Journal of
Kerman University of Medical Sciences

https://jkmu.kmu.ac.ir
10.34172/jkmu.2024.48
Vol. 31, No. 6, 2024, 312-317

Original Article

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Viral Infections and Spontaneous Abortion in Women: A Case-Control Study

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Abstract
Background: Spontaneous abortion before the 20th week, a prevalent pregnancy complication, is often linked to intrauterine viral infections. This study aims to identify DNA viruses in placental tissue from women with spontaneous abortion and full-term pregnancies, establishing a correlation with first-trimester spontaneous abortion.
Methods: This case-control study was conducted in Kerman city between 2021 and 2022. Placental tissue samples were collected from 50 women who had experienced spontaneous abortion in the first trimester and 50 women with normal pregnancies. The PCR method was employed to assess the presence of specific viruses.
Results: In the case group, the average age was 28.74 ± 6.11, while in the control group, it was 31.08 ± 6.48. The study identified cytomegalovirus (CMV), human papillomavirus types (HPV) 16 and 18, herpes simplex virus-1 (HSV-1), and herpes simplex virus-2 (HSV-2) genomes in varying percentages in the case group compared to the control group. Notably, the parvovirus B19 genome was not detected in either group. However, statistical analysis did not indicate a significant difference in the presence of these viruses between the case and control groups.
Conclusion: Consistent with some prior research, this study did not find a correlation between viral placental infections and an increased risk of spontaneous abortion. Further research should be conducted with larger sample sizes across different regions to ascertain the true role of viral infections in abortion.
Keywords: Spontaneous abortion, Viral infection, Normal pregnancy

Citation: Yarabbi M, Nakhaie M, Jafari Maskouni E, Mousavi E, Karimi N, Arabzadeh AM, et al. Viral infections and spontaneous abortion in women: a case-control study. *Journal of Kerman University of Medical Sciences*. 2024;31(6):312–317. doi: 10.34172/jkmu.2024.48

Received: February 14, 2024, **Accepted**: July 13, 2024, **ePublished**: December 17, 2024

Introduction

Miscarriage, a prevalent complication in early pregnancy, profoundly impacts women not only physically but also socially and psychologically. Defined as the unintentional loss of the product of conception before the 20th to 22nd week, with over 80% occurring in the first trimester, it remains a complex and inadequately understood phenomenon (1). Despite extensive research on the subject, in most cases, the precise causes of miscarriage, are still unknown. Etiological determination of spontaneous abortion stands as a pivotal challenge in childbirth. Genetic abnormalities, primarily fetal chromosomal issues, contribute to almost 50% of early pregnancy losses. Additional factors include maternal anatomical abnormalities, endocrine and immune-related causes, environmental exposures (e.g., caffeine, nicotine), infections, and advanced parental age—especially notable among women who have experienced early pregnancy loss (1). Contradictory findings in various studies challenge the general acceptance of uterine infections as a primary cause of miscarriage. Instead, emerging evidence highlights the pivotal role of viruses, especially in developed countries where most congenital infections with known causes are viral in nature.

Viral intrauterine infections are recognized as potential contributors to abortion. Viral trophoblast infections are particularly implicated, disrupting trophoblast function and influencing implantation and mating abnormalities (2). Numerous viruses are linked to spontaneous abortion, including cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), and herpes simplex virus-2 (HSV-2), Epstein-Barr virus (EBV), human papillomavirus (HPV), Zika virus, rubella virus, and parvovirus (3-7). While CMV is the most common viral infection transmitted through the placenta (8), it is noteworthy that parvovirus B19 can also be transmitted from an infected mother to the fetus. Viral infections, such as parvovirus B19 and HPV, pose significant risks during pregnancy. Parvovirus B19 infection can lead to spontaneous abortion, fetal anemia, non-immune hydrops, congestive heart failure, and fetal demise. Immediate diagnosis and timely fetal blood transfusion are crucial to mitigate these risks (9,10). Several studies highlight the association between HPV presence in the cervix and placenta with spontaneous abortion and premature birth (6). Rubella infection in pregnant women, particularly during the first trimester, is linked to miscarriage, stillbirth, and congenital rubella syndrome. This syndrome involves the rubella virus infecting the placenta, spreading to the fetus, and disrupting organ formation, resulting in a range of congenital defects such as cataracts, hearing loss, mental retardation, and congenital heart defects (1). In addressing these concerns, the current research aimed to investigate and diagnose abortion-associated viruses. The ultimate goal was to derive practical solutions to reduce the incidence of abortion based on the findings of this research.

