KIR3DL1 gene genotype in patients with spontaneous recurrent abortion

Genotipo del gen KIR3DL1 en pacientes con aborto espontáneo recurrente

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Abstract

Introduction and objective: The mechanism of natural killer (NK) cells is based on the recognition of insider cells from alien cells according to inhibitory and activator responses of the receptor. One group of natural killer cell receptors are the killer immunoglobulin-like receptors (KIRs). It seems that decreased inhibitory activity or increased stimulatory activity of these receptors can play a key role in recurrent abortions. Thus, this study aimed to investigate the KIR3DL1 gene genotype in patients with spontaneous recurrent abortions due to its significance.

Methodology: This case-control study was conducted on 40 patients with recurrent abortion, who were referred to Kamali Medical Center of Karaj in 2018 and 40 people with normal pregnancies. Genotypes of KIR genes were isolated using standard kits and two groups were compared in terms of KIR3DL1 genotype frequency. Data were analyzed through SPSS software using descriptive statistics (mean and standard deviation) and inferential statistics of paired t-test and Wilcoxon with a p-value less than 5%.

Results: The KIR3DL1 genotype in the control group was found in 33 people (82.5%) and it was negative in 7 people (17.5%). The KIR3DL1 genotype in the case group was seen in 23 people (57.5%) and it was negative in 17 people (42.5) (p=0.012). The odds ratio (OR) was 0.287 with 95% confidence interval (0.102-0.802) and p= 0.0174.

Conclusion: The results of this study revealed that the KIR3DL1 inhibitory genotype frequency was significantly lower in patients with recurrent abortion than that in those with normal pregnancy.

Keywords: Spontaneous recurrent abortion, Natural killer NK cells, KIR3DL1 gene, Karaj.

Resumen

Introducción y objetivo: El mecanismo de las células asesinas naturales (NK) se basa en el reconocimiento de células internas a partir de células extrañas según las respuestas inhibidoras y activadoras del receptor. Un grupo de receptores de células asesinas naturales son los receptores similares a las inmunoglobulinas asesinas (KIR). Parece que la disminución de la actividad inhibidora o el aumento de la actividad estimuladora de estos receptores pueden desempeñar un papel clave en los abortos recurrentes. Por tanto, el objetivo de este estudio fue investigar el genotipo del gen KIR3DL1 en pacientes con abortos espontáneos recurrentes.

Metodología: Este estudio de casos y controles se realizó en 40 pacientes con aborto recurrente, que se remitieron al Centro Médico Kamali de Karaj en 2018 y 40 personas con embarazos normales. Los genotipos de los genes KIR se aislaron utilizando kits estándar y se compararon dos grupos en términos de frecuencia del genotipo KIR3DL1. Los datos se analizaron mediante el software SPSS utilizando estadística descriptiva (media y desviación estándar) y estadística inferencial de la prueba t pareada y Wilcoxon con un valor de p menor al 5%.

Resultados: El genotipo KIR3DL1 en el grupo control se encontró en 33 personas (82,5%) y fue negativo en 7 personas (17,5%). El genotipo KIR3DL1 en el grupo de casos se observó en 23 personas (57,5%) y fue negativo en 17 personas (42,5) (valor de p = 0,012). La razón de posibilidades (OR) fue de 0,287 con un intervalo de confianza del 95% (0,102-0,802) y el valor de p fue de 0,0174.

Conclusión: Los resultados de este estudio revelaron que la frecuencia del genotipo inhibidor de KIR3DL1 fue significativamente menor en pacientes con aborto recurrente que en aquellas con embarazo normal.

Palabras clave: Aborto recurrente espontáneo, células NK asesinas naturales, gen KIR3DL1, Karaj.

Introduction

Recurrent abortion is one of the most common problems during pregnancy. It occurs in 1 to 3% of pregnancies and the fetus is aborted in the first trimester of pregnancy and usually before week 20[1,2]. Recurrent abortion refers to three or more consecutive abortions before week 20 of pregnancy. Researchers attribute various factors such as environmental, pathologic, and genetic factors to this complication[3,4]. Recurrent abortion occurs in two primary and secondary types. In the primary type, several consecutive abortions occur immediately, but in the secondary type, consecutive abortions begin after one or more successful pregnancies[5]. Idiopathic recurrent pregnancy loss (RPL) is a condition that results in emotional and physical complications. Moreover, when these patients have a successful pregnancy, they will be at the risk of prenatal complications such as growth restriction, preeclampsia, and preterm labor. Frequent attempts to get pregnant with subsequent abortions lead many patients to do numerous tests and procedures to clarify the individual cause of RPL[6]. Although patients may attribute uterine abnormalities, hormonal imbalances, or karyotype abnormalities to their RPL, approximately 50% have remained idiopathic with a RPL diagnosis. To get rid of this epidemic diagnosis by discovering other causes of RPL is crucial not only for the scientific community but also for the patients themselves. It provides the patient's response about their condition. We hope that it can yield newer and more targeted treatments[7].

