Immunogenetic study of parvovirus B19 infection and IL12 gene polymorphism in women with recurrent miscarriage

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Abstract
Viral infections during pregnancy have been associated with adverse pregnancy outcomes and birth defects in the offspring. Viruses rarely cross the placental barrier, but when the virus does reach the fetus, it can result in severe birth defects such as microcephaly or even fetal death. Human parvovirus (B19) is thought to cause severe complications is spontaneous abortion. Normal pregnancy is related to the successful transition from type 1 cellular immunity to type 2 cellular immunity. Interleukin (IL)-12 induced the production of type 2 cytokines. These experiments were conducted to study the effect of human B19 virus on the pregnant women and cause abortion. This case control study was done for 100 patients including different ages that range from 18-42 age that sever recurrent miscarriage. Also, the study include 50 apparently healthy control (AHC) this age were similar with the patients age. They were collected from different general hospitals in hilla; Baghdad as well as Mid-Euphrates Governorates of Iraq, during the period from February 2021 to September 2021. Endometrium; Cervical swabs; fetal fluids swabs as well as Blood specimens were collected and processed to extract viral genome and total DNA gene for screened human B19 virus by using PCR and IL-12(rs 3212227) polymorphism by PCR and sequencing. In addition, estimation serum IL-12 concentration by enzyme-linked Immunosorbent assay (ELISA). Out of 150 Endometrium; Cervical swabs; fetal fluids swab as well as Blood specimens involved in this study 48 % (72 out of 150) were found to have a viral infection with abortion & RPL. In women with RPL, the most commonly affected age stratum infected with DNA – B19 was (30-39 years) which constituted 48% (12 out of 25 cases), while the age stratum (17-29 years) was constituted 32% (8 out of 25 cases), followed by 20% (5 out of 25) in age stratum (40 – 49 years).Statistical comparison of these B19 in the Patients with Women With RPL according to age stratum revealed significant differences ($p< 0.05$). The percentage of a single band (162 bp) of the target sequence of IL-12 rs3212227 gene. The positive result, according to PCR amplification of a single band (162 bp) of IL-12 rs3212227 gene in women patients with RPL and AHC were 36.7% (55 of 150 cases) and 20% (5 of 50 cases), respectively. While the negative results were in women patients with RPL and AHC were 63.3% (95 of 150 cases) and 90% (45 of 50 cases), respectively. Two types of mutation in IL-12 rs3212227 gene was found transversion and transition. The frequency of transversion mutation (A\C; A\T; T\G; T\A and T\C) more than the transition mutation (A\G). The mean of serum IL-12 concentration for AHC and women patients with RPL groups were 8.00 ±0.31pg./ml and 14.00 ±0.59 pg./ml, respectively. Statistically, significant difference ($p<0.05$) was found on comparing the mean of serum IL-12 concentration among these study groups.
Keywords: recurrent miscarriage; health; IL12 gene polymorphism

1. Introduction

Miscarriage is considered as the most common complication in pregnancy, which is defined as the spontaneous loss of a fetus during the first 24 weeks of gestation. Several possible causes of miscarriage include genetic abnormalities of the fetus (more than 50% of miscarriages), anatomical abnormalities of the uterus, endocrine and immunological causes, environmental agents and infections (Carlo et al.,2020).

The likelihood of a live birth in the successive pregnancy in untreated women with RPL has been reported to range 42–86% after three miscarriages and decreases with increasing the number of pregnancy losses, reaching only 23–51% after ≥5 losses. This observation suggests that the number of miscarriages—a likely indicator of the gravity of the condition—is a major determinant of the reproductive success of women with recurrent miscarriage (RM); in fact, it has been reported that the live birth rates in the successive pregnancy in women with two consecutive losses is around 75% (Christiansen,2020).

Several studies have confirmed the role of some intrauterine infections (including listerosis, syphilis, CMV, HSV, adeno-associated viruses (AAVs) and parvovirus B19) as a cause of miscarriage, especially during the second trimester of pregnancy. However, the role of some other infections is still questionable. (Zahra et al.,2019).

Human parvovirus B19 (B19), a single-stranded DNA virus, is the causative agent for erythema infectious or fifth disease. B19 is mainly transmitted by respiratory droplets, but also through blood or vertically from mother to fetus. The proportion of pregnant women susceptible to B19 infection ranges from 34% to 65% in various parts of the world. The

doi.org/10.31838/hiv23.01.183 Received: 15.09.22, Revised: 11.10.22, Accepted: 28.12.22

incidence of seroconversion during pregnancy is estimated at between 1% and 1.5% in the endemic period, increasing to 13% in the epidemic period (Yi-quan et al.,2019).

