Study Imbalance of Some Cytokines and Vaginitis Agents in Women with Recurrent Abortion Women

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Abstract

Background: Cytokines play a key role in the success or failure of pregnancy. On the other hand, a plethora of bacterial and viral infectious agents have been associated with an increased risk of recurrent abortion (RA). **Objective:** This search focused on the association between cytokines imbalance and vaginitis infection with RA cases. **Materials and Methods:** A case-control study was designed on 65 aborted women attending AL-Hakeem Hospital in AL-Najaf province suffering from at least two or more aborting cases and 40 healthy delivery women as the control group. Two milliliters of blood samples were collected from all participants to measure interleukin-17 (IL-17), interleukin-23 (IL-23), and interleukin-10 (IL-10) levels by enzyme-linked immunosorbent assay (ELISA). High vaginal swabs were taken from all patients, cultured on differential media for 24 h at 37°C, and then positive bacterial growth re-culturing to obtain a pure single colony. **Results:** The results showed that the mean age of aborted women was 29.8 vs 27.4 years, and 33% have a positive family history; according to bacterial infection, the results found that positive bacterial growth appeared in 23 (35.3%) of high vaginal swabs (HVSs), while 42 (64.7%) gave negative results. The prevalence of bacteria was as follows: *E. coli* was isolated from 8/23 (34.8%) patients, followed by *P. aeruginosa* from 6/23 (26.0%). The results confirmed that IL-17 and IL-23 were higher in aborted women (126.4 ± 34.8, 84.9 ± 23.7 pg/ml) compared to delivered women (37.5 ± 11.2, 26.8 ± 9.6 pg/ml). Also, infected women had highly elevated levels of both cytokines (180.2 ± 28.5, 104.7 ± 43.4 pg/ml) compared to non-infected women (72.8 ± 24.9, 67.3 ± 18.5 pg/ml), while IL-10 decreased in aborted women compared to healthy women. **Conclusion:** Cytokines imbalance leads to impaired state of pregnancy, and there is a highly significant relationship between IL-17, IL-23, and IL-10 levels with abortion, especially in women who suffer from bacterial vaginitis

Keywords: Cytokine, recurrent abortion, Th1/Th2/Th17, vaginitis

Introduction

Abortion is a significant health problem that causes medical distress to the mother in addition to fetal loss, especially when it recurs. It has obstetric, psychologic, and economic implications.[1] Recurrent pregnancy loss (RPL) refers to two or more consecutive pregnancy losses before the 20th week of gestation from the last menstrual period, occurring in approximately 1–5% of women of reproductive age.[2]

Pregnancy is accompanied by dramatic changes in the maternal immune system to allow the coexistence of a genetically distinct fetus. These changes include the mother and placental barrier that helps to suppress the maternal immune response. The proper homeostatic balance between Th1 and Th2 cytokines is important for a stable maintenance of pregnancy and other reproductive events.[3] Dysregulated immunity has been proposed as a potential mechanism underlying recurrent spontaneous abortion (RSA).

The ability of embryos to avoid immune rejection is partially facilitated by the presence of fundamental

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cytokines in the peripheral blood and/or at the maternal-fetal interface.[4]

The interleukin-17 (IL-17) family consists of multiple related cytokines ranging from IL-17A to IL-17F and is a pro-inflammatory cytokine that plays an important role in immunoinflammatory responses. The possible role of IL-17 in spontaneous abortion has received increasing interest in recent years.[5]

Interleukin-10 (IL-10) is a Th2 cytokine that suppresses the Th1 immune response by reducing the production of TNF-α, IFN-γ, and interleukin-1 (IL-1).[6] IL-10 interacts with different factors and cells, playing a central role in pregnancy by modulating the maternal immune response and promoting embryo development by maintaining immunological tolerance.[7]

Bacterial vaginitis has been closely related to abortion and premature birth, alterations in the uterine microbiota usually result in altered cytokine levels.[8] This search was designed to detect the correlation between IL-10, IL-17, IL-23, and bacterial infection in RSA.

Materials and Methods

Patients

A case-control study was designed with 65 RSA women who were admitted to AL-Hakeem Teaching Hospital. Their ages ranged from 18 to 44 years and 40 apparently healthy delivered women as the control group with an age range between 20–44 years in the period from March–July 2022.

All physiological structural, hormonal, and chronic diseases such as diabetes and hypertension that lead to abortion or abortion with known cause were excluded, while all aborted women with more than two aborted cases were included.

