(RESEARCH ARTICLE)

Molecular detection of human parvovirus B19 DNA amongst pregnant women attending antenatal clinic in Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria

EUCHARIA A DILIBE [1, 2, *], NNEKA. R AGBAKOBA [2], SAMUEL I OGENYI [3], IFEOMA G NWAFULUAKU [4] and CHIDOZIE V UDEOGU [3]

[1] Department of Medical Microbiology, Faculty of Basic Clinical Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka Campus. Anambra State, Nigeria.
[2] Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[3] Department of Medical Microbiology, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[4] Department of Medical Microbiology and Parasitology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Abstract

Background: Parvovirus B19 infection causes a wide range of complication in pregnant women including spontaneous abortion, severe fetal anaemia, non-immune hydrops fetalis and even intrauterine fetal death, erythema infectiosum (5th) disease and aplastic crisis.

Objective: To perform molecular detection of human Parvovirus B19 among pregnant women attending antenatal clinic in Nnamdi Azikiwe Teaching Hospital Nnewi, Anambra State, Nigeria.

Materials and Methods: This is a cross-sectional study conducted among 92 consented pregnant women, between the ages of 20-40 years who are attending antenatal clinic in Nnamdi Azikiwe University Teaching Hospital Anambra State, Nigeria, from May 2020-November 2020. A total of 92 blood specimen was collected from the pregnant women.

Result: Out of the 20 IgM positive samples analyzed for Parvovirus B19 DNA, 13(65.0%) had Parvovirus B19 DNA and 7(35.0%) were negative for Parvovirus B19 DNA. Prevalence of Parvovirus B19 DNA in relation to gestational age was highest (69.2%) among pregnant women who were in their 2nd trimester and the prevalence was lowest (0.0%) among pregnant women who were in their 3rd trimester. Prevalence of Parvovirus B19 in relation to history of blood transfusion was highest (100.0%) among pregnant women who have had transfusion history and the prevalence was lowest (0.0%) among pregnant women who had no transfusion history.

Conclusion: The frequency rate of Parvovirus B19 DNA seropostivity was highest (65.0%) among the pregnant women investigated. The association of Parvovirus B19 DNA seroposivity with gestation age and history of blood transfusion was statistically significant.

Keywords: Molecular detection; Parvovirus B19 DNA; Real-time PCR; Pregnant women; NAUTH Nnewi

* Corresponding author: EUCHARIA A DILIBE
Department of Medical Microbiology, Faculty of Basic Clinical Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka Campus. Anambra State, Nigeria.

1. Introduction

Manifestations of Parvovirus B19 infection vary with the immunologic and haematologic status of the host. Parvovirus B19V is a common community acquired respiratory pathogen that does not have ethnicity, gender, age, socio-economic or geographic boundaries. Cossart and his colleagues were the first scientist that discovered human Parvovirus B19 in the sera of asymptomatic hepatitis B patients. The name originates from the coding of a serum sample number 19 in panel B. It gave anomalous results when it was tested by counterimmuno electrophoresis and radioimmuno assay [14].

Human Parvovirus (HPV-B19 is a small non- enveloped single stranded DNA (ssDNA) virus of the family Parvoviridae, the sub family Parvovirinae and the genus Erythrovirus. It is known to be a human pathogen. The human Parvovirus B19 type specie is among the smallest DNA-containing viruses. It's virion diameter is 18-26nm, hence the name 'Partum' meaning small. It's genome contains about 2500 base pairs, encoding for three major proteins. Two structural proteins VP1 and VP2 make up the viral capsid. The viral capsid consists of 95% of VP2while the remaining 5% is VP1 [11].

B19 exhibits a marked tropism to human bone marrow (BM) and replicates only in erythroid progenitor cells [5].

Parvovirus B19 infection have been reported to cause erythema infectiosum, arthropathy, non-immuned hydro fetalis (NHF), spontaneous abortion, fetal death, preterm birth, intrauterine growth restriction, birth defects, including abnormalities of the central nervous system, ophthalmologic manifestations, congenital heart defects, various haematological disorders including aplastic crisis, chronic anaemia and idiopathic thromobocytopeniapurpura [7]. This type of adverse effects may vary with gestational age at the time of [15]. Most infections, however leave no identifiable trace of damage.

