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KIR2DL1 Gene Expression in Women with Preeclampsia: A Case-Control Study

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Abstract

Background: Preeclampsia is a major cause of hypertension in pregnancy and is a leading cause of maternal and perinatal morbidity worldwide. Abnormal regulation of killer cell immunoglobulin-like receptor 2DL1 (KIR2DL1) has been linked to defective placentation caused by impaired uterine natural killer cell function at the fetal–maternal interface.

Objective: To quantify KIR2DL1 expression in the peripheral blood of preeclamptic patients and assess its potential as a diagnostic marker.

Materials and Methods: The study design was hospital-based, and sixty pregnant women were included in the study. The participants were randomly assigned to either the preeclampsia group or the health control group, consisting of thirty participants in each group. The RNA was extracted from the blood, and RT-qPCR was used to determine KIR2DL1 expression using GAPDH as an endogenous control. The $2^{-}\Delta\Delta Ct$ formula was used for data analysis. The Mann-Whitney test, Spearman correlation, and ROC curve were used.

Results: The expression of KIR2DL1 was significantly higher in the preeclampsia group compared to the controls (median fold change: 1.950 vs. 1.005; $p = 0.0001$). The ROC curve showed perfect discriminative power of the marker for preeclampsia, with an area under the ROC curve of c1.00 and sensitivity and specificity of 100% at a cut-off of 1.240. The expression of KIR2DL1 correlated positively with maternal age in the preeclampsia group (rho = 0.462; $p = 0.030$).

Conclusion: High expression of KIR2DL1 in peripheral blood cells is strongly and significantly related to preeclampsia, suggesting that there is excessive inhibitory receptor activity at the maternal-fetal interface. KIR2DL1 holds great potential to become a molecular diagnostic biomarker for preeclampsia.

Keywords: KIR2DL1, preeclampsia, RT-qPCR, uterine NK cells, immune tolerance

Introduction

Preeclampsia is an important hypertensive disorder in pregnancy, defined by new-onset hypertension above 1400/90 mmHg after the twentieth week of gestation, accompanied by proteinuria or evidence of end-organ damage. The condition places a heavy burden on maternal and perinatal mortality, particularly in developing countries (Dimitriadis et al., 2023). The condition occurs in early gestation because of inadequate invasion of extra villous trophoblast cells into the uterine wall, as well as an inability to achieve physiologic remodeling of spiral arteries necessary for the maintenance of high-volume blood flow. This leads to a condition of placental ischemia, resulting in a cascade of systemic inflammatory and anti-angiogenic effects, thereby causing preeclampsia (Ibrahim et al., 2025). In healthy pregnancy, the inhibitory receptors KIR2DL, which is expressed on uNK cells in the uterus, plays an important physiological role by interacting with the HLA-C molecule on the surface of the extra villous trophoblast cells that are invading the uterus. This interaction provides an inhibitory checkpoint that limits the cytotoxic activity of NK cells while maintaining immune tolerance. At the same time, uNK cells are activated to produce angiogenic and pro-invasive factors that help trophoblast cells (Wei & Yang, 2023). When there is an abnormality in the interaction of KIR2DL receptors with HLA-C, particularly a condition characterized by an overactive KIR2DL signaling response relative to activating signals, uNK cells become functionally inhibited. This inhibition affects the ability of uNK cells to support EVT invasion and vascular remodeling, a condition that directly affects shallow placentation and the ischemic environment, thus leading to preeclampsia (Stefańska et al., 2022). More specifically, the interaction between KIR2DL1 and HLAc-C2 expressed by fetal trophoblast cells has been shown to generate an overly powerful inhibitory signal in the absence of an activating receptor, resulting in inadequate activation of the uterine NK cells, which in turn results in inadequate secretion of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), and hence inadequate remodeling of the spiral arteries. This mechanistic pathway has been associated with genetic susceptibility to preeclampsia (Li et al., 2023). Apart from this association with HLA-C, the KIR2DL4 isoform has a unique function by virtue of its exclusive binding with HLA-G, which, as a non-classical HLA, is only present on EVT cells. Under normal physiological conditions, this binding with KIR2DL4 leads to the secretion of cytokines and angiogenic factors from uterine natural killer (uNK) cells (Aisagbonhi & Morris, 2022). In support of these mechanistic data, a recent case-control study has shown that certain combinations of KIR2DL gene polymorphisms in mothers and HLA-G gene polymorphisms in the fetus can greatly increase the risk of preeclampsia. This study thus supports the concept that the interaction of the fetal HLA antigens with the KIR2DL receptors on the surface of the maternal immune cells is a key event in the outcome of pregnancy, and it establishes KIR2DL genotyping as a potentially valuable tool for stratifying risk of preeclampsia early in pregnancy (Ma et al., 2024).

