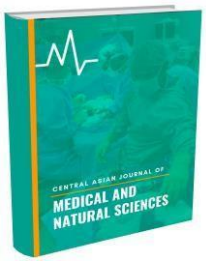

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Article

Immunological Study of Unexplained Recurrent Spontaneous Abortion

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Abstract: Miscarriage poses a significant health concern, with a substantial proportion attributed to immunological factors, particularly maternal immune responses towards the fetus. This study aimed to identify immunological biomarkers associated with unexplained recurrent abortions. Vaginal swabs and blood samples were collected from 100 women with recurrent abortions and 80 control women with previous normal pregnancies. Analysis revealed significantly higher T-helper 1 cytokine levels and lower T-helper 2 cytokine levels in women with recurrent abortions compared to controls. The imbalance in T-helper cell responses, indicated by elevated Th1:Th2 ratios and IFN-γ:IL-4 and IFN-γ:IL-10 ratios, suggests a potential mechanism underlying pregnancy loss. These findings underscore the importance of immune modulation in pregnancy outcomes and highlight the need for further research to elucidate underlying mechanisms and develop targeted interventions for optimizing maternal-fetal tolerance.

Keywords: RSA, T helper, IL-37, Vaginitis

1. Introduction

One of the most significant health issues with human reproduction is miscarriage. Fifteen percent of pregnancies end in spontaneous pregnancy loss. It is characterized as a fetal weight of less than 500 g or a fetal loss occurring before 20 weeks of gestation. A miscarriage occurs in around 12–15% of cases for women throughout their lives. About 5 percent of women lose two pregnancies in a row, and 1 percent lose three or more [1,2]. Although the prevalence of 0.5–5%, the significance of infectious illnesses in recurrent miscarriages remains unclear [3].

Candidate infectious disorders include measles, CMV, rubella, herpes simplex virus, Listeria monocytogenes, and Toxoplasma gondii. The following processes, including direct infection of the uterus, fetus, placenta, placental insufficiency, chronic endometritis, endocervicitis, amnionitis, and intrauterine miscellaneous infections, can result in pregnancy loss due to infectious disorders [4]. Abortion may be caused by mycoplasma, ureaplasma, chlamydia trachomatis, L. monocytogenes, or HIV infections.

In an immunocompromised patient, the most significant risk factor for subsequent miscarriages resulting from the acute stage is a chronic illness. For some individuals, evaluation for persistent infections may be necessary. In general, preventing infectious infections is not required, but it can help RPL patients feel less anxious [5]. One theory for the reason of unexplained pregnancy loss is immunological rejection of the fetus by the

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mother immune system as a consequence of detection of maternal antigens, which leads to aberrant immune cells and cytokine production [6]. Historically, cytokines have been categorized into families based on their immune cell of origin and the impact they have on the immune system [7]. The primary immune cells responsible for producing cytokines are CD4+ T-helper cells, which can be further subdivided into functional groups according to the cytokines they produce.

T-helper 1 (Th1) cells are the primary producers of interferon-gamma (IFNg), IL-2, and tumor necrosis factor beta (TNFb), which are the primary mediators of the cell-mediated immune response. Interleukin (IL)-4, IL-5, IL-6, and IL-10 are produced by T-helper 2 (Th2) cells and are the primary effectors of antibody-mediated humoral responses [6]. The two most significant of these functions are angiogenesis and trophoblastic invasion in placental proliferation. It is impossible to exclude out these cytokines' harmful consequences, though. Generally speaking, Th1 cytokines, such as TNF-α, are factors that cause abortions [8,9].

The pro-abortogenic effects of TNF-α have been attributed to a number of mechanisms, including trophoblast invasion and placentation and the induction of pro-apoptotic gene expression in human fetal membranes, which in turn speeds up membrane degradation and raises the risk of premature rupture. TNF-α has also been reported to indirectly promote miscarriage through NK cell or macrophage activation [7,9]. Because it is the seventh member of the IL-1 family to be found and shares its structural pattern, interleukin-37, a member of the IL-1 superfamily, was first known as IL-1 family member 7 (IL-1F7). Most cytokines in the IL-1 family, including IL-1, IL-18, and IL-33, are generated in response to tissue injury and pathogen infection, and they have pro-inflammatory properties[10]. On the other hand, cytokines that promote inflammation are suppressed by IL-37 both in terms of production and function. IL-37 production is stimulated and triggered throughout the inflammatory process in a way similar to other cytokines in the IL-1 family.