The specialized cells found in the uterus and placenta released a massive multitude of cytokines which are moderately responsible for the alteration of the immune response from Th1 to Th2. This shifting and increased production of Th1 cytokines may create embryonic condition more susceptible to inflammation and has been directly linked to spontaneous abortion (AlJameil et al.,2018).

Alternatively, pro-inflammatory mediators including in this category is IL-12, and other chemokine that fascinate inflammatory cells are involved in multidirectional processes such as development of endogenous pyrogens (IL-1, IL-6, TNF-α), readjust the integration of pro-inflammatory cytokines and various secondary mediators by the action of both macrophages and mesenchymal cells like fibroblasts, epithelial and endothelial cells and reassure the production of acute phaseproteins.Interleukin (IL) IL-12 is involved in uterine NK cells control of uterine vascular development. Polymorphisms in the IL-12/IL-18 genes could modify the cytokine balance, which might result in an increased susceptibility to recurrent spontaneous abortion (RSA) (Tyagi and Alharthi,2020).

2. Materials and Methods

Patients Population

This case control study was done for a one hundred-fifty specimens collected from female patients subjected to recurrent pregnancy loss and apparently healthy persons as control group from general hospitals as well as many private clinical in Middle Euphrates -Iraq. The age range of the study population was 18 years to 42 years. The specimens were collected during period from February 2021 to September 2021.

Endometrium; Cervical swabs; fetal fluids swabs as well as blood from each study group of female patients suffering from recurrent pregnancy loss should be enrolled, that classify into: -.

1.One hundred – fifty endometrium; cervical swabs; fetal fluids swabs as well as blood specimens from women suffering from abortion as well as recurrent Miscarriage.

2. Fifty blood and cervical swabs specimens of apparently healthy persons as control group.

All these specimens were submitted for genetic part for screening Parvovirus (B19) in women patients and apparently healthy control groups by polymerase chain reaction (PCR). However, the second part for detection SNPs of IL-12 genes polymorphism by sequencing.

Specimens Collection

Endometrium and\or cervical swabs; fetal fluids swab as well as blood specimens were collected patients and AHC by using two swabs for each patient:

The first is the flocked swab regular for endometrium ; cervical swabs collection, according to Catalog Number 21031 (Heinz, Herenz; Germany).

The second one is the flocked swab for fetal fluids swabs collection, according to Catalog Number 80503CS (Copan, Italy).

The two swabs were taken and mixed together in a 3-ml universal

transport medium (UTM) tube which provided with the flocked swab

regular Catalog Number 21031 (Heinz, Herenz; Germany).

Each specimen was aliquot into three 1.8 cryotube (Nunc-Kamstrup, Denmark) and stored at (-20°C) at the Virology Research Unit, College of Science, Babylon University. 5ml venous blood were collected aseptically from all patients by using gel tubes and EDTA tubes for gating blood serum and buffy coat, respectively; then stored at (-20°C).

I.Inclusion Criteria

Women with age ranged from 18 to 42 years old with unexplained miscarriage till 24 weeks of pregnancy were taken as cases, while women with full-term pregnancy during the conduction of cesarean operation have more than one successful pregnancy were taken as controls.

II.Exclusion Criteria

Women with other causes of miscarriage such as endocrine disorder (diabetes mellitus, thyroid disorder), anatomical causes acquired or congenital thrombophilia and other infection causes miscarriage such as toxoplasmosis, cytomegalovirus infection, rubella, Humam Herpes Virus-6\7 and herpes simplex-1 and 2 (HSV1 &HSV2).

3.4.1: Detection of Parvovirus (B19) by Real Time Polymerase Chain Reaction (RT-PCR)

Real-time PCR (qPCR) is based on two major processes: Firstly, isolation of viral genome (DNA\ RNA) from specimens, and Secondly, Real Time amplification for each sample. In real-time PCR (qPCR), the accumulating amplified product can be detected at each cycle with fluorescent dyes. This increasing signal allows to achieve sensitive detection and quantification of pathogens.

Detection of IL-12 rs3212227 SNPs by Sequencing:

Total DNA for SNPs of IL-12 rs3212227) polymorphism were extracted from peripheral blood and swabs of female patients using sequencing technique.

Evaluation of IL-12 Concentration in Blood Serum of Patients and AHC.

The concentration of IL-12 in the serum of female patients with recurrent miscarriage were evaluated by enzyme linked immunosorbent assay (ELISA).

The positive result according to RT-PCR shows 37.5 % (27 out of 72 cases) as positive while 62.5% (45 out of 72 cases) as negative, as shown in Table (4-6) as well as Figure (4-3). Statistically significant differences (p = 0.04) among patients' group.

3. The Results

Detection of Parvovirus B19 By RT-PCR:

Table 4.6 Percentage of B19 Positive Signals in Women Patients with RPL by Using qRT.PCR Technique.

