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Investigation of Human Parvovirus B19 Seroprevalence among Pregnant Women Experiencing Spontaneous Abortion in Kirkuk City, Iraq

Sangol M.Najeeb¹, Mohammed Y. Nooruldeen²

¹ Kirkuk directorate of health, Ministry of Health and Environment /Iraq.

² Medical Laboratory Techniques Department /College of Health and Medical Techniques/ Kirkuk / Northern Technical University / Iraq.

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Corresponding author:

Name: Sangol Mohammed Najeeb

Affiliation : Northern Technical University / Iraq

Email: Sangol92@ntu.edu.iq

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ABSTRACT

Human parvovirus B19 (HPV B19) may cross the placenta from mother to the fetus and cause major problems like non-immune hydrops fetalis, severe fetal anemia, as well as fetal mortality. The aim of the study was to determine the seroprevalence of HPV B19 in pregnant women with spontaneous abortion in Kirkuk. From November 2022 to the April 2023, blood samples from 135 aborted women aged 15-45 and 45 women with successful pregnancies as a control group were taken. Using the ELISA Technique, the sera from the samples were tested for HPV B19 -specific IgM and IgG antibodies. The result showed that 13 (9.63%) tested positive for HPV B19 IgM, 28 (20.74%) tested positive for IgG, $P < 0.01$. The highest seroprevalence of HPV B19 IgM antibodies was observed in the 15-25 age group (61.54%). HPV B19 IgG antibodies highest prevalence in the 26-35 age groups (50%) ($P < 0.05$). The study showed that in the 1st trimester, 11 cases (84.62%) tested positive for HPV B19 IgM antibodies, while 20 cases (71.43%) tested positive for IgG antibodies. Among women with three abortions, a higher proportion tested positive for HPV B19 antibodies, with 76.92% testing positive for HPV B19 IgM and 82.14% testing positive for IgG, among women residing in rural areas, a higher proportion tested positive, with 76.92% testing positive for HPV B19 IgM antibodies and 75% testing positive for HPV B19 IgG antibodies. In the normal weight category, 69.23% tested positive for HPV B19 IgM antibodies, and 82.14% tested positive for IgG antibodies. Aborted women have a higher prevalence of Parvovirus HPV B19 IgM and IgG than control groups, suggesting a potential association between B19 infection and pregnancy loss.



Introduction

The B19 virus is a single-stranded DNA virus that is very small. It is in the family Parvoviridae and the genus Erythrovirus [1]. In 1975, Cossart and colleagues in England found the first human parvovirus (HPV B19) infection. They did this by looking at serum from a healthy blood donor [2]. The HPV B19 virus can affect people of all ages and cause a number of diseases. Infection affects children with the Fifth Disease, adults with polyarthropathy syndrome and people with chronic hemolytic anemia who have a temporary aplastic crisis [3]. HPV B19 is often spread through the airways, through blood, through organ transplants, or from a mother to her unborn child at a rate of 25–50%. [4]. Infections during pregnancy can cause severe anemia and nonimmune hydrops fetalis (NIHF), which can cause a spontaneous miscarriage or the death of the fetus inside the uterus [5], [6]. Only a small number of pregnant women who are at risk for getting HPV B19 will actually get it. Throughout the first and second trimesters of pregnancy, particularly between weeks 10 and 20, the fetus appears to be most vulnerable to HPV B19 infection. This is when the erythroid precursors are developing the most. The HPV B19 can get into erythroblasts in particular [7]. The total number of red blood cells in the developing fetus increases thirty times throughout the third and sixth months of pregnancy. If the fetus gets parvovirus B19, it could get anemia. By the third trimester, the fetus can make a stronger immune response to the virus, which may explain why fetal loss is less likely at this point in the pregnancy [8]. Based on different studies, the chance that a fetus will die from an acute HPV B19 infection during pregnancy is between 3 and 38 %, with an average of 10 %. Serologic tests for HPV B19 IgM and IgG-specific antibodies can find out if a woman has Parvovirus B19 while she is pregnant. This means that the virus won't be able to spread to the fetus. There are a number of things that make it more likely for pregnant women to get it [3]. For example, pregnant women with one child are three times more likely to get sick than women who have never given birth. The number of infections and fetal deaths does go down as the pregnancy goes on [6].

