Comparative analysis of advanced polymerase chain reaction-based detection of Parvovirus B19 in first-trimester pregnant women at Thumbay Hospital, Ajman, United Arab Emirates

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ABSTRACT

Human parvovirus B19 (B19V) is considered a prevalent single-stranded DNA virus belonging to the *Parvoviridae* family. It can be vertically transmitted from mother to fetus and is primarily spread by respiratory droplets. The clinical presentation of B19V infection varies based on the viral strain and the individual's age and immune status. This study aims to determine the prevalence of B19V infection among pregnant women in the first trimester and investigate the association between the virus and infected pregnancy results. A descriptive cross-sectional study was conducted at Thumbay Hospital, Ajman, United Arab Emirates. Pregnant women in their first trimester who sought antenatal care were included in the study. The enzyme-linked immunosorbent assay was used, and the results were confirmed with polymerase chain reaction (PCR). All data were analyzed using SPSS statistical methods. The study population consisted of pregnant women in the first trimester attending Thumbay Hospital. The study focused on B19V prevalence among 87 pregnant women in the first trimester and its association with risk factors. Results showed almost 9% overall prevalence of B19V infection, with higher rates among younger women and women with previous pregnancies. The B19V PCR detects only 5 counts, with 5.7% of samples infected with B19V associated with a higher risk of fetal loss. Early gestational age was detected to have a significant association with a p-value of 0.006. There was an insignificant association between B19V immunoglobulin M PCR infection and others (gravidity along with history of miscarriage and history of blood transfusion). This study provided valuable insights into the prevalence and risk factors associated with B19V infection among pregnant women in the first trimester. The findings highlighted the importance of early detection and appropriate management of B19V infection to prevent severe complications and improve pregnancy outcomes.

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Introduction

Human parvovirus B19 (B19V) is a single-stranded DNA virus that belongs to the *Parvoviridae* family. The virus was discovered in 1975 in a group of healthy blood donors in the UK.[1] B19V, formally named primate *Erythroparvovirus* by the International Commission on Taxonomy of Viruses,[2] is an etiological agent of erythema infectiosum, a familiar childhood illness. Known as the tropism of B19V for erythroid progenitor cells, it is also associated with a wide range of moderate to severe clinical manifestations related to anemia, depending on the immunological and hematolog-

ical condition of the host.[3] B19V is a prevalent human pathogen with different seroprevalence rates worldwide. Estimates indicate that the virus ranges from 30% in children to 85% in adults.[4] The virus is primarily transmitted *via* respiratory droplets, and it can be vertically transmitted from the mother to the fetus *via* the placenta. The viral strain involved in the individual's age and immune status determines the clinical presentation of B19V infection.[5] Depending on the age and immunity status of the infected individual and the viral strain involved, the clinical presentation of B19V infection can be diverse. However, the most common clinical presentation of B19V infection is erythema infectiosum, also known as the fifth disease. This mild and self-limited illness is characterized by a rash appearing on the face, such as a slapped cheek and a lacy maculopapular rash on the trunk and limbs. Erythema infectiosum is prevalent in children but can also be seen in adults who may experience the diseases associated arthropathy.[6] In the case of a pregnant woman infected with B19V, the implications for the fetus can be significant, such as fetal loss along with hydrops fetalis, fetal anemia, and congenital anomalies considered as some of the serious consequences that will arise as a result of the infection, and about 50% of pregnant women may be vulnerable to the virus depending on the restricted circulation. B19V infection is endemic-epidemic; seasonal outbreaks are registered in late winter through early summer and the scourge peaks every 4 to 6 years. Vertical diffusion occurs in 25-50% of cases, and the hazard of fetal harm is 2-18% depending on the sequence studied, the discovery methods, and the incidence of the epidemic outbreak.[7] The severity of the outcome depends on the timing of the infection, with first-trimester infections being associated with the highest risk of fetal loss, leading to rates as high as 10%.[8]

Although some protection against infection can occur in the fetus through transplacental transfer of B19V-specific immunoglobulin (Ig) G antibodies from the mother, the timing of maternal infection affects its efficiency.[9] It is now known that in a proportion of cases after the acute phase of infection and the mounting of the specific immune response, the viral genome remains detectable for weeks to several months or up to 2 years.[10]