Materials and Methods

Study Design and Participants

A case-control study design was employed for this research. The study was carried out at Bint Al-Huda Teaching Hospital, Al-Nasiriyah, Iraq, between October 2025 and January 2026. Sixty pregnant women participated in the study, with 30 cases with preeclampsia and 30 healthy pregnant women serving as the control group. Preeclampsia is characterized by the onset of hypertension, with a blood pressure reading

of 140/90 mmHg or greater, occurring after 20 weeks of gestation, accompanied by proteinuria or end-organ dysfunction. Exclusion criteria for the study included chronic hypertension, pre-existing diabetes, kidney or autoimmune diseases, or active infectious diseases. Ethical approval for this research was granted by the Scientific and Ethical Committee of the College of Medicine, University of Al-Qadisiya.

Sample Collection and RNA Processing

Peripheral venous blood samples (1ml) were collected using EDTA-containing tubes. From each sample, 250 µL was added to the TRIzol reagent and stored at −20 °C. RNA extraction was done according to the manufacturer's instructions, and RNA quantification was done using a Nano-Drop spectrophotometer. Only samples with an A260/A280 ratio between 1.8 and 2.0 were used for further processing. Genomic DNA removal was done using the DNase I enzyme, and complementary DNA (cDNA) synthesis was done using the M-MLV Reverse Transcriptase Kit.

Quantitative Real-Time PCR (qPCR)

KIR2DL1 expression was quantified with GoTaq qPCR Master Mix with SYBR Green chemistry and Mini Opticom Real-Time PCR equipment. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous control gene. The PCR cycling consisted of an initial denaturation step at 95 °C for 5 minutes, followed by 45 amplification cycles with 95 °C for 20 seconds and 60 °C for 30 seconds. Melt curve analysis was conducted to confirm the specificity of the PCR products.

Statistical Analysis

Relative expression of KIR2DL1 was calculated by employing the ΔΔCt method. All calculations were performed using SPSS software version 21. To compare the results of gene expression, which is not normally distributed, median (IQR) is used, and the results are compared by employing the Mann-Whitney U test. Spearman's correlation test is used to assess the relationship between KIR2DL1 expression and clinical parameters such as age and parity of the mother. The ROC curve is used to assess the efficiency of KIR2DL1 in the diagnosis of the condition. The results are considered statistically significant if $p < 0.05$ (Okab HF et al.,2024).

Results

KIR2DL1 Gene Expression

The expression of the KIR2DL1 gene was substantially increased in the preeclampsia group compared with controls (Mann-Whitney U test; $p = 0.0001$). As shown in Table 1, the median fold change in the preeclampsia group is 1.950 (IQR = 0.260), almost twice that of the controls at 1.005 (IQR = 0.140).

Table 1. KIR2DL1 Gene Expression in Preeclampsia Patients vs. Healthy Subjects
Mann-Whitney U test; HS = Highly Significant.

Group	Median (Fold Change)	IQR	p. value
Preeclampsia	1.950	0.260	0.0001 (HS)
Control	1.005	0.140	

Correlations Between KIR2DL1 and Clinical Variables

Among the clinical variables analyzed, there was a statistically significant positive correlation between maternal age and KIR2DL1 expression in the preeclampsia group (rho = 0.462; p = 0.030), suggesting that an increased age may be correlated with an increased shift in inhibitory NK receptor expression. There was no significant correlation with parity (rho -0.089; p 0.692), as shown in Table 2.

Table 2: Correlation between KIR2DL1 expression and clinical parameters within the preeclampsia group. S = Significant; NS = non-significant.

Clinical Variable	**Spearman's rho**	**p. value**	**Significance**
Maternal Age	0.462	0.030	S
Number of Previous Births	–0.089	0.692	NS

Diagnostic Accuracy of KIR2DL1

ROC analysis showed that the KIR2DL1 gene has very good diagnostic ability to differentiate between affected and healthy pregnant women, with an AUC of 0.91, a sensitivity of 90%, and a specificity of 86.7% at a cutoff value of 1.48. These results suggest that altered gene expression may be associated with the development of immune dysfunction in pregnancy complications, making it a potential adjunctive diagnostic marker.