Two ml of blood were taken from all participants and placed in gel tubes to separate serum for measuring IL-17, IL-23, and IL-10 concentration by ELISA assay. Also, high vaginal swabs (HVSs) were taken from all aborted women by physicians to culture on blood and MacConkey media and incubated at 37°C for 24 h for isolation bacterial agents. The bacterial isolates were identified using biochemical tests and confirmed by Vitek system.[9]

Ethical approval

This study was conducted in accordance with ethical principles in the Declaration of Helsinki and approved by Ethical Committee of Kufa Faculty of Science document number 8379 in 11/6/2022. Approval and permission were taken from Al-Najaf health precedence. Members of all groups were informed and instructed about the aims of the study, and their verbal acceptance was obtained before taking samples and ruling out any verifiable cause of missed abortion before inclusion into the study.

Results

Demographic characteristics

A case-control study included 65 women with a history of two or more spontaneous repeated abortions. The age of the women ranged from 18–44 years, with a mean age of 29.8 ± 9.6 years, whereas 40 apparently healthy women as the control group with ages ranged from 20–44 years, with amean age of 27.4 ± 8.5 years. The results demonstrated that there was no significant difference between the women in the aborted and control groups depending on age, and all data about family history and number of abortions were shown in Table 1.

The results illustrate that positive bacterial growth appeared in 23 (35.3%) of HVS, while 42 (64.7%) gave negative results. The prevalence of bacteria was *E. coli* isolated from 8/23 (34.8%) patients, followed by *P. aerogenosa* in 6/23 (26.0%) isolate, *S. aureus* in 5/23 (21.7%) patients, and *Proteus spp* in 4/23 (17.4%) aborted women, as shown in Figure 1.

Estimation of IL-17 and IL-23 levels in patients and controls

The results indicated a significant difference in the serum levels of IL-17 and IL-23 between aborted and healthy women with highly increased in aborted women (126.4 ± 34.8, 84.9 ± 23.7 pg/ml) compared to delivered women (37.5 ± 11.2, 26.8 ± 9.6 pg/ml) as shown in Figure 2. Additionally, there was a highly elevated in IL-17 and IL-23 serum levels in infected aborted women (180.2 ± 28.5, 104.7 ± 43.4 pg/ ml) compared to non-infected women (72.8 ± 24.9, 67.3 ± 18.5 pg/ ml).

Estimation of IL-10 levels in patients and controls

The results show that the serum level of IL-10 decreased (11.8 ± 5.4 pg/ml) in aborted women compared to the healthy control group (26.5 ± 10.0 pg/ml) with a significant

Table 1: Demographic distribution of aborted and healthy women

Parameters	Aborted women	Healthy women	*P* value
Range of years	18–44	20–44	
Mean	29.8	27.4	0.0351
S.E	9.6	8.5	
Total no.	65	40	
Family history			
Yes	21 (32.3)		0.001
No	44 (67.7)		
No. of abortion			
2 abortion	34 (52.3)		0.0001
3–4 abortion	25 (38.4)		
5 or more	6 (9.3)		
+ve bacterial growth	23 (35.3)		
−ve bacterial growth	42 (64.7)		

difference. Also, the results indicated a decreased serum level of IL-10 in both infected and non-infected aborted women (14.1 ± 6.3, 9.5 ± 2.7 pg/ml), respectively, compared to the healthy group [Figure 3].

Discussion

The results showed that the mean age of patients was 29.8 years, which is consistent with a local study,[10] which observed a range of patients aged 20–49 years with a mean age of 24.26 ± 1.84. Additionally, 54% of patients had a family history of recurrent abortion (RA). Other studies confirm that advancing maternal age is associated with an increased risk of miscarriage as[11] suggest that aborted women were over 40 years of age and consider risk factor for RA. Also, the study[12] noticed that the age of aborted women ranged between 19 and 45 years (median 30 years) and a high rate had a family history of RA (0.0003).

This study found that about four genera of pathogenic bacteria are associated with HVS infection. These results are nearly similar to a local study in Iraq,[13] which showed that the ratios of the isolated bacterial samples were *E. coli* (54.4%), *S. agalactiae* (17%), *S. aureus* (12%), Klebsiella (8.5%), *P. aeruginosa* (5.3%), and Proteus (1%) and concluded that bacterial infections increase the risk of miscarriage. The study[14] found that *E. coli* and *S. aureus* were main causes of urinary tract infection, which are important factors in abortions and premature births.