Numerous studies have shown that IL-37 is an important regulator of inflammation and has been shown to significantly suppress inflammation and innate immunity in a number of disorders. It's possible that IL-37 inhibits the inflammatory process and mediates the synthesis of other cytokines, such IL-6[11]. By inhibiting Toll-like Receptor (TLR) signaling through the down-regulation of NF-κB brought on by either TLR2 or TLR4, IL-37 effectively suppresses inflammatory disorders[12].

Apart from impeding Dectin-1 signaling subsequent to pathogenic antigen recognition in APC, it also results in the suppression of mTOR signaling, which in turn inhibits pro-inflammatory cytokines such IL-1, IL-6, IL-17, TNF-α, and IFN-γ[13]. As a result, elevated levels of many inflammatory cytokines during pregnancy may play a key role in the emergence of infection, including miscarriage[14]. Consequently, the preservation of pregnancy depends critically on the anti-inflammatory cytokine IL-37[11]. The purpose of this study is to identify the immunological biomarker causes in women with RSA that is not explained.

2. Materials and Methods

The period of this case-control research was from early November 2023 to late February 2024 in Mosul City.

In Mosul General Hospital's obstetrics and gynecology department, 100 women between the ages of 20 and 35 who had recurrent abortions and viable singleton pregnancies between 5 and 12 weeks were included in the research.

As controls for the research, 80 women with two or more healthy pregnancies and no history of abortion were also included. Each patient completed an informed consent form and got comprehensive research information prior to taking part in the trial :

1. Between the ages of 15 and 45.
2. A singleton pregnancy that is viable at five to twelve weeks.

The exclusion criteria :

1. Obesity.
2. Ectopic or molar pregnancy.
3. Patients with chronic diseases, hypertension, diabetes, and thyroid disorders.
4. History of using any pertinent hormone therapy either before or during the present pregnancy.
5. Past cervical cerclage and genitourinary infection symptoms in the clinical setting Isolation of bacteria from the vagina.

Vaginal swabs were collected from each women enrolled in the study with the following steps:

1. The swab package had been partially opened.
2. The swab was gently twisted for 10 to 30 seconds after being introduced into the vagina 2 inches (5 cm) past the introitus.
3. The swab was removed without contacting the skin once it made contact with the vaginal walls and began to absorb moisture.
4. The lab received the swabs an hour after they were collected.

Blood collection using a five milliliter syringe, each woman had her vein punctured to get five milliliters of blood. Using the Enzyme-linked immunosorbent assay (ELISA) technique, blood samples were placed into plane tubes, allowed to clot for 30 minutes at 37 °C, and centrifuged for 15 minutes at 3000 rpm. The sera were then aspirated and transferred into Eppendorf tubes for the determination of T helper 1 cytokines (IL-2, TNF-α, and IFN-γ), T helper 2 cytokines (IL-4, IL-5, IL-6, and IL-10) and IL-37.

Statistical analysis.

The IBM SPSS ver 23.1 statistics application was used to conduct computerized statistical analysis. To do the comparison, T-Test and Chi-square (X2) were used. P-values less than 0.01 were regarded as extremely significant, whereas P-values more than 0.05 were regarded as statistically non-significant. The result was deemed statistically significant if the P-value was greater than 0.05.

3. Results

In the present research, pregnant women with previous abortions exhibit higher mean levels of IL-2 (247.3 pg/ml vs. 217.4 pg/ml), TNF-α (2293.7 pg/ml vs. 2033.4 pg/ml), IFN-γ (915.0 pg/ml vs. 784.6 pg/ml), and the overall T-helper 1 mean (1152.1 pg/ml vs. 1007.3 pg/ml) compared to the control group. Hence, pregnant women with previous abortion history had the highest mean T-helper 1 cytokine levels among the studied groups with p-values of 0.001.

Table 1: Means of T-helper 1 cytokines among the studied groups.

