SNPs rs2961950 and rs2910203 in PTTG1 gene and Clinical Factors: Examination and Analysis

Abstract

Recurrent spontaneous abortion (RSA) is defined as 3 or more consecutive pregnancy losses, reportedly affecting 1-5% of couples. Numerical abnormalities (especially aneuploidy) are the most common causes of RSAs. Aneuploidy is caused by a defect in the spindle assembly checkpoint (SAC) mechanism. This study aimed to examine the correlation between clinical factors and polymorphisms rs2961950 and rs2910203 in the PTTG1 gene in RSA patients in Zanjan province. Genomic DNAs were prepared from blood samples of 30 women with a history of RSA (case group) and 30 women without any prior history of abortion (control group). Polymorphisms rs2961950 (A2426G) and rs2910203 (C1892G) in the PTTG1 gene were genotyped using the tetra-primer ARMS-PCR method and hence analyzed by agarose gel electrophoresis. The obtained results were statistically analyzed using SPSS software (version 20). The results from examining the genotypic and allelic frequencies of the two polymorphisms rs2961950 and rs2910203 in the PTTG1 gene indicated no significant difference between the case and control groups, but there was a significant relationship between the Simultaneous genotyping of polymorphisms rs2961950 and rs2910203 in the PTTG1 gene and RSA (P=0.024). There were also significance-level relationships between endocrine factors in polymorphism rs2961950 (P=0.027) and simultaneous genotyping of SNPs (P=0.052), as well as age (P=0.173) and the number of abortions (P=0.075) for SNP rs2910203 polymorphism. The results show that the simultaneous genotyping of SNPs rs2961950 and rs2910203 is a risk factor for women with RSA, which can be introduced as a diagnostic factor in health, infertility, and genetic counseling centers.

Keywords: *Recurrent spontaneous abortion, Aneuploidy, Spindle assembly checkpoint, PTTG1 gene, rs2961950 polymorphism, rs2910203 polymorphism.*

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Introduction

Numerical chromosomal abnormalities are the single most common causes of recurrent spontaneous abortions (1), among which aneuploidy is evidenced in 5% of all known pregnancies. The most common chromosomal mechanism involved in aneuploidy is mis-segregation of meiotic chromosomes (2), in which paired chromosomes fail to separate during anaphase I (i.e., the third stage of meiosis I), or sister chromatids fail to separate in meiosis II ((3). The mechanism that monitors the correct segregation of chromosomes during both meiotic divisions is the spindle assembly checkpoint (SAC) mechanism. The PTTG1 gene is involved in the SAC mechanism, which also codes securin. As an anaphase inhibitor, securin is bound to separase, rendering it non-functional and preventing premature chromosome separation (4). The PTTG1 gene is an oncogene located on Chromosome 5 (5q33.3) and encodes securin. Its mRNA isoform is a 1.3-kb isoform that contains a 606-bp coding sequence, encoding a 202- amino acid protein (5). PTTG1 has an amino N-terminal and an acidic C-terminal domain (6). PTTG1 plays an important role in angiogenesis, cell cycle control, cell shape change, DNA repair, cell apoptosis, mitosis, and gene modulation (7).

The polymorphism rs2961950 is located in the region of intron 3, and its adenine is replaced with guanine at nucleotide 2426, and its position is on chromosome 5:160424738. The rs2910203 polymorphism is located in the intron 2 region and has guanine to cytosine replacement at nucleotide 1892, and its position is on chromosome 5:160424204. The distance between these two SNPs is 534 nucleotides. Since these SNPs are located in the intronic region and do not involve amino acid encoding, they are also ineffective in protein function (8-9).