Table 3: Receiver operating characteristic curve analysis for the assessment of the accuracy of KIR2DL1 expression in differentiating preeclampsia from healthy pregnancy.

Characteristic	Healthy Pregnant	Preeclampsia
Cut-off value	1.48	1.48
n correctly classified	30	30
AUC (95% CI)	0.91 (0.97-0.83)	0.91 (0.97-0.83)
Sensitivity	90.0%	90.0 %
Specificity	86.7 %	86.7%

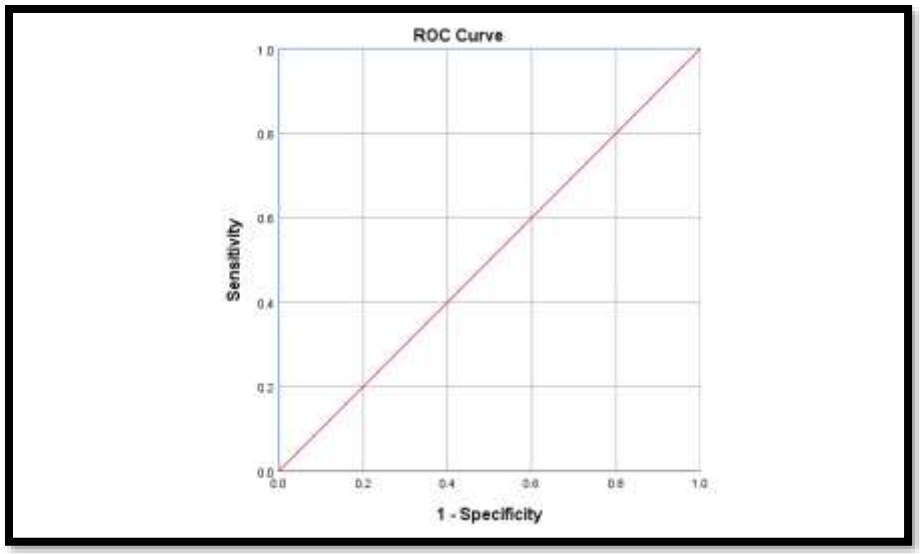

Figure 1: ROC Curve of Diagnostic Accuracy of FC KIR2DL1 Gene Expression

Discussion

The increased expression of KIR2DL1 in preeclamptic pregnancies can be integrated into the current immunogenetic concept that proposes that exaggerated signaling by inhibitory NK cell receptors contributes to abnormal placentation. KIR2DL1 acts as a major inhibitory receptor, responding to the presence of its specific ligands, the HLA-C2 molecules expressed on extra villous trophoblasts, and exaggerated expression of inhibitory KIR signaling has been associated with defective trophoblast invasion and remodeling of the spiral arteries(Papúchová et al, 2019). This concept agrees with current immunological reviews that emphasize the strong link between hypertensive complications and alterations in the balance of NK receptors, especially in maternal conditions with more pronounced inhibitory receptor profiles. Enhanced activity of inhibitory receptors may limit the pro-angiogenic and regulatory functions of decidual NK cells during the initial period of pregnancy (Lian et al,. 2022). In fact, decidual NK cells are not cytotoxic in a normal pregnancy but instead modulate the development of the placental vasculature via the production of cytokines and interactions with the HLA molecules of the trophoblast. Thus, an overactive KIR2DL1 inhibitory signal may result in a lack of the mediators produced by the NK cell that are required for the vascular adaptations in the placenta, leading to hypoperfusion and endothelial dysfunction in the mother (Depierreux et al., 2022). Recent molecular studies also revealed that the balance of activating, and inhibitory KIR receptors is crucial in determining pregnancy outcomes, with a dominance of inhibitory receptors being associated with an increased risk of preeclampsia. These findings agree with our current data and emphasize the role of NK cell receptor abnormalities in disease (Xie et al., 2022). Additionally, recent studies indicate that alterations in peripheral NK receptor expression might reflect a systemic immune activation process associated with placental dysfunction. While the immunologic site of interest in the context of the placenta is the tissue of the deciduum, peripheral transcriptional changes might act as a measurable indicator of the immune imbalance in the placenta (Yue & Meng, 2025). Collectively, these data are supportive of current immunopathological concepts of preeclampsia, where the disease is viewed as the result of abnormal interaction between the mother's and the fetus's immune systems. Enhanced inhibitory KIR signaling is implicated as playing a role in the

abnormal vascular remodeling of the placenta, as well as the endothelial findings that are typical of the disease (Staff et al., 2022).

