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

Dawalet Abed Albari Khudair[1], Shakir H. Mohammed Al-Alwany[2]
[1]Al-ZAHRAWI UNIVERSITY COLLEGE, Iraq
[2]Biology Dept, College of Science, University of Babylon/Iraq

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. TNFalpha, 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 includes 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 TNF-α (rs 361625) polymorphism by PCR and sequencing. In addition, estimation serum TNF concentration by enzyme-linked Immunosorbent assay (ELISA). The obtained results of this study are summarized as follows: The mean age of the patients with RPL was (32.70±12.41 years) was mor than the mean age of the apparently healthy control AHC (30.67 ± 11.17 years). There are non-significant statistical differences (p=0.47) between Abortion; RPL and AHC. A strong positive relationship (with highly significant correlation) was found between number of participants; number abortion; week of abortion and Maternal age (P< 0.001). However, there are no significant correlation between number of participants with control maternal age. 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 (317 bp) of the target sequence of TNF-α -238G/A (rs361625) gene. The positive result, according to PCR amplification of a single band (317 bp) of TNF-α -238G/A (rs361625) gene in women patients with RPL and HC were 23.3% (35 of 150 cases) and 14% (7 of 50 cases), respectively. While the negative results were in women patients with RPL and AHC were 76.7% (115 of 150 cases) and 86% (43 of 50 cases), respectively. The mean of serum TNF-α concentration for AHC and women patients with RPL groups were 6.25 ±0.78 pg./ml and 12.50 ±1.60 pg./ml,respectively. Statistically, significant difference (p<0.05) was found on comparing the mean of serum TNF-α concentration among these study groups.

Keywords: recurrent miscarriage; parvovirus B19 infection; Immunogenetic study; TNF alpha gene

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

doi.org/10.31838/hiv23.01.28 Received: 19.09.22, Revised: 14.10.22, Accepted: 22.12.22

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 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. (Tyagi and Alharthi,2020).

The parental carriers of balanced structural chromosomal rearrangements including balanced reciprocal and robertsonian translocations are responsible for 2-4% of RPL cases. Tumor necrosis factor-α (TNF-α) a key pro-inflammatory cytokine, is secreted by macrophages and plays an important role in the implantation, placentation and pregnancy outcome (Elsokkary et al,2018). There are several common single nucleotide polymorphisms (SNPs) in TNF-α which can regulate the transcription and production of TNF-α. To date, several promoter region SNPs in TNF-α have been reported, among which namely -308G>A (rs1800629) and -238G>A (rs361625) ;(rs361525), are most frequently studied in RPL (Alijotas-Reig et al,2017; Aslebahar et al,2019).

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 Adeno-associated Virus (AAV)and 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 and TNF-α 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 specimens 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).

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 TNF-α -238G/A (rs361625) SNPs by Sequencing

Total DNA for SNPs of TNF-α -238G/A (rs361625) polymorphism were extracted from peripheral blood and swabs of female patients using sequencing technique.

Evaluation of TNF-α Concentration in Blood Serum of Patients and AHC.

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

3. Result

Detection of Parvovirus B19 By RT-PCR

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.

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	

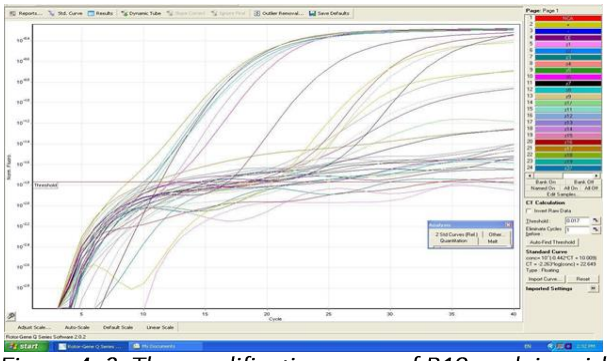

Figure 4. 3: The amplification curve of B19 nucleic acid.

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%	

4.4.3. Genotyping of TNF-α -238G/A (rs361625) Gene in RPL and AHC

For TNF-α -238G/A (rs361625) genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions as mentioned in the table (4-10).

The results revealed that the presence a single band (317 bp) of the target sequence of TNF-α -238G/A (rs361625) gene in agarose gel Figure (4-7).

