



## Original Article

# The Relationship between Single Nucleotide Polymorphism of Interleukin -10 Gene Promoter (-1082 A/G) with Recurrent Spontaneous Abortion in Iraqi Women

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### ABSTRACT

**Background:** Pro-inflammatory and anti-inflammatory cytokines and polymorphisms of their genes have been described to be involved in the pathogenesis of recurrent spontaneous abortion (RSA).

**Objective:** To investigate the association between RSA and single nucleotide polymorphism of interleukin -10 (-1082 A/G) in Iraqi women.

**Materials and Methods:** The study included 350 aborted women who had history of RSA as case group, In all cases full history and complete examinations including body weight, age and body mass index were done. Furthermore, the patients were screened for various known causes of miscarriages.

**Results :** Based on the screening test results (31.4 %) of RSA cases are unknown cause, So out of 350 only 110 case were enrolled in this study to investigate the association between RSA and promoter Polymorphisms of interleukin 10 gene (IL-10) (-1082 A/G) compared with 108 healthy controls. The frequency of the gene mutations in the patients and controls was determined using PCR-RFLP. In addition, the ELISA was conducted to investigate cytokine (IL-10) serum levels in women with RSA and healthy women. The result was shown significantly lower levels ( $p < 0.05$ ) in serum of IL-10 in RSA patients group than mean serum level for control group, in addition results showed no significant difference in the prevalence of IL-10 (-1082 A/G) genotype and allele frequency among women with recurrent spontaneous abortion and healthy controls in Iraqi women.

**Conclusion:** Our findings indicate that SNP of Interleukin -10 gene promoter (-1082 A/G) is not a risk factor for RSA in Iraqi women, a suggestion which requires further studies on other cytokines.

## Introduction

Recurrent pregnancy loss (RPL) is a common disease during early conception.

RPL is defined as the occurrence of three or more repeated spontaneous miscarriage prior



to 20 weeks from the last menstrual period with or without previous live births<sup>1-3</sup>. Recurrent Spontaneous pregnancy loss is one of the common complications of gestation, affecting 15% of conception the women. Despite sundry well established etiologic factors of RPL, the cause of RPL cannot be determined in nearly 50% of patients. It was suggested that these unexplained RSM might be due to immunologic factors<sup>4</sup>. The etiology of RSM is extremely heterogeneous, including genetic disorder, anatomical abnormalities, endocrine dysfunctions, immune factors, acquired and inherited thrombophilia in additional environmental factors<sup>5</sup>. blood group incompatibility, infections and uterine anomalies smoking and alcohol consumption can lead to occurrence of repeated spontaneous abortion<sup>6</sup>. Interleukin-10 is a potent immunoregulatory produced by T-helper (types-2) that inhibits production of pro-inflammatory cytokines especially TNF-a, IFN-g by inhibition proliferation of T-helper (type-1) lymphocytes and regulation of the survival and proliferation of B lymphocytes and T-helper 2 lymphocytes.

It down regulates cytotoxic inflammatory response<sup>7-9</sup>. Interleukin-10 is a multifunctional anti-inflammatory cytokine and immune-suppressive substance that is produced within the body by various cells including monocytes, macrophages, B cells, mast cells, T cells, dendritic cells, natural killer (NK) cells, in additional cells found at the maternal-foetal interface, namely endothelial, placental trophoblast and decidual stromal cells<sup>10-13</sup>. Interleukin-10 plays the role in the regulation of immune responses. It is secreted by antigen-presenting cells, promotes the evolution of immunologic tolerance, IL-10 is expressed early in gestation and remains elevated throughout the second trimester<sup>14</sup>. Single nucleotide

polymorphisms of IL-10 are associated with the evolution of preeclampsia. Some IL-10 gene promoter polymorphisms linked with cytokine regulation seem to be constitutional risk factors for early embryonic pregnancy failure.

An increase in the production of IL-10 early after embryo implantation is relevant to the success of pregnancy, So Higher serum levels of IL10 were detected in women having normal delivery than in cases with RSA at the time of abortion<sup>15</sup>. IL-10 plays the key role in Th2 immunity and The IL-10 gene is contained of 5 exons, spans almost 5.2kb and is located on the long (q) arm of chromosome 1 between positions 31 and 32. (1q31-q32) 02. Many of (SNPs) are reported in proximal region (-1082A/G; -819T/C; -592A/C) and distal region (the promoter region) of the IL-10 gene affecting in IL-10 transcription rate and also in production level<sup>16-20</sup>.