Thus, to improve the analysis and management of confirmed cases, it is necessary to raise awareness amongst healthcare workers for early suspicion and enlarged testing. For that, epidemiological risk factors, particularly exposure to children at home or work, and the knowledge of the local seasonal/annual circulation of the virus by virologic surveillance to identify periods of heightened community transmission as well as a broad case definition with clinical signs such as the abnormal value of maternal anemia can be considered.[11] The diagnosis and detection of B19V-specific IgM antibodies are considered the most common method for diagnosing an acute infection, although false-negative results may occur at an early stage of infection. The need for more accurate and precise results stems from the discrepancy between the IgM test and polymerase chain reaction (PCR), the latter being a more sensitive diagnostic method. This is particularly crucial in cases where IgM antibodies may not be detectable. It is also useful in testing amniotic fluid when fetal infection is suspected.[12] The detection of IgM indicates a recent infection, and accurate pregnancy monitoring must be made to evaluate fetal hydrops and predict fetal anemia through Doppler measurement of the middle cerebral artery peak systolic velocity.[13] On the other hand, concerning the detection with PCR, the study mentioned that B19V DNA remained at low levels in two subsequent determinations evidencing the virus' persistence in the patient. Finally, the infection was self-limited, and the virus became undetectable 18 months after the acute phase. Upon the first positive result by PCR, a follow-up was decided every 6 months on the advice of the treating medical team given the patient's concern. However, among healthy blood donors, the detection rate of B19V DNA has been reported to vary from 0.02-21%.[14]

Materials and Methods

Our study spanned a significant duration, from October 2022 to June 2023, allowing for a comprehensive analysis of the chosen subject. Thumbay Hospital is well-known for its exceptional medical services that provide high-quality care to its patients. The serum samples of 87 pregnant women were collected in plain containers, separated, and stored at -20ºC. The collection of specimens was an essential step in the diagnosis of B19V infection in pregnant women. A 5-mL blood sample was collected from each participant through venipuncture. The study included pregnant women of different ages in the first trimester who attended the antenatal clinic and signed consent forms. We arranged for written and verbal consent to be used previously. Also, the exclusion criteria were non-pregnant women, pregnant women in the second and third trimesters, and those aged below 17 or above 45 years and failure to sign a consent form according to ethical approval for this study, which was obtained from the Research and Ethical Committee at Gulf Medical University. All participants were informed about the study and consented verbally before enrollment.

Blood samples (enzyme-linked immunosorbent assay and polymerase chain reaction)

Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) test kit (Abcam, Cambridge, Massachusetts) provides a semi-quantitative *in vitro* assay for human antibodies of the class IgM against B19V *in serum* or plasma. The test kit contains microtiter strip wells coated with parvovirus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies (also IgA) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labeled antihuman IgM (enzyme conjugate) catalyzing a color reaction. The BioTek ELx800 Microplate Reader, manufactured by Agilent Technologies (Santa Clara, California, USA), was used in the methodology. This automated system is highly sensitive as well as specific; it is specifically designed to detect B19V. To facilitate rapid and quantitative analysis, it is capable of high throughput due to the use of microplate-based assays. An ELISA detector was used to ensure accurate and reliable results. With its easy-to-use interface along with its power and performance with real-time monitoring capabilities, the BioTek ELx800 proved to be the best choice in our research to enable effective and accurate detection of smallpox B19 infection.

Polymerase chain reaction

The RealStar® Parvovirus B19 PCR Kit was developed by Altona Diagnostics (Segrate, Italy). It has proven necessary in our research to detect B19V. This highly sensitive and reliable kit used real-time PCR technology to ensure accurate and rapid results for our study. It contains improved raw materials sifted and mixed using a specifically targeted for the B19 little ancestor. The easy-to-use protocol and comprehensive documentation provided by the RealStar® kit make it a valuable tool for the accurate diagnosis and monitoring of smallpox B19 infection. We used the RealStar® Parvovirus B19 PCR Kit to validate the ELISA results and compare them, as it offers a robust and reliable detection of B19V. The PCR test provided an additional layer of specificity and sensitivity, which enhanced the accuracy of the results. Using real-time PCR technology and optimized reagents, the RealStar® kit served as a valuable tool for comprehensive analysis and confirmation of the B19V disease, complementary to the ELISA test results.

