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Detection of Parvovirus B19 in Bad Obstetric History by Using Real Time PCR

Lina F. Abdulhassan[1] *MSc,* **Huda D. Hathal**[1] *PhD*, **Thuraya H. Abdullah**[2] *CABOG, FICOG*

[1]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Dept. of Gynecology and Obestetric, Al- Imamein Al-Kadhimein Medical City, Baghdad, Iraq

Abstract

Background Human *parvovirus* B19 (B19V) is a small single-stranded DNA virus. Infection during pregnancy can cause a variety of signs of fetal damage. The risk of adverse fetal outcome is increased if maternal infection occurs during the first two trimesters of pregnancy but may also happen during the third trimester.

Objective to determine the screen of *parvovirus* B19 in pregnant women with bad obstetric history by real time polymerase chain reaction (PCR).

Methods Two hundred Plasma and 200 placental tissue samples were collected from all pregnant women enrolled in this study. Three ml whole blood was collected in sterile EDTA-blood tube. Plasma was obtained by centrifugation of whole blood. Twenty-five grams of the placental tissue was homogenized with 10 ml of PBS by using tissue homogenizer for about 1 min at 4 °C.

Results 40 (20%) out of 200 plasma samples were real time PCR positive, the remainder 160 (80%) were real time PCR negative. Nineteen (9.5%) out of 200 placental tissue samples were positive for B19 real time PCR the remainder 181 (90.5 %) were real time PCR negative. All placental tissue positive (n=19) were positive by real- time PCR in plasma samples (n=40). Out of 40 pregnant women presented with positive parvoviruses results in current study demonstrated 21 (52.5%) gave abortion in first trimester and only 8 (20%) gave abortion in second trimester.

Conclusion *Parvovirus* B19 is common and highly distributed among pregnant ladies in this study and there is a significant association between B19 positivity and adverse pregnancy outcome.

Keywords *Parvovirus* B19, bad obstetric history, adverse pregnancy outcome, non-immune hydrops fetalis

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List of abbreviations: ANOVA = One-way analysis of variance, BOH = Bad obstetric history, CMV = Cytomegalovirus, EI = Erythema infectiosum, HB19V = Human *parvovirus* B19, NIHF = Non-immune hydrops fetalis.

Introduction

Human *parvovirus B19* (HB19V) is a small single-stranded DNA virus. Human *parvovirus B19* is the causative agent of erythema infectiosum (EI), a disease common in children. Studies have shown that intrauterine HB19V infection is related to non-immune hydrops fetalis (NIHF) and stillbirth [1, 2].

Approximately 1-3% of susceptible pregnant women without a known history of exposure to HB19V will seroconvert during pregnancy. Up to 50% of susceptible pregnant women exposed to HB19V through household contacts will develop seropositivity, while the rate of seroconversion is 20-30% when the exposure occurs in day care centers or school [3]. The risk of transmission to the fetus has been reported in the range of 17-33% but infection remains

asymptomatic in most cases. However, a wide range of adverse outcomes have been reported in association with intrauterine HB19V infection including: spontaneous abortions (rate of 14.8% if infected prior to 20 weeks gestation and 2.3% if the infection occurs after 20 weeks gestation); occurrence NIHF; congenital anomalies including central nervous system, craniofacial and eye anomalies [4]. Infection during pregnancy can cause a variety of other signs of fetal damage. The risk of adverse fetal outcome is increased if maternal infection occurs during the first two trimesters of pregnancy but may also happen during the third trimester [5].

HB19V can causes severe fetal anemia as a result of fetal erythroid progenitor cells infection with shortened half-life of erythrocytes, causing high output cardiac failure and therefore NIHF [5].

This study was carried out to determine the associated of HB19V in pregnant women with bad obstetric history.

