



2023; 19(1):179-184

بسم الله الرحمن الرحيم
Omdurman Islamic University Journal (OIUJ)

مجلة جامعة أم درمان الإسلامية

<https://journal.oiu.edu.sd/index.php/oij>

[https:// DOI 10.52981/oij.v19i2.3078.](https://doi.org/10.52981/oij.v19i2.3078)



ISSN: 5361-1858

Association between IL-6 -174G/C Polymorphism and unexplained Recurrent Abortion among Pregnant Ladies

Emtinan Elradi Ali Elradi¹*, . Nour Mahmoud Abdelateif².

¹Heamtology Department, Faculty of Medical Laboratory Sciences, University of Science & Digital Technology, Khartoum, Sudan

²Heamtology Department, Faculty of Medical Laboratory Sciences, AL-Neelain University, Khartoum, Sudan

Corresponding Email address: ...Tanton129@gmail.com

To cite this article:

Emtinan Elradi Ali Elradi¹*, . Nour Mahmoud Abdelateif². Association between IL-6 -174G/C Polymorphism and unexplained Recurrent Abortion among Pregnant Ladies in Khartoum-Sudan ,2022, Omdurman Islamic University Journal

ISSN: 5361-1858

[https:// DOI 10.52981/oij.v19i2.3078.](https://doi.org/10.52981/oij.v19i2.3078)

ABSTRACT

Objective: The aim of this study was to determine the association between interleukin 6 (IL-6 -174G/C) with susceptibility to Recurrent abortion (RA) in Sudanese pregnant ladies.

Methods: case control study, the genetic polymorphism of IL-6 -174G/C were studied by SSP-PCR in the DNA of a total of Forty (30) Sudanese ladies with the history of abortion and (30) ladies with normal pregnancy to three or more birth and without a previous abortion in the first trimester. Statistical analysis was performed using Chi square, correlation to spearman test to set differences were considered statistically significant with a $p < 0.05$.

Results: CG genotyping prevalence were significantly higher in cases than in controls, $P = 0.01$, respectively. On the other hand, GG genotype prevalence were higher among control than in RM patients, $P = 0.01$, respectively.

Conclusions: This study has proven a possible association of IL-6 -174 G/C polymorphism with susceptibility recurrent abortions.

Keywords: IL-6, Polymorphism, RA, SSP-PCR.

1. INTRODUCTION

Recurrent pregnancy loss refers to repeated and consecutive (≥ 3 times) spontaneous abortion events that occur before the 20th gestational week of pregnancy. This condition causes substantial

emotional stress to the patient and her family. It is estimated that as many as 1–2% of healthy women experience recurrent pregnancy loss (Diejomaoh, 2015). Several factors are known to contribute to this condition, such as advanced maternal age, abnormalities of the uterus and endocrine diseases (Garrido-Gimenez and Alijotas-Reig, 2015). However, these factors can only explain the etiology of the condition in a small subset of patients. Up to 60% of recurrent pregnancy losses have an unknown or unexplained cause, and represent a major challenge in reproductive medicine (Diejomaoh, 2015).

Genetic polymorphisms may be at least partially responsible for the incidence of these unexplained recurrent pregnancy losses (Daher et al., 2012). Cytokines, particularly interleukins, are important mediators in many reproductive events (Prins et al., 2012; Cheng and Sharma, 2015; Southcombe et al., 2015). IL-1B, for example, is crucial for the regulation of immune responsiveness of Th1/Th2 cells, which is necessary for a successful pregnancy (Agrawal et al., 2012; Sykes et al., 2012). IL-6 is involved in the modulation of embryonic implantation and placental development (Prins et al., 2012). IL-10 actively suppresses the maternal immune system to avoid rejection of the fetal allograft (Thaxton and Sharma, 2010; Cheng and Sharma, 2015). On the other hand, IL-18 may stimulate the production of interferon-gamma, which is needed for the remodeling of the blood vessels during pregnancy (Weissenbacher et al., 2014). Given the importance of these interleukins in pregnancy, a number of studies have investigated the association between polymorphisms in interleukin genes and the risk of recurrent pregnancy loss. Although the results obtained are often contradictory, the studies do provide some indications that functional polymorphisms, which are known to influence the expression of their respective genes, can influence the risk of recurrent pregnancy loss (Daher et al., 2012).