Methods

Study population

This case-control study involved the analysis of 50 placenta tissue samples from women who experienced spontaneous abortion of unknown cause (intrauterine death before 20 weeks) and 50 placenta tissue samples from women with normal pregnancies in the postpartum period (38–42 weeks). The research was conducted in Kerman city between 2021 and 2022. Patient sampling was conducted with the consent of the individuals or their relatives, and their information was securely preserved.

Inclusion criteria

- Patients residing in Kerman city.
- Mentally alert patients capable of providing written informed consent.
- Patients with spontaneous abortion of unknown cause.

Exclusion criteria

- Patients with a history of diseases such as diabetes, hypertension, pre-eclampsia, kidney disease, and thyroid disease.
- Patients with chromosomal disorders.
- Patients with placenta and umbilical cord anomalies.
- Patients with placental events such as decollement, placental abruption, etc.
- Patients with antiphospholipid syndrome.

Collection and storage of samples

Placenta tissue samples from postpartum pregnant women were carefully collected in sterile plastic containers with lids. These samples were then stored in the vicinity of nitrogen and promptly transported to the laboratory within 24 hours. Upon arrival, the samples were immediately stored in a freezer at -70 °C until extraction. Additionally, placenta tissue samples from spontaneous abortion cases were obtained as paraffin blocks from the archive department of the pathology laboratory at Afzalipur hospital in Kerman, Iran.

Sample preparation

In this study, 25 mg of placenta tissue underwent initial homogenization with 10 mL of phosphate-buffered saline using a homogenizer. The resulting homogenate was centrifuged at 5000 rpm and (2–8) degrees Celsius for approximately 15 minutes. The supernatant was carefully collected and subjected to DNA extraction.

DNA Extraction

DNA extraction from placenta tissue followed the manufacturer's protocol using the SinaClon tissue DNA extraction kit (Iran). For paraffin-embedded placenta tissue samples, a deparaffinization process was initiated on 10 µm tissue sections using xylene. The subsequent ethanol treatment removed the xylene, and the sample was transferred to a 1.5 mL microtube. The microtube then received 10 µL of pre-lysis buffer and 20 µL of ribonuclease enzyme, followed by incubation at 55 °C for 1–3 hours and then at 72 °C for 10 minutes. These extraction steps were mirrored in both fresh and paraffin-embedded samples. Post-extraction, the concentration and purity of the DNA were assessed by measuring the absorption of UV light at wavelengths of 260 and 280 nm. The light absorption ratio at 260 nm to 280 nm determined the purity, with an acceptable range of 1.8–2.

Polymerase chain reaction (PCR) for detection of viruses genome

The quality evaluation of the extracted genome involved amplifying the human β-globin gene using PCO3/PCO4 primers (Table 1). Subsequently, further investigations were conducted on samples that exhibited positive results. These DNA extracts were then used as templates for detecting the genome of the studied viruses (CMV, HSV-1, HSV-2, HPV-16, HPV-18, and B19) through PCR. The primers used for different regions of viral genes are presented in Table 1. Each reaction mixture consisted of 15 µL of master mix, 0.5 mL of each primer, 4–8 µL of PCR-grade H_2O, and 1–5 µL of template, resulting in a final volume of 25 µL. The PCR reaction followed specific thermal cycling conditions presented in Table 2 for each viral gene.

Data analysis

The results were analyzed statistically using SPSS 26

Table 1. Oligonucleotide sequence of primers used in this study

Virus and Internal control	Primer sequences	Gene	Product size (bp)	References
CMV	5´- CATAATCTCATCA GGGGAGC- 3´ 5´-TTGGGCTAACTATGC AGAGC- 3´	UL123	305	(11)
B19	5´-AATGCAGATGCCCTCCACG-3´ 5´-ATGATTCTCCTGAACTGGTCCA-3´	NS1	193	(9)
HSV-1	5´-CCGGAGAGGGACATCCAGGACTT-3´ 5´-GGGCCATGAGCTTGTAATACACCGT-3´	UL30	112	(12)
HSV-2	5´-TACGCTCTCGTAAATGCTTC-3´ 5´-GCCCACCTCTACCCACAA-3´	gpG	120	(13)
HPV-16	5´- CCCAGCTGTAATCATGCATGGAGA -3´ 5´- GTGTGCCCATTAACAGGTCTTCCA -3´	E6/E7	272	(14)
HPV-18	5´- CGACAGGAACGACTCCAACGA -3´ 5´- GCTGGTAAATGTTGATGATTAACT -3´	E6/E7	202	(15)
β-globin	5' ACACAACTGTGTTCACTAGC 3' 5' CAACTTCATCCACGTTCACC 3'	PCO3 PCO4	110	(16)

