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Research Article

Detection of Parvovirus B19 DNA in pregnant Sudanese women attending The Military hospital using Nested PCR technique

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ABSTRACT

***Background*:** Parvovirus B19 is a human pathogenic virus associated with a wide range of clinical conditions. During pregnancy congenital infection with parvovirus B19 can be associated with poor outcome, including miscarriage, fetal anemia and non-immune hydrops.
***Objective*:** The study aimed to determine the prevalenceof Parvovirus B19 DNA in pregnant women attending the Military hospital in Khartoum, demonstrating the association between the virus and poor pregnancy outcomes.
Subjects and methods: This study was a cross sectional study, testing pregnant Sudanese women whole blood samples (n= 97) for the presence of Parvovirus B19 DNA using nested PCR technique.
***Result*:** Two samples were found positive for Parvovirus B19 DNA out of the total number of samples screened.
***Conclusions*:** The prevalence of Parvovirus B19 DNA among pregnant women attending the Military hospital was 2.1%.

Introduction

Human parvovirus (B19V) was first described in 1975(1). The structural proteins determine many of the biological properties of the virus, including binding to cell receptor, haemoagglutination and induction of neutralizing responses (2). NS1 is an activating transcription factor for the single promoter of B19. In addition, NS1 nicks the replicative form of the viral genome at the origin of replication, allowing for replication of the viral DNA (3). Noscomial transmission has also been documented. The incubation period of the infection ranges from four to 14 days but can last as long as 21 days (4).

Although vaccine development has shown promising initial results, there is no currently vaccine available against parvovirus B19 (5).

Infection with parvovirus during pregnancy is not associated with increased risk of fetal malformation. However, infection during pregnancy is an important cause of intrauterine fetal death, stillbirth, and non-immune hydropsfetalis(6).

The fetus is particularly vulnerable to B19 infection because it has a rapidly expanding red-cell volume and relatively short red cell life span and because it may be unable to mount an effective immune response (7). The first association between parvovirus B19 infection in pregnancy and poor outcomes was reported in 1984, when hydropic fetuses were shown to have anti-B19 immunoglobulin M (IgM) (8). By increasing gestation age, the incidence of infection and fetal death decrease. If the mother has B19-specific antibodies, immunoglobulin G (IgG) against the virus, there will be no possibility of virus transition to the fetus (9).

In early studies, acute B19 infection was determined by demonstrating virus in serum by counter current immunoelectrophoresis (CIE) and immune electron microscope (IEM), tests that require serum specimens to be collected during the initial phase of infection when viral titer is high(10). In vitro the virus can be cultured in some erythromegakaryoblastoid cell lines, but replication is very inefficient (11).

Although B19 can be detected in serum by electron microscope (EM), B19 antigen enzyme linked immune sorbent assays (ELISA), and even hemoagglutination, B19 virus is usually detected by isolation of viral DNA by direct hybridization or Polymerase chain reaction (PCR) (12).The sensitivity of DNA hybridization tests can be increased by amplification of either target or the detector system. The most widely used method is amplification of the target by the polymerase chain reaction (13).

There is no much published data concerning the determination of the prevalence of parvovirus B19 among Sudanese pregnant women using nested PCR, in a study conducted by Adam. et al in Sudan (2015), which was based on serology and B19 DNA was not detected in any of the samples (14), another study conducted by Maksheed M. et al in Kuwait (1999) (15), which was also based on detecting B19 antibodies only. In addition to a study conducted by Barros De Freitas in Brazil (1999) which detected B19 DNA in only one mother(16), and B19 antibodies were also detected in pregnant women under different gestation trimesters by Mirambo MM. et al in Tanzania (2017) (17).

Subjects and methods

This study is across-sectional study conducted in the Military hospital, Khartoum state during the period from August 2020 – August 20221. According to the hospital's annual patient's records and the statistical equations, a sum of 97 samples was selected as a sample size. Probability sampling type and simple random sampling technique were applied. A minimum of 2ml venous whole blood samples were collected in EDTA containers from each of the pregnant women participating in the study.

DNA extraction:

DNA was extracted from whole blood samples using the chemical method, Guanidine Chloride Method using Roche High Pure kits.

Polymerase chain reaction:

To detect parvovirus B19 DNA, the virus B19 VPI coding gene was amplified using nested PCR employing two sets of outer and inner primers pair;

1.The outer primers pair were;(P1) 5'-CAAAAGCATGTGGAGTGAGG-3'(sense), (P2)5'-CTACTAACATGCATAGGCGC-3'(antisense).