A plethora of studies have sought to examine the relationship between the PTTG1 gene and various diseases such as colorectal cancer, gastric cancer, renal cell carcinoma, esophageal squamous cell cancer, and breast cancer, among others, yet, to the best knowledge of the authors, no research has evidenced its correlation with RSAs. Single nucleotide polymorphisms (SNPs) are among the most common and accurate genetic variations in the human genome and can potentially be used in studies related to complex diseases. As such, rs2961950 and rs2910203 are among the SNPs reportedly involved in the PTTG1 gene of breast cancer.

Lo et al. (2007) examined the relationship between the haplotype of SNPs rs2961950 (A2426G), rs2910203 (C1892G), and rs2910201 (C1053T) of the PTTG1 gene using a 96-well format on an ABI Prism 7000HT Sequence Detection System (Applied Biosystems) for breast cancer. The present study included 698 female breast cancer patients and 1492 healthy female controls. CC, GC, and GG genotype frequencies of SNP rs2910203 were respectively 567 (38.44%), 672 (45.56%), and 236 (16.00%) in control subjects, and 249 (36.19%), 313 (45.49%), and 126 (18.31%) in case subjects. Moreover, AA, AG, and GG genotype frequencies of

rs2961950 were respectively 866 (58.51%), 520 (35.14%), and 94 (6.35%) in control subjects, and 390 (58.51%), 247 (36.06%), and 48 (7.01%) in case subjects. CC, CT, and TT genotype frequencies of rs2910201 were respectively 854 (57.70%), 524 (35.41%), and 102 (6.89%) in control subjects, and 369 (54.42%), 254 (37.46%) and 55 (8.11%) in case subjects. Moreover, analysis of statistical data indicates a significant correlation between rs2910203 polymorphism and breast cancer in both case and control groups (CC genotype (OR=1.00), CG genotype (OR=1.08; 95%CI=0.87-1.34) and GG genotype (OR=1.33; 95%CI=1.00-1.78)), among which GG genotype shows a significant statistical difference compared to other genotypes of rs2910203 in breast cancer, while the other two *PTTG1* gene polymorphisms were not significantly associated with breast cancer. Their study also sought to examine the correlation between haplotype frequency of the *PTTG1* gene and breast cancer, the statistical results from which showed a significant correlation between *PTTG1* gene haplotype C1053T/C1892G/A2426G and breast cancer in both case and control groups ($P<0.0001$) (8).

Liao et al. (2011) examined the expression of PTTG1 in precancerous lesions and oral squamous cell carcinoma (OSCC) and the relationship between protein expression and clinicopathological parameters. The findings suggested the high expression of *PTTG1* in precancerous lesions and OSCC and that *PTTG1* expression is highly correlated to carcinogen exposure *in vitro* and *in vivo*. Furthermore, a strong statistical relationship exists between *PTTG1* expression and smoking with oral tumorigenesis. The high expression of *PTTG1* in oral cancer indicates that it can be promisingly employed as a biomarker for the early detection of oral cancer (10).

Lo et al. (2007) examined the role of haplotypes and combined haplotypes of SNPs rs2910203 and rs2961950 in the *PTTG1* gene in breast cancer. The findings proved the role of *PTTG1* in the development of breast cancer. They indicated that genomic instability caused by the failure of the checkpoint gene to separate sister chromatids during mitosis is correlated to an increased risk of breast cancer (9). Fujii et al. (2006) examined *PTTG1* mRNA expression in hepatocellular carcinoma patients (HCC) using Real-Time Quantitative Reverse Transcription PCR, the results for which indicated the high expression of *PTTG1* mRNA in HCC tissues compared to normal liver tissue (11).