T-helper 1 cytokines (Mean±SD)	Pregnant women		P-value*
	With previous abortion	Control group	
IL-2 (pg/ml)	247.3±43.8	217.4±38.9	0.001
TNF-α (pg/ml)	2293.7±509.5	2033.4±589.8	0.001
IFN-γ (pg/ml)	915.0±101.8	784.6±129.1	0.001
T helper 1 mean	1152.1±164.9	1007.3±80.54	0.001

* P-value was calculated using an unpaired T-test.

In the current study, pregnant women with previous abortions exhibit lower mean levels of IL-4 than normal control women (206.0 pg/ml vs. 245.9 pg/ml), IL-5 (109.3 pg/ml vs. 135.43 pg/ml), IL-6 (879.65 pg/ml vs. 1940.1 pg/ml), IL-10 (464.7 pg/ml vs. 525.2 pg/ml), and the overall T-helper 2 mean (414.9 pg/ml vs. 671.2 pg/ml) compared to the control group (p=0.001).

Table 2: Means of T-helper 2 cytokines among the studied groups

T-helper 2 cytokines (Mean±SD)	Pregnant women		P-value
	With previous abortion	Control group	
IL-4 (pg/ml)	206.0±33.24	245.9±10.01	0.001
IL-5 (pg/ml)	109.3±50.25	135.43±29.81	0.001
IL-6 (pg/ml)	879.65±662.0	1940.1±574.19	0.001
IL-10 (pg/ml)	464.7±132.2	525.2±100.48	0.001
T helper 2 mean	414.9±143.1	671.2±228.5	0.001

Pregnant women who had previously had abortions showed a substantially higher Th1:Th2 ratio (2.96±0.60) in the current research than did the control group (1.87±0.59) (p=0.013), suggesting that this group's T-helper 1 to T-helper 2 cytokine ratio was greater. Regarding the IFN-γ:IL-4 ratio, there was no statistically significant difference found between the two groups (p=0.081). The results for the control group were 4.42±0.53 and 4.52±0.69 for pregnant women who had previously had an abortion. In contrast, the control group's IFN-γ:IL-10 ratio (4.02±2.49) was substantially higher than that of the pregnant women who had previously had an abortion (2.17±0.76) (p=0.001), as shown in Figure 1.

Table 8 : Means of T-helper 1/2 cytokines rations among the studied groups

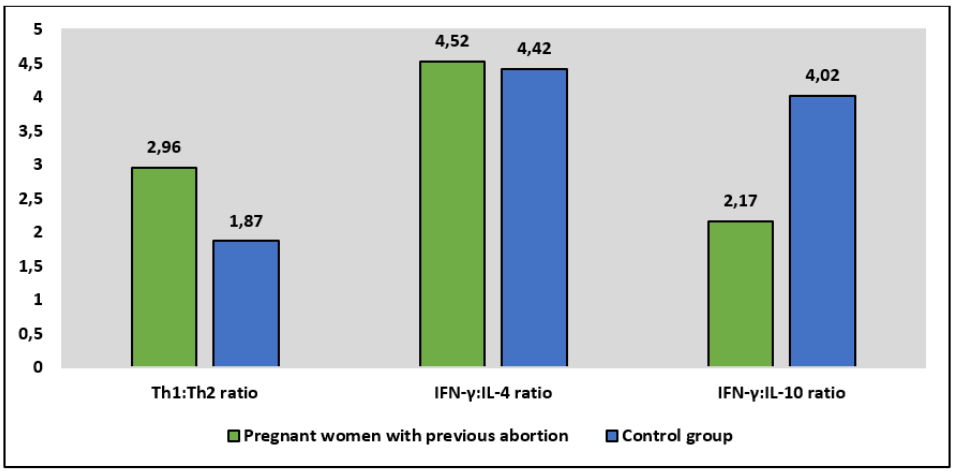

Figure 1: Means of T-helper 1/2 cytokines rations among the studied groups

In the current study, Pregnant women with previous abortions exhibited a significantly lower mean level of IL-37 (29.55±22.26 pg/ml) compared to the control group (65.04±20.02 pg/ml) (p=0.001), Figure 2.

