World of Science: Journal on Modern Research Methodologies

*Volume 2 Issue 10, Year 2023*
*ISSN. 2835-3072*
*https://univerpubl.com/index.php/woscience*

# The Role of Interleukin 18 (IL-18) Gene Regulation in Recurrent Spontaneous Abortion

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**Article Information**

Received: Aug 23, 2023
**Accepted:** Sep 26, 2023
**Published:** Oct 28, 2023

**Keywords**

IL-18 gene, recurrent spontaneous abortion, q RT-PCR, IL-18 ELISA

**ABSTRACT**

*Recurrent abortion during pregnancy is considered one of the most health issues in women around the world. Therefore, understanding the biological events and activities that are involved in the repeated loss of fetus and is the focus of many research laboratories. However, the involvement of pro-inflammatory interleukins such as IL-18 in the pregnancy loss is still poorly studies. This study hypothesized that this IL-18 gene regulation could drive the inflammation and the tissue damage in placenta and the embryo. This study aims to investigate the role of IL-18 gene in women with repeated pregnancy loss. Results shows significant increase of IL-18 gene expression when RT-qPCR was used. These results were confirmed at the IL-18 protein levels as well when using ELISA technique, indicative of a strong relation and contribution of IL-18 in recurrent abortion. Further research is suggested to better investigate the mechanistic pathways that could contribute in this effect.*

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## Introduction

Recurrent abortion is a physiological process that is wildly spread amongst pregnant women. It is estimated that one in four women suffer from spontaneous abortion, with recurrent

miscarriage (Wang and Jiang, 2022). In spite of the modern technologies, there is no direct cause of recurrent abortion that is fully discovered yet. Many reasons apply here, for example, genetic variation, immune factors, pathological and infections with parasite or bacteria (Salam Khalid Mutlag *et al.*, 20l9) (Megersa *et al.*, 2020). Recently, research showed promising evidence in the contribution of interleukins in immune response regulation, IL-18 recorded as one of the altered cytokines in abortion (Ihim *et al*., 2022).

IL-18 is a pro-inflammatory cytokine that contribute in the innate and adaptive immune response (Ihim *et al.*, 2022). It can be produced by many immune cells such as macrophages, T-cells and natural killer cells (Poznanski *et al.*, 2017). Il-18 drives cells to produce interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α (R. Mahmood arid & R. Sarhat, 2018). Furthermore, IL-18 enhance the differentiation of Th1 and Th2 (Fang *et al*., 2022, Harrison *et al*., 2015).

Studies conducted by different researchers showed IL-18 upregulation in women with repeated abortion history (Yang *et al.*, 2022, Al-Ghamdi *et al.*, 2011, Blom and Poulsen, 2012). In this regard, IL-18 affects the balance between Th1/Th2 as a result of programmed cell death in the trophoblast cells, thus, triggering cells to produce other cytokines (Nakanishi, 2018, Tan and Tang, 2021). These are correlated with tissue damage which intern leads to the fetus loss. Moreover, genetic factors that are linked to the IL-18, also appear to be involved in embryological developments.

Taking together the role of IL-18 in the pathogenesis of abortion, understanding the mechanisms underlying its regulation and its potential as a therapeutic target is of great interest. This study aims to summarize the available knowledge on the role of IL-18 in the regulation of immune responses during pregnancy and its potential as a therapeutic target for preventing pregnancy loss.

**Materials and methods:**

Study Design: The study included 40 women aged 20-40 years who attended the Gynecological and pediatric Hospital in Kirkuk city. Additionally, a control group of ten women with at least two previous normal pregnancies were taken.

**Sample Collection:** A volume of 3m1 of blood were collected from each enrolled woman by vein puncture using a syringe. The blood samples were immediately transferred into sterile test tubes and centrifuged at 3000 rpm for 15 minutes. After centrifugation, the obtained sera were carefully aspirated using air automatic micropipette and transferred to Eppendorf tubes. The serum samples were then stored in a -20 freezer for subsequent analysis.

**1. (PCR) amplification**

IL-18 gene expression was investigated using real time PCR technique to ensure the quality

## 2. Sample preparation for expression study

Cervical swabs collected from women in this study, RNA were extracted from swabs using (Promega kit). In order to quantify the mRNA transcripts of IL-18 gene, RNA was first transformed to complementary DNA (cDNA) using (promega ) which were used for the RT-PCR. Primers sequences that were used in this study shown in table 1.primers were obtained from macrogene and β-globin primers were used as a housekeeping gene.