The goal of this study was to find out how common HPV B19 IgM and IgG are in pregnant women with spontaneous abortion because of problems caused by HPV B19 infection during pregnancy and a inadequate research about how common this infection is in Kirkuk province.

Materials and Methods

The cross-sectional hospital-based study was carried out in Kirkuk city from the November 2022 to the April 2023. The study involving 135 pregnant women who underwent spontaneous abortions and visited gynecological and pediatric hospitals and private laboratories aged 15–45 was conducted in Kirkuk. the study additionally included 45 pregnant women with successful births as a control group. This study excluded individuals who had chronic illnesses and all women with abortion-related bacterial or viral infections, including Rubella, Cytomegalovirus , Herpes simplex virus , and toxoplasma parasites.

Blood sampling

Five milliliters of blood were drawn from each participant's vein using a syringe after a vein puncture in the study. Blood samples were centrifuged at 3000 rpm for 15 minutes in sterile test tubes to create serum. After that, the sera were transferred after being sucked with an automated micropipette, and put into eppendorf tubes, where they were maintained at -20 °C in a deep freezer. After that, the ELISA technique (SunLong Biotech china) kit was employed to determine HPV B19 IgM and IgG levels.

Detection of Human parvovirus B19 IgM and IgG using ELISA technique Principle

The ELISA is based on the qualitative enzyme immunoassay technique. The Microplate provided in this kit has been pre-coated with an antigen specific to B19-IgM or IgG make it to solid-phase antigen. Samples are added to the Microplate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for B19-IgM or IgG is added to each Microplate well and incubated, so the antigen-antibody-Enzyme labeled antigen complex is formed. Following a wash to remove any unbound reagent, then the TMB substrate solution is added to each well. Only those wells that contain B19-IgM and HRP conjugated B19 antigen will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The qualitative determination of B19-IgM or IgG is determined by comparing with the CUTOFF value.

Procedure

- 1- There were left two wells for negative controls and two wells for positive controls, and one well was left empty as a blank control in the Micro Elisa strip plate. Number: A negative control and a positive control sample for each micro porosity hole on a board were set, one for each sequential number. 2 holes, 1 hole, ck (ck hole without samples and HRP-Conjugate reagent, the rest of the same step operation)
- 2- Adding samples: Negative and positive control in a volume of 50 μ l were added to the negative and positive control wells respectively. In sample wells, 40 μ l Sample dilution buffer and 10 μ l sample were added. Samples were loaded onto the bottom without touching the well wall. Well mixed with gentle shaking.
- 3- Incubation: The plates were incubated for 30 min. at 37°C after being covered with a Closure Plate membrane.
- 4 - Dilution: Distilled water is applied to dilute the concentrated washing buffer (30 times for 96test).
- 5- Washing: The membrane of the closure plate was carefully removed, aspirated, then wash solution was added in its stead. Discard the wash solution once it has settled for 30 seconds. the washing procedure were repeated for 5 times.
6. 50 μ l HRP-Conjugate reagent were added to each well except the blank control well.
7. Incubation as described in Step 3.
- 8- Washing as described in Step 5.
- 9- Coloring: 50 μ l Chromogen Solution A and 50 μ l Chromogen Solution B were added to each well, mixed with gently shaking and incubated at 37 °C for 15 minutes.
10. Termination: 50 μ l stop solution were added to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm a Microtiter Plate Reader was used. The OD value of the Blank control well is set as zero. Assay carried out within 15 minutes after adding stop solution.

Determine the result

Test performance: the average of positive control value was ≥ 1.00 , whereas the average negative control value was ≤ 0.10 .

Calculating the crucial value (CUT OFF) :critical value is equal to the average of the negative control value + 0.15.

Negative assessment: If the OD value < CUT OFF, the sample is negative HPV B19 (IgM or IgG) tests.

positive assessment: If the OD value >CUT OFF, the sample is positive HPV B19 (IgM or IgG) tests.

Ethical consideration

Each woman's husband or other family members had to give their informed consent before she could join the study. Each subject was given a thorough explanation of the procedure prior to the blood sample being obtained to ensure they were aware of every step. The subjects were also informed that declining to participate in the study would not have any negative consequences. Kirkuk's health directorate has accepted this according to document numbered 48025 in October 31, 2022.

