Original Article

Comparison Between Serological and Molecular Detection of Human Parvovirus B19 Infection Among Pregnant Women with Spontaneous Abortion in Kirkuk, Iraq

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Abstract

Background: Human parvovirus B19 (HPV19) is a widespread virus that primarily affects youngsters in their school years. Acute infection is a known concern for pregnant women and their unborn children since the mother's immune system is suppressed during pregnancy. This can have devastating effects on both the mother and the unborn child. Infection with HPV19 during pregnancy has been linked to a variety of adverse outcomes, including congenital defects, stunted development, abortion, and even stillbirth. **Objective:** The objective of this study was to compare serologic and molecular methods in the detection of HPV19 infection in pregnant women who had spontaneous abortion in Kirkuk city. **Materials and Methods:** Blood samples were collected from 135 aborted women were attended Gynecological and Pediatric Hospital and private laboratories in Kirkuk city from the period between the beginning of November 2022 to the end of April 2023. The sera obtained from samples were analyzed for IgM- and IgG-specific antibodies of HPV19 using ELISA technique and detection of viral DNA using real-time PCR. **Results:** A total of 135 aborted women aged 15–45 were enrolled in this study. The result showed high rate of women with spontaneous abortions were within the age group 26–35 year, with normal weight, had abortions more than two times and most of the women were from the urban area and aborted in the first trimester of pregnancy. The results indicate that out of the 13(9.63%) IgM+ve cases of aborted pregnant women samples evaluated, 11 cases (84.62%) were found to be positive for HPV19 DNA through real-time PCR testing. Furthermore, among the cases with 28 (20.74%) positive IgG results, 1 case (3.57%) was also positive for HPV19 DNA. This indicates that a small proportion of IgG-positive cases were also found to have detectable Parvovirus B19 DNA. On the other hand, none of the cases classified as ELISA-negative were positive for B19DNA. **Conclusion:** The findings demonstrate a high prevalence of B19 DNA detected through PCR analysis in IgM-positive cases, supporting the association between HPV19 infection and the presence of viral genetic material. The presence of HPV19 DNA in a small proportion of IgG-positive cases suggests the possibility of ongoing viral replication or persistence. The absence of HPV19 DNA in ELISA-negative cases indicates the specificity of the PCR test in detecting the viral genetic material.

Keywords: Human parvovirus B19, molecular, spontaneous abortion

Introduction

B19 infection is a global problem that was initially identified by Cossart and colleagues in 1975 in England while testing a serum from a healthy blood donor.[1] HPV19 is the smallest and simplest DNA virus currently known. It has a single-stranded DNA genome that is 5.5 kb in size with a diameter of 22–24 nm.[2] HPV19 is the only member of the Parvoviridae family that is pathogenic for humans.[3] B19V replication occurs in erythroid-precursor cells and has been associated with a number of diseases, including erythema infectious (also known as the "Fifth Disease"), underlying hemolysis, which is most common in children and can be asymptomatic, polyarthralgia-arthritis syndrome, transient aplastic disorder in