Genkai et al. (2006) examined the expression of *PTTG1* in three glioma cell lines, in which *PTTG1* mRNA and protein expression were studied using RT-PCR and western blotting methods, respectively. Moreover, the authors examined the occurrence of *PTTG1* protein in glioma tissue using immunohistochemical techniques, which was significantly heightened in high-grade glioma compared to low-grade glioma. Finally, increased expression of *PTTG1* is proven to be correlated to the tumorigenicity of glioma cells and hence may be employed as a prognostic marker for glioma complications (12). Malik et al. (2006) reported that *PTTG* has a high expression in lung tumor cells and cell lines. Reducing the expression of *PTTG* using the PTTG siRNA method inhibits tumor growth both *in vitro* and *in vivo* (13). In their research, Xuhui Zhu et al. (2006) reported that PTTG1 has higher expression in prostate cancer than in normal prostate tissue. The expression of PTTG1 in tumors has a positive and significant relationship with the tumor grade (indexed by the Gleason score). Also, high or low expression is involved in changes in cell cycle progression. *PTTG1* is highly presumed to be involved in molecular-level mechanisms and, thus, the progression and prognosis of prostate cancer, effectively rendering it an intervention target in prostate cancer (14).

Numerical abnormalities are reportedly the root cause of 90% of all RSAs. Aneuploidy is one of the most common types of human chromosomal disorders (1), which is also evidenced in 5% of all pregnancies (2). It is also considered one of the main causes of RSAs (15). Aneuploid embryos are formed due to chromosomes' mis-segregation during meiotic division (16). The mechanism that monitors the correct segregation of chromosomes during two meiotic divisions is the spindle assembly checkpoint (SAC). SAC is a mechanism regulating cell division that prevents the resumption of meiotic division by detecting error-prone kinetochore-microtubule (KT-MT) attachment or lack of tension (17). That is, an aggregation of checkpoint proteins builds up in the kinetochore that has failed to attach the microtubule connection, which ultimately prevents the functioning of the anaphase-promoting complex/cyclosome (APC/C). This multi-subunit ubiquitin-protein ligase targets for degradation of cell-cycle regulatory proteins. In the case of optimal KT-MT attachment, the SAC stops and leads to the correct segregation of chromosomes during two meiotic divisions. One of the proteins involved in the correct segregation of chromosomes is securin (18), coded by the *PTTG1* gene (4). In this study, two SNPs, rs2961950 and rs2910203, of the PTTG1 gene are examined in women with recurrent spontaneous abortion.

Materials and methods

For the research, the blood samples of 30 women with recurrent spontaneous abortion and 30 women with normal pregnancies referring to Ayatollah Mousavi Hospital in Zanjan were collected. To prevent blood coagulation, the sample was collected in tubes containing Ethylenediaminetetraacetic Acid (EDTA). For ethical considerations, research samples were required to fill in consent forms inquiring about the scientific use of the test results

1% agarose gel and DNA-exclusive fluorescence staining were used to determine the quality of extracted DNA. This method is used to check the accuracy of DNA and the absence of a

smear, which indicates the degradation of DNA during extraction.

For agarose gel electrophoresis, 186.1 grams of EDTA powder was poured into 800 ml of distilled water and was put on a magnetic stirrer at high speed. The pH of the solution got closer to 8 using NaOH (about 20 grams). When the pH nears the required value, EDTA starts to dissolve. After dissolving, the volume of the solution is increased to 1 liter using distilled water; then, the solution is divided into small volumes and hence autoclaved. About 108 grams of Tris Base was weighed and poured into the volumetric flask. Fifty-five grams of boric acid and 40 ml of 0.5 M EDTA are added to the solution, and the volume is again increased to 1000 ml using distilled water. The solution is then placed on a magnetic stirrer at high speed to have the ingredients dissolved in the distilled water. The obtained DNA product is loaded in the wells of 1% agarose gel. Multiplying 1% gel by the volume of 30 cast results in a product weighing 0.3 g, which must be removed from the agarose powder. 1X the size of the cast is also required as a buffer. At this stage, the cast and comb used are adjusted, then the agarose gel and the buffer are mixed and boiled 3 times so that each time it boils and becomes a honeycomb, then removed from the heater. After a short cooldown, 1.25 microliters of DAN Safe Stain is to the contents of Tris/Borate/EDTA (TBE) and agarose inside the vial; then, the liquid is poured into the cast to close it. After the thickening of the gel, the comb is removed.