Recurrent Miscarriage	Positive		Negative		Chi-Square (P-value)
	N	%	N	%	
Once (n=30)	10	13.9	20	27.8	P=0.03 sign. (P<0.01)
Twice (n=32)	14	19.4	18	25	
Three Times and Above (n=10)	3	4.2	7	9.7	
Total	27	37.5	45	62.5	

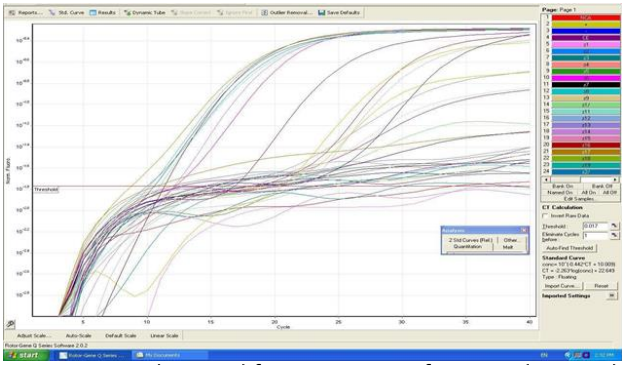

Figure 4. 3: The amplification curve of B19 nucleic acid.

4.3.5 The Results of B19 in the Patients with Women with RPL According to the Age Stratum.

In women with RPL, the most commonly affected age stratum infected with DNA – B19 was (30-39 years) which constituted 48% (12 out of 25 cases), while the age stratum (17-29 years) was constituted 32% (8 out of 25 cases), followed by 20% (5 out of 25) in age stratum (40 – 49 years).

Statistical comparison of these B19 in the Patients with Women with RPL according to age stratum revealed significant differences (p< 0.05) Table (4-7).

Table 4. 7. Frequency of B19 RT-PCR Signal Among the Patients with Women With RPL According to the Age Stratum

	Years	B19			P value
		No.	Positive	Negative	
Age Stratum	17-29	23	9	14	Anova test P=0.02 S. (P<0.05)
		31.9%	12.5%	19.4%	
	30-39	34	14	20	
		47.2%	19.4%	27.8%	
	40-49	15	4	11	
		20.8%	5.6%	15.3%	
Total		72	27	45	
		100%	37.5%	62.5%	

4.4.2. Genotyping of IL-12 rs3212227 Gene in RPL and AHC

For IL-12 rs3212227 genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal condition as mentioned in the table (3-7). The results revealed that the presence a single band (162 bp) of the target sequence of IL-12 rs3212227 gene in agarose gel Figure (4-5).

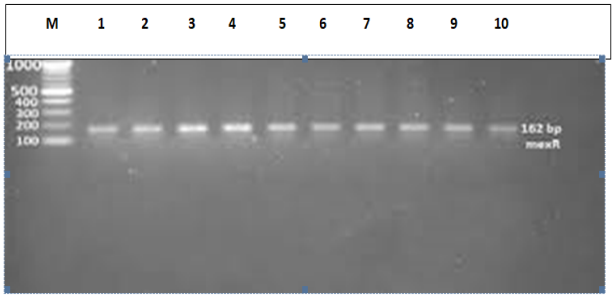

Figure 4.5: Agarose gel electrophoresis of an amplified product patterns of IL-12 rs3212227 exon5 region. 1.5 % Agarose Gel Electrophoresis, TBE 1X , at Voltage 75 Volt for 45 min.

Table (4-8) show the percentage of a single band (162 bp) of the target sequence of IL-12 rs3212227 gene. The positive result, according to PCR amplification of a single band (162 bp) of IL-12 rs3212227 gene in women patients with RPL and AHC were 36.7% (55 of 150 cases) and 20% (5 of 50 cases), respectively. While the negative results were in women patients with RPL and AHC were 63.3% (95 of 150 cases) and 90% (45 of 50 cases), respectively as shown the Table (4-8).

Table 4.8: Percentage of IL-12 rs3212227 signals in patients' women with RPL and AHC groups by PCR technique.

IL-12 rs3212227 gene band	RPL No.(%)	AHC No.(%)
Positive	55 (36.7%)	5 (20%)
Negative	95 (63.3%)	45 (90%)
Total	150 (100%)	50 (100%)

4.4.2.1. Genotyping of IL-12 rs3212227Among Study Groups.

The results showed that DNA polymorphism distribution were DNA polymorphism distributions according to A\C; G\T; T\G; G\C; T\A; T\C and G\A haplotypes were 9%; 7% ; 9% ; 6% ; 9% ; 5% and 4%, respectively in patients women with RPL and 4% ; 0% ; 8% ; 2% ; 3% ;4%and 2%, respectively in AHC group. In addition, was found two types of mutation in IL-12 rs3212227 gene transversion and transition. The frequency of transversion mutation (A\C; A\T; T\G; T\A and T\C) more than the transition mutation (A\G) Table (4-9). The frequency of transversion mutation (A\C; G\T; T\G; G\C; T\A; T\C) more than the transition mutation (A\G).