Table 2. Thermal cycling conditions used for the PCR reaction of different viral genes

Stage	Temperature (° C)	Time
Initial denaturation	95 ° C	4 min
denaturation	95 ° C (denaturation)	30 s
Cycling stage (35 cycles) (annealing)	HSV-1: 58 °C HSV-2: 54 °C CMV: 54 °C HPV-16: 60 °C HPV-18: 55 °C B19: 56 °C	30 s
Extension	72 °C (Extension)	30 s
Final extension	72 °C (Extension)	10 min

software. Chi-square and Fisher's exact tests were applied for the statistical analyses.

Results

Demographic information of patients

In this case-control study, the average age of patients in the case group, including women with spontaneous abortion before the 20th week of pregnancy, was 28.74 ± 6.11 years. In the control group, including women with a normal fourth-trimester type 2 pregnancy of 38 weeks, it was 31.08 ± 6.48 years (Table 3).

Frequency of different viruses in the case and control groups

Following the PCR examination of samples from the case and control groups, the presence of viral DNA was observed as follows: CMV: 6 cases in the case group and 3 cases in the control group, HSV-1: Only 1 case in the case group, HSV-2: 3 cases in the case group, HPV-16: 2 cases in the case group and 1 case in the control group, HPV-18: Only 1 case in the case group, Parvovirus B19: Not identified in any case or control group (Table 4).

Discussion

Termination of pregnancy before the 20th week is defined as abortion, with miscarriage being the most prevalent complication of pregnancy, associated with various causes. Understanding the prevalence of abortion in the community aids as an indirect gauge of maternal health. Awareness of its causes allows for inspection and adjustment. While genetic factors and fetal abnormalities are widely accepted as causes of abortion (17,18), recent studies suggest intrauterine infections as significant contributors (19). Infections with bacterial, viral, and parasitic agents are implicated in abortion, and they often appear as influential factors in spontaneous abortion. Intrauterine infections by various viruses, including HSV, mumps, rubella, parvovirus, EBV, HPV, and polyomaviruses, are associated with both spontaneous abortion and premature births (4-7). Given the considerable prevalence of genital infections and the established connection between viral infections and abortion, coupled with the insufficient information on virus prevalence during pregnancy in Kerman province and in the whole country, this study attempted to explore the molecular prevalence of specific viruses. Specifically, CMV, HSV-1, HSV-2, HPV-16, HPV-18, and parvovirus B19 were scrutinized in placental tissue samples from both spontaneous abortion cases and term pregnancy cases. The study revealed varying percentages of viral genome presence in placental tissue samples. In the case group, the CMV genome was detected in 12%, HPV-16 in 2%, HPV-18 in 2%, HSV-1 in 2%, and HSV-2 in 6%. Conversely, the control group exhibited lower percentages for each virus: 6% for CMV, 1% for HPV-16, and no detection for HPV-18, HSV-1, and HSV-2. Notably, the parvovirus B19 genome was absent in both the case and control groups. Despite a higher frequency of CMV in cases of spontaneous abortion compared to term pregnancy, no significant association was found between the detection of CMV in the placenta and an increased risk of miscarriage. Consistent with our study, a report by Charostad and colleagues in Tehran in 2020 documented a prevalence of 8.4% and 4.9% for this virus in 83 cases of spontaneous abortion and 80 cases of therapeutic abortion, respectively, without finding a significant

Table 3. Demographic information between case and control groups

Demographic parameters	Groups	Number	Mean (year)	SD	Median	*P* value
Age	Case	50	28.74	6.11	28.00	0.066
	Control	50	31.08	6.48	31.00	

Table 4. The number of positive cases of viruses among the case and control groups

