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

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Research Article

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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.

Materials 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 was first described in 1975(1). Human parvovirus B19 (B19V) is a human pathogenic virus, a member of erythrovirus genus in the parvoviridea family (2). B19 is a small, non-enveloped, ssDNA virus and, like all parvoviruses, the capsid proteins are arranged with icosahedral symmetry. B19 is 20-25 nm in diameter and has a genome of 5-6 kb (3). The B19 capsid is composed of two capsomer proteins, VP1 and VP2 which are encoded by overlapping reading frames (4). The structural proteins determine many of the biological properties of the virus, including binding to cell receptor, haemoagglutination and induction of neutralizing responses (5). A number of nonstructural proteins has been identified, the major nonstructural protein NS1(4). 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 (6). The virus is transmitted through exposure to infected droplets or blood products and vertically from mother to fetus. 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 (7).

B19 infection is common infection. Its seroprevalence increases with age, from 2-10% of children under 5 years old, to 40-60% in adults more than 20 years old, and up to 85% in the elderly population. Infection is more common in the late winter and early summer, with epidemics peaks every 3-4 years (8).

The infection can occur asymptomatically or can be associated with a wide range of clinical features, such as erythema infectiosum, post infectious arthropathy and transient aplastic crises in patients with hemolytic anaemia. Chronic infections can also occur mainly in immunocompromised patients (9).

Generally, erythema infectiosum is self-limited and does not require treatment. Patients with arthralgia may require nonsteroidal anti-inflammatory drug treatment. Patients in transient aplastic crises may require erythrocyte transfusion while the marrow recovers. Chronic red cell aplasia, if severe may require intravenous immunoglobulin therapy. This treatment may improve anemia symptoms, but it may precipitate a rash or arthropathy. Intravenous immunoglobulin also has been in several case reports of severe illness (7). Although vaccine development has shown promising initial results, the is no currently vaccine available against parvovirus B19 (10).

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(11).

Maternal infection in pregnancy is a potential hazard to the fetus because of the virus ability to infect fetal erythroid precursor cells and fetal tissues (9). 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 (12). 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) (13). 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 (14).

The role of innate immunity in contrasting B19 virus infection has not been investigated in detail. Antibodies are the hallmark of the adaptive immune response to B19. In naïve individuals, B19 specific antibodies are produced early after infection and are assumed to be able to neutralize viral infectivity and progressively lead to clearance of infection. Immunoglobulin M (IgM) are produced first and can usually last about 3-6 months following infection, soon followed by production of immunoglobulin G (IgG) that is assumed to be long-lasting. Immunoglobulin A (IgA) can also be detected in body fluids (2).

If laboratory testing is needed, there are two types of diagnostic tests to confirm parvovirus infection: B19 specific antibody testing or viral DNA testing (7). 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(15). In vitro the virus can be cultured in some erythromegakaryoblastoid cell lines, but replication is very inefficient (16).

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)(17).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 (18).The advent of PCR has greatly increased the sensitivity of DNA detection in serum and tissue samples, although it poses a great propensity of contamination (17).

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 (24), another study conducted by Maksheed M. *et al* in Kuwait (1999) (25), 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(26), and B19 antibodies were also detected in pregnant women under different gestation trimesters by Mirambo MM. *et al* in Tanzania (2017)(27).

Materials And Methods

This study is across-sectional study conducted in the Military hospital, Khartoum state. 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.

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. Positive and negative controls were processed using the same protocol and included with each batch of the tested samples. The final PCR products were analyzed using gel electrophoresis on 2% (w\v) agarose gel in TAE buffer and stained with ethidium bromide. The amplicons of 288 base pairs (bp) were visualized by using the gel documentation system Syngene,Bioimaging,UK(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.

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.

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.

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).

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%	

Discussion

During pregnancy congenital infection with parvovirus B19 can be associated with poor outcome, including miscarriage, fetal anemia and non-immune hydrops(20). Importance of B 19 investigation in pregnant women is due to vertical transmission of this virus (21). Infection during pregnancy can cause a variety of other signs of fetal damage .The risk of adverse fetal outcomes increases if maternal infection occurs during the first two trimesters of pregnancy but may also happen during the third trimester (22). Maternal and public health concern about association of B19 infection during pregnancy and morbidity of the birth is affected by risk of fetal loss (2).

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% (23).

Some studies results showed disagreement with this result, such as a study done by Adam. *et al* in Sudan (24), 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 (25), 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 (26), 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 (27), 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%.

Abbreviations

B19V
Human parvovirus B19
CIE
current immunoelectrophoresis
ELISA
enzyme linked immune sorbent assays
EM
electron microscope
IEM
immune electron microscope
IgA
Immunoglobulin A
IgG
immunoglobulin G
IgM
immunoglobulin M
NS1
nonstructural protein
PCR
Polymerase chain reaction

SPSS
Scientific Package for Social Sciences

Declarations

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Authors' contributions

SHO and SSA conceived the design and carried out the experiments. AAI and RMA and AMA and EKA obtained, analyzed and interpreted the data. MMA and HAH and RAE wrote and revised the manuscript. MAH provides financial support for all experiments. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Each participant was asked to sign a written ethical consent form during the interview, before the specimen was taken. The informed ethical consent form was designed and approved by the ethical committee of the Faculty of Medical Laboratory Research Board, National University-Sudan.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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