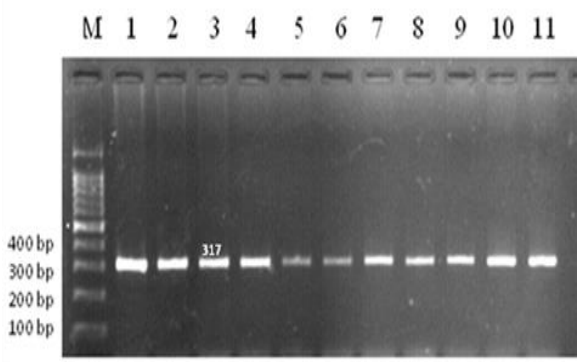

Figure 4.7. Agarose gel electrophoresis of an amplified product patterns of TNF-α -238G/A (rs361625) Exon5 region. 1.5 % Agarose Gel Electrophoresis, TBE 1X, at Voltage 75 Volt for 45 min.

Table (4-10) show the percentage of a single band (317 bp) of the target sequence of TNF-α -238G/A (rs361625) gene. The positive result, according to PCR amplification of a single band (317 bp) of TNF-α -238G/A (rs361625) gene in women patients with RPL and HC were 23.3% (35 of 150 cases) and 14% (7 of 50 cases), respectively. While, the negative results were in women patients with RPL and HC were 76.7% (115 of 150 cases) and 86% (43 of 50 cases), respectively as shown the Table (4-10).

Table 4.10: Percentage of TNF-α -238G/A (rs361625) signals in patients with RPL and AHC groups by PCR technique.

TNF-α -238G/A (rs361625) band	RPL No.(%)	AHC No.(%)
Positive	35(23.3%)	7 (14%)
Negative	115 (76.7%)	43 (86%)
Total	150 (100%)	50 (100%)

4.4.3.1. Genotyping of TNF-α -238G/A (rs361625) Among study groups

The results showed that DNA polymorphism distribution were DNA
polymorphism distributions according to A\C; A\T; A\G; T\G; T\A and T\C
haplotypes were 9% ; 7% ; 9% ; 6% ; 9% and 5%, respectively in women patients with RPL and 4% ; 0% ; 8% ; 2% ; 3% and 2%, respectively in HC group.

4.5. Evaluation of Serum TNF-α concentration By ELISA Among Study Population

While, the table (4-13) was showed the mean of serum TNF-α concentration for AHC and women patients with RPL groups were 6.25 ±0.78 pg./ml and 12.50 ±1.60 pg./ml,respectively. Statistically, significant difference ($p<0.05$) was found on comparing the mean of serum TNF-α concentration among these study groups (Table 4-13).

Table 4.13: Results of serum TNF-α concentration by ELISA for AHC and women patients with RPL

TNF-α	AHC (pg/ml)	Women with RPL (pg/ml)
Mean± SE	6.25 ±0.78	12.50 ±1.60
LSD	3.59	
P value	*P*<0.05 (0.039) *	

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% (Bascietto et al,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 TNF-α -238G/A (rs361625) Gene in RPL and AHC

In pregnancy, the immune system plays an important role. The normal development thereof is the result of a balance between the cytokines produced by TH1 immune response (TNFa, TNFb, IFNg, IL2, IL12 etc.), and those produced by TH2 immune response (IL4, IL5, IL6, IL 10, IL13, etc.) (Aslebahar et al,2019; Estuardo et al,2021). A disruption of said balance may cause alterations before and during pregnancy. The Single Nucleotide Polymorphisms of TNFa G238A, and G308A, that modify TNFa and Lymphotoxin a (LTα) concentrations are an example of this, which, in turn, in many studies have been associated to an increase of pregnancy diabetes, spontaneous abortion, pregnancy loss, recurrent pregnancy loss, preeclampsia, and premature delivery (Drews et al,2019; El-Bassyouni et al,2019).

In the current study, the distributions of TNF-α -238G/A (rs361625) according to frequencies of A\C; A\T; A\G; T\G; T\A and T\C haplotypes were 9%; 7%; 9%; 6% ; 9% and 5%, respectively in women patients with RPL and 4% ; 0% ; 8% ; 2% ; 3% and 2%, respectively in HC group. These results consistent with Estuardo et al, (2021) was found an increase in prevalence in TNFa, G238A compared with TNFa G308A and LTa A252G (31.9 vs 25.4 and 26.5%). The heterozygous form was higher in prevalence compared with the homozygous. In 50.3% no mutations of TNFa G238A, TNFa G308A and LTa A252G were found; the number of patients that only presented one polymorphism was 23.2%, with 2 polymorphisms represent 21%, and presented 3 polymorphisms (5.3%). Also, these results agreement with result of Sudhir et al, (2016) was reported a higher rate of TNFa G308A in women with RPL compared with a control group (29.57% vs 16.22%).