## Material and Methods

This study was carried out in the department of obstetrics and gynecology at Al-Elwiya and Al-yarmouk teaching hospitals in Baghdad, between October 2013 and June 2014. The patient group comprised 350 Iraqi women with a history of at least three repeated spontaneous miscarriage The healthy control group involved 108 Iraqi women who have formerly had at least two normal pregnancies with no history of ectopic pregnancy, miscarriage, or stillbirth.

All subjects were fully investigated for routine analysis at the hospital laboratory were performed to exclude known causes of abortion and including the anticardiolipin antibodies (IgM), antiphospholipid (IgM), Activated partially prothrombin time (APPT), Coagulation factors involved protein S, protein C, activated protein C resistance, Antithrombin III and investigation of toxoplasmosis antibodies (IgM) and cytomegalovirus antibody (IgM),

rubella antibody (IgM), as well homocysteine level, TSH and progesterone hormones. One hundred and ten cases of the repeated spontaneous miscarriage were recruited from the outpatient clinic after exception of the possible etiological factors. Cytokine IL10 levels were measured in the cases and control serum ELISA kit from Peprotech Company.

Total genomic DNA from peripheral anticoagulant blood was extracted by use the blood DNA isolation kit of promega company. Sequence amplification was performed by using polymerase chain reaction (PCR). The primers were decided by using specific Primer F:5'-TTC CCC AGG TAG AGC AAC ACT-3', R:5'-GAT GGG GTG GAA GAA GTT GAA-3'. PCR was performed in final total volume of 25  $\mu$ l (12.5  $\mu$ l) of Green Master mix 1X, (9.5  $\mu$ l) of doubled distilled water, (0.5  $\mu$ l) of each primer and (2  $\mu$ l) of genomic DNA). The cycling conditions were as follows: an initial denaturation at 94°C for 5 min, followed by 38 cycles at 94°C for 45 sec, and 72°C for 1.5 min, with a final extension step of 10 min at 72°C in the last cycle.

The PCR products and the ladder marker (25bp) were run on electrophoreses on 2% agarose gel for 2 h and gel viewed under UV trans-illuminator after staining with ethidium bromide stain. The PCR products were incubated at 37°C for 15 min with MnII restriction endonuclease enzyme (Biolabs Inc. company) Digestion products were run on 3% agarose gel electrophoresis for time 2.5 hrs. Then stained with use ethidium bromide, after that it was visualized under UV light using ultraviolet transilluminator, to detect the IL-10 alleles. Three genotypes were founding in this our study: genotype A/A, have one fragment (238bp); genotype A/G, have three fragments (238bp, 136bp and 102bp); genotype G/G, have two fragments (136bp and 102bp). For statistical analysis, Chi-

square test was used to compare the genotype and allele frequencies. Means and standard deviations (SD) are presented for describing variables with continuous distribution. The odds ratio (OR) was used to recognition the ratio of the risk of RSM among patient group with various allele and genotype to the risk among control group. The 95% confidence interval (CI) for OR was calculated using confields' method.

## Results

TIV and The clinical characteristics of cases and controls are listed in Table (1). No significant alterations were observed in mean age, Menarche (years), and mean Body mass index (BMI) (kg/m<sup>2</sup>) between RSA cases and control ( $p > 0.05$ ), while higher in irregular menstrual history (%) and number of pregnancies were seen in the RSA group.

Although they did not constitute strong risk factors of RSA, they were selected as the covariates that were controlled for in subsequent analyses. The experimental group consisted of women with a history of three or more spontaneously repeated first trimester spontaneous miscarriage. These included 350 cases of RSA. The control group consisted of women with one or more live births and no history of first trimester spontaneous miscarriage and that included 108 normal women.

This study applied for the detection of many of Immunological and coagulation assays for 350 female with RSA and the result were as shown in table (2).

Based on the results screening test 31.4 % of RPL cases are unknown cause, So out of 350 only 110 case were enrolled in this study to investigate the relationship between RPL and interleukin 10 (IL-10) (-1082 A/G) promoter gene, Polymorphisms and compared with 108 healthy controls. The frequency of the gene mutations was

determined in the patients cases and controls using PCR-restriction fragment length polymorphism (PCR-RFLEP).

In additional, the ELISA was utilized to investigate cytokine (IL-10) serum levels in women with RSA and control women. The result demonstrate the estimated levels of serum IL-10 for recurrent pregnancy lose women were ( $43 \pm 15.82$  pg/ml) which was significantly lower ( $p < 0.05$ ) than mean level of serum for control group ( $74.17 \pm 17.78$  pg/ml).