Methods

Blood and placental tissue samples were collected from 200 pregnant women with bad obstetric history, attending the Gynecology outpatient clinics, wards and emergency unit in Al-Imamein Al-Kadhimein Medical City, and Baghdad Teaching Hospital during the period from December 2015 to May 2016. Three ml whole blood was collected in EDTA-blood tubes and plasma was obtained by centrifugation of EDTA-blood tube at 5,000 rpm for 5 min. While placental tissue twenty-five grams was homogenized with 10 ml of phosphate buffer solution by using tissue homogenizer for about 1 min at 4°C [6]. The resulting suspension was subjected to two freeze-thaw cycles to further break the cell membranes. After that, the homogenate centrifuged for about 15 min at a speed of 5000 rpm and temperature of (2-8) °C. The supernatant then collected carefully, both samples were stored at (-20°C) till DNA extraction.

Exclusion Criteria

Patient with a diabetic through HbA1c level, anti-phospholipid syndrome through anti-cardiolipin test and Rh, ABO incompatibility were excluded from this study.

Molecular method for diagnosis of Parvovirus B19

DNA was extracted from placental tissue and plasma using DNA isolation kit ((DNA-sorb-B (Sacace)/Italy) Kit) according to the manufacturer's instruction. The concentration and purity of the purified DNA was quantified by the use of nanodrop instrument following the instruction of the manufacturer, RT-PCR TaqMan assay using real time amplification with fluorescent reporter dye probes specific for HB19V and Internal Control (IC) were used in current study.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) was used for all statistical analysis; Chi-square used for categorical variables (Fishers exact test was used when expected variables were less than 5) and t-test was used to compare between two means. One-way ANOVA analysis was used to compare between more than two means. A two-sided significant level of 0.05 was considered to indicate a statistically significant difference.

Results

Molecular diagnostic method for detection of Parvovirus B 19 by real-time PCR

Results improved that 40 (20%) out of 200 plasma samples were real-time PCR positive, the remainder 160 (80%) were real-time PCR negative. Nineteen (9.5%) out of 200 placental tissue samples were positive for HB19V real-time PCR the remainder 181 (90.5 %) were real- time PCR negative.

All placental tissue positive (n= 19) were positive by real-time PCR in plasma samples (n=40). Real-time PCR was performed in duplicate for each sample that gave positive results.

The prevalence of positive HB19V in relation to age groups is presented in Table (1) and table

(2). In current study, 40 pregnant women proved as HB19V positive in plasma and 19 in placental tissue out of 40, the highest prevalence was observed in age group (30-39) years in both plasma 17 (28.3 %) and placental tissue 7 (11.7%). The association between age and positive pregnant women to parvoviruses was statistically significant in plasma (P=0.002) and highly significant in placental tissue (P<0.001).

Table 1. Correlation between B19 positive plasma cases and age of the pregnant women

Age	Positive viremia		Negative viremia		χ^2	P
	No.	%	No.	%		
<20 years	2	10.5	17	89.5	14.6	**0.002 Significant**
20-29 years	15	13.6	95	86.4		
30-39 years	17	28.3	43	71.7		
≥40 years	6	54.5	5	45.5		

Table 2. Correlation between B19 positive tissue cases and age of the pregnant women

Age	Positive viremia		Negative viremia		χ^2	P
	No.	%	No.	%		
<20 years	1	5.3	18	94.7	29.8	**<0.001 Highly Significant**
20-29 years	5	4.5	105	95.5		
30-39 years	7	11.7	53	88.3		
≥40 years	6	54.5	5	45.5		

Parvoviruses B19 in plasma and tissue with pregnancy number and pregnancy outcome

There was highly statistical significant between HB19V positive in plasma in associated with pregnancy number (P<0.001) and highly significant association (P<0.001) with pregnancy outcome table (3).

Table 3. Correlation between *Parvovirus* B19 in plasma and gravidity

Variable		Positive plasma		Negative plasma		χ^2	P
		No.	%	No.	%		
Pregnancies number	1-2	7	10.0	63	90.0	17.2	**<0.001 Highly Significant**
	3-4	12	15.8	64	84.2		
	>4	21	38.9	33	61.1		
Pregnancies outcome	Normal	2	2.7	73	97.3	22.5	**<0.001 Highly Significant**
	Adverse	38	30.4	87	69.6		

The association between HB19V positive in placental tissue compared to the number of pregnancy and pregnancy outcome were presented in table (4), there was a statistical significant association (P<0.006) between increased pregnancies number compared to positive parvoviruses and highly significant differences (P<0.001) with pregnancy outcome.