Bahmani et al, (2018) was observed frequencies of GG and GA genotypes of TNF-α-308G/A polymorphism were respectively 84% and 16% in the patient group and 81% and 19% in the control group. AA genotype was not observed in any of the groups.

In addition, Puppala et al, (2016) and Ma et al, (2017) have shown prevalence of the homozygous TNFa G238A polymorphism from 0 to 8%, and the heterozygous one from 3 to 31%; and the homozygous TNFa G308A polymorphism from 0 to 12.3%, and the heterozygous one from 6.6 to 48%, respectively.These results compatible with present results of current study.

Furthermore, Jung et al, (2020) in a meta-analysis of 21 studies with 3437 cases and 4016 controls, compared several TNFa polymorphisms with RPL and found an association of TNFa G308A with a higher risk of RPL in the heterozygous form. Similarly, Hui et al, (2016) in a meta-analysis that included 1430 patients with RPL, and 1727 controls, reported that the TNFa G308A polymorphism is correlated to a high risk of RPL in Asian women.

A study in Alkhuriji et al, (2013) showed a significant

association between TNFα-308G/A polymorphism and RPL in Saudi Arabia.

Aboutorabi et al, (2018) who found significant differences in the distribution. This research confirmed that TNF-α polymorphisms might suspect factor of recurrent pregnancy loss cases,and TNF-α polymorphisms were the possible genetic problem of pregnancy loss.

In contrast, the results of current study were disagreement with study of Mariya et al, (2020) who explained no statistical difference in allele frequencies of the TNF-α -308 G/A polymorphism was established between controls and study patients (p=0.78). Similarity,Liu et al,(2010) who observed no significant relationship between TNFα-308G/A polymorphism and RPL in women patients in China.

In humans, tumor necrosis factor (TNF)-α inhibit embryonic and fetal development as well as the proliferation of human trophoblast lines.It is also demonstrated that enhanced uterine expression of pro-inflammatory cytokines such as TNF has been associated with embryo loss (Ru-Xin et al,2015).

The TNF-α polymorphisms might suscept factor of recurrent pregnancy loss cases. Therefore, the TNF-α polymorphisms were the possible genetic problem of pregnancy loss (Aboutorabi et al, 2018).

Placental cytokines such as TNF α have been reported to play roles in necrotic and apo necrotic trophoblast cell death by influencing caspase and endothelial cell activation activities. TNF α are thought to play important roles in early pregnancy and also to be elevated in inflammatory states (Ali and Khan, 2020).

The maternal overexpression of the TNF alpha gene may increase the recruitment of inflammatory leucocytes in the maternal-fetal interface, resulting in uteroplacental perfusion deficiency, development of thrombotic events and placental hypoxia, finally abortion of embryos (Baud and Karin, 2001).

Certain immune-genetic factors which initiate activate immune system abnormally may play a crucial role and might be responsible for recurrent pregnancy loss.

The findings in the present study were different from those of other RPL studies. The reason for the difference in the results of gene polymorphism studies can be due to genetic and geographical differences and a small sample size.

The mean of serum TNF-α concentration for AHC and women patients with RPL groups were 6.25 ±0.78 pg./ml and 12.50 ±1.60 pg./ml,respectively. Statistically, significant difference (p<0.05) was found on comparing the mean of serum TNF-α concentration among these study groups. These results agreement with Tersigni et al, (2018) who found the level of TNF- α in women patient group had high significant differences (P 0.003) in comparison with the control group.

These results agreement with study of Baud and Karin, (2001) who found the level of TNF- α aborted women has increased significantly (p < 0.05) compared to controls (Li et al,2017).

Raghupathy and Kalinka, (2008) have been reported there is a significant increase in the median values of the TNF- α (12.40pg/mL) in the subjects with threats of miscarriage, when compared with the apparently healthy pregnant subjects (TNF- α, 1.00pg/ mL) and apparently healthy non-pregnant subjects (TNF-alpha, 0.70pg/mL and IL-2, 0.75pg/mL) (p<0.05).

The TNF-alpha is primarily expressed by mononucleo macrophage, CD4 + Th1, cell NK, etc; These immune cells, certain reproductive tissues can also express this cytokine. The expression of TNF-α DNA decreased significantly in normal delivery compared to that of abortion (Fares et al,2021).