4.4.2.2. Sequences alignment fragment results of IL-12 rs3212227 gene, Exon-5 region by Bio Edit program version 7.2.5

The sequencing results observed that many SNPs between the one resolved haplotype and between the IL-12 rs3212227, exon5 for Primer3 plus reference sequences. The results appeared in the

presence of nineteen SNPs Figure (4-6). Which revealed that which located at position 51 a substitution mutation (A→C); position68 & 124 substitution T→ G ; position 63 substitution G→ T; position 84 substitution G→ C; position 99 substitution T→ A; position 105 substitution T→ C and last one at position 114 substitution G→ A Figure (4-6) according to the reference sequence alignment of the human IL-12 rs3212227 gene.

Evaluation of Serum IL-12 concentration By ELISA Among Study Population:

Table (4-12) was showed the mean of serum IL-12 concentration for AHC and women patients with RPL groups were 8.00 ±0.31pg./ml and 14.00 ±0.59 pg./ml, respectively. Statistically, significant difference ($p<0.05$) was found on comparing the mean of serum IL-12 concentration among these study groups (Table 4-12).

Table 4.12: Results of serum IL-12 concentration by ELISA for AHC and women patients with RPL

IL-12	AHC (pg/ml)	Women with RPL (pg/ml)
Mean± SE	8.00 ±0.31	14.00 ±0.59
LSD	5.65	
P value	*P*<0.05 (0.001) *	

4. Discussion

Risk Factors of Recurrent Pregnancy Loss

About 21 to 41% of premature parturitions are due to intrauterine infections. The access of microorganisms to the embryo before parturition causes the inflammatory response syndrome of the fetus against producing microbial products leading to premature labor. The consequences are multiple organ dysfunction syndrome (MODS) in the embryo and an increase in the mortality rate of the fetus (Nasirpour et al.,2017). Viral intrauterine infections are among the key etiological causes of spontaneous abortions (Charostad et al.,2020).

Established risk factors include anatomical, genetic, endocrine, and hemostatic alterations. With around 50% of idiopathic cases, immunological risk factors are getting into the scientific focus, however international guidelines hardly take them into account (Vomstein et al.,2021).

In more than half of the cases, the causes of abortion have been genetic disorders and chromosomal abnormalities (Singh et al., 2018). Nevertheless, other factors affecting abortion are as follows: uterine abnormalities, infectious diseases and untreated diseases of the mother,the age of the mother during pregnancy, previous history of abortion, age at the first menstruation, menstrual disorders, use of contraceptive drugs, environmental conditions and mother's lifestyle such as smoking and use of caffeine, being exposed to cigarette smoke, stress, exposure to mobile phone radiation, and low socioeconomic and employment status, which are effective in the occurrence of abortion (Volgsten et al.,2018 ; Poustchi et al.,2018).

Detection of Parvovirus B19 by RT-PCR

Although the outcome of fetuses affected by PB19 infection has been generally reported to be good, several complications such as miscarriage, intrauterine death (IUD) and hydrops can potentially occur. Identification of the maternal serological status is the first step in stratifying the risk of fetal infection. If the mother is immune, she can be reassured that she will not develop the infection and that exposure will not result in adverse consequences. On the other hand, appropriate follow-up of those mothers showing seroconversion due to PB19 is fundamental in order to identify fetuses with complications. The risk of vertical transmission of PB19 has been estimated to be around 25% (Basciettoetal.,2018). Determination of etiology of the anemia and hydrops is very important. If

the cause of hydrops is B19V, the intrauterine blood transfusion is very critical, and chance of fetal survival can improve 60% to 80%; in other hand, in untreated cases this rate is 15% to 30% (Cosmi et al.,2002; Nyman et al.,2002).

The positive result of current study according to RT-PCR shows 37.5 % positive, while 62.5% as negative. These result disagreement with previous studies, which revealed parvovirus B19 DNA–positive fetal or placental tissue samples in 7.5%–15% of fetuses from IUFDs (Lottie et al.,2002) Parvovirus B19 –DNA was detected in 5 fetuses with gestational ages 14,22,23,30, and 39 weeks; these included fetuses from 4(2.4%) of the 169 IUFDs and 1 (0.8%) of the 120 miscarriages (Anita et al.,2008).