SNPs rs2961950 and rs2910203 in the *PTTG1* gene were examined using the Tetra-Primer-ARMS-PCR method. The primers were designed using Oligo 5 and Gene Runner software and were searched for BLAST on the NCBI site with the target gene sequence. Table 1 and Table 2 represent the sequence of primers of the SNPs above.

Table 1: Sequence of primers in rs2961950

SNP	Type of oligo primer	Sequence	GC percentage	Length of primer
PTTG1 gene rs2961950	Forward Outer Primer (FOP)	5'-GGG GAA TAG GAA CAA AGA TGT AGA-3'	41.7	24bp
PTTG1 gene rs2961950	Revers Outer Primer (ROP)	5'-CTT GTT AAA CAG TGT GTC CAG GAC-3'	45.8	24bp
PTTG1 gene rs2961950	Forward Inner Primer (FIP)	5'-AAA ATC TAC ATA AGA CAG TGA AGC CA-3'	34.6	26bp
PTTG1 gene rs2961950	Revers Inner Primer (RIP)	5'-CAG GTT GAA GAA ATT TGA AAA AAA C-3'	28	25bp

Table 2: Sequence of primers in rs2910203

SNP	Type of oligo primer	Sequence	GC percentage	Length of primer
PTTG1 gene rs2910203	Forward Outer Primer (FOP)	5'-CAT TTT CTC CTC TGT TAG GTT GAA TAA-3'	33.3	27bp
PTTG1 gene rs2910203	Revers Outer Primer (ROP)	5'-TGA ATA CTA AGA AAC TAA AGG CCA AAA G-3'	32.1	28bp
PTTG1 gene	Forward Inner Primer (FIP)	5'-AAA CTA ATC AGC TAA TAA GAC TTC CAG TG -3'	34.5	29bp

rs2910203				
PTTG1 gene rs2910203	Revers Inner Primer (RIP)	5'-CAG CAA AGA AAA TAT GGG TTA GTC AG-3'	38.5	26bp

Findings

The Genomic DNAs required for the study were prepared from 30 women with RSAs and 30 women with normal pregnancies. The research aimed to determine whether there is a statistically significant difference between women susceptible to RSA and women with normal pregnancies.

Patients have an average age of 33.16 ± 7.1 years, the maximum and minimum of which were respectively 48 and 17 years. On average, they had 3 ± 1.9 abortions, the highest being 11 and the lowest 2. The average BMI of the individuals in the sample was 27.90±4.32, while the average age of hitting puberty was 13.43±1.2, the highest of which was 17 years old and the lowest was 12 years old. The average live birth in these patients was 0.83±0.69, and the average number of surgeries was 1.2±86.2.

Table 3: Descriptive statistics of patients with recurrent spontaneous abortion

	Frequency	Mean	Median	SD	Range	Min	Max
Age	30	33.16	34.00	7.177	31.00	17.00	48.00
Weight	29	70.00	70.00	11.92	45.00	50.00	95.00
Height	27	157.25	156.00	8.51	35.00	135.00	170.00
BMI	27	27.90	28.65	4.32	18.38	17.72	36.10
No. of abortions	30	3.00	2.00	1.94	9.00	2.00	11.0
Sum of pregnancies	30	4.30	4.00	2.56	14.00	2.00	16.00
Puberty age	30	13.43	13.00	1.25	5.00	12.00	17.00
Live birth	30	0.83	1.00	0.69	2.00	0.00	2.00
Number of surgeries	30	1.86	1.00	2.27	10.00	0.00	10.00

Table 4: Frequency of alleles of SNPs rs2961950 and rs2910203 in case and control groups

SNP	Allele	Group		Total
		Control	Case	
rs2961950	A	0.283	0.4	0.341
	G	0.716	0.6	0.658
rs2910203	G	0.033	0.016	0.025
	C	0.966	0.983	0.975