Statistical analysis

The Chi-square test was applied with conventional equations to compare data. In accordance with the established levels of significance, the results were published with a P value, p-value was regulated significant the level of < 0.05 and highly significant as the level<0.01[9].

Results

Table 1 shows the results of HPV B19 IgM and IgG among study groups (aborted women and the control groups). The reults showed that 13 (9.63%) tested positive for HPV B19 IgM, 28 (20.74%) tested positive for HPV B19 IgG. And out of the total 45 control samples examed, non of these samples tested positive for HPV B19 IgM (0%), and only 1 (2.22%) tested positive for B19 IgG.And the remaining 44(97.78%) from the control group tested negative for both HPV B19 IgM and IgG antibodies, P<0.01.

Table 1: Seroprevalence of HPV B19 in aborted women and the control groups

Studied groups	HPV B19 IgM +ve		HPV B19 IgG +ve		Negative		Total Number	
	No.	%	No.	%	No.	%	No.	%
Aborted women	13	9.63	28	20.74	94	69.63	135	100
Control group	0	0	1	2.22	44	97.78	45	100
			Chi-Square: 13.1		P-value: <0.01			

Table 2 shows that the seroprevalence of B19 IgM antibodies in aborted women according to age group. The highest seroprevalence of B19 IgM antibodies was observed in the 15-25 age group (61.54%), followed by the 26-35 age group (30.77%), and the lowest in the 36-45 age group (7.69%). Conversely, the seroprevalence of B19 IgG antibodies showed a decreasing trend across the age groups, with the highest prevalence in the 26-35 age group (50%) and the lowest in the 36-45 age group (14.3%), (P<0.01).

Table 2: Distribution of HPV B19 seropositive aborted women according to age group

Age groups (Years)	Women with abortion					
	HPV B19 IgM +ve		HPV B19 IgG +ve		HPV B19 IgM-ve and IgG-ve	
	No.	%	No.	%	No.	%
15 - 25	8	61.54	10	35.7	26	27.65
26 – 35	4	30.77	14	50	41	43.62
36 - 45	1	7.69	4	14.3	27	28.72
Total	13	100	28	100	94	100
			Chi-square: 13.4		P-value: <0.01 (Significant)	

Table 3 summarizes the frequency of HPV B19 -IgM and IgG antibody in regard to trimester of spontaneous aborted women. The study showed that in the 1st trimester, 11 cases (84.62%) tested positive for HPV IgM antibodies, while 20 cases (71.43%) tested positive for HPV IgG antibodies. In the 2nd trimester, 2 cases (15.38%) tested positive for HPV IgM antibodies, while 8 cases (28.57%) tested positive for HPV IgG antibodies, not statistically significant was observed between the groups (P>0.05).

Table 3: The seroprevalence of HPV antibodies in relation to trimester of abortion

Trimester of abortion	Women with abortion					
	HPV B19 IgM +ve		HPV B19 IgG +ve		HPV B19 IgM-ve and IgG-ve	
	No.	%	No.	%	No.	%
1st trimester	11	84.62	20	71.43	68	72.34
2nd trimester	2	15.38	8	28.57	26	27.66
Total	13	100	28	100	94	100
			Chi-square Value: 0.92		P-value: >0.05 (non-significant)	

Table 4 presents the relationship between B19 virus and the number of abortions among women with recurrent pregnancy loss (RPL). Among women with three abortions, a higher proportion tested positive for B19 antibodies, with 76.92% testing positive for B19 IgM antibodies and 82.14% testing positive for B19 IgG antibodies. The study indicated a statistically significant association between the number of abortions and B19 seropositivity among women with RPL (P<0.01).