Statistical analysis

In this cross-sectional study, the data collected was analyzed using the SPSS version 29 (IBM, Armonk, NY, USA). The analysis involved the use of the *t*-test and the Chi-Square test as well as descriptive results along with the p-values that obtain statistical significance. A p-value of ≤0.05 was considered to be statistically significant. This means that there was a low probability of the results being due to chance, and there was strong evidence to support the findings.

Results

A total of 87 apparently healthy pregnant women who attended Thumbay University Hospital during the period from October 2022 to June 2023 were enrolled in this study to determine the frequency of B19V. The age range was between 17-45 and most of them (33/87, 37.9%) were aged between 25-35 years (Table 1 and Figure 1). Antibody detection of B19V was positive for IgM in 7 women (8%) and negative for IgM in 80 women (92.0%). For the PCR test, the results were 5/87 (5.7%) positive detections and 82/87 (94.3%) negative, showing better and more precise results using the PCR test (as shown in Table 2).

Most of the pregnant women in the first trimester had anti-B19V IgM antibodies within the second month, and there was no association between gestational age and seropositivity of anti-parvovirus IgM (p=0.130). PCR showed the same results but with a significant association with p=0.006 (Table 3 and Figure 2).

In terms of gravidity, 33 (37.9%) pregnant women with primigravida had negative anti-B19V IgM antibodies, while 47 (54.0%) women with multigravida had negative IgM antibodies. Moreover, 7 (8%) pregnant women with multigravida had positive anti-B19V IgM antibodies. There was no association between gravidity age and seropositivity anti-parvovirus IgM (p=0.409). Comparing the IgM antibodies with the PCR test, almost the same results of gravidity were obtained, but, with a lower count in the positive multigravida (3, 3.4%) and no significance (p=0.683) (Table 4).

Most women who had a history of blood transfusion were positive for anti-B19V IgM antibodies, and there was no association between a history of blood transfusion and seropositivity of anti-parvovirus IgM (p=0.720); however, it showed more difference in positive cases, with 2 counts less in the PCR test in blood transfusion and also with no significance (p=0.564) (Table 5).

Among pregnant women with a history of miscarriage, 2 (2.3%) tested positive for anti-B19V IgM antibodies, implying recent or acute infection with parvovirus B19. There was no association between a history of miscarriage and seropositivity of anti-parvovirus IgM (p=0.446). The PCR test showed fewer counts in positive values in miscarriage with 0 (0.0%) and non-statistical significance (p=0.304) (Table 6).

Discussion

B19V is a widespread infection that may affect 1-5% of pregnant women, mainly with normal pregnancy outcomes.[14] Infection during pregnancy can cause a variety of other signs of fetal damage. The risk of adverse fetal outcomes is increased if maternal infection occurs during the first two trimesters of pregnancy but infection may also happen during the third trimester.[15] In this study, B19V IgM antibodies were detected in seven cases which is interestingly close to other studies carried out in Sudan [which reported that out of 93 participants, 8 (8.6%) were positive for B19 IgM],[16] in Libya [which reported that among 150 pregnant women tested, 8 (5%) were positive for IgM to B19V],[17] and in Iran.[18]

Table 1. Distribution of age groups among apparently healthy pregnant women.

Age groups	Frequency	Percentage (%)
Less than 25 years	28	32.2
25-35 years	33	37.9
More than 35 years	26	29.9
Total	87	100

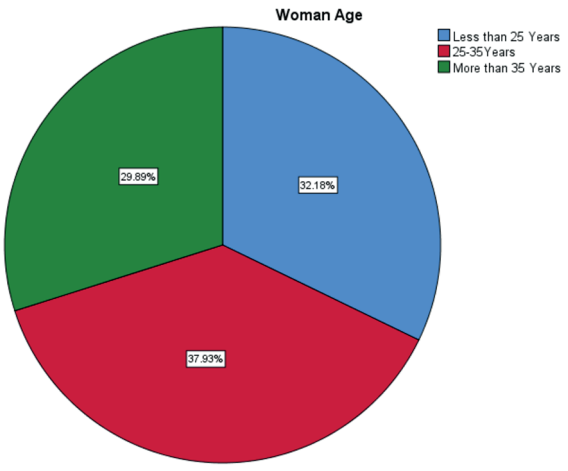

Figure 1. Distribution of age groups among apparently healthy pregnant women.