Many studies on TNFα showed that it is a pleiotropic proinflammatory cytokine involved in a wide variety of cellular processes and, moreover, has contradictory effects ranging from cell proliferation to cell death. First, it was described that TNFα was involved in immune system regulation and was mainly secreted by cells such as monocytes, macrophages, natural killer (NK) cells, T lymphocytes, mast cells, and neutrophils, but later several works showed that it is also produced by non-immune cells like endothelial cells, adipocytes, neurons, fibroblasts, and smooth muscle, among others (Baud and Karin, 2001; Mercogliano et al, 2021).

TNFα can regulate several pathological and physiological processes beyond the immune system. The duality of many members of the TNF superfamily, as ligand and receptor, gives rise to the particular phenomenon of reverse signaling when acting as a receptor, tmTNFα can signal outside-to-inside back to the tmTNFα expressing cell. Pleiotropic effects of TNFα can be due not only to its two forms but also to the low homology of the ligand binding domain and no homology in the intracellular domain of the receptors, which have no enzymatic activity and therefore have to recruit scaffold proteins to unleash the signaling cascade (Mercogliano et al, 2021).

Placental cytokineTNF α, have been reported to play roles in necrotic and apo necrotic trophoblast cell death by influencing caspase and endothelial cell activation activities. TNF α are thought to play important roles in early pregnancy and also to be elevated in inflammatory states (Ali and Khan, 2007).

Anderson, (2007) who explained the high levels of pro-inflammatory molecules TNF a, and inflammatory leukocytes (macrophages, neutrophils, lymphocytes) were found in women with recurrent pregnancy loss (RPL) compared to women with normal pregnancy.

The level of TNF alpha protein in the placental tissue of the aborted ewes has increased compared to controls. The development of excessive pro-inflammatory cytokine (TNF-a) would assess fetal rejection (Baud and Karin, 2001).

The lower TNF-α concentrations could improve pregnant women's energy metabolism and their embryo development, increase synthetic progestagen and choriogonadotropin levels, and

stimulate trophoblast to generate urokinase-type plasminogen activator (uPA), It promotes the deterioration of decidual cell ectomatrix and placenta implantation and eventually plays a role in pregnancy sustainability (Fares et al, 2021).

Chaouat et al, (2003) reported that PBMCs were accumulated locally through a series of complicated mechanisms at the maternal-fetal interface and secreted a larger amount of TNF-α in the form of paracrine or autocrine, possibly resulting in abortion. The higher TNF-α concentrations can lead to abortion by the promotion of trophic cell apoptosis, the elevation of synthetic PG E2, excitation of uterine smooth muscle,stimulation of Th1 type of immunological reaction, rejection of embryonic tissue, coagulative system activation leading to placenta trophic vessel thrombosis. Thus, the proportion of CD4 + T cells in the peripheral blood of early pregnant women had decreased significantly, presumably as a result of physiological changes in several hormones during pregnancy contributing to certain changes in the maternal immune system and ensuring the development of relatively lower levels of TNF-α in pregnant maternity.

Refrences

Aboutorabi, R, Behzadi, E, Sadegh, M. J, Fatehi, S. P, Semsarzadeh, S, Zarrin, Y, & Mostafavi, F. S. (2018). The study of association between polymorphism of TNF-α gene's promoter region and recurrent pregnancy loss. Journal of Reproduction & Infertility, 19(4), 211.

Ali, R. and I. Khan (2006). "Mycotic abortion in cattle." Pakistan Veterinary Journal 26(1): 44.

Ali, R, & Khan, I. H. (2020) Mycotic abortion in cattle. Pak Vet J, 26:44-46.

Alijotas-Reig, J, Esteve-Valverde, E, Ferrer-Oliveras, R, Llurba, E, & Gris, J. M. (2017). Tumor necrosis factor-alpha and pregnancy: focus on biologics. An updated and comprehensive review. Clinical reviews in allergy & immunology, 53(1), 40-53.

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.

Alkhuriji, A. F, Alhimaidi, A. R, Babay, Z. A, & Wary, A. S. (2013). The relationship between cytokine gene polymorphism and unexplained recurrent spontaneous abortion in Saudi females. Saudi medical journal, 34(5), 484-489.

Anderson, R. (2007). Thematic content analysis (TCA). Descriptive presentation of qualitative data, 1-4.

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

Aslebahar, F, Neamatzadeh, H, Meibodi, B, Karimi-Zarchi, M, Tabatabaei, R. S, Noori-Shadkam, M, & Dehghani-Mohammadabadi, R. (2019). Association of tumor necrosis factor-α (TNF-α)-308G> A and-238G> A polymorphisms with recurrent pregnancy loss risk: a meta-analysis. International Journal of Fertility & Sterility, 12(4), 284.