2.The inner primers pair were;(P3) 5'-CCCAGAGCACCATTATAAGG-3'(sense), (P4) 5'-GTGCTGTCAGTAACCTGTAC-3'(antisense).

The amplification was performed as the following protocol: A- In the first round of amplification: outer primers (P1andP2) were used, 30 cycles were programmed in the thermocycler machine. Denaturation, annealing, and primers extension were set at 94C° for 1 minute, 55C° for 2 minutes and 72C° for 1 minute respectively, and then final extension was made for further five minutes. B: In the second round of amplification the inner primers (P3andP4) were used and cycling parameter of this round was the same as the first round (18, 19).

Data analysis:

The final data were analyzed using the computer programme, Statistical package for social sciences (SPSS), version 20. Chi square test was applied on the data to determine the association between the variables of interest, and p value of ≤ 0.05 was considered the borderline of significance.

Ethical consideration:

Ethical approval for this conduction was obtained from Sudan international university management. Informed consent was taken individually and orally from the eligible participants, information's including age, gestation period and number of miscarriages were gathered in a questionnaire and the final results were handed back to them. Permission for sample collection was obtained from the military hospital authorities.

The informed ethical consent form was designed and approved by the ethical committee of the Faculty of Medical Laboratory Research Board, National University-Sudan.

Results

A total of 97 whole blood samples were collected from pregnant women (of different trimesters). Out of them, 12 were in the first trimester (4-12) weeks, 26 in the second trimester (16-24) weeks, and the remaining 59 pregnant women were in the third trimester (28-36) weeks. There was no statistical significance difference between parvovirus B19 and gestation trimester (p value = 0.5).

The age of these pregnant women ranged from 20-40 years, and it was classified into 4 categories: 20-25 years, 26-30 years, 31-35 years and 36-40 years. The distribution of the participant pregnant women among these categories was: 31, 36, 18, and 12 respectively. Twenty-nine pregnant women had a history of previous miscarriage which was estimated to be 1-2 miscarriage, 5 had previous miscarriage of 2-3, 1 woman had more than 3 previous miscarriages, and the remaining 62 pregnant women had no previous miscarriages. There was a strong statistical non significance between B19 DNA and previous miscarriage (p value = 0.9).

Two cases from the evaluated 97 pregnant women samples were found to be positive (2.1%) for B19 DNA using nested PCR. There was a strong statistical non significance between PCR positive and negative samples (p value = 0.9).

Out of the 29 samples for pregnant women with 1-2 previous miscarriage, 1 revealed positivity (3.4%) for B19 DNA, and the other positive sample was for a pregnant woman with no history of previous miscarriages (1.6%), however both positive samples were for pregnant women in third trimester (3.4%) out of the 59 third trimester pregnant women.

Table 1: Frequency of PCR results

PCR result	Frequency	Percent
positive	2.0	02.1%
negative	95	97.9%
Total	97	100%

Table 2: Correlation between PCR results and age

Age		PCR results		Total	P.value
		positive	negative		
20-25	Count	0.1	30	31	0.5
	%	3.2%	96.8%	100.0%	
26-30	Count	0.0	36	36	
	%	0.0%	100.0%	100.0%	
31-35	Count	0.1	17	18	
	%	5.6%	94.4%	100.0%	
36-40	Count	0.0	12	12	
	%	0.0%	100.0%	100.0%	
Total	Count	0.2	95	97	
	%	02.1%	97.9%	100.0%	

Table 3: Correlation between PCR results and trimester

Trimester		PCR result		Total	P.value
		positive	negative		
1st	Count	0.0	12	12	0.5
	%	0.0%	100.0%	100.0%	
2nd	Count	0.0	26	26	
	%	0.0%	100.0%	100.0%	
3rd	Count	2.0	57	59	
	%	3.4%	96.6%	100.0%	
Total	Count	2.0	95	97	

Table 4: Correlation between PCR results and miscarriages

miscarriages		PCR result		Total	P.value
		positive	negative		
1-2	Count	1.0	28	29	0.9
	%	3.4%	96.6%	100.0%	
2-3	Count	0.0	5.0	5.0	
	%	0.0%	100.0%	100.0%	
>3	Count	0.0	1.0	1.0	
	%	0.0%	100.0%	100.0%	
no	Count	1.0	61	62	
	%	1.6%	98.4%	100.0%	
Total	Count	2.0	95	97	
	%	2.1%	97.9%	100.0%	

Out of the 2 B19 positive cases, 1 (3.2%) belonged to a pregnant female from the age group of 20-25 year, and the other 1 (5.6%) was for a pregnant women of an age group of 31-35 years.There was no statistical significance difference between age and B19 DNA (p value = 0.5).