B19V transmission occurs through the respiratory route vertically from mother to fetus, and through transfusion or transplantation and most often during the viremia that proceeds clinical presentation. Household day care, and school transmissions are common. Viral levels as of high as 10^{13} genome equivalents (greq)/ml are often found in the blood of asymptomatic individuals during the early phase of acute infection [8]. There are numerous reports of transmission by pooled plasma-derived products including clothing factor concentrates despite solvent detergent (S/D) treatment, heat treatment and or other viral inactivation methods [21]. Infectivity has been correlated with a high concentration of B19V prompting plasma fractionators to implement screening for B19V DNA by nucleic acid testing (NAT) to exclude high titer donations from entering manufacturing pools. Blood products advisory committee, 2010); [5]

B19V infection transmitted by transfusion has already been described as a cause of chronic anaemia [9]; [13] as well as transient heart failure [3] in affected individuals.

However, Parvovirus B19 is common in developed countries and studies indicated that about 15% of pre-school children 50% of adults and 85% of elderly people had serological evidence of past infection. Seasonal outbreaks of Parvovirus B19 infection occur every 3-5 years. During school outbreaks, 10-60% of exposed children developed symptoms consistent with Parvovirus B19 infection. The incidence in pregnancy is about 1-5% and it can cause complication in 3% of infected pregnant women. There is no specific antiviral therapy or vaccine for HPV-B19 infections. However specific human vaccine is necessary to prevent aplastic crisis in patients with underlying disorders and pregnancy complication in sero-negative women of the child bearing age. A lot of studies had highlighted the problems of diagnosis of HPV-B19 based on the characteristics facial rash, particularly in pregnant women. Otherwise, polymerase chain reaction (PCR) or alternative nucleic acid amplification technology (NAT) may be a better diagnostic tool during acute B19 V infection [18].

There is no published data on the epidemiology of Parvovirus B19 in NAUTH Nnewi, Anambra State, Nigeria. To the best of our knowledge, this study was the first report published on the frequency rate of Parvovirus B19 DNA in NAUTH Nnewi, Anambra State, Nigeria. The aim of this study was to detect the frequency rate of Parvovirus B19 DNA among pregnant women and to assess the frequency rate of Parvovirus B19 DNA with risk factors.

2. Material and methods

This was a cross sectional study conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State, Nigeria from May, 2020 to November, 2020. Data were tabulated and analyzed using Epi-info (2000, CDC, Atlanta, Georgia, USA) software packages, [26]. The Pearson chi-square test was used to analyzed qualitative data and was compared with values on the chi-square table at 95% confidence interval, P values <0.05 was considered statistical significant. Confidentiality of information obtained from the participants investigated was maintained. Verbal consent

of the participants was taken before being enrolled in the study. Laboratory results of specimens collected were handed to all participants included in the study. Permission to collect the specimens and ethical approval to run the study was obtained from Ethical Committee Nnamdi Azikiwe University Teaching Hospital, (NAUTH) Nnewi (Approval No. NAUTH/CS/66/Vol.12/051/2019/032). Complete information regarding risk factors, if any was handed to all participants under the study without concealment. Sampling was a non-probability purposive sampling type and sample strategy was convenience where participants were chosen on the basis of accessibility. Demographic and obstetrical data were obtained by direct interviewing questionnaire from the pregnant women.

All blood specimens were collected from the pregnant women attending antenatal clinic at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State, Nigeria. A 5 ml blood specimens were collected from a total of 92 pregnant women in EDTA containers and centrifuged at 3000 rpm for 5 mins. Plasma was separated into sterile Eppendorf tubes and stored at -20°C until assayed.

2.1. Molecular detection of Parvovirus B19 DNA

Out of the 44 samples that were positive for Parvovirus B19 IgM, 20 samples were randomly selected for Parvovirus B19 DNA extraction, due to scarcity of resources.