Some pathologic factors include immunologic factors such as autoimmune diseases, anti-phospholipid syndrome, Systemic lupus erythematosus (SLE), anatomical abnormalities including the bicornuate uterus, uterine adhesions referred to as Asherman syndrome, endometrial polyps, Sub-locus fibroids, polycystic ovary syndrome, infectious agents of endocrine problems such as diabetes, thyroid disorder, and luteal phase dysfunction[8,9]. Other studies have shown that spontaneous recurrent abortions in humans are a common phenomenon and major cytogenetic abnormalities are one of its important causes[10,11]. However, the causes of half of the spontaneous recurrent abortions are still unclear[13,14].

NK cells play a crucial role in identifying immunity in pregnancy[15]. NK cells include about 10 to 15 percent of bloodstream lymphocytes[16]. In the first trimester of pregnancy, these cells express CD3-CD16-CD56 phenotypes, stimulating the decidual cells[17]. Studies have suggested that these cells play a crucial role by enhancing and interacting directly with invasive trophoblasts in a healthy pregnancy[18,19]. 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) - the identification cards of self-cells. These interactions usually result in immune tolerance under normal conditions[20]. A group of NK cell receptors is killer immunoglobulin-like receptors (KIRs)[21]. These receptors are glycoproteins and consist of three parts of extracellular immunoglobulin, one intracellular segment, and one short or long intracellular segment. KIR gene family is located within the leukocyte receptor cluster on chromosome 19q13.4. Out of the 16 genes found, 8 are inhibitor genes, 6 are activator genes, and 2 are pseudogenes. In this regard, the KIR3DL1 gene is placed within the group of inhibitor genes[22,23]. It seems that decreased inhibitory activity or increased stimulatory activity of these receptors can play a key role in recurrent abortions[20-22]. Due to the lack of sufficient studies in this field, the present study was conducted to investigate the genotype of the KIR3DL1 gene in patients with spontaneous recurrent abortions, referred to Kamali Hospital in Karaj in 2018.

Materials and methods

This research was conducted as a case-control study. The study population consisted of all patients with spontaneous recurrent abortions, referred to Kamali Hospital in Karaj in 2018. The sample size was calculated to be 40 based on the prevalence of recurrent spontaneous abortion in previous studies. The convenient sampling method was used in this study. A total of 40 women with three or more spontaneous recurrent abortions with unknown reasons were included in the patient group. All routine tests were performed to determine the cause of the abortion. These tests include a couple of karyotyping, serological tests, hormone tests, investigation of uterine abnormalities, anticardiolipin and anti-coagulant tests, and microbial tests. The control group included 40 non-pregnant women who had a normal pregnancy and had at least two healthy children. Five mL of blood samples of all participants were preserved in tubes containing etilendiaminotetraenoic acid (EDTA) in 2–8 °C for genetic evaluation. KIR genotyping was performed using the KIR SSO polygon capsule type kit (Tepnel Lifecodes Ref: 545110, Connecticut, USA). Data were analyzed through SPSS software using descriptive statistics (mean and standard deviation). Differences between two groups in the genotype were estimated by paired t-test and Wilcoxon test and $p<0.05$ was considered statistically significant.

Results

Descriptive results: The mean age in the control group was 27.87 with a standard deviation of 4.06. The youngest of them was 21 years old and the oldest of them was 36 years old (Figure 1).

The mean age of the patients in the case group was 26.57 years with a standard deviation of 3.16. The youngest of them was 20 years old and the oldest of them was 33 years old (Figure 2).

KIR3DL1 genotype in the control group was found in 33 people (82.5%) and it was negative in 7 people (17.5%) (Figure 3).

KIR3DL1 genotype was also found in 23 people (57.5%) and it was negative in 17 cases (42.5%) (Chart 4).