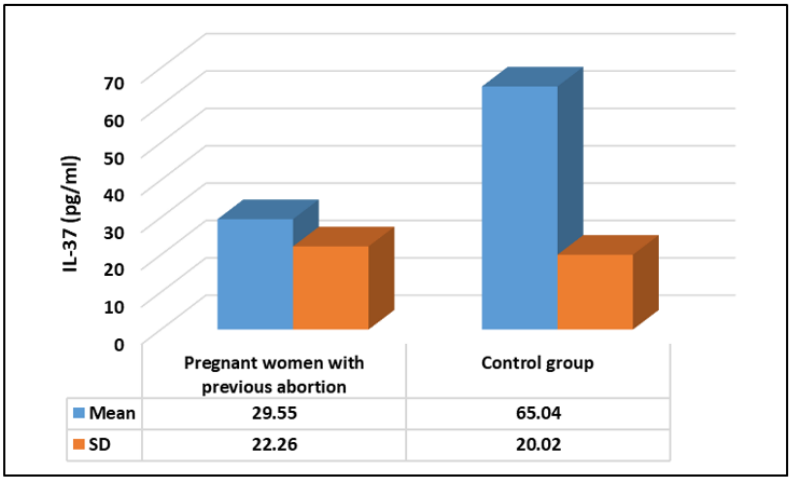

Figure 2 : Means of IL-37 in the studied groups
P-value : 0.001

Table 4 displays the mean concentrations of IL-37 and T-helper cytokines among pregnant women with previous abortions about vaginitis, categorized by positive and negative culture results. In terms of T-helper 1 cytokines, including IL-2, TNF-α, and IFN-γ, no significant differences were observed between pregnant women with positive and negative culture results (p>0.05 for all). However, for T-helper 2 cytokines, pregnant women with positive culture results demonstrated significantly lower mean levels of IL-5 (85.9±40.78 pg/ml) compared to those with negative culture results (132.6±48.2 pg/ml) (p=0.032), and significantly lower mean levels of IL-6 (831.6±599.6 pg/ml) compared to those with negative culture results (927.6±722.1 pg/ml) (p=0.022). Moreover, pregnant women with positive culture results exhibited significantly lower mean levels of IL-37 (27.17±20.6 pg/ml) compared to those with negative culture results (34.92±23.9 pg/ml) (p=0.001).

Table 4 : Means of IL-37 and T-helper cytokines in Pregnant women with previous abortion about vaginitis

cytokines (Mean±SD)		Pregnant women with previous abortion		P-value
		Positive culture	Negative culture	
T-helper 1 cytokines	IL-2 (pg/ml)	247.6±44.7	247.0±43.9	0.87
	TNF-α (pg/ml)	2282.3±587.7	2305.0±422.8	0.036
	IFN-γ (pg/ml)	909.2±96.26	914.7±108.0	0.041
T-helper 2 cytokines	IL-4 (pg/ml)	209.5±37.87	212.5±27.81	0.067
	IL-5 (pg/ml)	85.9±40.78	132.6±48.2	0.032
	IL-6 (pg/ml)	831.6±599.6	927.6±722.1	0.022
	IL-10 (pg/ml)	463.13±118.5	466.3±145.7	0.71
IL-37 (pg/ml)		27.17±20.6	34.92±23.9	0.001

In the current study, in terms of T-helper 1 cytokines, women who had subsequent abortions exhibited higher mean levels of IL-2, TNF-α, IFN-γ, and the overall T-helper 1 mean compared to those who had normal deliveries. Similarly, for T-helper 2 cytokines, women with subsequent abortions had lower mean levels of IL-4, IL-5, IL-6, IL-10, and the overall T-helper 2 mean compared to those with

normal deliveries. Furthermore, the Th1:Th2 ratio, IFN-γ:IL-4 ratio, and IFN-γ:IL-10 ratio were all significantly higher in women with subsequent abortions compared to those with normal deliveries, indicating an imbalance in T-helper cell responses favoring Th1 cytokines in the former group. Interestingly, the mean level of IL-37 was also significantly higher in women with subsequent abortions compared to those with normal deliveries (p=0.001).