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patients with hemolytic anemia, chronic anemia in immune-compromised people, and hydrops fetalis,[2] especially in those with immune system compromises or hematological abnormalities, as well as pregnant women.[3] B19V is mostly spread by the respiratory method, blood transfusions, organ and bone marrow transplantations, and mother-to-child transmission.[4] The incubation period for the illness ranges from 4 to 14 days, although it can go up to 21 days.[5] Every 3 to 5 years, there are seasonal outbreaks. High rates of transmission were observed from late spring to summer, while other investigations found the largest peaks to be in late winter and early spring. B19V seroprevalence frequencies range from 29% to 72% in the overall adult population.[6] When an infection impacts a pregnant woman, it may result in severe anemia and nonimmune hydrops fetalis (NIHF), which may induce spontaneous abortion and intrauterine fetal mortality.[1] Only a small percentage of pregnant women who are vulnerable to B19 will actually become infected with the virus. According to various research, the probability of fetal death linked to acute HPV19 infection during pregnancy is estimated to be 10%, ranging from 3 to 38%.[1] Symptoms including erythema infectiosum, a mild fever, headaches, and impatience may appear 10–14 days after the infection. It is estimated that 25% of viruses pass from the mother to the fetus. The biggest chance of a virus transformation occurs 1 week after infection, when the mother's blood has the highest virus content and IgM antibodies first appear in circulation. The likelihood of infection and fetal death declines as gestational age rises. This is due to passive antibody transmission to the fetus beginning in week 25 of pregnancy and continuing beyond, as well as a decrease in the appearance of P-antigen or globaside in the villous trophoblast layer with increasing gestational age.[7] Recently, popular HPV19 infection detection techniques included nucleic acid tests (NAT) for HPV19-DNA and enzyme-linked immunosorbent assays (ELISA) for anti-HPV19 immunoglobulin G (IgG) and anti-HPV19 immunoglobulin M (IgM) antibodies.[4] As there are presently no approved vaccines to prevent parvovirus B19 infection, it is useful to assess community propensity to human parvovirus when evaluating the risk to pregnant women. However, the absence of the classic rash pattern in the majority of patients with parvovirus B19 infection and the clinical similarities to other rash disorders, particularly rubella, underscores how challenging it is to diagnose B19 infection just through clinical examination.[2]

The aim of the study was an attempt to compare the serological and molecular methods in the detection of anti-HPV19 IgM and anti-HPV19 IgG using enzyme-linked immune sorbent assay (ELISA) technique. And detects viral DNA by real-time PCR among pregnant women with spontaneous abortion in Kirkuk city, Iraq.

Materials and Methods

Study design

A cross-sectional study was carried out from the beginning of November 2022 to the end of April 2023 on 135 pregnant women with spontaneous abortions who attended Gynecological and Pediatric Hospital and private laboratories in Kirkuk city.

Study population

The study population involved 135 pregnant women who had spontaneous abortions attended Gynecological and Pediatric Hospital and private laboratories aged 15–45 years. All women with abortion-related bacterial or viral infections, such as rubella, cytomegalovirus (CMV), herpes simplex virus (HSV), and toxoplasma parasites. Clinical evidence of genitourinary infection and patients with chronic disease were excluded from this study.

Analytical methods

Each woman in the study had her vein punctured to obtain 5 mL of blood using a syringe. Serum was prepared by centrifuging blood samples at 3,000 rpm for 15 min in sterile test tubes. The sera were then aspirated using an automatic micropipette and transferred to Eppendorf tubes, where they were then kept in a deep freezer at −20°C, then used to determine human parvovirus HPV19 IgM, human parvovirus HPV19 IgG antibodies using ELISA technique (SunLong Biotech, China) kit. HPV19 DNA was extracted from the patient's serum according to a commercial DNA extraction kit (Magnetic Bead Method) No. 6 (Hamburg, Germany). HPV19 DNA in human serum was detected using (Bosphore, Turkey) human parvovirus B19 kit.

Ethical consideration

Before each woman was enrolled in the study, informed agreement was acquired from her husband or other family members as well. Before the blood sample was taken, the process was fully described to each person to make sure they knew exactly what would happen. Kirkuk's health directorate has accepted this.

Statistical analysis

A specific statistical test was used to detect the relation between the studied variables which is the chi-square test and *P* value was regulated significantly at the level of <0.05 and highly significant at the level <0.01.