2.2. Parvovirus B19 DNA Extraction for PCR

The extraction of the Parvovirus B19 DNA was done using a Zymo research biological fluid quick DNA miniprep plus Kit (Lot no: ZRC 202764 Inquaba biotec, West Africa Ltd) according to the manufacturer instruction

2.3. DNA Extraction Protocol

A 200 μl volume of red blood cell sample from anticoagulated blood sample was added to a microcentrifuge tube. After that a 200 μl volume of Biofluid & cell buffer (Red) was added. A 20 μl volume of proteinase K was added, then the mixture was thoroughly mixed and then the tubes were incubated at 55°C for 10 mins. A 420 μl volume of genomic binding buffer was added to the digested sample and mixed thoroughly. The mixture was transferred to a "Zymo spin" 11C..XL column in a collection tube and was centrifuged at 12,000g for 1 minute and the collection tube was discarded with flow through. A 400 μl DNA pre-wash buffer was added to the column in a new collection tube and it was centrifuged for 1 minute and the collection tube was emptied. A 700 μl g-DNA wash buffer was added and it was centrifuged for 1 minute, and the collection tube was emptied. A 200 μl g-DNA wash buffer was added and it was centrifuged for 1 minute and the collection tube with the flow through was discarded. After that, in order to elute the DNA it was transferred to a clean microcentrifuge tube and A 50 μl volume DNA elution buffer was added, it was then centrifuged for 1 minute.

2.4. Detection of Parvovirus B19 DNA [12]

Polymerase chain reaction (PCR) method was used for detection of Parvovirus B19 DNA.

2.4.1. Principle of PCR (Polymerase Chain Reaction) Detection Method.

Parvovirus B19 DNA detection by the polymerase chain reaction (PCR) method, was based on the amplification of the pathogen specific region, using special Parvovirus B19 primer in the real time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. The real time monitoring of the fluorescence intensities during the real time PCR allows the detection of accumulated product without re-opening the reaction tubes after the PCR run.

2.4.2. Amplification of Human Parvovirus B19 DNA

The extracted DNA was used as a template for the detection of B19V DNA by PCR. Negative controls consisting of phosphate buffered saline (PBS) were extracted concomitantly with the plasma during the extraction step.

Amplification was performed using quick-load one tag one step PCR master mix (2x) with standard buffer (Biolabs, incorporation, New England) specific primers for a conserved region of the NSI gene were designed based on the complete genome of B19V (Inqaba Biotec, West Africa Ltd), [27]: forward and reverse nucleotide sequences were: PARVO V B19-F$_1$ sequences: 5´-A ATACACTGTGGTTTTATGGGCCG-3´ and PARVO V B19-R$_1$ Sequences:

5´-CTAAAATGGCTTTTGCAGCTTCTAC-3´; respectively [27].

2.4.3. Protocol for DNA Amplification using the Genomic guided sequence method [17].

The DNA was amplified based on the protocol published by [17], with modification to optimize the PCR reaction [17]. The PCR master mix (2x) was gently vortex and briefly centrifuge after thawing. The thin walled PCR tube was placed on ice and the following components for each 25 µl reaction was added accordingly. A 12.5 µl of PCR master mix (2x) was added to 21 PCR tubes. After that 2.5 µl of forward primer (10 µm) was added to 21 PCR tubes. A 2.5 µl of reverse primer was added to 21 PCR tubes. Then the samples were gently vortexed and spun down and A 5 µl of the template DNA was added to 20 PCR tubes only except the control. A 2.5 µl of nuclease free water was also added to make it up to 25 µl. One round of amplification was performed on the 21 PCR tubes using a DNA thermal cycler (Applied Biosystem PCR Machine, United States of America) using the recommended thermal cycling condition outlined below,

Amplification conditions were as follows: Initial denaturation was performed for 3 min at 94^0C and was followed by 40 cycles of denaturation at 94^0C for 30 sec and annealing was performed at 49.4^0C for 30 sec and extension was performed at 72^0C for 1 min. Final extension was performed at 72^0C for 15 mins. Holding was performed at 4^0C for 1 hr 40 mins. The DNA amplification was programmed for 45 cycles for 2 hrs in the thermocycler machine.

2.5. Internal Quality Control Measures

Negative controls containing the components except the DNA extracted was included in each cycle to exclude non-specific amplification.