Among the most commonly studied interleukin polymorphisms with regard to their relationship with recurrent pregnancy loss risk are the IL1B rs16944, IL1B rs1143634, IL6 rs1800795, IL6 rs1800796, IL10 rs1800896 and IL18rs187238 polymorphisms (meta-analyzed in Agrawal et al., 2012; Chen et al., 2015; Lee et al., 2015; Woods et al., 2015; Su et al., 2016). Except for IL1B rs1143634, all other polymorphisms occur in the promoter regions of their genes and are thought to affect the rate of gene transcription. On the other hand, IL1B rs1143634 is predicted to disrupt the splicing process (Woods et al., 2015), which can then influence the normal function of IL-1B and, as a result, the risk of recurrent pregnancy loss. The association of these polymorphisms has been investigated in several populations, but few studies have been conducted in the Chinese population (Agrawal et al., 2012; Chen et al., 2015; Lee et al., 2015; Woods et al., 2015; Su et al., 2016).

For the IL1B rs16944 polymorphism, two studies have demonstrated an association between the variant allele and the risk of recurrent pregnancy loss in a Korean population (Kim et al., 2014) and a Chinese population (Ma et al., 2017). However, another study conducted in China showed a lack of association, which indicates that the effect of the polymorphism differs even among distinct geographical areas of the same population (Ma et al., 2012). In addition, a study from the UK also failed to find an association between the polymorphism and risk of recurrent pregnancy loss (Linjawi et al., 2005). Besides, the association of the IL1B rs1143634 polymorphism and recurrent pregnancy loss risk has been investigated in two populations in China (Ma et al., 2012, 2017). Similarly, for IL6 rs1800795 and rs1800796 polymorphisms, contradictory findings have been observed in several reports. For the former, Demirturk et al. (2014) and Parveen et al. (2013) demonstrated an association of the polymorphism with risk of recurrent pregnancy loss in Turkish and northern Indian populations, respectively. However, no significant association was found in Saudi Arabian (Alkhuriji et al., 2013), Iranian (Bahadori et al., 2014) or Romanian populations (Bohiletea and Radoi, 2014).

The relationship between genetic factors and the interleukin 6 -174G/C is not cleared, genetic polymorphisms as the one that is studied may represent markers for selecting the therapeutic

options and for counselling patients with recurrent spontaneous abortions. Several studies published, showed that there are no association between interleukin 6 -174G/C and Recurrent miscarriage. while other studies have proven a possible association of polymorphism IL-6 174G/C and the increased frequency of recurrent abortions.

The aim of this study was to determine the association between interleukin 6 (IL-6 -174G/C) with susceptibility to Recurrent abortion (RA) in Sudanese pregnant ladies.

2.Methodology

Study population: This a case control study, a total of 30 ladies with RM and 30 normal controls will be analysed for carrying IL-6 -174G/C. Thirty (30) Sudanese ladies with the history of abortion and (30) ladies with normal pregnancy to three or more birth and without a previous abortion in the first trimester. And this study was conducted in Omdurman Maternity Hospital located in Khartoum state this study will be conducted between Dec 2022 to Dec 2023.

Sample Collection: Peripheral blood samples will be collected under sterile conditions into tubes containing K⁺ ethylene diamine tetra acetic acid (EDTA).

DNA extraction by Guanidine Chloride method:

RBCS will be Hemolyzed by alkaline solution (Red Cells lysis buffer) ,then the membranes will be digested by solution containing detergent and proteases (White Cells Lysis buffer), then protein will be precipitated out by Guanidine hydrochloride (GuHCL) and centrifugation , finally DNA will be precipitated by absolute ethanol ,washed by 70% ethanol and eluted in 50 µL of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 .