Table 2. Frequency of parvovirus B19 immunoglobulin M results and polymerase chain reaction results in pregnant women.

Tests		Frequency	Percentage (%)
IgM result	Positive	7	8.0
	Negative	80	92.0
PCR Result	Positive	5	5.7
	Negative	82	94.3
Total		87	100.0

IgM, immunoglobulin M; PCR, polymerase chain reaction.

Table 3. Association between first trimester and immunoglobulin M results, and PCR results among apparently healthy pregnant women.

Tests		First Trimester				p
		First month (%)	Second month (%)	Third month (%)	Total (%)	
IgM results	Positive	1 (1.1)	5 (5.7)	1 (1.1)	7 (8.0)	0.130
	Negative	11 (12.6)	28 (32.2)	41 (47.1)	80(92.0)	
Total		12 (13.7)	33 (37.9)	42 (48.3)	87 (100)	
PCR results	Positive	0 (0.0)	5 (5.7)	0 (0.0)	5 (5.7)	0.006
	Negative	12 (13.7)	28 (32.2)	42 (48.3)	82 (94.3)	
Total		12 (13.7)	33 (37.9)	42 (48.3)	87 (100)	

IgM, immunoglobulin M; PCR, polymerase chain reaction.

Table 4. Association between gravidity and immunoglobulin M results, and polymerase chain reaction results among apparently healthy pregnant women.

Tests		IgM result		p
		Positive (%)	Negative (%)	
Gravidity	Primigravida	2 (2.3)	33 (37.9)	0.409
	Multigravida	5 (5.7)	47 (54.0)	
Total		7 (8.0)	80 (92)	
PCR results	Primigravida	2 (2.3)	33 (37.9)	0.683
	Multigravida	3 (3.4)	49 (56.4)	
Total		5 (5.7)	82 (59.8)	

IgM, immunoglobulin M; PCR, polymerase chain reaction.

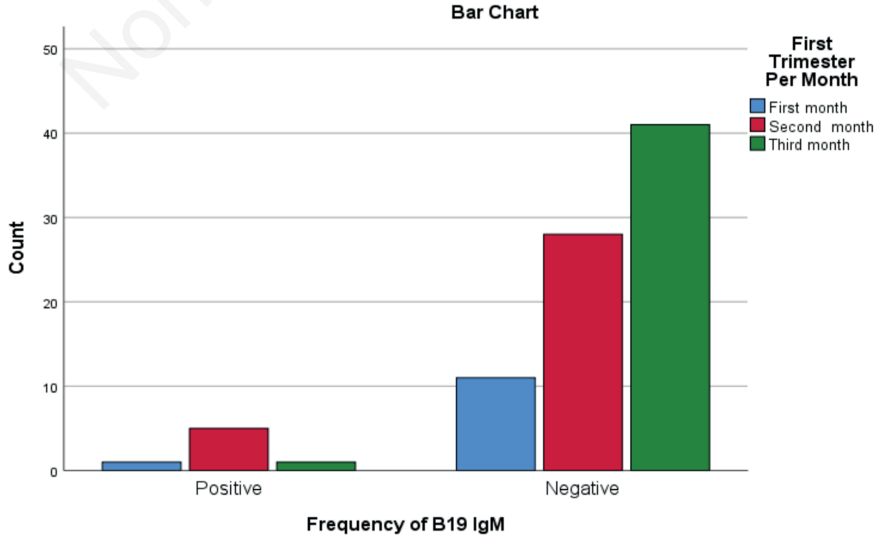

Figure 2. Association between first trimester, immunoglobulin M (IgM) results and polymerase chain reaction results among apparently healthy pregnant women.

Table 5. Association between history of blood transfusion and immunoglobulin M results and polymerase chain reaction results among apparently healthy pregnant women.