2.6. Agarose gel electrophoresis

The amplified products were analyzed by gel electrophoresis on a 1.0% (w/v) agarose (ultra-pure Agarose, invitrogen Life technologies, and stained with Ethidium bromide (0.5 µg/ml) at 120 volts and 60 Amp for 20 mins). The DNA was visualized using a gel documentation system (Gel Imaging system, Alpha.Innotech USA). The size of each amplicon was 100 bp [10].

2.6.1. Internal Quality Control Measures

The negative control were in parallel with water as template. The negative control (NC) and the positive control (PC) were included in each run. A 10 µl of ready to use DNA ladder (molecular marker) mixed with loading dye was loaded in the first well of the solidified gel immersed in TBE buffer in gel electrophoresis chamber, to know the size of the band.

2.7. Statistical Analysis

Data were tabulated and statistically Analyzed using EPI-inf (2000, CDC, Altanta, Georgia, USA) software package, [26]. Quantitative data were expressed as numbers and percentages. The pearson chi-square test was used to analyzed qualitative data and was compared with values on the chi-square table at 95% confidence interval, P value<0.05 was considered the borderline of significance and spearman's rank order correlation coefficient (r_s) was also used to test the relationship between two continuous or ordinal variables to determine the strength and direction of a monotonic relationship. It was then used to know whether they are significant or not significat P-value<0.05 was considered statistically significant.

3. Results

3.1. Demographic Characteristics of Study Population

In this study, a total of 92 pregnant women who are attending antenatal clinic at Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra Nigeria, within the period of study from May, 2020-November, 2020, who are within the age range between 20-40years were investigated serologically for Parvovirus B19 IgM and IgG antibodies, molecular investigation for Parvovirus B19 DNA was carried out by random selection of 20 samples out of the 44 samples that were positive for Parvovirus B19 IgM and they were analyzed for Parvovirus B19 DNA using real time PCR.

3.2. Prevalence of Parvovirus B19 DNA by age among pregnant women in NAUTH Nnewi using Pearson Chi-Square test

Out of the 20 IgM positive Samples randomly selected and analyzed for Parvovirus B19 DNA, 13 (65.0%) had Parvovirus B19 DNA and 7 (35.0%) were negative for Parvovirus B19 DNA. Prevalence of Parvovirus B19 DNA was highest (38.5%)

and (38.5%) among pregnant women aged (26–30 years) and 31–35 years respectively. The prevalence of Parvovirus B19 DNA was lowest (0.0%) among pregnant women aged 36 –40 years.

There was no statistically significant association (DNA: X^2 = 5.274 ; P = 0.153) between Parvovirus B19 DNA and age of the pregnant women (Table 1).

Table 1 Prevalence of Parvovirus B19 DNA By Age Among Pregnant Women In NAUTH Nnewi

Age (years)	Number analyzed	B19 DNA Status	Chi-Square	p-value
s21 – 25	19	3 (23.0)		
26 – 30	24	5 (38.5)		
31 – 35	26	5 (38.5)	5. 274	0.153
35 – 40	23	0 (0.0)		
Total	92	13 (100.0)		

3.3. Prevalence of Parvovirus B19 DNA by risk factors among pregnant women in NAUTH Nnewi using Pearson Chi-Square test

There was a highly statistically significant association (DNA: X^2 = 9.204; p = 0.000) between Parvovirus B19 DNA

and gestational age. The prevalence of Parvovirus B19 DNA was highest (69.2%) among pregnant women who were in their 2nd trimester and the prevalence of Parvovirus B19 DNA was lowest (0.0%) among pregnant women who were in their 3rd trimester.

There was no statistically significant association (DNA: X^2 = 2.485; P = 0.478) between Parvovirus B19 DNA and History of complication. The prevalence of Parvovirus B19 DNA was highest (38.5%) among pregnant women who have had miscarriage, followed by those pregnant women who have had still birth (30.8%), followed by those pregnant women who had none (23.1%) and lowest among pregnant women who have had both (miscarriage and still birth) (7.7%).

There was no statistically significant association (DNA: X^2 = 0.890; p = 0.0641) between Parvovirus B19 DNA and no. of children in a household. Parvovirus B19 DNA prevalence was highest (46.2%) among pregnant women who have had 0 – 2 children in a household followed by those who had 3 – 4 (38.5%) children in a household and lowest (15.3%) among pregnant women who had 5 and above children in a household, gradually decreasing prevalence values were observed between the prevalence of Parvovirus B19 DNA and no. of children in a household.