**Table 1: Primer designs**

| Primer name | Forward Primer (5' – 3') | Reverse Primer (5' – 3') |
|---|---|---|
| IL-18 | '5-GATAGCCAGCCTAGAGGTATGG-3' | '5-CCTTGATGTTATCAGGAGGATTCA-3' |
| Bglobin | '5-ACACAACTGTGTTCACTAGC-3' | '5-CAACTTCATCCACGTTCACC-3' |

## 3. Real time-PCR was performed following manufactures instructions (promega).

The RT-qPCR assay was performed using 2x master mix (promega). Into each PCR tube, 19µl of master mix was added in addition of 1µl cDNA to the final volume of 20µl. The Go Tag master mix contained 10µl, one microliter from each forward and reverse primer, and 7.27µl nuclease free water, Go sript RT mix 0.4µl, CXR Dye 0.33µl. The PCR reaction was performed in the Qtower' G touch PCR from analytika jena. The PCR conditions were first, reverse transcription at 37ºC for l5mint. Second reverse transcription inactivation 95ºC for l0 mint. 40 cycle denaturation of 95ºC for 10 sec., annealing 60ºC for 30sec, extension 72ºC for 30sec.

**Quantification method:**

Quantified method was used to quantify real-time RT-qPCR results (Livak and Schmittgen, 2001).

**Immunological study:**

(Sunlong Elisa kit SL0980Hii) was used in order to determine IL-18 protein levels.

Dilution of standard: standards were prepared following the procedure provided with the kit and slandered concentration were (90, 60, 30, 15, 7.5)pg/ml. In to each well (standards wells) 50 µl was added, then 40µl of sample dilution buffer mixed with l0µl of the serum samples and the plate were then incubated for 30 minutes at 37ºc. after the incubation time the plate was washed five times and then the washing buffer was aspirated and 50µlof the HRP-conjugate reagent was added to each well except the blank wells and incubated for 30 minutes at 37 ºC followed by five times washing processes. After that, 50µl: 50µl of chromogen solution A to chromogen solution B were added to each well. After a gentle shake, the plate covered with foil and incubated for 15 minutes. Stop solution 50 µl was added to each well to stop the reaction and the plate was then placed in the ELISA plate reader, the absorbance was read at

450nm. the standard curve was conducted and the linear regression fit was performed to ensure the experiment and to measure IL-18 concentrations in the samples accordingly.

**Statistical analysis:** Graph pad prism software was used and paired t- test was used unless otherwise stated.

**Results:**

In this study, results show the present of IL-18 gene in the conventional PCR product (figure not shown). After this q RT- PCR was performed in order to investigate levels of IL-18 in RNA transcripts in recurrent spontaneous abortion cases as shown in figure 1 which showed significant increase ($p<0.001$) in IL-18 in mRNA concentrations when compared with controls.

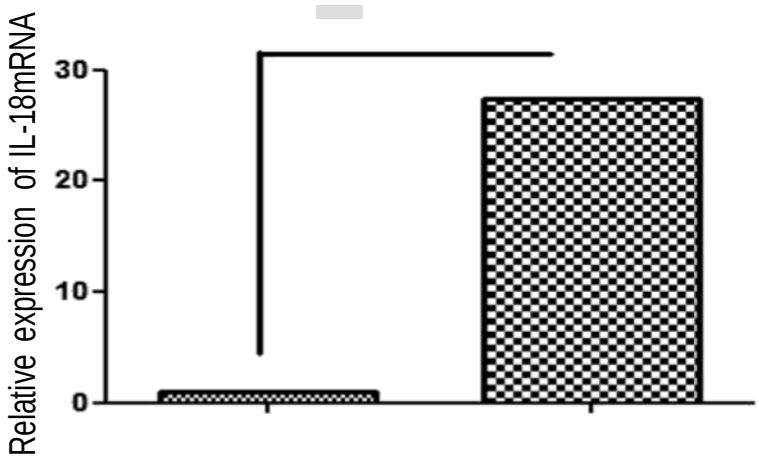


**Figure 1: The effect of IL-18 gene expression in recurrent abortion.**

IL-18 mRNA were normalized using β-globin as a reference gene and the fold change was measured. *** represents significance when $p<0.001$ paired t-test.

The gene expression results were confirmed at the protein levels which in turn investigated using ELISA technique. The results conducted by this research revealed significant elevation in levels of IL-18 proteins in patients with spontaneous abortions when compared with controls were means ±SE recorded 0.27±0.002 in patients versus 0.138±0.007 in controls as shown in figure 2.