Table 4: Relationship between the HPV B19 and the incidence of abortion in RPL women

No. of abortion	Total No.	Women with abortion					
		HPV IgM +ve		HPV IgG +ve		ELISA total negative	
		No.	%	No.	%	No.	%
2	17	2	15.38	5	17.86	10	10.64
3	60	10	76.92	23	82.14	27	28.72
≥4	58	1	7.69	0	0	57	60.63
Total	13	13	100	28	100	94	100
			Chi-square Value: 45.3		P-value: <0.01		

Table 5 summarized the distribution of seropositive of B19 virus in aborted women. Among women residing in urban areas, 23.08% tested positive for HPV19 IgM antibodies and 25% tested positive for HPV19 IgG antibodies. In contrast, among women residing in rural areas, a higher proportion tested positive, with 76.92% testing positive for HPV19 IgM antibodies and 75% testing positive for HPV19 IgG antibodies. The p-value of 0.71 suggests that there is no significant association between residence (rural or urban) and the seropositivity for virus B19 among aborted women.

Table 5: Residence distribution of B19 virus-positive aborted women

Residence	Women with abortion					
	HPV19 IgM +ve		HPV19 IgG +ve		HPV19 IgM-ve and IgG-ve	
	No.	%	No.	%	No.	%
Urban	3	23.08	7	25	22	23.40
Rural	10	76.92	21	75	72	76.59
Total	13	100	28	100	94	100
Chi- Square: 0.92				P-value: >0.05		

The results of HPV19 seropositivity in aborted women with obesity were summarized in Table6, In the normal weight category, 69.23% tested positive for HPV19 IgM antibodies, and 82.14% tested positive for HPV19 IgG. With overweight women, 7.69% tested positive for B19 IgM antibodies, and 7.14% tested positive for HPV19 IgG antibodies. However non significant difference was noticed (P-value>0.05).

Table 6: Relation of HPV B19 seropositivity to obesity of aborted women

Obesity	Women with abortion					
	HPV19 IgM +ve		HPV19 IgG +ve		HPV19 IgM-ve and IgG-ve	
	No.	%	No.	%	No.	%
Under weight	3	23.08	3	10.71	25	26.60
Normal	9	69.23	23	82.14	68	72.34
Over weight	1	7.69	2	7.14	1	1.06
Total	13	100	28	100.00	94	100
P-value: >0.05				Chi- Square: 0.80		

Discussion

The current study revealed the following: the frequency of HPV B19 IgM 13(9.63%) and IgG 28(20.74%) with total positive 41(30.37%) among pregnant women who had previously experienced abortions. This prevalence is similar to what has been reported in pregnant women in Iraq [5].and Arabic countries such as Egypt [10]. Libya [11]., and Tunisia [12]. and greater than that noted among expectant mothers in several other countries 2.2% in Kuwait[13]. 3.0% in Germany [14]. and 6.0% in Nigeria [15]. but less than those reported in Tanzania (32.8%) [16]. and Iran (57%) [17].

Also, the present study documented that that 9.63% of the participants had specific IgM antibodies for parvovirus B19 at the time of abortion, demonstrating primary acute infection. Many pregnant women appear to be susceptible to this virus during pregnancy. This result is consistent with findings reported by Zhou et al [18]. reported rate of 9.31% for HPV B19 IgM .However, our result was lower than the Jensen et al [19].and Rahbar et al [20].)who revealed seropositivity rates of 12.9% and 18.1%, respectively. Another study by Iyanda et al [21]. reported a low rate of 4% for HPV B19 IgM, while El-Sayed et al [10].found a higher rate of 48%.

In our study, we found that 20.74% of the aborted women who were tested had antibodies against HPV B19 IgG. This result is in line with what Iyanda et al. [21]. and Hasan et al. [22], Iyanda et al. [21]. found that 20% of pregnant women had positive cases, and Hasan et al. [22]. found that 24.7% of healthy pregnant women in Diyala province had the virus. Emiasegen et al [23]. also found that 27.5% of people had it. The rate of anti-HPV B19 IgG found in our study is lower than what has been found in pregnant women in Nigeria (22.5%) [24], Syria (61.2%) [25], Sudan (61.4%) [26], the Netherlands (70%) [27], Russia (26%) [28]. and other places (75.3%).

Some studies Documented the prevalence of HPV B19 is in Iraq. In a study done in Baquba-Diyala province, Iraq, HPV B19 -specific IgM antibodies and IgG antibodies were found in 10% and 13% of pregnant women who had had abortions before [29]. In another study done in Erbil, B19-specific IgM antibodies were found in 9 percent of people and IgG antibodies in 39 % of people [2]. In Thi-Qar province, B19-specific IgM antibodies were found in 34% of pregnant women who had had abortions before, and IgG antibodies were found in 38% [1]. The differences between the current study and other study may due to technical reasons such as uses

different methods between our study and others could be caused by different methods (immunological vs. molecular), sample sizes, populations studied in different places, length of incubation, immune status of each person, and demographic and geographical differences, among other things [30].