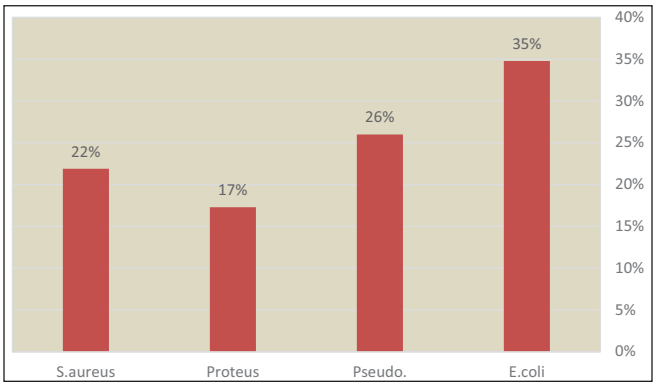

Figure 1: Bacterial frequency in recurrent abortion women

Bacteria can spread hematogenously to the fetoplacental unit and cause abortion, usually late in gestation[15] found that the main causative agents of HVS were *E. coli* (31.5%), *Enterobacter* spp. (18.4%), *K. pneumoniae* (12.5%), *E. faecalis* (11.6%), *S. aureus* (10.2%), and *P. aeruginosa* (8.4%).

This result revealed an important role for IL-17 and IL-23 cytokines in RA. Cytokines have multifunction including regulation of embryo implantation, placental development, cytotrophoblast proliferation, vascular remodeling, trophoblast invasion, cell death, and the induction of embryo tolerance in the uterus.[16,17] They mentioned that the pro-inflammatory cytokine levels related to Th1 (IL-1, IL-17, IL-12, and IL-23) immune response were significantly higher, and Th2-related cytokines (IL-10, IL-6, IL-4, and IL-33) were significantly lower in women with a history of recurrent miscarriage compared to normal pregnant controls with a positive relationship between Th cells and pregnancy loss andthat this might help to adopt the preventive and therapeutic methods for solving the recurrent miscarriage issues in patients with no other reason for their problem.

Similar to our results, the study[18] found that IL-17 serum levels were significantly increased in non-pregnant women with RPL compared to fertile women, suggesting that women with RPL have a propensity of pro-inflammation via Th1 and Th17 immunity and decreased immune

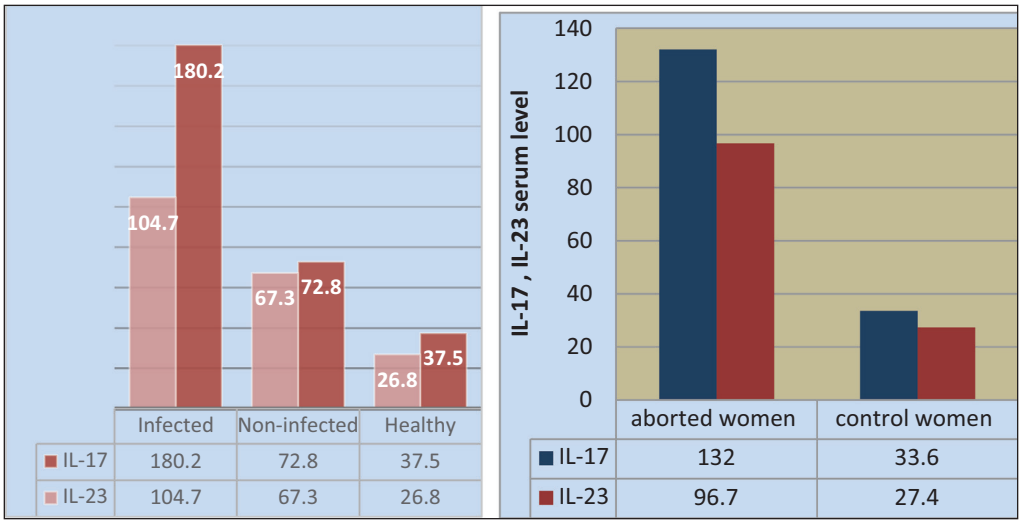

Figure 2: IL-17 and IL-23 levels in A-aborted and healthy women, B-infected and non-infected aborted women