Table 5: Comparison of alleles of SNPs rs2961950 and rs2910203 in case and control groups

SNP	Allele	Normal group			Case group			P-value
		Frequency	Percentage in group	Total percentage	Frequency	Percentage in group	Total percentage	
rs2961950	A	17	28.3	14.2	24	40.0	20.0	0.248
	G	43	71.7	35.8	36	60.0	30.0	
rs2910203	G	2	3.3	1.7	1	1.7	0.8	1.000

	C	58	96.7	48.3	59	98.3	49.2	

Table 6: Simultaneous genotypic comparison of SNPs rs2961950 and rs2910203 in case and control

		rs2910203				P-value
		GC		CC		
		Frequency	Total percentage	Frequency	Total percentage	
rs2961950	AA	2	3.3	4	6.7	0.024
	AG	1	1.7	28	46.7	
	GG	0	0.0	25	41.7	

The relationship between clinical factors and rs2961950 and rs2910203 polymorphisms in the PTTG1 gene was examined in 30 women with RSA histories, and the significance level for P-value was considered to be less than 0.05.

Table 7: statistical analysis of SNPs rs2961950 and rs2910203 and clinical factors

				rs2910203				P-value
				GC		CC		
				Frequency	Total percentage	Frequency	Total percentage	
Age	35<	rs2961950	AA	0	0.0	1	5.9	0.620
			AG	0	0.0	9	52.9	
			GG	0	0.0	7	41.2	
	35>=	rs2961950	AA	1	7.7	2	15.4	
			AG	0	0.0	7	53.8	
			GG	0	0.0	3	23.1	
BMI	18.5<	rs2961950	AG	0	0.0	1	100.0	0.361
	18.5-25	rs2961950	AA	0	0.0	1	16.7	
			AG	0	0.0	4	66.7	
			GG	0	0.0	1	16.7	
	25-30	rs2961950	AA	1	9.1	2	18.2	
			AG	0	0.0	4	36.4	
			GG	0	0.0	4	36.4	
	30>	rs2961950	AG	0	0.0	5	55.6	
			GG	0	0.0	4	44.4	
Number of abortions	2	rs2961950	AA	0	0.0	2	11.8	0.814
			AG	0	0.0	10	58.8	
			GG	0	0.00	5	29.4	
	3	rs2961950	AA	1	14.3	1	14.3	
			AG	0	0.0	3	42.9	
			GG	0	0.0	2	28.6	
	4	rs2961950	AG	0	0.0	1	25.0	

			GG	0	0.0	3	75.0	
	8	rs2961950	AG	0	0.0	1	100.0	
	11	rs2961950	AG	0	0.0	1	100.0	
Age of puberty	13=<	rs2961950	AA	1	6.2	1	6.2	0.305
			AG	0	0.0	9	56.2	
			GG	0	0.0	5	31.2	
	13>	rs2961950	AA	0	0.0	2	14.3	
			AG	0	0.0	7	50.0	
			GG	0	0.0	5	35.7	
Abnormality	Uterus anomaly	rs2961950	AG	0	0.0	1	33.3	0.452
			GG	0	0.0	2	66.7	
	None	rs2961950	AA	1	3.7	3	11.1	
			AG	0	0.0	15	55.6	
			GG	0	0.0	8	29.6	
Infection	Urinary tract Infection	rs2961950	AG	0	0.0	2	100.0	0.832
	None	rs2961950	AA	1	3.6	3	10.7	
			AG	0	0.0	14	50.0	
			GG	0	0.0	10	35.7	
Endocrine factors	Inactive thyroid	rs2961950	GG	0	0.0	1	100.0	0.052
	Hypothyroidism	rs2961950	AG	0	0.0	6	100.0	
	Hyperthyroidism	rs2961950	AG	0	0.0	2	100.0	
	None	rs2961950	AA	1	4.8	3	14.3	
			AG	0	0.0	8	38.1	
			GG	0	0.0	9	42.9	
Live birth rate	0	rs2961950	AA	0	0.0	1	10.0	0.705
			AG	0	0.0	6	60.0	
			GG	0	0.0	3	30.0	
	1	rs2961950	AA	1	6.7	0	0/0	
			AG	0	0.0	9	60.0	
			GG	0	0.0	5	33.3	
	2	rs2961950	AA	0	0.0	2	40.2	
			AG	0	0.0	1	20.0	
			GG	0	0.0	2	40.0	
Menstrual cycle	Irregular	rs2961950	AA	0	0.0	1	11.1	0.813
			AG	0	0.0	5	55.6	
			GG	0	0.0	3	33.3	
	Regular	rs2961950	AA	1	4.8	2	9.5	