According to a study done in Syria on 273 pregnant women, the highest rate of IgM antibodies was found in women between the ages of 22 and 26 (9.4%), and the lowest rate was found in women over the age of 37 (0%). [25]. Another study of 231 pregnant women in Oyo State, Nigeria, found that the highest rate of HPV B19 IgM antibody (10.8%) was in women aged 22–27, and the lowest rate was in women aged 38 and older [31]. Many studies had shown that the number of people with IgM decreased with age, and they also showed that younger women had never been infected before, while older women had [2,22].

The current results showed that the presence of HPV B19 IgM antibodies changed depending on how far along the pregnancy was when it ended on its own. This study found that in the first trimester, HPV B19 IgM antibodies were found in 84.62 % of cases, which shows that the infection happened recently. In the second trimester, HPV B19 IgM antibodies were found in 15.38% of cases, which is less than in the first trimester the exact reasons for these differences are not known, but they may have something to do with damage to many organ systems (30). During the second trimester, the fetus is at a high risk because its red blood cell (RBC) mass is growing quickly and its RBCs don't live very long [32]. When a fetus is infected with the parvovirus B19 in the first half of pregnancy, there is a high risk of miscarriage, fetal hydrops, and death. When a mother is known to have an acute infection, it is best to keep a close eye on the fetus through weekly sonographic exams for 12 weeks to check for hydrops or signs of anemia through Doppler waveforms [33].

The Present study shows that the chances of testing positive for HPV IgM and IgG antibodies go up as the number of abortions goes up. This result was the same as what the Sudanese study [34]. and the Iran study [35]. found. But it was higher than what the study carried out in Sudan found [26]. This difference could be because of the different ways of diagnosing, the differences in population and location, the type of samples used, and the population that was involved in the study [3].

Overall, the results show how important it is to think about HPV seropositivity when there are repeated pregnancy losses and how more research is needed to find out what effect B19 infection has on reproductive health. The current study didn't agree with Hussien et al [36]. study, which didn't find a significant difference between the number of abortions and the incidence of HPV B19. However, he did say that his results could be due to a small sample size. This difference in results could be due to a number of things, such as differences in how the study was set up, the size of the sample, the characteristics of the population, the testing methods, or even just luck. There was a significant difference between the two groups, which may have been caused by the fact that the current study had a larger sample size or used different testing methods.

The present investigation revealed that women in rural areas were more likely to have an abortion caused by Parvovirus B19 than women in urban areas, even though the difference wasn't very big. Even though the difference wasn't big enough to be statistically important, this result is in line with what other studies have found about similar trends. This finding backs up what other studies have found: that the difference in lifestyle leads to a higher rate of abortion, which mostly affects people who live in rural areas [12, 13]. Observed differences in abortion rates between cities and rural areas could be caused by a number of things. Access to health care is one of these factors, since people in cities usually have better access to hospitals and specialized care. Socioeconomic factors may also play a role, since economic opportunities and resources in cities are often different from those in rural areas [37, 38]. The difference in abortion rates could also be caused by the fact that people in cities and rural areas live and get health care in different ways.

The non-significant difference observed in the study (P -value > 0.05) indicates that there is no statistically significant association between B19 seropositivity and obesity based on the data that was looked at. In other words, the study did not find enough evidence to suggest that the prevalence of B19 seropositivity is significantly different between underweight, normal weight, and overweight women. Obesity is usually linked to a number of health problems, such as an increased chance of getting some infections [39]. B19 seropositivity means that you have antibodies against human parvovirus B19, which is known to cause fetal infections and miscarriages, among other symptoms [40].

Conclusions

The incidence of HPV B19 IgM and IgG is higher in women who have had abortions, pointing to a possible association between infection and miscarriage. there is a higher chance of testing positive for B19 IgM and IgG antibodies as the number of abortions rises. The present study showed that the seroprevalence of B19 IgM antibodies varies with age, with higher rates seen in younger age groups.

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