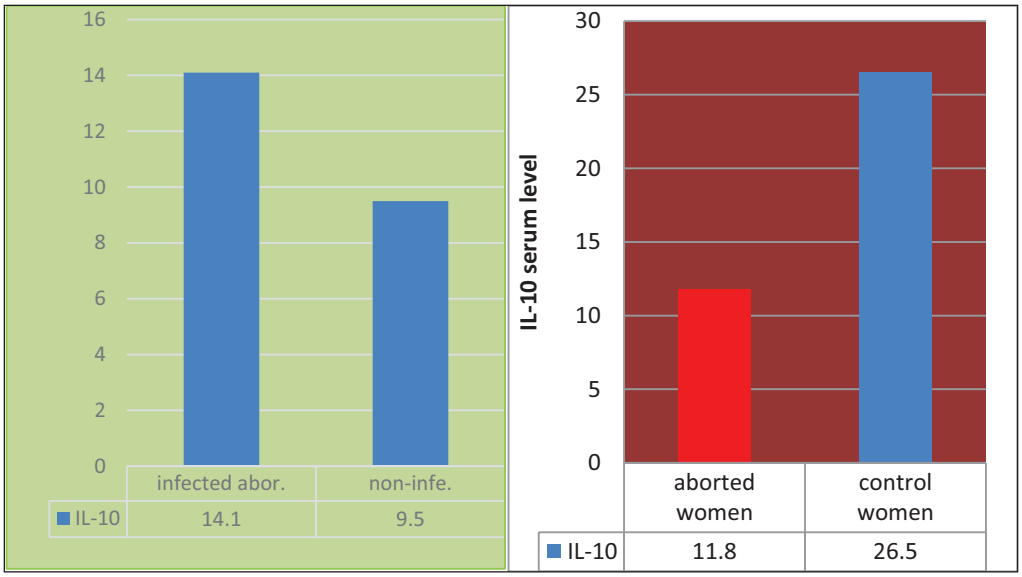

Figure 3: IL-10 levels in A-aborted and control healthy women, B-infected and non-infected aborted women

regulatory function by Foxp3$^+$ regulatory T cells that produce IL-10. Also, the study[19] reported higher Th17 cells and IL-17 in abortion women and observed that a higher expression of IL-17 seems to be involved in the pathogenesis of endometriosis, which may cause reproductive failure, including RM. Similarly,[20] they hypothesized that higher Th17 cell counts and elevated Th1/Treg ratio would trigger an inflammatory response that would ultimately aid RA development.

In this search, IL-23 increased in aborted women. This may be related to the explanation by the study,[21] who stated that when the IL-23 level is increased above the initial levels, Th17 cells expand dramatically, resulting in an imbalance of Treg/Th17 cells and consequential embryo rejection. Also confirmed by the study,[22] who suggested that both peripheral blood and decidual levels of IL-23 were considerably higher in RPL women than in normal pregnancies, indicating that IL-23 may be involved in the pathophysiology of RPL.

The action of IL-23 induces the formation and expansion of Th17, which leads to the release of pro-inflammatory IL-17, and this underlines the importance of dendritic cells presenting the antigens in the induction of inflammatory processes via the expression of IL-23 and consequently IL-17.[23]

IL-23 increases the local concentration of matrix metallopeptidase 9 (MMP-9) and stimulates angiogenesis, which makes it an extremely important element in a proper implantation. However, clinical studies showed increased expressions of IL-23 in patients with RPL.[24]

According to bacterial infection, the results confirmed elevated levels of IL-17 and IL-23 in infected aborted women, and this is consistent with the study,[25] who indicated that pathogenic *E. coli* infection was also distinctly different between the control and RSA groups. It has been reported that *E. coli* infection of the female reproductive tract is an important factor contributing to severe uterine inflammation and disturbance of the profile of cytokines, including leukotrienes, IL-17, TNF, and IL-6. Also, the study[26] found that abundances of several bacterial infections were statistically significantly negatively related to the expression levels of Th1/Th2/Th17 cytokines and led to increased IL-17, IL-6, and IL-22 serum levels. This may be a result of shifts in the microbial community structure resulting in changes in metabolic and inflammatory status .The study[27] revealed that the concentration of IL-17A in RA and RA with microbial infection was elevated versus healthy women, with a significant difference ($P < 0.05$).

Kaislasuo *et al.*[28] mentioned that women with normal pregnancies show a more anti-inflammatory profile (IL-10 and IL-4) compared to both non-pregnant women and those with a diagnosed miscarriage. They observed that the success of implantation and pregnancy depends on the maintenance of a balance between pro- and anti-inflammatory signals and is a process regulated by both the maternal local environment and the implanting blastocyst. Also, the study[29] confirmed that IL-10 concentrations in normal pregnancies were significantly higher than in pregnancies ending in first-trimester loss and provided evidence in support for the relevance of the shift from inflammation to anti-inflammation necessary for the maintenance of the pregnancy. In line with this,[30] they found that IL-10 serum levels were significantly decreased in women with RPL compared to normative pregnant women.

An abnormal Th1-type cellular immune response is a recent hypothesis for immunological reproductive failure in women. In cases of pregnancy loss, deregulation of

the mother's immune system could be responsible for the failure, where the implanting embryo is recognized as foreign and is thus rejected.[31] The change in the cytokine balance synthesis in favor of those synthesized by Th2 cells with an increase of IL-6 and IL-10 secretion is considered essential for maintaining a normal pregnancy.[32]

Conclusion

Cytokines imbalance leads to impaired state of pregnancy, and there is a highly significant relationship between IL-17, IL-23, and IL-10 levels in abortion, especially in women suffering from bacterial vaginitis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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