Viruses		Groups	
		Control, n (%)	Case, n (%)
CMV	Negative	47 (94.0)	44 (88.00)
	Positive	3 (6.00)	6 (12.00)
HSV-1	Negative	50 (100.0)	49 (98.0)
	Positive	0 (0.0)	1 (2.0)
HSV-2	Negative	50 (100.0)	47 (94.0)
	Positive	0 (0.0)	3 (6.0)
HPV-16	Negative	49 (98.0)	48 (96.0)
	Positive	1 (2.0)	2 (4.0)
HPV-18	Negative	50 (100.0)	49 (98.0)
	Positive	0 (0.0)	1 (2.0)
B-19	Negative	50 (100.0)	50 (100.0)
	Positive	0 (0.0)	0 (0.0)

correlation (20). Another study in China in 2015 reported CMV infection prevalence rates of 9.1% in abortion cases and 6.7% in normal cases, with no significant relationship found between CMV infection and abortion (21). While some studies have suggested a higher prevalence of CMV in intrauterine infections and its potential role in abortion, the present study's results do not fully align with these findings (22,23). The exact mechanism remains unclear, but some studies propose that CMV might induce fetal death and abortion by causing a systemic disease in the mother and involving the placenta and fetus. Moreover, these findings suggest that genital CMV infection in the first trimester could influence pregnancy outcomes and contribute to miscarriage. In the current study, no significant relationship was found between the prevalence of HSV-1, HSV-2, and spontaneous abortion. A study in Iran by Borhani et al involving 35 placental tissues with spontaneous abortion and 35 placental tissues with term delivery detected HSV in only 2.8% of cases, and no significant difference was observed between the case and control groups (24). Similarly, in a study by Charostad and colleagues on 83 spontaneous abortion cases compared to 80 abortion treatment cases, no HSV-1 and 2 were detected in aborted cases (20). Contrary to the present study, several other studies have reported a high prevalence of HSV-1 and HSV-2 in aborted placental tissue. For instance, a study in Brazil in 2020 by Avila et al reported a prevalence of 37.5% for HSV-1 in placenta samples (10). Additionally, in a 2017 study in Iraq, Hassan et al found the presence of HSV-2 in 19 spontaneous abortion placenta tissues (19%) out of 100 placenta tissues (13). These studies suggest that intrauterine infections with HSV-1 and HSV-2 may lead to fetal growth restriction, premature birth, and an increased risk of spontaneous abortion. The present study also investigated the presence of DNA from HPV-16 and 18, with both types showing a low frequency of around 2%. Similarly, a study in Italy by Tognon et al on 80 spontaneous abortion placental tissues and 80 voluntary abortion placental tissues reported the detection of HPV in only 2.5% of voluntary abortion cases, with no DNA detected in cases of spontaneous abortion (25). Contrastingly, some authors have suggested an increased prevalence of HPV during pregnancy, its transmission through the placenta, and its potential negative impact on the fetus. Bober et al reported a prevalence of HPV in 18% of spontaneous abortion cases and 7% of term pregnancy cases. Their results indicate that HPV infection in the first trimester of pregnancy may adversely affect pregnancy outcomes and elevate the risk of miscarriage (26). In the examination of the last virus in this study, parvovirus B19, no detection was made in any of the case and control samples. In a study similar to the present one, Mirzaei et al reported a prevalence of 10% for parvovirus B19 in the case group and 1.4% in the control group. However, they did not observe a significant relationship between the prevalence of parvovirus B19 and abortion (27).

Conclusion

In summary, the findings of this study indicate that CMV, HSV-1, HSV-2, HPV-16, HPV-18, and B19 had no significant association with increased risk of spontaneous abortion. Discrepancies in sample types (fresh tissue samples versus paraffin tissue samples), the size of the studied population (larger sample size), and variations in the sensitivity and specificity of virus detection methods may contribute to the absence of a clear association between the mentioned viral infections and spontaneous abortion. The study suggests that further investigations should be conducted on a much larger sample size, encompassing diverse populations within the country.

Acknowledgments
This study was performed as part of a master's thesis by Maryam Yarabbi. We also thank the individuals and organizations that participated in this research.

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Competing Interests

The authors declared no conflict of interest.

Ethical Approval

The project received approval from the Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran, under number IR.KMU.AH.REC.1400.208.

Funding

This study was financially supported by Kerman University of Medical Sciences, Kerman, Iran, by grant number 400000114.

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