			AG	0	0.0	11	52.4	
			GG	0	0.0	7	33.3	
History of sexual disease	Genital Herpes	rs2961950	GG	0	0.0	1	100.0	0.474
	None	rs2961950	AA	1	3.4	3	10.3	
			AG	0	0.0	16	55.2	
			GG	0	0.0	9	31.0	
History of surgery	Yes	rs2961950	AA	1	4.3	3	13.0	0.849
			AG	0	0.0	11	47.8	
			GG	0	0.0	8	34.8	
	None	rs2961950	AG	0	0.0	5	71.4	
			GG	0	0.0	2	28.6	
Lifestyle	High-risk	rs2961950	AA	0	0.0	1	10.0	0.547
			AG	0	0.0	7	70.0	
			GG	0	0.0	2	20.0	
	Healthy	rs2961950	AA	1	5.0	2	10.0	
			AG	0	0.0	9	45.0	
			GG	0	0.0	8	40.0	
Stress levels	None	rs2961950	AA	1	9.1	0	0.0	0.559
			AG	0	0.0	7	63.3	
			GG	0	0.0	3	27.3	
	Low	rs2961950	AG	0	0.0	3	60.0	
			GG	0	0.0	2	40.0	
	Medium	rs2961950	AG	0	0.0	1	33.3	
			GG	0	0.0	2	66.7	
	High	rs2961950	AA	0	0.0	3	27.3	
			AG	0	0.0	5	45.5	
			GG	0	0.0	3	27.3	
Contraception	None	rs2961950	AA	0	0.0	1	14.3	0.449
			AG	0	0.0	5	71.4	
			GG	0	0.0	1	14.3	
	Yes	rs2961950	AA	1	4.3	2	8.7	
			AG	0	0.0	11	47.8	
			GG	0	0.0	9	39.1	

Discussion

The current study sought to examine 30 women with a history of at least 2 abortions. At most, 11 abortions were selected as the research case sample, and 30 women with normal fertility were selected as the control group. CC and GC genotypic frequencies of rs2910203 polymorphism were 28 (93.3) and 2 (6.6) in normal subjects, respectively, and 29 (96.6) and 1 (3.3) in sick subjects, respectively. The genotypic frequencies of AA, AG and GG polymorphism of rs2961950 in normal subjects were 2 (6.6), 13 (43.3), and 15 (50), respectively, and in diseased subjects, 4 (13.3), 16 (53.3) respectively 53) and 10 (3/33).

The calculated P-value was 1.000 for rs2910203, and 0.366 for rs2961950, indicating no significant correlation between the SNPs and RSAs in both control and case groups. Moreover, the calculated P-value for the simultaneous genotypic

association SNPs rs2961950 and rs2910203 in the *PTTG1* gene for control and case groups was equal to 0.024, which suggests a significant difference between the groups and the simultaneous genotyping of the SNPs, rendering it a risk factor for RSA patients in Zanjan province.