Results

In total, 135 women who had abortions participated in the study, and they were tested for HPV19 IgM and IgG antibodies as well as HPV19 DNA. In Table 1, the general characteristics of studied women, the study showed a high rate of women with spontaneous abortions were within the age group 26–35 years, with normal weight, had an abortion

Table 1: General characteristics of women with spontaneous abortion

Characteristics	Aborted women (n = 135)		Statistics
	No.	%	
Age (years)			
15–25	44	32.6	Chi-square = 17.150
26–35	59	43.70	P value = <0.001
36–45	32	23.70	
Total	135	100	
BMI			
Underweight	31	22.96	Chi-square = 8.050
Normal	100	74.0	P value = <0.01
Overweight	4	2.96	
Total	135	100	
Residence			
Rural	32	23.8	Chi-square = 6.711
Urban	103	76.2	P value = <0.01
Total	135	100	
No. of abortion			
2	17	12.6	Chi-square = 5.200
3	60	44.4	P value = <0.05
≥4	58	43.0	
Total	135	100	
Trimester of abortion			
1st trimester	99	73.3	Chi-square = 8.178
2nd trimester	36	26.7	P value = <0.05
Total	135	100	

Table 2: Comparison between ELISA and PCR in the detection of human parvovirus B19 in women with abortion

PCR result	ELISA +ve				ELISA -ve		P value
	HPV IgM+ve		HPV IgG+ve		HPV IgM-ve and IgG-ve		
	No.	%	No.	%	No.	%	
Positive	11	84.62	1	3.57	0	0	<0.001
Negative	2	15.38	27	96.43	94	100	
Total	13	100	28	100	94	100	

Sensitivity of PCR/HPV19 IgM: 84.62%, and the specificity is 100%

Sensitivity of PCR/HPV19 IgG: 7.1%, and the specificity is 100%

more than two times and most of the women were from an urban area and aborted in the first trimester of pregnancy. Table 2 indicates that out of the 13 IgM+ve cases of aborted women samples evaluated, 11 cases (84.62%) were found to be positive for HPV19DNA through PCR testing. Furthermore, among the cases with positive IgG results, 1 case (3.57%) was also positive for HPV19 DNA. This indicates that a small proportion of IgG-positive cases were also found to have detectable HPV19 DNA. On the other hand, none of the cases classified as ELISA-negative were positive for HPV19DNA.

Discussion

When a B19V infection throughout pregnancy is suspected, the diagnosis first depends on the serology of the mother's serum, and specific IgM and IgG tests should be done quickly. Within 10 days of transmission, IgM becomes detectable in an immunocompetent person, and a few days later, IgG levels rise, providing lifelong immunity.[8]

It is advised that the diagnosis of the acute infection be made during pregnancy using a multi-factorial approach, which includes the concurrent identification of both immunological and virological variables in serum from the mother's peripheral blood. This is true even though the finding of specific IgM is confirmatory. B19V genome determination in maternal serum is indicated as it can increase diagnostic precision and help track the stage of infection.[9]

The majority of women who experienced recurrent pregnancy loss (RPL) were under 35 years old, as reported

by Nguyen *et al*.[10] The majority of cases in the current study also had a history of first-trimester abortions, which is in line with earlier research by Fadhil,[11] who claimed that the first and second trimesters were the most common times for spontaneous abortions. In addition, a few of the study's participants had a history of miscarriage, stillbirth, or multiple births. According to Shan *et al.*,[12] the majority of the study's sample had an abortion during the first trimester of pregnancy, which is consistent with the findings of Hussien *et al*.[13] Similarly, Ali *et al.*[14] reported that 60% of women with abortions had undergone three or more abortions. Huss[15] found a significant correlation between the number of abortions and previous miscarriages and suggested that women who have experienced multiple abortions are more likely to have had previous miscarriages as well. The study also found that the rate of abortion more frequently occurred in women who belonged to urban areas than in rural areas. This finding is consistent with different studies done earlier that the difference in lifestyle and higher prevalence of abortion primarily affect urban.[16,17] Factors such as access to healthcare services, socioeconomic conditions, and differences in lifestyle and reproductive healthcare practices between urban and rural areas could potentially contribute to variations in abortion rates.[12]