There was a highly statistically significant association (DNA: X^2 = 20.574; p = 0.000) between Parvovirus B19 DNA and history of blood transfusion. The prevalence of Parvovirus B19 DNA was highest (100.0%) among pregnant women who have had transfusion history and prevalence of Parvovirus B19 DNA was lowest (0.0%) among pregnant women who had no history of blood transfusion.

There was no statistically significant association (DNA: X^2 = 3.555; p = 0.314) between Parvovirus B19 DNA and occupation. The prevalence of Parvovirus B19 DNA was highest (53.8%) among pregnant women who were civil servant followed by pregnant women who were housewives, followed by the pregnant women who were business women and prevalence of Parvovirus B19 DNA was Lowest (7.7%) among pregnant women who were students (Table 2)

Table 2 Prevalence of Parvovirus B19 DNA By Risk Factors Among Pregnant Women In NAUTH Nnewi

S/NO	Risk factors	Total (n)	Positive Sera n %	Chi-Square	P-Value
Gestational age	1st trimester	28	4 (30.8)		
	2nd trimester	34	9 (69.2)	9.204	0.010
	3rd trimester	30	0 (0.0)		
	Total	92	13 (100.0)		

History of complication	None	28	3 (23.1)	2.485	0.478
	Miscarriage	26	5 (38.5)		
	Still birth	18	4 (30.8)		
	Both	20	1 (7.7)		
	Total	92	13 (100.0)		
No. of children in a household	0 – 2	43	6 (46.2)	0.890	0.0641
	3 – 4	27	5 (38.5)		
	5 and above	22	2 (15.3)		
	Total	92	13 (100.0)		
History of blood transfusion	Yes	39	13 (100.0)	20.574	0.000
	No	53	0 (0.0)		
	Total	92	13 (100.0)		
Occupation	Student	12	1 (7.7)	3.555	0.314
	Civil Servant	29	7 (53.8)		
	House wife	28	3 (23.1)		
	Business	23	2 (15.4)		
	Total	92	13 (100.0)		

3.4 plasmid DNA profile of Parvovirus B19

Results of the Plasmid profile of Parvovirus B19 revealed that Parvovirus B19 harbour Plasmids with different molecular weights. L is the DNA molecular marker that shows the position of the expressed Parvovirus B19 genes while NC is a non-template control. The samples show positive and negative bands for the expressed Parvovirus B19 genes at 100bp to 1000bp. (figure 1 & 2).
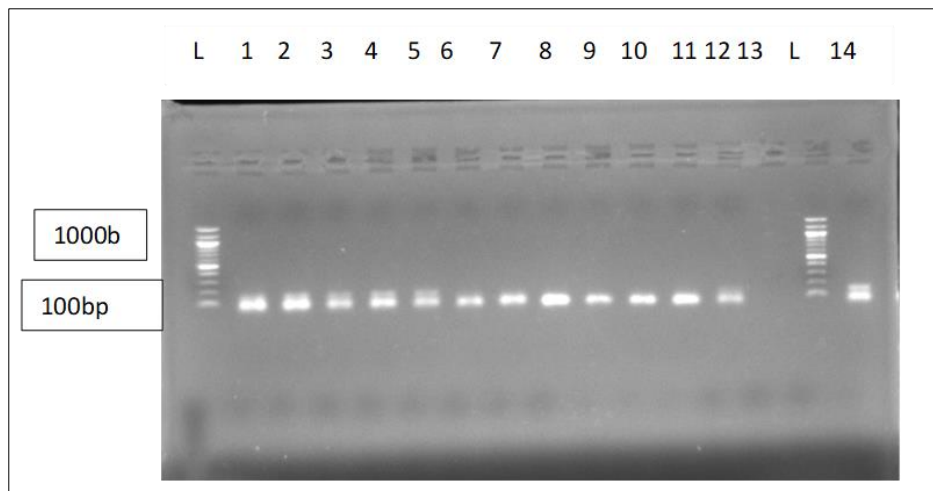