Genotyping of Interleukin-6

The SSP-PCR (sequence-specific primer-polymerase chain reaction) method was applied for genotyping (12) ; PCR mixture of 20 µl was prepared using premix master mix tubes (MaximeTMPCR premix Kit{i-Taq}) for each sample (see Table 1), genomic DNA will be amplified in two different PCRs for each polymorphism; each reaction employed a generic antisense primer and one of the two allelespecific sense primers (see table 2). To assess the success of PCR amplification in both reactions. The PCR reaction will be carried out in a Thermal Cycler (Techne, UK), with the following program: 1min at 95°C followed by 10 cycles of 30 sec at 95°C, 50 sec at 58°C, 40 sec at 72°C, followed by 20 cycles of 20 sec at 95°C, 50 sec at 54°C and 50 sec at 72°C, with 5min at 72°C as final extension.

Demonstration of PCR product:

Five µl of the PCR product (ready to load) will be electrophoresed on 1.5% agarose gel, and stained with ethidium bromide, 1X TBE buffer will be used as a running buffer. The Voltage applied to the gel will be 100 volt with time duration of 30S minutes. 100 pb DNA ladder will be used as molecular weight marker with each patch of samples .Finally, PCR product will be demonstrated by gel documentation system.

Table 1: master mix tube preparation:

Reagent	Volume
ddH ₂ O ₂	13.5µl
IL6(-174) C primer	0.5µl
IL6(174) G primer	0.5µl
IL6 Generic	0.5µl

Template DNA	5µl
Total reaction	20µl

Table 2. Primer Sequences Used for the IL6 SSP Genotyping Method:

Primer position	Primer sequence	Product size
IL6(-174) C primer	5'-CCC CTA GTT GTG TCT TGC C-3'	240 bp
IL6(174) G primer	5'-CCC CTA GTT GTG TCT TGC G-3'	240 bp
IL6 Generic	5'-GCC TCA GAG ACA TCA CCA GTC C-3'	240 bp

Statistical analysis:

All the statistical analyses will be performed using the statistical package for the social sciences (SPSS) software version 17.0 for Microsoft windows. The result will be compared between cases and controls by Chi-square test. Correlations between the IL-6 (-174G/C) were calculated by spearman test.

3. Results

In this study, 30 cases of Recurrent miscarriage were targeted, the age with range of (20-40 years). The genotype and allele frequency for both patients and control are listed in (table 2), showing highly statistically difference in the genotype distribution between cases and control. CG genotyping prevalence were significantly higher in cases than in controls, $P = 0.01$ ($OR = 4$; 95% $CI = 1.4 - 17.8$). On the other hand, GG genotype prevalence were higher among control than in RM patients, $P = 0.01$, respectively.

4. Discussion

The ethology of Recurrent miscarriage remains largely unclear. The possible immunologic etiologist of pregnancy failure has been intensively investigated. Many investigators have assessed possible associations between etiologist of recurrent pregnancy loss and gene polymorphisms, including a family of enzymes responsible for metabolism of environmental toxins, glutathione S-transferase (GST) (Imseis et al., 1997).

Anti-inflammatory cytokines such as IL-6 and IL-10 considered as crucial factors for maintaining a normal pregnancy. Among potential co-factors, functional SNPs in which alter the expression of IL-6 play a decisive role at various stages of recurrent pregnancy loss development. The main findings of this study were an important positive association between IL-6 (-174G/C) with recurrent abortion risk. In Iran, several studies on the relationship among various cytokine polymorphism and RPL were reported. In one study, Torabi et al. reported that there are no substantial variations between RPL cases and controls, when they investigated the role of [-592A/C, -819C/T, -1082A/G (IL-10)], -174C/G (IL-6), and -197G/A (IL-17) in the risk of recurrent pregnancy loss (Torabi et al., 2009). But, Nematollahi et al investigations failed to discover the connection between the prevalent polymorphisms in the IL-6 SNP and RPL risk (Nematollahi et al., 2015). In comparison to other populations, the Caucasian Northern Ireland people is the only one with a lower frequency of IL-6 174 allele (Velez et al., 2007) .