While in Molecular Experiment, Results of polymorphism analysis between patient and control group were recorded in table (3) that illustrated the genotype frequencies of the IL-10 (1082 A/G) polymorphism among RSA patients compared with healthy group. The frequency of the wild type AA was (76.36%), and the heterozygote AG was (17.27%) while, of the homozygote for the polymorphic allele GG was (6.36%) in RSA patients, whereas in healthy women the frequency the genotype of the wild type AA was (75.92%), the frequency of the heterozygote AG was (18.51%) while, of the homozygote for the polymorphic allele GG was (5.55%).

Results from table (3) illustrates also allele frequency for A allele in RSA cases and controls were (85.0%) and (85.18%) and allele G were (15.0%) and (14.81%), respectively. There was not statistically significant difference in genotype distribution of allele frequency in recurrent abortion patient and controls. Statistical analysis revealed that IL-10 -1082 A/G polymorphism in the present sample is not linked with repeated miscarriages.

### Discussion

Physiologic adaptations was required in pregnancy of all maternal systems, including the immune system. This process is sophisticated and includes alteration at

different levels of the maternal immune system. Successful gestation is characterized by a shift toward T-helper(type-2) immune response and suppression of adaptive immune responses to ensure acceptance of the semi-allogenic fetal graft; While, susceptibility to recurrent abortion is probably mediated by Th1 type immune response with pronounced expression and secretion of proinflammatory cytokines like TNF-a and IFN-gamma paralleled with decreased production of anti-inflammatory cytokines like IL-10<sup>21</sup>.

The current study was investigated the linked the IL-10 gene polymorphisms and idiopathic RPL and institute whether this SNP are associated with RPL. Our results don't show any a role for - 1082 A/G IL10 promoter polymorphisms in repeated miscarriages in studied group. While in other study refers to Genotype and frequencies the allele of cytokine polymorphisms are significant differences among different populations.

These differences reveal that inheritance of certain cytokine gene polymorphisms is strongly linked with ethnicity. Cytokines production is under genetic control and IL-10 promoter polymorphisms were implicated in repeated pregnancy loss pathogenesis<sup>22</sup>. Our results were agreed well with Rezaei and Dabbagh<sup>23</sup> who measured levels of cytokines in serum and that demonstrate, higher serum levels of IL10 were detected in women having normal delivery than in cases with repeated pregnancy loss furthermore, study by Abdullah and Mahdi, was disagree with presence study and indicate to significantly higher levels of IL-10 in the aborted women as compared with the controls was detected between women with miscarriage and control groups<sup>24</sup>.

IL-10 is located on chromosome 1and encoded by the IL10 gene and many SNPs have been reported in proximal (at

position -819T/C, -1082A/G and -592A/C) and distal regions of the gene. Several polymorphisms are reportedly included in IL-10 transcription rate, thereby directly affecting with regulation of IL-10 production levels<sup>13,19</sup>.

The role of IL-10 levels in idiopathic RPL pathogenesis still controversial. It was suggested that increased IL-10 expression was linked to successful pregnancy, whereas low levels were linked with recurrent abortion<sup>25</sup>. IL-10 concentrations in Serum are low in preeclampsia, another common disorder of pregnancy; thus, IL-10 could be an important anti-inflammatory cytokine contributing to the outcome of pregnancy<sup>14</sup>. During this study, present results show that the -1082A/G polymorphism in the IL10 gene is presence in RSM cases and controls, with different result.

This difference was not well marked and was statistically non significant, about this our results consistent with several studies which demonstrate that of A/G polymorphism of IL-10 were not associated with RSA<sup>26-27</sup> but contradiction with the findings of other study that showed relationship between IL-10 polymorphisms among Tunisian RSM cases<sup>28</sup> and that emphasis of the Interleukin -10 was have an important role in the survival of gestation<sup>29</sup>.

There is clear evidence to suggest that the immune system for mother during gestation can inhibit or enhance the evolution of the fetoplacental unit. Some cytokines produced by both T cell and non-T cell favor embryo survival and growth. In spite of the complexity of the cytokines network, it appears that cytokines favoring the maintenance of embryo survival belong to the Th2 pathway<sup>30</sup>.

In addition to that, the findings of the current study are in disagree to the research revealed that indicated a significant linked between the IL-10 polymorphism and occurrence of RPM in Iranian female<sup>22,31</sup>.

The presence Result agrees with (34) who indicate to do not significant relationship between the - 1082 A/G promoter polymorphism in human IL-10 gene and occurrence of RSA.