In contrast, a study from Northern Ireland by Watt et al., (2013) was examined 3921 women of reproductive age and 33.5% of them were at risk of infection as they had no antibodies against B19V. Silingardi et al., 2009 who found 50% of women are at risk of developing infection during pregnancy, which can lead to non-immune hydrops fetalis, a well-established cause of fetal death.In an study of 72 pregnant women with B19V, it was noted that the risk of vertical transmission is higher if infection occurs by gestational week 20. Six out of eight cases of fetal loss observed were 'attributed to B19V infection' without further elucidation (Bonvicini et al., 2011). Also, in a study from Nigeria, B19V prevalence among pregnant women was estimated at 40.7%, as 111 out of 273 patients in the study had detectable levels of either IgG or IgM antibodies, however these were not associated with a history of miscarriage (Emiasegen et al., 2011).

A higher percentage of IgM antibodies indicating recent infection was observed in women with adverse pregnancy outcomes (22.72%, n = 88) compared with 4.5% observed in 88 control healthy pregnant women (Brkic et al., 2011). Interestingly,

anti B19V IgG antibodies were higher in controls than cases (70.5 and 53.4% respectively, P = 0.046). An important limitation of this study is that the adverse pregnancy outcome included miscarriage, non-immune hydrops fetalis and intrauterine fetal death, thus the association of miscarriage alone with B19V is not clear.

Jensen et al., (2000) reported that 65.7% (1881/2859) of pregnant women were B19 IgG seropositive at their frst antenatal visit. In addition, of the IgG-negative women, 10.3% (101/978) were found to have an acute B19 infection during pregnancy.

In pregnant women suspected of contracting B19 infection during pregnancy, the infection rate was higher and reported up to 21% (100/478) in a prospective study (.Chisaka et al., 2006).

Whether gestational age at infection influences the risk of fetal complications is crucially important in clinical practice. The highest risk of fetal loss appears to follow maternal infection during weeks 9 to 16 of pregnancy; risk is reduced with infection in the second half of pregnancy and is rare if infection occurs in the last 2 months (Ornoy and Ergaz,2017).

In a large prospective cohort study including 1018 pregnant women with acute B19 infection, Enders et al., (2004) reported the incidence of fetal loss associated with maternal infection at 0–8, 9–12, 13–16, and 17–20 weeks of gestation (WG) was 17.2%, 9.9%, 12.7%, and 5.7%, respectively. A high incidence of fetal hydrops was observed at 13–16, 17–20, and 21–24 WG with 7.3%, 7.0%, and 5.2%, respectively.

Co-infection of B19 and other infective pathogens is common. Although a significant risk of B19 infection for fetal loss was observed in our study, most of the included studies did not state whether pregnant women co-infected with B19 and another infectious pathogen were excluded. However, a large proportion of women (about 34%–65%) are susceptible to

B19 during pregnancy; and, after acute B19 virus infection, adverse pregnancy outcomes are elevated. The beneft of comprehensive screening of B19 IgG and IgM in all pregnant women needs further sociological and economic studies to confirm its value (Yi-quan et al.,2019).

From above we thought, maternal parvovirus B19 infection during pregnancy increased the risk of fetal loss, spontaneous abortion, and stillbirth. A high incidence of fetal loss and fetal hydrops was observed in pregnant women following infection. Whether infectious symptoms in pregnant women increase the risk of adverse pregnancy outcomes needs further investigation.

Genotyping of IL-12 rs3212227 Gene Polymorphisms in RPL and AHC

The IL-12 cytokine regulate the maternal-embryo interactions during the implantation process in a stage-specific fashion and is expressed the human uterus control of implantation and transformation of uterine vasculature.It was hypothesized that some cases of recurrent implantation failure are probably related to the absence of an adequate vascular remodeling (Pantos et al.,2022).

The frequencies of current study A\C; G\T; T\G; G\C; T\A; T\C and G\A haplotypes for IL-12 were 9%; 7%; 9%; 6%; 9%; 5% and 4%, respectively in women patients versus and 4% ; 0% ; 8% ; 2% ; 3% ;4%and 2%, respectively in AHC group. These results disagreement with Ostojic et al., (2007) who found the frequencies of DD, ID, II for IL-12 were, 25.6%, 52.8% and 21.6% respectively, in patients versus 21.3%, 51.5% and 27.2% respectively in controls were not associated with RSA in our women.

In addition, the current results were disagreement with Peterlin et al, (2007) who found no association between IL12 gene polymorphism and women with RSA.

In addition, Ostojic et al, (2003) who have demonstrated the importance of the role of IL-12 in abortion. These suggested that abnormal cytokine profiles might cause defective local vascularization and affect trophoblast cell growth and function. IL-12 has an important immunoregulatory function, as it promotes Th1 cells⁄ cytokines differentiation with downregulation of Th2 cytokines (Rutigliano et al.,2022).

