of *HLA-C* was a risk factor ($P<0.05$; OR = 2.25) (Table 6). The maternal and paternal genotypes of *HLA-C* are shown in Tables 7 and 8. No significant relation was found for maternal *KIR+HLA* combinations (Table 9). In addition, maternal *KIR AA* genotype in combination with paternal *HLA* heterozygote was a risk factor ($P<0.05$; OR = 3.24) (Table 10). Medical diagnosis accuracy components (sensitivity, specificity and PPV) are shown in Table 11.

Discussion

This case-control study aimed to investigate maternal *KIR*, and both maternal and paternal *HLA-C* genes in preeclamptic and healthy couples in order to find a sensitive and specific method for prediction of preeclampsia (cohort approach) before marriage. As we hypothesized, the inhibitory interactions could be more

Table 6. Distribution of Parental HLA Ligand Genotypes in the Couples With Preeclampsia and Healthy Couples

Maternal HLA Ligand Genotypes	Paternal HLA Ligand Genotypes	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)
C1 or C2	C1 or C2	25	34	NS	NA
C1 or C2	C1 and C2	30	16	0.0289*	2.25 (1.13-4.46)
C1 and C2	C1 or C2	22	18	NS	NA
C1 and C2	C1 and C2	23	32	NS	NA

*Significant at $P<0.05$. [a] NS: non-significant. [b] NA: not applicable.

Table 7. Distribution of Maternal HLA Ligand Genes in the Couples With Preeclampsia and Healthy Couples

Maternal HLA Ligand Genotypes	HLA-C1	HLA-C2	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)
1 (C1C1)			22	32	NS	NA
2 (C2C2)			33	18	0.0231*	2.2 (1.16-4.33)
3 (C1C2)			45	50	NS	NA

*Significant at $P<0.05$. [a] NS: non-significant. [b] NA: not applicable.

Table 8. Distribution of Paternal HLA Ligand Genes in the Couples With Preeclampsia and Healthy Couples

Paternal HLA Ligand Genotypes	HLA-C1	HLA-C2	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)
1 (C1C1)			20	31	NS	NA
2 (C2C2)			27	21	NS	NA
3 (C1C2)			53	48	NS	NA

[a] NS: non-significant. [b] NA: not applicable.

Table 9. Distribution of Maternal *KIR* and *HLA-C* Genotypes

KIR Genotype	HLA-C Genotype	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)
AA	C1 or C2	20	13	NS	NA
	C1 and C2	12	15	NS	NA
AB or BB	C1 or C2	35	37	NS	NA
	C1 and C2	33	35	NS	NA

[a] NS: non-significant. [b] NA: not applicable.

Table 10. Distribution of Maternal *KIR* With Paternal *HLA-C* Genotypes

KIR Genotype	HLA-C Genotype	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)
AA	C1 or C2	10	20	NS	NA
	C1 and C2	22	8	0.0100*	3.24 (1.36-7.69)
AB or BB	C1 or C2	37	32	NS	NA
	C1 and C2	31	40	NS	NA

*Significant at $P<0.05$. [a] NS: non-significant. [b] NA: not applicable.

associated with preeclampsia, because of lower activity of the NKs having a high capacity of cytokines, angiogentic and adhesive molecules. Our results supported the hypothesis for maternal *KIR2DL1* in combination with paternal *HLA-C2* (sensitivity = 79%) as well as maternal *AA* genotype in combination with paternal heterozygosity (specificity = 92%; PPV = 13%) (Table 11).

The first investigation was done in 2004 by Hiby et al in which maternal *KIR* and fetal *HLA-C* genes had been assayed. The authors found that the mothers lacking activating KIRs were more at risk of preeclampsia (27). Yu et al found that in Han Chinese, mothers with *KIR A* haplotype (*AA* genotype) (the haplotype consisting inhibitory KIRs) were more at risk of preeclampsia (28). Another study on Han Chinese showed that lack of activating KIRs is associated with preeclampsia (29), a condition occurring in *AA* patients. As described by Moffett et al in a commentary, risk of preeclampsia was higher if HLA-C2 of trophoblast was derived from paternal side than of the maternal (30).