In conclusion, it is suggested that women with IL-6 -174 G/C polymorphism have susceptibility of recurrent abortions.

References:

1. Agrawal, S., Parveen, F., Faridi, R. M., and Prakash, S. (2012) Interleukin-1 gene cluster variants and recurrent pregnancy loss among North Indian women: retrospective study and meta-analysis. *Reprod. Biomed. Online* 24, 342–351.
2. Alkhuriji, A. F., Alhimaidi, A. R., Babay, Z. A., and Wary, A. S. (2013) The relationship between cytokine gene polymorphism and unexplained recurrent spontaneous abortion in Saudi females. *Saudi Med. J.* 34, 484–489.
3. Bahadori, M., Zarei, S., Zarnani, A. H., Zarei, O., Idali, F., Hadavi, R., and Jeddi-Tehrani, M. (2014) IL-6, IL-10 and IL-17 gene polymorphisms in Iranian women with recurrent miscarriage. *Iran. J. Immunol.* 11, 97–104.
4. Bohiltea, C. L., and Radoi, V. E. (2014) Interleukin-6 and interleukin-10 gene polymorphisms and recurrent pregnancy loss in Romanian population. *Iran. J. Reprod. Med.* 12, 617–622.
5. Chen, H., Yang, X., Du, J., and Lu, M. (2015) Interleukin-18 gene polymorphisms and risk of recurrent pregnancy loss: a systematic review and meta-analysis. *J. Obstet. Gynaecol. Res.* 41, 1506–1513.
6. Cheng, S. B., and Sharma, S. (2015) Interleukin-10: a pleiotropic regulator in pregnancy. *Am. J. Reprod. Immunol.* 73, 487–500.
7. Daher, S., Mattar, R., Gueuvoghlian-Silva, B. Y., and Torloni, M. R. (2012) Genetic polymorphisms and recurrent spontaneous abortions: an overview of current knowledge. *Am. J. Reprod. Immunol.* 67, 341–347.
8. Demirturk, F., Ates, O., Gunal, O., Bozkurt, N., Aysal, T., and Nacar, M. C. (2014) IL-6 gene promoter polymorphisms: genetic susceptibility to recurrent pregnancy loss. *Bratisl. Lek. Listy.* 115, 479–482.
9. Diejomaoh, M. F. E. (2015) Recurrent spontaneous miscarriage is still a challenging diagnostic and therapeutic quagmire. *Med. Princ. Pract.* 24, 38–55.
10. Imseis, H.M., Greig, P.C., Livengood, C.H., Shunior, E., Durda, P. and Erikson, M. (1997). Characterization of the Inflammatory Cytokines in the Vagina During Pregnancy and Labor and With Bacterial Vaginosis. *Journal of the Society for Gynecologic Investigation*, 4(2), pp.90–94. doi:<https://doi.org/10.1177/107155769700400208>.
11. Garrido-Gimenez, C., and Alijotas-Reig, J. (2015) Recurrent miscarriage: causes, evaluation and management. *Postgrad. Med. J.* 91, 151–162.
12. Kim, J. O., Lee, W. S., Lee, B. E., Jeon, Y. J., Kim, Y. R., Jung, S. H., Chang, S. W., and Kim, N. K. (2014) Interleukin-1β -511T>C genetic variant contributes to recurrent pregnancy loss risk and peripheral natural killer cell proportion. *Fertil. Steril.* 102, 206–212.
13. Lee, Y. H., Choi, S. J., and Ji, J. D. (2015) Association between IL-6 -174 G/C, IL-6 -634 G/C, and IFN-γ +874 A/T polymorphisms and susceptibility to recurrent pregnancy loss: a meta-analysis. *J. Assist. Reprod. Genet.* 32, 1421–1427.
14. Linjawi, S., Li, T. C., Laird, S., and Blakemore, A. (2005) Interleukin-1 receptor antagonist and interleukin-1 β polymorphisms in women with recurrent miscarriage. *Fertil. Steril.* 83, 1549–1552.
15. Ma, J., Zhang, X., He, G., and Yang, C. (2017) Association between TNF, IL1B, IL6, IL10 and IFNG polymorphisms and recurrent miscarriage: a case control study. *Reprod. Biol. Endocrinol.* 15, 83.
16. Ma, X., Xu, L. J., Wang, J., Xian, M. M., and Liu, M. (2012) Association of IL-1β and IL-6 gene polymorphisms with recurrent spontaneous abortion in a Chinese Han population. *Int. J. Immunogenet.* 39, 15–19.
17. Nematollahi, Z., Hadinedoushan, H., Aflatoonian, A., Eslami, G. and Ghasemi, N. (2015). The association between single nucleotide polymorphism in interleukin-27 gene and recurrent pregnancy loss in Iranian women. *Iranian Journal of Reproductive Medicine*, [online] 13(4), pp.209–214. Available at: <https://pubmed.ncbi.nlm.nih.gov/26131009/> [Accessed 1 Mar. 2023].
18. Parveen, F., Shukla, A., and Agarwal, S. (2013) Cytokine gene polymorphisms in northern Indian women with recurrent miscarriages. *Fertil. Steril.* 99, 433–440.