Molecular-based techniques, such as PCR, have proven valuable in diagnosing various infectious diseases caused by different agents.[18] Serum samples can detect low levels of viral DNA up to four months post-infection, while in other tissues, this period can extend to years.[19] To diagnose chronic or acute infections, a combination of standard real-time PCR along with serological evaluations is recommended.[20]

The current findings emphasize the value of molecular testing, specifically PCR, in detecting the presence of HPV19 DNA in aborted pregnant women samples. The high proportion of HPV19 DNA detection in IgM-positive cases supports the use of IgM antibodies as an indicator of recent infection, while the presence of HPV19 DNA in a subset of IgG-positive cases raises the possibility of ongoing viral activity. These results contribute to a better understanding of the relationship between serological markers and the detection of viral genetic material, providing important insights into the diagnosis and management of parvovirus B19 infections in the context of aborted pregnancies.[21,22]

Studies involving volunteers have demonstrated that IgM antibodies can be detected for up to 12–14 weeks after incubation, whereas IgG antibodies can be present throughout the patient's life.[6] The IgM measurements can be useful for several years, while IgG levels can be monitored indefinitely.[7,23] B19 virus can be identified in serum using EM, ELISA for B19 antigen, or even hemagglutination, but the most common method is isolation of viral DNA using direct hybridization or Polymerase chain reaction.[7] Amplification of either the target or the detection system can boost the sensitivity of DNA hybridization testing. Amplification of the target by PCR is the most used technique.[24]

In line with our findings, Abdulhassan *et al*.[25] results revealed that among the 200 plasma samples tested, 40 (20%) were found to be positive for Parvovirus B19 through real-time PCR, while the remaining 160 (80%) were negative and all 19 samples that tested positive for Parvovirus B19 in the real-time PCR analysis were also positive in IgM ELISA. While study done by Abdelkabeer *et al.*[26] found that using PCR, 2 cases (2.1%) from the examined 97 samples from aborted women tested positive for HPV19 DNA, which was less than our finding as we found that out of the tested samples, 12 (8.89%) were positive for HPV19 DNA through PCR analysis.[27-30] The differences in the observed prevalence rates may be due to the characteristics of the study populations, including geographic location, demographics, or other factors, which might account for the contrasting results. Parvovirus B19 infection rates can vary among different populations and regions due to variations in exposure, and immune status. It is also important to consider the sensitivity and specificity of the PCR assay used in each study. Variations in the performance of the PCR assay, including differences in primer selection, amplification methods, and laboratory protocols, could influence the detection rates of B19 DNA.

Additionally, the sensitivity and specificity values for PCR/B19 IgM and PCR/B19 IgG are as follows: PCR/B19 IgM (sensitivity 84.62% and specificity 100%) while PCR/B19 IgG (sensitivity 7.1% and specificity 100%).

These values indicate the performance characteristics of the tests in terms of their ability to correctly identify true positive cases (sensitivity) and true negative cases (specificity). For PCR/B19 IgM, the test demonstrates a sensitivity of 84.62%, indicating that it can accurately detect B19 IgM antibodies in individuals who are truly positive for the virus. The specificity is 100%, meaning that the test shows no false-positive results and correctly identifies individuals without B19 IgM antibodies as negative. In contrast, PCR/B19 IgG exhibits a sensitivity of 7.1%, suggesting that it has a lower ability to detect B19 IgG antibodies in individuals who are truly positive for the virus. However, the specificity remains at 100%, indicating that the test does not produce false-positive results and correctly identifies individuals without B19 IgG antibodies as negative.

Conclusions

The findings demonstrate a high prevalence of HPV19 DNA detected through PCR analysis in IgM-positive cases, supporting the association between B19 virus infection and the presence of viral genetic material. The presence

of HPV19 DNA in a small proportion of IgG-positive cases suggests the possibility of ongoing viral replication or persistence. The results provide valuable insights into the presence and prevalence of B19 virus in aborted pregnant women samples and highlight the importance of molecular-based methods like PCR in diagnosing B19 virus infection.

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Conflicts of interest
There are no conflicts of interest.

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