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Original Article

The Single Nucleotide Polymorphism rs2305957 is not Associated with Recurrent Pregnancy Loss

Mohammed J. Ashour[1], Fadel A. Sharif[1*]

1. Department of Medical Laboratory Sciences, Islamic University of Gaza, Gaza, Palestine.

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This study was conducted in order to investigate the association between the single nucleotide polymorphism (SNP) rs2305957 G/A and recurrent pregnancy loss (RPL) in a group of Palestinian women residing in Gaza strip. A retrospective case-control study was carried out during the period of May to August 2015. A total of 380 females including 190 recurrent pregnancy loss (RPL) patients and 190 control women without previous history of RPL, aged 20–35 years were included in the study. The SNP was analyzed by tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR). No statistically significant difference was found between RPL cases and controls in terms of allelic and genotypic distribution of rs2305957 G/A. SNP rs2305957 G/A does not represent a risk factor for RPL in the investigated population.

Keywords: Recurrent pregnancy loss (RPL), rs2305957, single nucleotide polymorphism (SNP), Gaza Strip, Palestine

Recurrent pregnancy loss (RPL), which is currently defined as two or more consecutive pregnancy losses before the 20th week of gestation, affects as many as one in 20 couples seeking parenthood. Although many known causes of RPL including uterine anatomic abnormalities (15%), infections (1%–2%), hormonal deficiencies (20%), immuneg--ical (20%), and genetic (2%–5%) defects have been identified, a significant number of cases (approxim--ately 40%–50%) do not have known causes (1-7).

Studies on mosaic embryos have shown that the occurrence of mitotic aneuploidies is common in human preimplantation embryos, and at least, some of those aneuploidies can lead to embryo loss (8, 9). Recently, McCoy et al., have identified a genetic variant, SNP rs2305957 G/A on maternal chromosome 4, that increases the aneuploidy risk in the embryos of women of European and East Asian ancestries (10). Several genes are located in the vicinity of that SNP, but the authors attributed the association to PLK4 gene (3).

Therefore, this study was designed in order to test whether this polymorphism is associated with RPL in Palestinian women.

Materials & methods

Study Samples

The study was conducted on 190 Palestinian women, 18–35 years old, who had at least two RPLs

*Correspondence: Department of Medical Laboratory Sciences, Islamic University of Gaza, Gaza, Palestine.
E-mail: fsharif@iugaza.edu.ps, Fadelsharif@gmail.com

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Table 1. Sequence of primers used for rs2305957 analysis

Primer	Sequence 5' ⟶ 3'	Amplicon Size (bp)
Forward inner (A allele)	TTTGATTTTCTGCTGTGGGAATCTTTA	Control: 378 allele A: 174 allele G: 260
Reverse inner (G allele)	TCCTTAATGCTTTTATCAAAAGACTTGAC	
Forward outer	CGCATCAGTAATTGAGAAGCAAAATT	
Reverse outer	AAGAACTTAGGAAAGAATTCCAGGTTCA	

≤20 weeks of gestation. 190 age and ethnicity matched women with at least two live births and without a previous history of abortion or pregnancy-associated complications served as control group. Informed consent was obtained from all participants.

DNA extraction and rs2305957 genotyping

DNA was isolated from whole blood samples using Wizard DNA extraction kit (Promega, USA) according to the manufacturer's instructions. The rs2305957 G/A polymorphism was analyzed by T-ARMS-PCR. The primers which were designed using primer1 software (http://primer1.soton.ac.uk/primer1.html) are presented in Table 1. PCR cycling was performed at 95 °C for 4 min followed by 32 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 45 s, extension at 72 °C for 45 s and a final extension at 72 °C for 8 min. The PCR products were separated by electrophoresis on 2% agarose gels and analyzed after ethidium bromide staining on a gel documentation system.

Statistical analysis

The genotype and allele frequencies in RPL patients and controls were analyzed by standard chi-square test with odds ratio (OR) for risk of RPL at 95% confidence intervals (CI). Hardy-Weinberg equilibrium was tested using a freely available software: (http: //www. oege. org/software/hwe-mr-calc.shtml).

Results

Genotype and allele frequencies of the tested polymorphism were not significantly different between RPL patients and controls (Table 2). Moreover, statistical analyzes of the genotypes under recessive and dominant models (data no shown) indicated no significant difference between the two study groups.

Analysis of the observed and the calculated expected genotype frequencies of SNP rs2305957 G/A polymorphism in the control group showed that the distribution of genotypes are in Hardy-Weinberg equilibrium.

Discussion

Recurrent pregnancy loss (RPL) is considered as a multifactorial complication of pregnancy, and despite extensive research worldwide, the cause of nearly half of the RPL cases remains elusive.

Genetic testing of miscarriage tissues offers the possibility to identify pregnancy losses caused by chromosome anomalies which may be responsible for lethal embryonic development.

RPL due to chromosomal aneuploidies may simply be a consequence of the dramatic increase of aneuploid ova associated with advanced maternal age. However, all the patients included in this study were relativrly young (≤35 years).

Some embryos may be at increased risk of chromosome aneuploidies as a result of genetic variations in the parental genomes promoting abnormal mitotic and/or meiotic chromosomal segregation. In their investigation on human embryos, McCoy et al. have shown that the maternal minor "A" allele of the SNP rs2305957 G/A is strongly associated with occurrence of post-zygotic mitotic aneuploidy (10).

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Table 2. Specific probes for R117H mutation detection

Genotype/ allele	Patients n= 190	Controls n= 190	Odds Ratio (95% CI)	P-value
GG	114 (60.0%)	118 (62.10%)	1.09 (0.72 to 1.65)	**0.674**
GA	68 (35.80%)	59 (31.05%)	1.24 (0.807 to 1.897)	**0.328**
AA	8 (4.20%)	13 (6.85%)	0.598 (0.242 to 1.479)	**0.266**
Allele G	296 (77.90%)	295 (77.60%)	0.985 (0.699 to 1.386)	**0.930**
Allele A	84 (22.10%)	85 (22.40%)		

Testing this polymorphism in our RPL patients and controls did not reveal any association between the rs2305957 polymorphism and RPL. The frequency of the minor "A" allele was very similar in both groups (Table 2). This lack of association could be due to ethnic genetic variation unrelated to the investigated SNP or most probably to linkage disequilibrium to other sequence variants in the vicinity of rs2305957.

In conclusion, results of the present study showed that the rs2305957 polymorphism does not contribute to the risk of RPL in Palestinian women.

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Conflict of interests

The authors declared no conflict of interests.

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