Tests		History of blood transfusion		Total	p
		No (%)	Yes (%)	No. (%)	
IgM results	Positive	6 (6.9)	1 (1.1)	7 (8.0)	0.720
	Negative	68 (78.2)	12 (13.8)	80 (92.0)	
Total		74 (85.1)	13 (14.9)	87 (100)	
PCR results	Positive	1 (1.1)	4 (4.6)	5 (5.7)	0.564
	Negative	12 (13.8)	70 (80.5)	82 (94.30)	
Total		13 (14.9)	74 (85.1)	87 (100)	

IgM, immunoglobulin M; PCR, polymerase chain reaction.

Table 6. Association between history of miscarriage and immunoglobulin M results, and polymerase chain reaction results among apparently healthy pregnant women.

Tests		History of miscarriage		Total	p
		Yes (%)	No (%)	No. (%)	
IgM results	Positive	2 (2.3)	5 (5.7)	7 (8.0)	0.446
	Negative	16 (18.4)	64 (73.6)	80 (92.0)	
Total		18 (20.7)	69 (79.3)	87 (100)	
PCR results	Positive	0 (0.0)	5 (5.7)	7 (8.0)	0.304
	Negative	18 (20.7)	64 (73.6)	80 (92.0)	
Total		18 (20.7)	69 (79.3)	87 (100)	

As we found in the current study, 7 (8%) women were positive for IgM, which is a higher value than the number reported in the study conducted in Sudan,[19] which found that the serum from women positive for IgM was positive in one woman, which may be due to the use of a different type of ELISA kit. Therefore, and for all the above, we find that it is difficult to prevent B19V infection because it is often asymptomatic, and exposure is common during epidemics. The prevalence of IgM was also low in the study conducted by Jamjoom *et al.*[20] who estimated that B19V was prevalent among pregnant women in the age group of 25-35 years and that the frequency of IgM antibodies increased with age, which is consistent with a previous study conducted by Arabzadeh *et al.*[21]

In this study, B19V seropositivity was low among the pregnant women who had an abortion and there was no association between a history of miscarriage and seropositivity of anti-parvovirus IgM (p=0.591), which means that there was a significant association, as previously reported in the study by Adam *et al.*[19] (p=0.834). Also, in a study performed by Mirzaei *et al*. to determine the prevalence of B19V in intra-uterine fetal death, virus DNA was observed in a few numbers of participants. A study by Nyman *et al.* revealed that DNA of B19V was observed negative in abortions in the first trimester and positive in the second trimester in fetal tissues; this difference may be related to the specimen population and the diagnostic techniques used.[22,23]

In the present study, there was no association between IgM results and abortion (p=0.591), which is not in line with a study carried out in Denmark in which first-trimester serum samples were tested for B19V IgM positivity.[22] B19V IgM positivity was associated with a high-rate increased risk of fetal loss. A strong statistical association was observed between the presence of B19V IgM antibody during pregnancy and spontaneous abortion.

The seropositivity of anti-B19V IgM antibodies among pregnant women who had a history of blood transfusion was presented as a low number. There was no association between a history of blood transfusion and seropositivity of anti-parvovirus IgM (p=0.959), which is similar to a previous study done in Nigeria that found no association between a history of blood transfusion and seropositivity of anti- parvovirus IgM (p=0.62).

Women in their second month of the first trimester of pregnancy had the highest frequency of B19V-IgM antibodies compared to those in their first month and third month. There was no association between gestational age and seropositivity of anti-parvovirus IgM (p=0.144). The frequency of B19V antibodies was higher in pregnant women with multigravida in which IgM was positive, but there was no significant association, which disagrees with the study done by Emiasegen *et al.*[24]

This study has several limitations that should be acknowledged. First, the sample size of 87 pregnant women, although sufficient for preliminary findings, limits the generalizability of the results to a broader population. Additionally, the study was conducted in a single hospital, which may not reflect the overall prevalence and risk factors associated with B19V infection in other regions or healthcare settings. The cross-sectional design also restricts the ability to establish causality between B19V infection and adverse pregnancy outcomes. Furthermore, the reliance on ELISA and PCR testing, while effective, may not capture all cases, particularly those with low viral loads or in the early stages of infection, leading to potential underreporting.

Conclusions

This study identifies a 9% prevalence of B19V infection among pregnant women in their first trimester, with notable associations related to age and previous pregnancies. The findings emphasize the need for early detection and monitoring of B19V due to its potential impact on adverse pregnancy outcomes, including fetal loss.

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