Table 4. *Parvoviruses B19* in placental tissue compared to pregnancy number and pregnancy outcome

Variable		Positive tissue No.	Positive tissue %	Negative tissue No.	Negative tissue %	χ^2	P
Pregnancies number	1-2	3	4.3	67	95.7	10.3	**0.006 Significant**
	3-4	5	6.6	71	93.4		
	>4	11	20.4	43	79.6		
Pregnancies outcome	Normal	0	0.0	75	100	12.5	**<0.001 Highly Significant**
	Adverse	19	15.2	106	84.8		

Type of abnormality of parvoviruses B19 in compared to gestational age and abortion

Out of 40 pregnant women presented with positive parvoviruses results in current study demonstrated 21 (52.5%) gave abortion in first trimester and only 8 (20%) gave abortion in second trimester there was highly statistical differences between gestational age and abortion in positive cases (P<0.005) (Table 5).

Table 5. Distribution of positive cases with gestational age and abortion in current pregnancy

Parameter		Positive tissue No.	Positive tissue %	Negative tissue No.	Negative tissue %	χ^2	P
Pregnancies number	1-2	3	4.3	67	95.7	10.3	**0.006 Significant**
Pregnancies outcome	Normal	0	0.0	75	100	12.5	**<0.001 Highly Significant**

A highly significant association was observed between women with high B19 viral load in plasma and in placental tissue and adverse pregnancy outcome (p<0.001) (Table 6).

Table 6. The association between abnormal pregnancy outcome and viral load

Variable		Normal No.	Normal %	Adverse No.	Adverse %	χ^2	P
Viral load in plasma	**Positive (40 cases)**	2	5.0	38	95.0	22.5	**<0.001 Highly Significant**
	Negative (160 cases)	73	45.6	87	54.4		
Viral load in tissue	**Positive (19 cases)**	0	0.0	19	100	12.5	**<0.001 Highly Significant**
	Negative (181 cases)	75	41.4	106	58.6		

Fishers exact test

Adverse pregnancy outcome and B19 viremia

Analysis of patients that were subsequently proved to be real-time PCR positive for HB19V in association with adverse pregnancy outcome in plasma and placental tissue was studied and the results was clarified a highly significant association as shown in table (7) with P<0.05 (P=0.0001).

Table 7. The relation between adverse Pregnancy outcome and B19 viremia

Type of adverse pregnancy outcome	Positive plasma		Positive tissue	
	No.	%	No.	%
Abortion	21	52.50	8	42.1
Still birth	13	13.00	6	31.5
Congenital abnormalities	3	7.50	3	15.7
Total	40	100%	19	100%
Chi-square	11.367		9.0294	
P-value	0.0001**		0.00216**	

** (Highly significant).

Discussion

Relation of demographic data in study group

The present study found highly significant association in studied group with adverse pregnancy outcome with different types such as stillbirth, abortion and congenital abnormality, adverse pregnancy outcome is any event which reduces the chance of having a healthy baby.

The mechanism of how some pregnancies lead to adverse maternal outcome is not fully understood though endothelial dysfunction [7], the findings of present study supported evidence are available in the study that reported that advanced maternal age and gestational hypertension has been suggested to play a role in adverse pregnancy outcome [7,8].

Relationship between maternal age and infection with Parvovirus B19

In this study, results showed increased age of women was associated significantly with positive infection with HB19V infection in plasma and placental tissue. This finding is consistent with results of Quemelo et al. [9], which showed that HB19V infection increases parallel with increased age of women.

Some postulate refer that older people may succumb to viral infection as a result to exaggerated immune responses, these age-elevated IL-17 responses induce a lethal immune pathology during viral infection. These responses synergize with defective Parvovirus B19 clearance with aging noted by impaired IFN-α responses [10].

Correlation of Parvoviruses B19 in plasma and tissue with pregnancy number and pregnancy outcome

Current study showed that women with increased number of multipara (>4) were significantly associated with HB19V infection of plasma and placental tissue. This is similar to results of Adam et al. [11]. It is known that infection occurs throughout adult life increasing the seroprevalence rate from ~60% at age 18 years to >80% in geriatric populations (10). Moreover, multigravida B19 virus-susceptible women are more liable to acquire HB19V infection from their living children [12].