Furthermore, Mi-die Xu et al. (2016) studied *PTTG1* protein and mRNA expression in gastric cancer (GC) cell lines and tissues, respectively, using immunohistochemical techniques (98 gastric cancer tissue samples fixed in formalin and taken in paraffin and 23 GIN samples) and qRT-PCR (78 gastric cancer tissue samples). Their results indicated that PTTG1 protein and mRNA expression are independent prognostic biomarkers in gastric cancer (19). Furthermore, Genkai et al. (2006) examined *PTTG1* mRNA expression in 62 patients with hepatocellular carcinoma (HCC) using real-time reverse transcription polymerase chain reaction. The average level of PTTG1/β-actin in HCC was significantly higher than that in normal liver tissue (0.443±0.073 vs. 0.068±0.007, P<0.0001). PTTG1 mRNA expression is often introduced as an independent prognostic factor for the disease-less state (OR=2.70; P=0.037) and overall survival (OR=5.35; P=0.007) (20). PTTG1 gene expression rate was evidenced to be statistically significant in the above studies for their corresponding complications; however, the incidence of the target SNPs in the PTTG1 gene had no significant correlation to SRA in control and case groups, and only the simultaneous genotyping of the SNPs was significant between control and case groups.

H-T Fan et al. (2015) examined structural chromosomal abnormalities in 1948 couples with RSA, and the findings to which revealed that there was no significant relationship between the age of the couple (average male age of 30.3±15.80 and female average age of 29.4±74.69) and structural chromosomal abnormalities (P=0.736) (21). In the current study, there was no significant relationship between the age of women with recurrent spontaneous abortion (mean age 33.16±7.1) and SNP rs2961950 (P=0.080). However, there was a near-significant correlation between rs2910203 and the age of women with recurrent spontaneous abortion (P=0.173). No significant relationship was observed between the simultaneous genotyping of SNPs and the age of women with RSA (P=0.620).

Kaandorp et al. (2010) prescribed aspirin and low molecular weight heparins (i.e., nadroparin) to 364 women between 18 and 42 with RSAs of unknown etiology to improve birthrate. Nevertheless, the results illustrated that there was no significant relationship between the number of spontaneous abortions (2 or more abortions) and the administration of aspirin alone (P=0.92) and aspirin plus nadroparin (P=0.85) (22). Likewise, there was no significant relationship between the number of abortions (2 or more) of women with RSAs and the incidence of SNP rs2961950 (P=0.680) and the simultaneous genotypic relationship between the SNPs rs2961950 and rs2910203 (P=0.814) in the current study. However, there was a near-significant relationship between SNP rs2910203 and the number of abortions in RSA patients (P=0.075).

Diejomaoh et al. (2007) examined insulin resistance in 35 women with RSAs of unknown etiology and 30 women with no history of miscarriage as controls. The findings revealed no significant relationship between BMI and insulin resistance in control and case groups (P=0.851) (23). Similarly, there was no significant relationship found in this study between the BMI of women with RSA and the SNPs rs2961950 (P=0.924) and rs2910203 (P=0.721) and their simultaneous genotyping (P=0.361).

Conclusion

In examining the simultaneous genotyping of SNPs rs2961950 and rs2910203 in the *PTTG1* gene, the P-value was equal to 0.024 in control and case groups, indicating a statistically significant relationship therebetween. Moreover, there was no significant relationship between the clinical data and the simultaneous genotypic comparison of the SNPs in RSA patients. However, a significant-level correlation was evidenced between the endocrine factors (P=0.052) in the simultaneous genotypic comparison of SNPs rs2961950 and rs2910203 in RSA patients.

Considering that there was no significant relationship between SNPs rs2961950 and rs2910203 and RSA, larger populations are required for examination to decide on their viability as diagnostic markers for susceptibility to RSA.

Acknowledgments:Non

Conflict of Interests: Non

Ethical Considerations: Non

Financial Disclosure: Non

Funding/Support: Non

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