Discussion

During pregnancy congenital infection with parvovirus B19 can be associated with poor outcome, including miscarriage, fetal anemia and non-immune hydrops (20).

This study revealed that the prevalence of B19 DNA among 97 pregnant women blood samples was 2.1%, which is agreed with the fact that parvovirus B19 prevalence in pregnancy is 1-5% , but in epidemic situation it receives to 10% (21).

Some studies results showed disagreement with this result, such as a study done by Adam. et al in Sudan (22), showed that the seroprevalence of B19 IgG among pregnant women (n =500) was 61.4% with one subject positive for IgM and B19 DNA was not detected using PCR. In addition another study done by Maksheed M. et al in Kuwait (23), aimed only to detect B19 IgG, and IgM using ELISA, and it was 53.3% and 2.2% for IgG and IgM respectively. This disagreement is probably due the relatively low specific methods of virus detection used by both researchers despite the large number of suspect involved.

In a study done by Barros De freitas. et al in Brazil (24), 42 B19 IgG and IgM negative and 5 IgG and IgM positive pregnant women samples which were previously screened by ELISA, were submitted to nested PCR and it was detected in one mother who seroconverted from IgG negative to IgG positive.From a comparison perspective, this low detection rate of B19 DNA is attributed to low number of samples screened knowing that a large portion of these samples were IgM negative (5samples).

In another study done by Mirambo. et al in Mwanza ,Tanzania (25), 258 pregnant women of a median age of 21 (19-25) years were tested for parvovirus B 19 IgM and IgG using ELISA.116 (44.96%) ,109 (42.25%), and 33 (12.79%) of these pregnant women were in the first, second and third trimester respectively.The prevalence of B19 IgM was 83 (32.8%) and IgG was 142 (55%).This study restricted the age range to a maximum of 25 years which is not corresponded with our age range that was relatively wide, and also the method used for screening the virus depended on serology only, however the researcher incorporated different gestation trimesters which is agreed with our study.

Conclusion

According to the study results parvovirus B19 DNA was screened, and positively detected in selected pregnant women with a percentage of 2.1%.

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Competing interests

The authors declare that they have no competing interests

References

[1] Giorgio E, De Oronzo MA, Iozza I, Di Natale A, Cianci S, Garofalo G, Giacobbe AM, Politi S. Parvovirus B19 during pregnancy: a review. J Prenat Med. 2010 Oct;4(4):63-6.

[2] Erdman DD, Durigon EL, Wang QY, Anderson LJ. Genetic diversity of human parvovirus B19: sequence analysis of the VP1/VP2 gene from multiple isolates. J Gen Virol. 1996 Nov;77 (Pt 11):2767-74.

[3] Poole BD, Zhou J, Grote A, Schiffenbauer A, Naides SJ. Apoptosis of liver-derived cells induced by parvovirus B19 nonstructural protein. J Virol. 2006 Apr;80(8):4114-21.

[4] Servey JT, Reamy BV, Hodge J. Clinical presentations of parvovirus B19 infection. Am Fam Physician. 2007 Feb 1;75(3):373-6..

[5] Mossong J, Hens N, Friederichs V, Davidkin I, Broman M, Litwinska B, Siennicka J, Trzcinska A, VAN Damme P, Beutels P, Vyse A, Shkedy Z, Aerts M, Massari M, Gabutti G. Parvovirus B19 infection in five European countries: seroepidemiology, force of infection and maternal risk of infection. Epidemiol Infect. 2008 Aug;136(8):1059-68.

[6] Khameneh ZR, Hanifian H, Barzegari R, Sepehrvand N. Human parvovirus B19 in Iranian pregnant women: a serologic survey. Indian J Pathol Microbiol. 2014 Jul-Sep;57(3):442-4.

[7] Török TJ, Wang QY, Gary GW Jr, Yang CF, Finch TM, Anderson LJ. Prenatal diagnosis of intrauterine infection with parvovirus B19 by the polymerase chain reaction technique. Clin Infect Dis. 1992 Jan;14(1):149-55.