Figure 1 PCR results for Parvovirus B19 genes analyzed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is a 100bp-1000bp DNA ladder (molecular marker). Samples 1, 2, 3, 4, 5, 6 7, 8, 9, 10, 11, 12 and 14 are positive bands for the expressed Parvovirus B19 at 100 bp. While sample 13 is negative for the expressed Parvovirus B19 gene
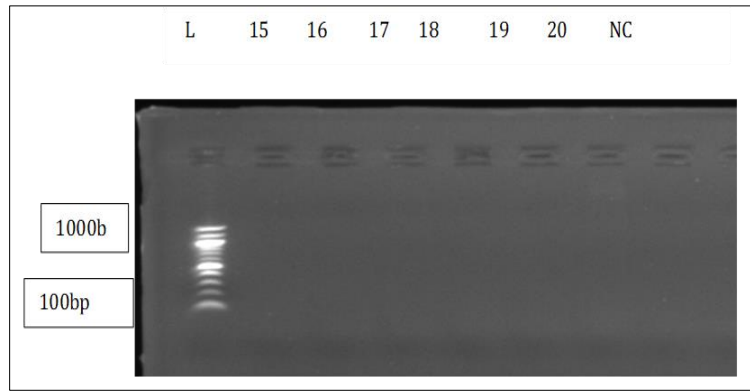
Figure 2 PCR results for Parvovirus B19 genes analyzed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is a 100bp-1000bp DNA ladder (molecular marker). Sample 15, 16, 17, 18, 19 and 20 are negative for the expressed Parvovirus B19 genes. NC is a Non template control

4. Discussion

Parvovirus B19 Infection can cause severe morbidity and mortality and acute infection is a high risk for pregnant women and fetus. This study was designed to determine the prevalence of Parvovirus B19 DNA using real time PCR among pregnant women attending Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria.

In this study, the seropositivity rate of Parvovirus B19 DNA was 13(65%). The result was higher to that reported by a study, the seropositivity rate of Parvovirus B19 DNA was 13(65%). This result was higher to that reported by a study conducted in Saudi Maternity Teaching Hospital Khartoum by [20] who reported a seropositivity rate of 43(43%). The result of this study was also higher to that reported by another study conducted in Sudan by [23], who reported a seropositivity rate of (41%). The result of this study is also higher than the result of the study reported in Libya by [16] who reported a seropositivity rate of 5%. However the result of this study was lower than the result of a study conducted in Italy by [24] who reported a seropositivity rate of (96.3%). These differences in the results obtained by different researchers may be attributed to endemicity of infection, different diagnostic techniques used, demographic and geographical variation, sample size and time of conduction.

Prevalence of Parvovirus B19 in relation to age of the pregnant women. There was no statistical significant association between the prevalence of Parvovirus B19 DNA and age of the pregnant women. In the present study highest seropositivity rate of Parvovirus B19 DNA (38.5%) and (38.5%) was recorded among the pregnant women aged (26-30 years) and (31-35) years respectively. Other studies reported varying findings e.g in Syria the highest seropositivity was (67.4%) in the age range 37-42 years, [22] and in Libya it was (57%) in the age range of 33-37 years [16]. However the prevalence of Parvovirus B19 DNA was Lowest (0%) among pregnant women aged 36-40 years.

Prevalence of Parvovirus B19 DNA in relation to gestational age. There was a highly significant association between the prevalence of Parvovirus B19 DNA and gestational age. In the present study, the findings revealed a high seroposivity rate of Parvovirus B19 DNA (69.2%) among pregnant women in their 2nd trimester, followed by those in their 1st trimester (30.3%) and lowest among those in their 3rd trimester (0%). This result is dissimilar to the study conducted in Sudan by [20] who reported a low seropositivity rate of (37.5%) among pregnant women in their second trimester. However, highest seropositivity (31.1%) was reported in Northeastern Europe by [5] among pregnant women in the second trimester and it was (50.3%) in Erbil Iraq by [2] among pregnant women in the first trimester. These differences may be due to the different diagnostic techniques used sample size, demographic and geographical variations. The possible explanation for high prevalence of Parvovirus B19 DNA among the pregnant women in their second trimester in this study, is that the fetus seems to be most susceptible to Parvovirus B19 infection during the first and second trimester of pregnancy and especially between week 10 and 20 which coincide with the major development of erythroid precursor [1].