Bahmani, A, Bonyadi, M. J, & Tagavi, S. (2018). The Association of the TNF-α-308G/A Gene with Recurrent Miscarriage Abortions in East Azarbaijan. Journal of Kermanshah University of Medical Sciences, 22(3).

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.

Baud, V. and M. Karin (2001). Signal transduction by tumor necrosis factor and its relatives. Trends in cell biology 11(9): 372-377.

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.

Drews-Piasecka, E, Seremak-Mrozikiewicz, A, Barlik, M, Kurzwińska, G, Wolski, H, Woyciechowska, A, & Drews, K. (2014). The significance of TNF-α gene polymorphisms in preterm delivery. Ginekologia Polska, 85(6).

El-Bassyouni, H. T, Raouf, S. M. A, Farag, M. K, Nawito, W. M, Salman, T. M, & Gaber, K. R. (2018). Dysregulation of tumor necrosis factor-α and interleukin-6 as predictors of gestational disorders. Middle East Journal of Medical Genetics, 7(2), 112.

Elsokkary, M, Elshourbagy, M, Labib, K, Mamdouh, A, El-Shahawy, Y, Nossair, W. S, & Abdelshafy, A. (2018). Assessment of hysteroscopic role in management of women with recurrent pregnancy loss. The Journal of Maternal-Fetal & Neonatal Medicine, 31(11), 1494-1504.

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.

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.

Fares, B. H, Hussain Al-tememy, H. A, Abdulmajeed, B. A, & Dakheel, M. M. (2021). Molecular Detection of Tumor Necrosis Factor-Alpha (TNF-a) Gene in Mycotic aborted Placenta of Ewes Using RT-PCR. Medico-legal Update, 21(1).

Hao Ma & Stephen Difazio. (2018). An efficient method for purification of PCR products for sequencing. BIOTECHNIQUESVOL. 44, NO. 7

Hui L, Xing X, Xing T, et al. Association of TNF-α genetic polymorphisms with recurrent pregnancy loss risk: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2016;14(6):1–10.

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.

Jung A, Cho H, Gwan G, et al. (2020) Tumour necrosis factor alpha gene polymorphisms in women with recurrent pregnancy loss: a meta-analysis. Human Fertility. 23(3):159–169.

Li, S, Wang, L, Xing, Z, Huang, Y, & Miao, Z. (2017). Expression level of TNF-α in decidual tissue and peripheral blood of patients with recurrent spontaneous abortion. Central European Journal of Immunology, 42(2), 156-160.

Liu, C, Wang, J, Zhou, S, Wang, B, & Ma, X. (2010). Association between -238 but not -308 polymorphism of Tumor necrosis factor alpha (TNF-alpha) v and unexplained recurrent spontaneous abortion (URSA) in Chinese population. Reproductive biology and endocrinology: RB&E, 8, 114. https://doi.org/10.1186/1477-7827-8-114

Liu, R. X, Wang, Y, & Wen, L. H. (2015). Relationship between cytokine gene polymorphisms and recurrent spontaneous abortion. International journal of clinical and experimental medicine, 8(6), 9786.

Mariya Levkova, Trifon Chervenkov, Mari Hachmeriyan, Lyudmila Angelova. Association between polymorphic markers human leucocyte antigen-G and tumour necrosis factor alpha and susceptibility to recurrent miscarriages among Bulgarian women. Turk J Obstet Gynecol 2020; 17:34-9.

Mercogliano, M. F, Bruni, S, Mauro, F, Elizalde, P. V, & Schillaci, R. (2021). Harnessing tumor necrosis factor alpha to achieve effective cancer immunotherapy. Cancers, 13(3), 564.

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.

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

Puppala M, Lakshmi V, Anuradha A, et al. Association of tumor necrosis factor-alpha and interleukin-6 gene polymorphisms with preeclampsia. International Journal of Bioassays. 2016;5(2):4744–4747

Raghupathy, R, & Kalinka, J. (2008). Cytokine imbalance in pregnancy complications and its modulation. Frontiers in Bioscience-Landmark, 13(3), 985-994.

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

Sudhir, N, Beri, A, & Kaur, A. (2016). Association of tumor necrosis factor-alpha 308G/A polymorphism with recurrent miscarriages in women. Journal of human reproductive sciences, 9(2), 86.

Tersigni, C, D'Ippolito, S, Di Nicuolo, F, Marana, R, Valenza, V, Masciullo, V, & Di Simone, N. (2018). Recurrent pregnancy loss is associated to leaky gut:

a novel pathogenic model of endometrium inflammation? Journal of translational medicine, 16(1), 1-9.
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.
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