Table 5: Means of IL-37 and T-helper cytokines in Pregnant women with previous abortion about pregnancy outcomes

Immunological parameters		Pregnant women with previous abortion		P-value
		Had subsequent another abortion (n:76)	Had subsequent normal delivery (n:24)	
T-helper 1 cytokine	IL-2 (pg/ml)	268.4±14.45	176.7±35.3	0.001
	TNF-α (pg/ml)	2318.1±77.6	2162.9±836.4	0.001
	IFN-γ (pg/ml)	934.0±105.0	920.3±94.4	0.001
	T helper 1 mean	1204.2±47.3	1120.0±275.2	0.001
T-helper 2 cytokines	IL-4 (pg/ml)	192.9±8.6	247.7±47.2	0.001
	IL-5 (pg/ml)	119.3±51.0	129.3±31.19	0.001
	IL-6 (pg/ml)	531.3±108.8	965.6±422.9	0.001
	IL-10 (pg/ml)	411.0±111.2	478.6±74.23	0.001
	T helper 2 mean	338.6±13.3	652.5±86.8	0.001
Th1:Th2 ratio		3.25±0.21	2.06±0.49	0.001
IFN-γ:IL-4 ratio		4.73±0.42	3.88±0.96	0.001
IFN-γ:IL-10 ratio		1.91±0.61	2.99±0.62	0.001
IL-37 (pg/ml)		27.17±20.6	34.92±23.9	0.001

4. Discussion

Per the current study data, women with abortions displayed higher levels of Th1 cytokine profiles compared to the group of women in the control. While we can see the difference in levels of Th2 cell cytokines in the case of the women in the experimental group compared to the women in the control group, who had both abortions and live births, no significant variations were observed in their Th1 response. Hence, such data indicates that female subjects who have got the abortions might have a repercussion on the Th1/Th2 immune responses inhibition.

In accord with these conclusions, among the Th2 cells which drudge lower in aborted women versus other controls, the cell of Th1 were higher among the women. The function of pro and anti-inflammatory cytokines on pregnancy outcomes is an issue that has attracted the scientific community's interest, as highlighted in the studies mentioned[2,3]. The study done by Raghupathy et al[4], showed evidence that when pregnant women receive IL-2, a Th-1 type cytokine that normally causes abortion, it leads to higher abortion rates than the normal numbers. Another fact is that the results of our investigation are in agreement with the earlier studies in which those women, who had an abortion previously, showed signs of elevated T-helper 1 cytokine levels in the study groups[1. 5].

The other point, Hisano et al[6], through flow cytometric analysis simply revealed that, from the previous history of abortion, the Th1/Th2 ratios were significantly higher when compared to the control group. The imbalance of uterine microenvironment was perceived as the cause of an adverse pregnancy outcome. On the other hand, the experiments carried out by Hisano et al on the placental cells of women who faced spontaneous abortion but made it finally to term showed a greater polarization towards the Th2 immune response among them than the Th2 immune response witnessed among women who had persistent miscarriages. This conclusion is supported by comparing the ratios of Th1 to Th2 cytokines.

These ratios provide insight into the balance between Th1 and Th2 immune responses. They suggested that this imbalance in the uterine microenvironment could potentially result in adverse pregnancy outcomes. The study also proposed that the cause of recurrent pregnancy loss (rPL) may be attributed to Th1-dominant immunity, which poses a significant threat to the fetus. In normal pregnancy, there is a natural shift in the immune response towards Th2 immunity, aiming to suppress excessive Th1 cytokine production and maintain a favorable environment for fetal development.

As a result, the current study emphasizes the connection between Th-1 cytokines (IL-2, IFN-γ, and TNF-α) and fetal rejection by showing a strong link between these cytokines and first-trimester pregnancy loss. Table 4.7 shows that the mean level of IL-37 was considerably lower in pregnant women who had previously had abortions (29.55±22.26 pg/ml) than in the control group (65.04±20.02 pg/ml) (p=0.001). Cytokines that contribute to inflammation are suppressed by interleukin 37, both in terms of production and activity. IL-37 production is stimulated and triggered throughout the inflammatory process in a way similar to other cytokines in the IL-1 family.

Numerous studies have shown that IL-37 is an important regulator of inflammation and has been shown to significantly suppress inflammation and innate immunity in a number of disorders. Because IL-37 regulates anti-inflammatory response in part by suppressing other cytokines such as IL-6 and acting as an endogenous inhibitor[7]. The results of those studies demonstrate that women who had abortion earlier have the risk of increasing the immunity alterations, and this might be the reason why they develop an ill predestination in their later pregnancies. IL-37, which has anti-inflammatory capabilities, is therefore a major player in the preservation of the immune system steadiness and in the putting in check of excessive inflammation which occurs in normal pregnancy[8].