[8] Xu J, Raff TC, Muallem NS, Neubert AG. Hydrops fetalis secondary to parvovirus B19 infections. J Am Board Fam Pract. 2003 Jan-Feb;16(1):63-8.

[9] Rahbar N, Vali Zadeh S, Ghorbani R, Kheradmand P. Prevalence of parvovirus B19 specific antibody in pregnant women with spontaneous abortion. Acta Med Iran. 2015;53(3):168-72.

[10] Heegaard ED, Brown KE. Human parvovirus B19. Clin Microbiol Rev. 2002 Jul;15(3):485-505.

[11] Brown KE. The expanding range of parvoviruses which infect humans. Rev Med Virol. 2010 Jul;20(4):231-44.

[12] Lamont RF, Sobel JD, Vaisbuch E, Kusanovic JP, Mazaki-Tovi S, Kim SK, Uldbjerg N, Romero R. Parvovirus B19 infection in human pregnancy. BJOG. 2011 Jan;118(2):175-86.

[13] Clewley JP. Polymerase chain reaction assay of parvovirus B19 DNA in clinical specimens. J Clin Microbiol. 1989 Dec;27(12):2647-51.

[14] Adam O, Makkawi T, Reber U, Kirberg H, Eis-Hübinger AM. The seroprevalence of parvovirus B19 infection in pregnant women in Sudan. Epidemiol Infect. 2015 Jan;143(2):242-8.

[15] Maksheed M, Pacsa AS, Essa SS, Ahmed MA, Monem RA, Surkouh M. The prevalence of antibody to human parvovirus B19 in pregnant women in Kuwait. Acta Trop. 1999 Oct 15;73(3):225-9.

[16] Barros De Freitas R, Buarque De Gusmão SR, Durigon EL, Linhares AC. Survey of Parvovirus B19 Infection in a Cohort of Pregnant Women in Belém, Brazil. Braz J Infect Dis. 1999 Feb;3(1):6-14.

[17] Mirambo MM, Maliki F, Majigo M, Mushi MF, Moremi N, Seni J, Matovelo D, Mshana SE. The magnitude and correlates of Parvovirus B19 infection among pregnant women attending antenatal clinics in Mwanza, Tanzania. BMC Pregnancy Childbirth. 2017 Jun 7;17(1):176.

[18] Yamakawa Y, Oka H, Hori S, Arai T, Izumi R. Detection of human parvovirus B19 DNA by nested polymerase chain reaction. Obstet Gynecol. 1995 Jul;86(1):126-9.

[19] Bergallo M, Merlino C, Daniele R, Costa C, Ponzi AN, Cavallo R. Quantitative competitive-PCR assay to measure human parvovirus B19-DNA load in serum samples. Mol Biotechnol. 2006 Jan;32(1):23-9.

[20] Butchko AR, Jordan JA. Comparison of three commercially available serologic assays used to detect human parvovirus B19-specific immunoglobulin M (IgM) and IgG antibodies in sera of pregnant women. J Clin Microbiol. 2004 Jul;42(7):3191-5.

[21] Habibzadeh S, Peeri-Doghaheh H, Mohammad-Shahi J, Mobini E, Shahbazzadegan S. The prevalence of parvovirus B19 infection among pregnant women of Ardabil in 2013. Iran J Microbiol. 2016 Jun;8(3):214-218.

[22] Adam O, Makkawi T, Reber U, Kirberg H, Eis-Hübinger AM. The seroprevalence of parvovirus B19 infection in pregnant women in Sudan. Epidemiol Infect. 2015 Jan;143(2):242-8.

[23] Maksheed M, Pacsa AS, Essa SS, Ahmed MA, Monem RA, Surkouh M. The prevalence of antibody to human parvovirus B19 in pregnant women in Kuwait. Acta Trop. 1999 Oct 15;73(3):225-9.

[24] Barros De Freitas R, Buarque De Gusmão SR, Durigon EL, Linhares AC. Survey of Parvovirus B19 Infection in a Cohort of Pregnant Women in Belém, Brazil. Braz J Infect Dis. 1999 Feb;3(1):6-14.

[25] Mirambo MM, Maliki F, Majigo M, Mushi MF, Moremi N, Seni J, Matovelo D, Mshana SE. The magnitude and correlates of Parvovirus B19 infection among pregnant women attending antenatal clinics in Mwanza, Tanzania. BMC Pregnancy Childbirth. 2017 Jun 7;17(1):176.

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