IL-12 were expressed at lower⁄different levels in feto-maternal interface compared to'non-abortion, IL-12 (ins⁄ del) genetic variant might be involved in abnormal uterine vascularization, which affects growth and function of trophoblast cells.Whereas too high IL-12 secretion is probably abortifacient because it promotes or co-induces (with IL-18) activation of the uNK cells, engaging them towards acytotoxic / cytostatic pathway, overriding the physiological control of their cytokine production by KIR⁄ trophoblast MHC interaction, and disregulating their IFN-gamma production (Ledee-Bataille et al.,2004).

As discussed above, the inconsistency in study results may be due to study size or to genetic and/ or geographical features of the population from which the subjects were recruited.

In the current results, the results revealed that the patient groups had high14.00 ±0.59 pg./ml in IL12 levels in comparison with the control group8.00 ±0.31pg./ml (Table 5). These results agreement with study of Kwiatek et al., (2021) who found significant differences in the distribution of that IL-12 concentration levels in patients with missed abortion are higher than that in normal early pregnant women. Tian and kang, (2014) who demonstrated the missed abortion patients demonstrated a significantly higher number of common stressors and higher serum cortisol levels compared to controls (both $p < 0.05$).

Also, Roda et al., (2004) found the IL-12 levels were significantly higher in RSA 66±25 pg./ml compared to control group 51±24 pg./ml ($P<0.05$).

In addition, Wilson et al, (2004) who found the expression profiles of IL-12 observed a significant association between higher levels of IL-12 in

peripheral blood and endometrium in women with RSA.

IL-12 is the most important cytokine in the differentiation of Th 1 cells and the immune-inflammatory responses mediated by Th 1-type cytokines.Since the percentage of Th 1 cells correlates with IL-12 levels(Glassman et al.,2021).

Some studies have been reported that the expression profiles of IL12 in women with repeated implantation failure markedly differed when compared to astandard profile in the control group of fertile women and that abnormal cytokine profiles correlated with abnormal angiogenesis (Rhoda et al., 2004).

Too high IL-12 secretion is probably abortifacient because it promotes or co-induces (with IL-18) activation of the uNK cells, engaging them towards a cytotoxic / cytostatic pathway, overriding the physiological control of their cytokine production by KIR/ trophoblast MHC interaction, and disregulating their IFN-gamma production (Ledee-Bataille et al., 2004).

Interestingly, IL12 cytokine were apparently required at lower doses for the successful development of local vascular remodeling, The an abnormal cytokine profile might cause defective local vascularization and affect trophoblast cell growth and function. Furthermore, they have demonstrated the importance of the role of IL-12 of abortion (Ostojic et al., 2003).These suggest that abnormal cytokine profiles might cause defective local vascular izationand affect trophoblast cell growth and function. IL-12 has an important immune regulatory function, as it promotes Th1 cells/ cytokines differentiation with downe rgulation of Th2 cytokines. IL-12 acts as an NK cytotoxicity activator, IFN-c inducer and inhibitor of angiogenesis (Pantos et al., 2022).

Th1 secreted cytokines including IFNy, TNF β, TNF α and IL-2 can directly damage the placenta or indirectly activate the immune cells. The TNF α factor can cause fetal excretion by creating necrosis in the embryo and increasing the contraction of the uterus. This cytokine has a poly-tropic property that induces the expression of cytokines and precursor chemokines and ultimately tissue degradation (Roumandeh et al.,2018).

The opinion of authors of current study that for a pregnancy to succeed there had to be down regulation of the maternal immune system with enhancement of Th2 type cytokine expression and suppression of the Th1 response at the maternal foetal interface. Stimulation of the Th1 response results in abortion, whilst Th2 dominance was beneficial to the pregnancy. Since then, there have been many studies supporting this hypothesis.

Refrences

AlJameil, N., Tabassum, H., AlMayouf, H., Alshenefy, A., Almohizea, M. M., & Ali, M. N. (2018). Identification of serum cytokines as markers in women with recurrent pregnancy loss or miscarriage using MILLIPLEX analysis. Biomed. Res, 29.

Anita Riipinen, Elina Väisänen, Mika Nuutila, Markku Sallmen, Riitta Karikoski, Marja-Liisa Lindbohm, Klaus Hedman, Helena Taskinen, Maria Söderlund-Venermo, Parvovirus B19 Infection in Fetal Deaths, Clinical Infectious Diseases, Volume 47, Issue 12, 15 December 2008, Pages 1519–1525, https://doi.org/10.1086/593190

Bascietto, F., Liberati, M., Murgano, D., Buca, D., Iacovelli, A., Flacco, M. E, & D'Antonio, F. (2018). Outcome of fetuses with congenital parvovirus B19 infection: systematic review and meta-analysis. Ultrasound in Obstetrics & Gynecology, 52(5), 569-576.

Bonvicini, F., Puccetti, C., Salfi, N. C., Guerra, B., Gallinella, G., Rizzo, N., & Zerbini, M. (2011). Gestational and fetal outcomes in B19 maternal infection: a problem of diagnosis. Journal of Clinical Microbiology, 49(10), 3514-3518.