Consequently, reduction in IL-37 in women that had a abortion before can be attributed to the disorders of the regulation of the immune system, thus may be one of the predispositions for the high inflammation rate, which can be the reason for a number of diseases of pregnancy[9]. Therefore, the potential role of a low grade of IL-37 in pregnant women with a history of incomplete first trimester outcomes can also be important for correct fetal development and functional placenta. Besides acting as a modulating factor of trophoblast infiltration and of the growth of vessels that are essential for the successful completion of placenta maturation, establishing an equilibrium in fetal growth, IL-37 plays an important role. Thus, a contention of IL-37 comes along with the risks of failure of placental maintenance, leading to placental insufficiency and the resulting pregnancy complications such as preeclampsia and fetal growth restriction[10].

As a result, IL-37 is an anti-inflammatory cytokine that ensures the overall maintenance of a full gestation phase. Statistically insignificant differences regarding T-cell 1 cytokines, especially IL-2, TNF-α, and IFN-γ, were present in pregnant women whose cultures were positive and negative (p>0. 05 for everyone). But in opposition, there is a statistically meaningful difference (p=0. 0005) in negative pregnancy culture results, with a significantly lower mean level of IL-5 (85). While a serving of noodle soup increased by 10% from 90 to 100 mg/100 grams in the Vietnamese relief campaign, the sodium content decreased by 22% during the Garifuna case (from 130 to 102 mg/100 grams).

Along with a decrease of adipose-stored TNF-α (760 pg/ml) and IL-6 (55. 71 pg/ml), these alterations resulted in the reduction of their mean levels. Procalcitonin levels in the second group of patients, who all happened to have negative culture results (927. pg/ml) were significantly higher (6 ± 599. pg/ml for) than those without infection (927 pg/ml). After 30 days the saliva levels of heavy metals in both the experimental group (6-722. 1 pg/ml) (p=0. 022). Additionally, pregnant women experiencing a positive culture showed significant differences in the IL-37 mean values (26). 924 Healthcare professionals should assume that HCV infection is identified in a patient who has an underlying or recent liver transplant and whose DAST score is 17±20. 6 pg/ml, unless they get a negative culture result (34). IL-4 level levels were found to be 82±23 (N. 5¯pg/ml) (p=0. 001) for the group

with positive culture results, but lower for the group with negative culture results (IL-4 level levels = 72±17. 6 pg/ml).

The higher level of T-helper 2 cytokines points to possible pregnant mothers that were recorded with the positive culture were found to have no significant mean levels of IL-5 and IL-6 compared to the ones that were registered with the negative culture. Possibly, an inhibition or disturbed T-helper 2 immunity is happening in a soldering vaginal infection, which may have consequences in the maternal immune environment and pregnancy consequences. Also, the detection of explicitly lower mean levels of IL-37 in pregnant women already assessed to have positive culture results added significantly. IL-37 remains famous for its properties as an anti-inflammatory and is one of the key regulating factors of the immune system. This observation of lower IL-37 levels in vaginitis indicates the possibility of the system's immune balance disturbance at the microbiological level where its anti-inflammatory property is probably inactive.

The study revealed that women who had a termination of pregnancies later had higher T-helper1 cytokine levels and a reduced concentration of T-helper2 cytokine in contrast to women who had normal deliveries. The Th1:Th2 ratio, and IFN-γ:IL-4 ratio as well as IFN-γ:IL-10 ratio in those with status of NCCS were considered to be high, thereby an imbalance to the Th-helper cell response. Profiling of maternal serum cytokine enables to collection of specific information on maternal inflammatory status, fetal distress, and early indications of immunological dysregulation[12]. Thus, to obtain a clearer picture of the immunological processes occurring, immunological profiling through simultaneous evaluation of multiple cytokines has higher sensitivity and more distinctly describes the ongoing inflammatory processes than single cytokine measurements as cytokines are part of complex functional networks (Workman & Murphy, 2013; O'Regan & Dorling, 2014).