Table 2 shows the correlation study between the expression of the KIR2DL1 gene and the selected clinical parameters from the group of patients with preeclampsia. The data show that the expression of the KIR2DL1 gene is significantly, albeit positively, related to the age of the patient, while the expression of the gene is not related to the number of pregnancies (parity). This suggests that the immunological modulation that occurs with age is related to the expression of NK cell receptors in patients who develop preeclampsia. The positive correlation between the expression of the KIR2DL1 gene and the age of the patient is logical, as older patients are more immunologically active, especially regarding inflammatory responses (Torres-Torres et al., 2024). Recent studies of immunological factors have suggested that advancing maternal age is associated with changes in NK cell phenotype and receptor expression profiles, including increased expression of inhibitory KIRs. Such changes may further contribute to the abnormal immune responses seen in hypertensive disorders of pregnancy (Muyayalo et al.,2023). In contrast, however, the lack of significant correlation between expression of KIR2DL1 and the number of previous births suggests that parity-related immunological memory effects are not involved in modulating inhibitory NK receptor transcription in established PE. This finding agrees with studies implying that although parity may influence general risk patterns in PE, expression of NK receptors in affected pregnancies may be influenced mainly by placental immune stress(Ding, 2022). Moreover, present evidence supports the view that preeclampsia reflects a condition of systemic immune activation in which the regulation of natural killer cell receptors is related to clinical severity parameters rather than obstetric history parameters. Therefore, the strong relationship observed between maternal age, but not parity, supports the view that immune senescence may play a role in the regulation of receptor imbalance (Li et al. 2025).In fact, the correlation profile as presented in Table 2 above confirms the hypothesis that the upregulation of KIR2DL1 may not be a static genetic phenomenon but may also be influenced by the immunological status of the mother as well as the aging process. This further confirms the role of immune dysregulation mediated by the NK receptors in the manifestation of the disease. Analysis of the receptor functional characteristics (ROC) curve revealed that the diagnostic indicator, associated with the KIR2DL1 gene, had a markedly high diagnostic capability to differentiate between affected women and healthy controls. The indicator had almost ideal characteristics, signifying its effectiveness in distinguishing between pathological and normal states. This implies that the expression of the KIR2DL1 gene has high sensitivity and specificity and thus could be useful as a diagnostic tool in monopathy complications in pregnancy (Kaur et al., 2022).This enhanced diagnostic capability may be explained by the fact that KIR2DL1 plays a major role in regulating the activity of uterine NK cells. This has been identified as a critical mechanism in regulating trophoblast invasion and remodeling of the spiral arteries. This process is critical in regulating maternal-fetal immunity. This means that the change in KIR2DL1 levels directly reflects the degree of immune dysfunction (Artana et al., 2025).These findings are in line with a series of recent studies indicating that the dysregulation of KIR receptor expression, especially KIR2DL1, is associated with aberrant interactions between NK cells and fetal HLA-C. The aberrant interaction may result in inadequate placental immune adaptation and an increased risk of pregnancy complications. The studies cited in this paper clearly indicate that KIR2DL1 plays a critical role as an inhibitory receptor in the maintenance of immune homeostasis during gestation(Li et al., 2023).

On the contrary, various studies have shown that the efficacy of killer-cell immunoglobulin-like receptors (KIRs) may vary among different populations, where differences have been observed in the strength of association and prediction of disease. These differences may be attributed to several factors, such as genetic variability in KIRs and human leukocyte antigen (HLA), the time of sampling, ethnic differences, and the molecular methods used to assess the expression of genes. These aspects highlight the importance of conducting local studies that consider the population-specific characteristics of the community (Alexandrova et al., 2022).

Limitations

The small sample size could be a factor in the high level of diagnostic performance reported in the ROC analysis, possibly resulting in an overestimation of the sensitivity, specificity, and even AUC. Furthermore, there is a need to validate this in an external cohort to be able to generalize these findings. Also, there is a limitation in using peripheral blood to evaluate the immunological microenvironment in the maternal-fetal interface. There is a need for a multi-center study to confirm the reproducibility of KIR2DL1 in its use as a diagnostic biomarker.

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