Brkic, S., Bogavac, M. A., Simin, N., Hrnjakovic-Cvetkovic, I., Milosevic, V., & Maric, D. (2011). Unusual high rate of asymptomatic maternal parvovirus B19 infection associated with severe fetal outcome. The Journal of Maternal-Fetal & Neonatal Medicine, 24(4), 647-649

Chaouat, G., Ledee-Bataille, N., Dubanchet, S., Zourbas, S., Sandra, O., & Martal, J. (2004). Reproductive immunology 2003: reassessing the Th1/Th2 paradigm? Immunology Letters, 92(3), 207-214

Charostad, J., Mokhtari-Azad, T., Yavarian, J., Ghavami, N., Khorrami, S. M. S., Behboudi, E, & Shafiei-Jandaghi, N. Z. (2020). Detection of human herpes viruses 1-5 in miscarriage: A case-control study. International Journal of Reproductive BioMedicine, 18(7), 501.

Chisaka, H., Ito, K., Niikura, H., Sugawara, J. I., Takano, T., Murakami, T, & Yaegashi, N. (2006). Clinical manifestations and outcomes of parvovirus B19 infection during pregnancy in Japan. The Tohoku Journal of Experimental Medicine, 209(4), 277-283

Christiansen, O. B. (2020). The epidemiology of recurrent pregnancy loss. Recurrent pregnancy loss, CRC Press: 2-12.

Cosmi, E., Mari, G., Delle Chiaie, L., Detti, L., Akiyama, M., Murphy, J, & Bahado-Singh, R. (2002). Noninvasive diagnosis by Doppler ultrasonography of fetal anemia resulting from parvovirus infection. American journal of obstetrics and gynecology, 187(5), 1290-1293.

Emiasegen, S. E., Nimzing, L., Adoga, M. P., Ohagenyi, A. Y., & Lekan, R. (2011). Parvovirus B19 antibodies and correlates of infection in pregnant women attending an antenatal clinic in central Nigeria. Memórias do Instituto Oswaldo Cruz, 106, 227-231.

Enders, M., Weidner, A., Zoellner, I., Searle, K., & Enders, G. (2004). Fetal morbidity and mortality after acute human parvovirus B19 infection in pregnancy: prospective evaluation of 1018 cases. Prenatal Diagnosis: Published in Affiliation with the