The data obtained in the research points out the cytokine profiles contrasts between the health-maintaining and struggling pregnant women who intended to give normal delivery. Of note, specific patterns of TH1 and TH2 cytokines were also observed along with that of IL37, suggesting a possible role of immunomodulation in the choice of the reproductive outcome. The reduced mean T-cell proliferation level by spontaneous proliferation among pregnant women who eventually went through a normal delivery instead of having any adverse outcome is evidence of a strong T-cell mediated immune response in successful pregnancies. This research corroborates with the findings of another study by Makhseed et al where they linked high levels of T-helper 1 cytokines to poor pregnancy outcomes,e. g. term deliveries and low risks of complications like preterm birth, and intrauterine growth restriction, among others[13].

The same goes for the lower TH1 and TH2 cytokine levels as compared to a normal childbirth process which is another favorable thing in the support of the appropriate TH1/ TH2 immune response that is needed in maintaining the restoration of pregnancy health. T-helper 2 cytokines have long been known to help the adaptive immune system avoid rejection of the fetus and protect it from harmful inflammation, thus ensuring a successful pregnancy. The higher T-helper 2 value in the group of women having a normal delivery highlights the importance of an optimal T-helper 2 response as a strategy to protect the health of the mother and her child[14]. The observed differences in the ratios of Th1:Th2, IFN-γ:IL-4, and IFN-γ:IL-10 are additional cytokines joining the comparison that underscore the keys to the immune system balance in pregnancy.

Higher ratios of Th1:Th2 and IFN-γ: IL-4/IL-10, which means a pro-inflammatory tendency is coupled to undesirable outcomes, and a lower ratio is found in grouping with positive progress to normal delivery[15]. Peng et al which led to the discovery of elevated levels of IL-6 being present in the unfavorable outcome group when compared to both aborters' pregnancy ($p < 0$)[16]. An illustration of 5 healthy community members and 5 healthy members of the normal pregnancy group ($p < 0$. Furthermore, the results of our study and these previous studies do coincide[17,18]. These studies from different parts of the world jointly suggest the same trend where the Th1 cytokines level is increased and on

the contrary, the Th2 cytokines level is decreased by URSA. The overwhelming evidence from this pool of studies of Th 1b skewed cytokine profile in URSA patients further validates the immune dysregulation discussed. The repeated reconfirmation of the findings confirms the essence of the understating of the aspects of immunology that cause URSA and it shows the possibilities of developing therapies that would restore the balance between the immune system components and improve the pregnancy outcomes in the affected individuals.

5. Conclusion

The findings of this study highlight significant immunological alterations among women with a history of previous abortions, characterized by elevated T-helper 1 cytokine levels and decreased Th2 cytokine levels, resulting in a higher Th1/Th2 ratio compared to controls. Additionally, a notable difference in IL-37 levels was observed between the two groups, suggesting its potential role in pregnancy outcomes.

The association between vaginal microbiota and cytokine levels among women with previous abortions further emphasizes the intricate interplay between immune modulation and pregnancy health. These findings underscore the importance of immune balance in maintaining maternal-fetal tolerance and preventing adverse pregnancy outcomes. Further research is warranted to elucidate the underlying mechanisms driving these immunological alterations and to explore targeted interventions aimed at optimizing pregnancy health in women with a history of recurrent abortions.

Recommendations:

The study's conclusions lead to the following suggestions being put forth:

1. Explore more on why and how immune dysregulation is seen in women who experienced recurrent abortions in a previous pregnancy. Discover the particular routes taken by the cytokines affected leading to Th1/Th2 imbalance and their effect on pregnancy outcomes.
2. Moreover, we need more studies to unravel the mechanisms behind immune changes reported to have occurred in pregnancies of women from patients with a history of abortion.
3. The healthcare providers should enlighten pregnant women on how to keep a close surveillance of their vaginal health and report to a medical facility in case of any suspicious symptoms to them. Women's education programs containing information about pregnancy health, in particular, can help women make a decision and actions to improve and maintain their health. Conduct longitudinal studies to assess the long-term effects of immune dysregulation on maternal health and subsequent pregnancies. Follow up with women with previous abortion history to evaluate the persistence of immune alterations and their implications for future reproductive outcomes.