18. Prins, J. R., Gomez-Lopez, N., and Robertson, S. A. (2012) Interleukin-6 in pregnancy and gestational disorders. *J. Reprod. Immunol.* 95, 1–14.
19. Saijo, Y., Sata, F., Yamada, H., Kondo, T., Kato, E.H. and Kishi, R., 2004. Single nucleotide polymorphisms in the promoter region of the interleukin-6 gene and the risk of recurrent pregnancy loss in Japanese women. *Fertility and sterility*, 81(2), pp.374-378.
20. Southcombe, J. H., Redman, C. W. G., Sargent, I. L., and Granne, I. (2015) Interleukin-1 family cytokines and their regulatory proteins in normal pregnancy and pre-eclampsia. *Clin. Exp. Immunol.* 181, 480–490.
21. Su, D., Zhang, Y., Wang, Q., Wang, J., Jiao, B., Wang, G., and Wu, X. (2016) Association of interleukin-10 gene promoter polymorphisms with recurrent miscarriage: a meta-analysis. *Am. J. Reprod. Immunol.* 76, 172–180.
22. Sykes, L., MacIntyre, D. A., Yap, X. J., Teoh, T. G., and Bennett, P. R. (2012) The Th1:Th2 dichotomy of pregnancy and preterm labour. *Mediators Inflamm.* 2012, 967629.
23. Velez, D.R., Menon, R., Thorsen, P., Jiang, L., Simhan, H., Morgan, N., Fortunato, S.J. and Williams, S.M. (2007). Ethnic differences in interleukin 6 (IL-6) and IL6 receptor genes in spontaneous preterm birth and effects on amniotic fluid protein levels. *Annals of Human Genetics*, [online] 71(Pt 5), pp.586–600. doi:<https://doi.org/10.1111/j.1469-1809.2007.00352.x>.
- Thaxton, J. E., and Sharma, S. (2010) Interleukin-10: a multi-faceted agent of pregnancy. *Am. J. Reprod. Immunol.* 63, 482–491.
24. Torabi, R., Ostad Karampour, M., Mohammadzadeh, A., Arefi, S., Keramatipour, M., Zarei, S., Zeraati, H. and Jeddi-Tehrani, M. (2009). The Relationship between Polymorphisms of Blood Coagulation Factor V Gene and Recurrent Pregnancy Losses. *Journal of Reproduction & Infertility*, [online] 9(4), pp.305–316. Available at: <https://www.jri.ir/article/342> [Accessed 1 Mar. 2023].
24. Weissenbacher, E. R., Wirth, M., Mylonas, I., Ledger, W. J., and Witkin, S. S. (2014) *Immunology of the Female Genital Tract*. Springer, Heidelberg.