All the 3 above studies had assayed maternal KIR and fetal HLA-C at the time of pregnancy, but we assayed paternal HLA-C instead of the fetal. This novelty of our work for preeclampsia enables us to predict preeclampsia before pregnancy. This approach can be used in cohort studies in future. This novelty has also been used in another study by Hiby et al for prediction of infant birth weight (31) and recurrent miscarriage (32).

Activating interactions result in activation of the UNKs having high capacity of cytokines, adhesive and angiogenic factors. These factors are necessary for implantation, arterial remodeling and placentation. Failure in these processes results in insufficient blood supply to fetus and hence preeclampsia (33). Interferon gamma (IFN-gamma) required for induction of apoptosis is necessary for implantation and early placentation (34,35) (of course, not pathologic apoptosis). As well, vascular endothelial growth factor (VEGF) is an angiogenic factor necessary for ovulation, implantation and placentation (36,37). Endometrial scratching have recently been used to induce such factors through inducing an artificial inflammation (38,39). All this evidence provides us with a multidimensional approach to pregnancy and reproduction complications. In the present study our approach was immunogenetic.

We had some limitations in our study. First, we could not recognize the number of copies of *KIR* genes via PCR-SSP; second, we could not recognize the exact subtypes of the genotypes (40); and third, lack of fetal genetic evaluations. Although this study was done as a case-control study, found sensitivities and specificities (Table 11) can be used in cohort and predictive approaches.

Table 11. Medical Diagnosis Accuracy of the Significant Findings

Significant Findings	Couples With Preeclampsia (n=100) No. (%)	Healthy Couples (n=100) No. (%)	*P* Value (Yate Correction)	OR (CI)	Medical Diagnosis Accuracy % (CI)			Accuracy
					Sensitivity	Specificity	PPV	
Maternal-2DL2/3+ Maternal-CI[a]	67	82	0.0231	0.44 (0.23-0.86)	82% (±6%)	33% (±6%)	9% (±1%)	57%
Maternal C1	67	82	0.0231	0.44 (0.23-0.86)	82% (±6%)	33% (±6%)	9% (±1%)	57%
Maternal C2C2	33	18	0.0231	2.24 (1.16-4.33)	33% (±6%)	82% (±6%)	9% (±1%)	57%
Maternal-2DL1+ Paternal-C2	79	64	0.0406	2.02 (1.07-3.81)	79% (±6%)	36% (±6%)	6% (±1%)	57%
Maternal-AA+ Paternal-C1/C2	22	8	0.0100	3.24 (1.36-7.69)	22% (±6%)	92% (±6%)	13% (±1%)	57%
Maternal-C1/C2+ Paternal-C1C2	30	16	0.0289	2.25 (1.13-4.46)	30% (±6%)	84% (±6%)	9% (±1%)	57%

[a] First and second rows show protecting effect (OR<1). Hence their sensitivities, specificities, PPVs and accuracies are based on this.

Conclusions

The inhibitory maternal-paternal combinations *KIR2DL1+HLA-C2* and *AA+HLA-C1C2* seem to be more associated with preeclampsia. The first combination was more sensitive and the second one was more specific. The main conclusion of the present study was prediction of preeclampsia with help of maternal *KIR* typing and parental *HLA-C* typing. Further studies on this hypothesis are necessary in other populations, because approving such hypotheses requires a meta-analysis.

Conflict of Interests

There is no conflict of interests. The named kits were used because of their previous validation and so there is no commercial interests.

Ethical Issues

The study was approved by the Ethics Committee of Lorestan University of Medical Sciences with registration number "lums.rec.1394,10". Written consent was obtained from all the participants.

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