Experimental studies on a hypertensive rat model have demonstrated that IL-10 helps in the normalization of blood pressure and endothelial function, and confirmed the importance of this cytokine in the inflammatory response<sup>34</sup>. There are other reports have also demonstrate that probably there is an equilibrium state between the anti-inflammatory and the pro-inflammatory cytokines which are produced at the time of pregnancy at local site and are critical for successful pregnancy.

None of the cytokines is singly included and alone critical; it is probably all of the inflammation-related genetic variants which shape the cumulative risk of repeated pregnancy loss<sup>35</sup>. Our known that the immune response of an individual change from person to another depended on genetic variation in the genes linked to the cytokine production, which is partly under genetic control. Therefore, early identification of immunologic alter would help in the prevention of pregnancy loss.

However, the immunologic profile of women gestation needs to be more clearly defined<sup>22</sup>, other study shown IL-10 production is under genetic control, it has been shown that adenine (A) at the site - 1082 in the promoter region of the IL-10 is linked with low production and guanine (G) with high production of IL-10. Since those factors contributing to IL-10 production appear to be important in RSA, so (-1082A/G) polymorphism in IL-10 detection was done with allele refractory mutation system (ARMS-PCR) among 38 Finnish female with RPL and 131 ethnically matched fertilize women controls. No significant differences in the -1082 genotype and allele frequencies were found between

controls and RSM women. The study suggested that IL-10 (-1082A/G) polymorphism have not a major genetic role in RSA<sup>36</sup>.

### Conclusion

In conclusion, our results suggest that the IL-10 (-1082A/G) gene polymorphism is not a risk factor for RPL in Iraqi women, While further studies are needed on other cytokines in comparisons of cytokine serum levels in their different polymorphism to explore any relationship with recurrent abortion.

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### Conflict of Interest

None.

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**Table 1.** The demographic data of the unexplained RSA patient and controls

Characteristic	Cases	Controls	P value
Mean age (years)	30.23±4.76	29.89±5.22	p>0.05
body mass index BMI(Kg/m <sup>2</sup> )	22.37±4.24	23.78±3.88	p>0.05
Menarche (years)	13.46±1.62	13.35±1.57	p>0.05
Irregular menstrual history %	65	15	p<0.01
Number of pregnancies	4.37±1.22	2.6±1.38	p<0.01
Abortion	3.7±0.78	0	p<0.001

**Table 2.** Results of screening tests for RSA cases and control

Parameter	Prevalence of +Ve %	Seroprevalence PRL cases	Seroprevalence Control	Normal value
TOXO(IgM)	4.28	27.51±2.5	7.33±1.3	<12 mIU/L
CMV(IgM)	6.85	21.4±2.12	6.21±1.3	<12 mIU/L
Rubella (IgM)	4.00	18.1±2.4	4.43±1.1	<11 mIU/L
Cardiolipin (IgM)	7.42	16.2±2.8	5.67±1.8	<12 mIU/L
Phospholipid (IgM)	7.14	23.1±2.9	4.15±1.8	<12 mIU/L
Protein C deficiency	6.00	67.85 ± 17.21	83.41 ± 9.62	70-130 %
Protein S deficiency	4.85	68.63 ± 17.4	93.35 ± 1.6	65-140 %
Homocysteine	8.85	18.9±6.8	7.31±2.21	7.1±2. mg/dl
TSH	3.14	7.21±1.8	3.8±1.4	0.5-5.0 mIU/L
Progesterone	4.85	5.8±1.92	31.81±2.9	30±2.2 ng/ml
APCR	5.14	>2	<2	<2
APTT	2.00	19 ±21	14±2	13±2 sec
Antithrombin III	4.00	67 ± 13	87±33	75-145 ng/ml



**Table 3.** Genotype and Allele frequencies of -1082 G/A polymorphism in the IL-10 gene promoter in women with recurrent spontaneous abortions and in the control group

IL10 -1082 (A/G)	Genotypes frequency (%)			Allele's frequency (%)	
	Homozygote Wild type A/A	Heterozygote A/G	Homozygote Mutant type G/G	(A) Allele	(G) Allele
Patients(N:110)	84 (76.36%)	19 (17.27%)	7 (6.36%)	187 (85.0%)	33 (15.0%)
Control(N:108)	82 (75.92%)	20 (18.51%)	6 (5.55%)	184 (85.18%)	32 (14.81%)
$\chi^2$	0.06	0.05	0.0026	0.0004	0.0017
P value	0.92	0.89	0.94	0.99	0.98
OR(95%CI)	0.86(0.41-1.8)	0.86( 0.41-2.8)	0.94(0.66-1.3)	1(0.70-1.67)	0.99(0.5-1.4)