REFERENCES

[1] R. Arora, A. Rathee, M. Sachdeva, and U. Agrawal, "Unexplained repeated pregnancy loss and T helper cells," European Journal of Obstetrics & Gynecology and Reproductive Biology, vol. 254, pp. 277-283, Nov. 2020.

[2] M. Jain, Z. Hassan, and S. Jain, "Study of Th 1 and Th 2 activity in women with threatened miscarriages," International Journal of Reproduction, Contraception, Obstetrics and Gynecology, vol. 4, no. 6, pp. 1918-1922, Nov. 2015.

[3] G. A. Abdullah and N. K. Mahdi, "The role of cytokines among women with spontaneous miscarriage," Medical Journal of Islamic World Academy of Sciences, vol. 21, no. 3, pp. 119-124, 2013.

[4] R. Raghupathy and J. Kalinka, "Cytokine imbalance in pregnancy complications and its modulation," Front Biosci., vol. 13, no. 1, pp. 985-994, Jan. 2008.

[5] M. P. Piccinni, R. Raghupathy, S. Saito, and J. Szekeres-Bartho, "Cytokines, hormones and cellular regulatory mechanisms favoring successful reproduction," Frontiers in Immunology, vol. 12, article 717808, Jul. 2021.

[6] M. Hisano, K. Nakagawa, J. Kwak-Kim, R. Sugiyama, H. Sago, and K. Yamaguchi, "Changes in the T-helper 1 and 2 cell populations during pregnancy in tacrolimus-treated women with repeated implantation failure and recurrent pregnancy loss," Human Fertility, vol. 25, no. 5, pp. 975-982, Oct. 2022.

[7] G. Cavalli et al., "The anti-inflammatory cytokine interleukin-37 is an inhibitor of trained immunity," Cell Rep., vol. 35, no. 1, article 108955, Apr. 2021.

[8] M. Kwiatek, T. Gęca, and A. Kwaśniewska, "Pro- and Anti-Inflammatory Cytokines in the First Trimester-Comparison of Missed Miscarriage and Normal Pregnancy," Int J Environ Res Public Health, vol. 18, no. 16, article 8538, Aug. 2021.

[9] F. Cunningham et al., "Williams Obstetrics," 23rd ed., McGrraw-Hill Companies, 2010.

[10] J. J. Oats and J. Boyle, "Llewellyn-Jones Fundamentals of Obstetrics and Gynaecology, E-Book," Elsevier Health Sciences, Nov. 4, 2022.

[11] M. Makhseed et al., "Th1 and Th2 cytokine profiles in recurrent aborters with successful pregnancy and with subsequent abortions," Human Reproduction, vol. 16, no. 10, pp. 2219-2226, Oct. 2001.

[12] M. D. Salazar Garcia et al., "Early Pregnancy Immune Biomarkers in Peripheral Blood may Predict Preeclampsia," J Reprod Immunol, vol. 125, pp. 25-31, 2018.

[13] I. C. Weel et al., "Association Between Placental Lesions, Cytokines and Angiogenic Factors in Pregnant Women With Preeclampsia," PLoS One, vol. 11, article e0157584, 2016.

[14] L. M. T. Stokkeland et al., "Serum Cytokine Patterns in First Half of Pregnancy," Cytokine, vol. 119, pp. 188-196, 2019.

[15] T. Spence et al., "Maternal Serum Cytokine Concentrations in Healthy Pregnancy and Preeclampsia," J Pregnancy, vol. 2021, article 6649608.

[16] Y. Peng and M. Wang, "Significance to investigate the TH1/TH2 cytokines and their proportions in peripheral blood of women with unexplained recurrent spontaneous abortion and normal women based on pregnancy status."

[17] Y. Gao and P. L. Wang, "Increased CD56(+) NK cells and enhanced Th1 responses in human unexplained recurrent spontaneous abortion," Genet Mol Res, vol. 14, no. 4, pp. 18103-18109, 2015.

[18] L. Y. Zhu, "Changes and clinical significance of peripheral blood helper T lymphocyte and natural killer (NK) cells in unexplained recurrent spontaneous abortion (URSA) patients after abortion and successful pregnancy," Clin Exp Obstet Gynecol, vol. 42, no. 1, pp. 62-XX.