In Valeur-Jensen study; they found that the seroprevalence of HB19V in mothers significantly increased with the number of their living children, particularly, children aged 5-7 years [13].

The higher risk of infection has to be due to a higher exposure rate among women with children at home. This fact most likely represents a mixture of a higher exposure rate,

as multigravida women generally have a higher rate of daily contacts compared with nulliparous women, and probably a reduced level of active immunity, because of the stress factor. Furthermore, serious medical disease may be found in multigravida women which causes clearly impairs the level of active immunity and thereby increases the susceptibility to infection [14].

Present study revealed a highly significant association between infected women with HB19V in plasma and abnormal pregnancy outcome ($p<0.001$). This finding is consistent with results of Kishore et al. [15], which reported that women with bad obstetric history (BOH) and/or pregnancy complications had a high frequency of TORCH and HB19V infections causing fetal wastage, intrauterine growth restriction, nonimmune hydrops fetalis and congenital malformations.

This study found a highly significant association between women with HB19V placental tissue infection and abnormal pregnancy outcome ($p<0.001$). This finding coincides with results of Lamont et al. [16], which revealed that if pregnant women develop HB19V infection, there is a 30% chance of fetal transmission, which is associated with adverse fetal outcomes.

Relationship between gestational age and Parvovirus B19 infection

The common abortion cases in present study including women infected with Parvovirus B19 infection were in first trimester. This is similar to results of Kishore et al. [15]. The risk of fetal complications depends largely upon the gestational age at the time of maternal infection with HB19V. It seems that the highest risk for fetal loss if maternal infection occurs during weeks 9-16 of pregnancy, then infection is reduced in the second half of pregnancy and rare if infection occurs in the last 2 months [17].

The possible explanation for these results because transplacental transmission is more likely to occur due to the presence of the P-antigen, which is a glycolipid (globoside) present in the trophoblast. This receptor is highly expressed in the first and second trimester, but virtually non-existent in the third trimester [18]. This period showed growing of fetus organ and hemopoiesis occurs in the fetal liver and due to the increased demand from the growing fetus, there is a 34-fold increase in red blood cell mass, These unique circumstances make the fetus especially vulnerable to any insult with respect to erythropoiesis [19].

Relationship between adverse Pregnancy outcome and B19 viremia

The current data showed highly significant association among infected women with Parvovirus B19 with different type of adverse pregnancy outcome. This finding is consistent with many studies done by Leduc et al. [20] and Watt et al. [21] who found that, the adverse pregnancy outcome of HB19V infection including abortion, still birth and congenital abnormalities increased in children of mothers with parvovirus infection in pregnancy.

Relationship between adverse pregnancy outcome and viral infection were analyzed by large group of authors who suggest that the type of response initiated in the placenta may determine the immunological response of the mother and consequently, the pregnancy outcome [22,23]. It is well accepted that in viral infection during pregnancy will lead to embryonic and fetal death, induce miscarriage or induce major congenital anomalies. However, even in the absence of placental transmission, the fetus could be adversely affected by the maternal response to the infection [24].

Viral infection of the placenta lead to stimulate high circulating levels of inflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF-α, which causes fetal inflammatory response syndrome (FIRS), even though the virus is not able to reach the fetus, which lead to adverse pregnancy outcome [25].

In conclusion, HB19V is common and highly distributed among pregnant ladies in this study. There is a significant association between HB19V positivity and adverse pregnancy outcome. The rate of abortion in positive HB19V is the most adverse pregnancy outcome followed by stillbirth.

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Authors Contribution
Abdulhassan: DNA extraction, molecular, real time PCR methods diagnosis, analysis and interpretation of result and statistical analysis. Dr. Hathal: drafting the article and revising it critically for important intellectual content. Dr. Abdullah: samples and patients selections.

Conflict of interest
The authors declare no conflict of interest.

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Correspondence to Lina F. Abdulhassan
E-mail: linaabdalhassan@gmail.com
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