International Society for Prenatal Diagnosis, 24(7), 513-518.
Estuardo, L. I. J., Carlos, D. M., Roberto, H. R., de Jesús, Á. P. F., Jorge, S. M., Antonio, Y. G. M, & Manuel, V. H. V. (2021). Delivery in water, experience in a population of Mexican women in Mexico City. Journal of Gynecological Research and Obstetrics, 7(3), 055-060.
Glassman, C. R., Mathiharan, Y. K., Jude, K. M., Su, L., Panova, O., Lupardus, P. J, & Garcia, K. C. (2021). Structural basis for IL-12 and IL-23 receptor sharing reveals a gateway for shaping actions on T versus NK cells. Cell, 184(4), 983-999.
Jensen, I. P., Thorsen, P., Jeune, B., Møller, B. R., & Vestergaard, B. F. (2000). An epidemic of parvovirus B19 in a population of 3596 pregnant women: a study of sociodemographic and medical risk factors. BJOG: An International Journal of Obstetrics & Gynaecology, 107(5), 637-643.
Lédée-Bataille, N., Dubanchet, S., Coulomb-L'hermine, A., Durand-Gasselin, I., Frydman, R., & Chaouat, G. (2004). A new role for natural killer cells, interleukin (IL)-12, and IL-18 in repeated implantation failure after in vitro fertilization. Fertility and Sterility, 81(1), 59-65.
Nasirpour, H., Key, Y. A., Kazemipur, N., Majidpour, M., Mahdavi, S., Hajazimian, S, & Taefehshokr, S. (2017). Association of rubella, cytomegalovirus, and toxoplasma infections with recurrent miscarriages in bonab-Iran: A case-control study. Gene, Cell and Tissue, 4(3).
Nyman, M., Tolfvenstam, T., Petersson, K., Krassny, C., Skjöldebrand-Sparre, L., & Broliden, K. (2002). Detection of human parvovirus B19 infection in first-trimester fetal loss. Obstetrics & Gynecology, 99(5), 795-798.
Ornoy, A., & Ergaz, Z. (2017). Parvovirus B19 infection during pregnancy and risks to the fetus. Birth defects research, 109(5), 311-323.
Ostojic, S., Dubanchet, S., Chaouat, G., Abdelkarim, M., Truyens, C., & Capron, F. (2003). Demonstration of the presence of IL-16, IL-17 and IL-18 at the murine fetomaternal interface during murine pregnancy. American journal of reproductive immunology, 49(2), 101-112.
Pantos, K., Grigoriadis, S., Maziotis, E., Pistola, K., Xystra, P., Pantou, A. & Simopoulou, M. (2022). The Role of Interleukins in Recurrent Implantation Failure: A Comprehensive Review of the Literature. International Journal of Molecular Sciences, 23(4), 2198
Peterlin B. (2007) Division of Medical Genetics. Department of Obstetrics and Gynecology,University Medical Centre Ljubljana
Poustchi, H., Eghtesad, S., Kamangar, F., Etemadi, A., Keshtkar, A. A., Hekmatdoost, A, & Malekzadeh, R. (2018). Prospective epidemiological research studies in Iran (the PERSIAN Cohort Study): rationale, objectives, and design. American journal of epidemiology, 187(4), 647-655
Roda, A., Pasini, P., Mirasoli, M., Michelini, E., & Guardigli, M. (2004). Biotechnological applications of bioluminescence and chemiluminescence. TRENDS in Biotechnology, 22(6), 295-303.
Roumandeh, N., Zare, A., & Saremi, A. (2018). Immunology of Recurrent Spontaneous Abortion. Sarem Journal of Medical research, 3(2), 121-126.
Rutigliano, H. M., Thomas, A. J., Umbaugh, J. J., Wilhelm, A., Sessions, B. R., Kaundal, R., & Davies, C. J. (2022). Increased expression of pro-inflammatory cytokines at the fetal–maternal interface in bovine pregnancies produced by cloning. American Journal of Reproductive Immunology, 87(3), e13520.
Silingardi, E., Santunione, A. L., Rivasi, F., Gasser, B., Zago, S., & Garagnani, L. (2009). Unexpected intrauterine fetal death in parvovirus B19 fetal infection. The American journal of forensic medicine and pathology, 30(4), 394-397
Singh, S., Hussain, R., Shekhar, C., Acharya, R., Moore, A. M., Stillman, M, & Ball, H. (2018). Abortion and unintended pregnancy in six Indian states: findings and implications for policies and programs.
Tian, C. F., & Kang, M. H. (2014). Common stress and serum cortisol and IL-12 levels in missed abortion. Journal of Obstetrics and Gynaecology, 34(1), 33-35.
Tyagi, P., & Alharthi, N. S. (2020). Evaluation of pro-inflammatory cytokine level in cases of idiopathic recurrent spontaneous miscarriage in Saudi Arabia. Biomedical and Biotechnology Research Journal (BBRJ), 4(3), 225.
Volgsten, H., Jansson, C., Darj, E., & Stavreus-Evers, A. (2018). Women's experiences of miscarriage related to diagnosis, duration, and type of treatment. Acta Obstetricia et Gynecologica Scandinavica, 97(12), 1491-1498.
Vomstein, K., Feil, K., Strobel, L., Aulitzky, A., Hofer-Tollinger, S., Kuon, R. J., & Toth, B. (2021). Immunological risk factors in recurrent pregnancy loss: guidelines versus current state of the art. Journal of Clinical Medicine, 10(4), 869.
Watt, A. P., Brown, M., Pathiraja, M., Anbazhagan, A., & Coyle, P. V. (2013). The lack of routine surveillance of parvovirus B19 infection in pregnancy prevents an accurate understanding of this regular cause of fetal loss and the risks posed by occupational exposure. Journal of medical microbiology, 62(1), 86-92.
Wilson, R., Moor, J., Jenkins, C., Miller, H., Walker, J. J., McLean, M. A. & McInnes, I. B. (2004). Abnormal first trimester serum interleukin 18 levels are associated with a poor outcome in women with a history of recurrent miscarriage. American Journal of Reproductive Immunology, 51(2), 156-159.
Yi-quan Xiong, Jing Tan, Yan-mei Liu, Qiao He, Ling Li, Kang Zou, Xin Sun. The risk of maternal parvovirus B19 infection during pregnancy on fetal loss and fetal hydrops: A systematic review and meta-analysis. Journal of Clinical Virology

114 (2019) 12–20.
Zahra Sayyadi-Dehno, Seyed Mahmood Seyed Khorrami, Nastaran Ghavami,Fahimeh Ghotbi-Zadeh, Maryam Khushideh, Mostafa Hosseini,Somayeh Shatizadeh Malekshahi, and Nazanin-Zahra Shafiei-Jandaghi. Molecular Detection of Adeno-Associated Virus DNA in Cases of Spontaneous and Therapeutic Abortion. FETAL AND PEDIATRIC PATHOLOGY 2019, VOL. 38, NO. 3, 206–214 https://doi.org/10.1080/15513815.2019.1576817