Figure 1. Age-frequency of patients in the control group

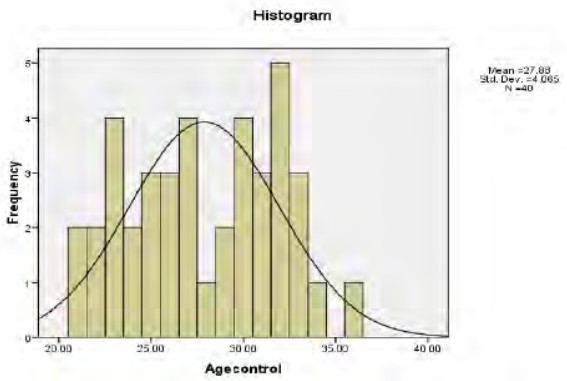


Figure 2. Age-frequency of case group patients

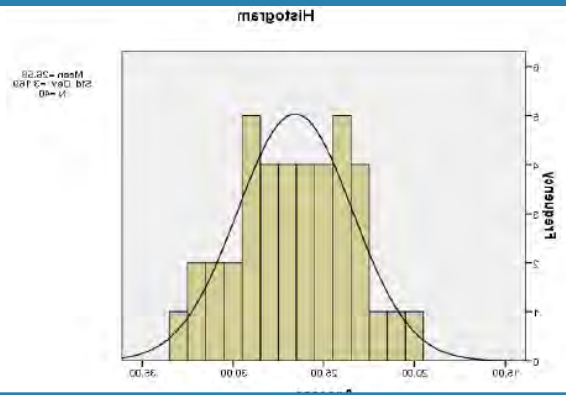


Figure 3. Frequency of KIR3DL1 genotype in the control group

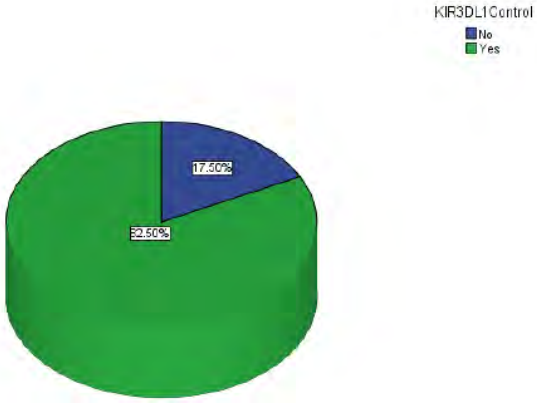
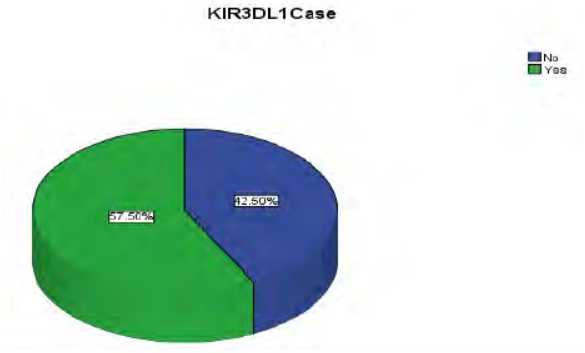


Figure 4. Frequency of KIR3DL1 genotype in the case group



Analytical results

The Kolmogorov-Smirnov test was used to determine the normal distribution of data, which was normal for patients' age in both groups (P> 0.05).

(Table 1) illustrates the comparison of the mean age of the two groups.

Table 1. Comparison of the mean age of patients in two groups

Variable	Control group	Case group	P-value
Mean age (SD)	(4.06) 27.87	(3.16) 26.57	0.180

The results presented in (Table 2) show that the frequency of the KIR3DL1 genotype was significantly lower in the case group than that in the control group (P = 0.012).

Table 2. Comparison of KIR3DL1 genotype frequency in two groups

Variable	Control group	Case group	P-value
KIR3DL1 genotype frequency	(82.5%) 33	(57.5%) 23	0.012

Moreover, the odds ratios (OR) were also calculated and were found 0.287 with a 95% confidence interval (0.102-0.802) and P= 0.0174.

Discussion

A conducted study in 2012 investigated the KIR family gene in patients with recurrent abortion, the results revealed that genotypes with activator function significantly increased in the group with recurrent abortion compared to that in the control group and this difference was not significant for the inhibitory genotypes 24. However, the KIR3DL1 inhibitory genotype in the present study was significantly lower in the case group than that in the control group. In another study carried out in 2018, the results revealed that the KIR3DL1 inhibitory genotype frequency was significantly lower in the case group than that in the control group, confirming the results of the present study[5-9]. In the mentioned study, the odds ratio for the KIR3DL1 inhibitory genotype was 0.277 (0.085-0.904) with a p-value of 0.025, while the OR value was 0.287 in the current study (0.102-0.802) with a p-value of 0.0174. In another study carried out in 2007, the frequency of inhibitory genotype (KIR2DL2 in this study) was significantly lower in the group of patients with recurrent abortion than that in the control group 25, confirming the results of the present study[16-20]. In general, numerous studies have been conducted on maternal KIR genes in spontaneous recurrent abortion. However, these studies suffer from some limitations, including very low sample size, a different methodology for KIR typing, multiple trials, similar controls, and different clinical criteria for examining patients 26. For example, not all studies have made a simple distinction between KIR A and KIR B haplotypes, but have analyzed KIR genes. There is no rationale at the current time to introduce KIR typing into the clinic until these problems are resolved. Cohort studies with good controls are needed to determine whether there is a particular KIR genotype with a failure of trophoblast in the first trimester.

Conclusion

The results of this study revealed that the frequency of KIR3DL1 inhibitory genotype was significantly lower in patients with recurrent abortion than that in those with normal pregnancy. However, further studies in this area are recommended. It is recommended that a multicenter cohort study be conducted to investigate the different genotypes of KIR genes and their association with spontaneous recurrent abortion to clarify this issue.

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