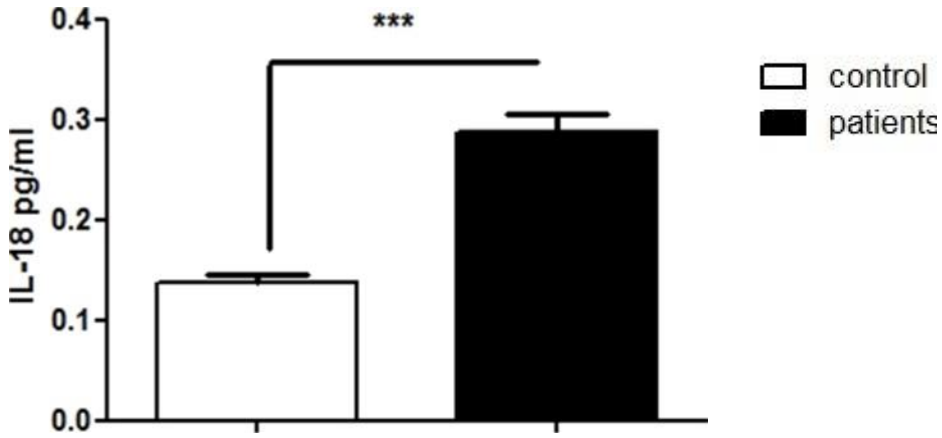


**Figure 2: Elevated IL-18 protein expression in patients with recurrent abortion.**

Serum samples were collected from control group and patients and IL-18 protein concentrations were detected as described above in the methods section. Error bars represents Mean+SE. P<0.001.Absorbance read at 450 nm.

**Discussion:**

IL-18 is produced by many immune cells as mentioned earlier and is so called works as a bridge between all immune cells because it's produced by the latter cells and also induces other immune cells such as T-helper and natural killer cells to produce interferon gamma IFN-γ. Results conducted in this study confirms the role of IL-18 in one of the problematic disorders that faces pregnancy which is recurrent abortion. Therefore, understanding the mechanistic pathways underlying this effect is considered a new approach in the immunotherapy of abortion. This effect may be due to the signaling pathway underlying the IL- 18 which is activated when binding with its receptor complex IL-18 RC which is composed of alpha and beta subunits. In this regards the downstream pathways such as MAPK and NFκB are mostly activated (Yasuda *et al.*, 2019, Rex *et al.*, 2020). The current research suggests a possible activation of these two pathways in women that have a history of spontaneous abortion indicative of a strong role of IL-18 gene regulation that can be further studied at the signal transduction level. Schematic representation of the signaling pathways that is activated upon IL-18 activation is showed in figure 3.

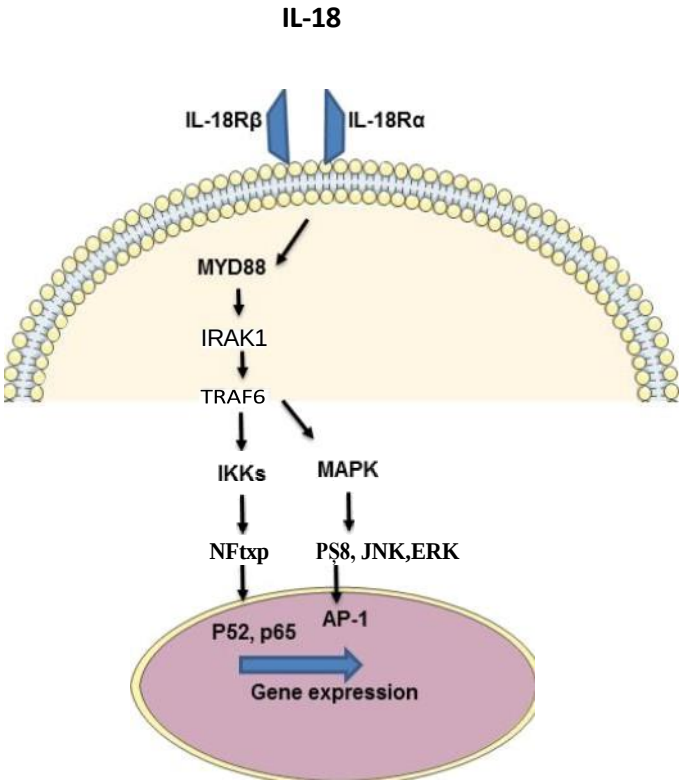


**Figure 3: Suggested signaling pathways that could be activated upon increased IL-18.**

Increased levels of IL-18 gene and protein could be due to the gene polymorphism which in turn contribute to the susceptibility of recurrent pregnancy loss (Salimi *et al.*, 2020).Therefore, they drive a pro-inflammatory scenario that affects surrounding tissues especially the placenta and the fetal sac leading to the loss of the embryo (Löb *et al.*, 2021).

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