Prevalence of Parvovirus B19 DNA in relation to history of complication. There was no statistical significant association between the prevalence of Parvovirus B19 DNA and history of complication. In the present study, the frequency rate of positive Parvovirus B19 DNA among pregnant women with history of complication was highest (38.5%) among pregnant women who had miscarriage followed by those who had still birth (30.8%), followed by those who has none (23.1%) and lowest among those who had both (7.7%). The result of this study was lower that the report of the study

conducted by [20] on Parvovirus B19 among pregnant women with history of complication who revealed that the frequency rate of positive Parvovirus B19 DNA was (57.9%) and it was considered statistically significant. The findings of the study was lower than the findings of the study in Iraq (61.1%) by [25] and also lower that the study conducted in Iran by [23]. However the result of this study was higher than the report of the study conducted in Iraq (11.1%) by [19]. The findings of this study was also higher than the report of the study conducted by [16] on Parvovirus B19 infection in pregnancy who reported that the spontaneous loss rate of fetuses affected with Parvovirus B19 in their first trimester was (13.0%) and higher than the spontaneous loss rate of fetuses affected with Parvovirus B19 in their second trimester (0.5%) [16]. the difference may be due to the different diagnostic techniques demographic and geographical variation, type of samples and the population investigated.

Prevalence of Parvovirus B19 DNA in relation to history of blood transfusion. There was a highly significant association between Parvovirus B19 DNA and history of blood transfusion. In the present study, the prevalence of Parvovirus B19 DNA was highest (100.0%) among pregnant women who have had transfusion history and Parvovirus B19 DNA was lowest (0.0%) among pregnant women who had no history of transfusion. The findings of this study is similar to the report by [20] on Parvovirus B19 among pregnant women in Saudi Arabia, who reported that the frequency rate was higher (65.5%) among pregnant women with past history of blood transfusion (33.8%) and in that study there was a statistically significant association between Parvovirus B19 DNA and history of blood transfusion. The findings of this study was similar to that reported in Sudan by [23] and not similar to that reported by a study conducted by [22] where the frequency rate of pregnant women with past history of blood transfusion was (3.3%) the findings of this present study is also not similar to the study in Northeastern Europe conducted by [5] where the frequency rate of pregnant women with past history of blood transfusion was (30.8%). On the other hand, the frequency rate of Parvovirus B19 among the pregnant women investigated might be influenced by various demographic and obstetrical variables. This findings supported the fact that to decrease the incidence of blood transfusion- associated Parvovirus B19 infection, it is needed to transfuse only blood products negative for B19V.

Parvovirus B19 DNA in relation to number of children in a household. There was no statistical association between Parvovirus B19 DNA and number children in a household. In the present study of the frequency rate of Parvovirus B19 was highest (46.2%) among pregnant women with (0-2) number of children in a household, followed by those with (3-4) number of children in a household (38.5%) and lowest(15.3%) among the pregnant women with (5 and above) number of children in a household. The result of this study is dissimilar to the report of the study conducted by [4] who reported that the risk of Infection in pregnant women with one child is 3 times more than nulliparous women, but the risk for women with three or more children are 7-5 time more.

Parvovirus B19 DNA in relation to occupation. There was no statistically significant association between the prevalence of Parvovirus B19 DNA and occupation. In the present study, the frequency rate of Parvovirus B19 DNA was highest (53.8%) among pregnant women who were civil servant, followed by those who were business women (15.4%) and lowest (7.7%) among pregnant women who were students. The result of the present study is lower than the report of the study conducted by [6] on Parvovirus B19 Infection in fetal deaths, who reported that 20.0% of mothers with a Parvovirus B19 DNA- positive fetus are civil servants that work with children. The report of [6] also indicated that (35.3%) of the mothers with antenatal Parvovirus B19 infection had occupational contact with children and the report of this present study is also higher than their result. The reason for this is that the risk of Parvovirus B19 infection during pregnancy is greatest in women who are in contact with preschool children [6]. However their sample size was too small to address the occupational risk. The result of the current study is contrary to the report of the study carried out by [5], who revealed that the risk of infection among susceptible adults seems to be higher (c.50%) following exposure to B19 at home compared to school or hospital exposure (c.20-30%) [5].

Application of PCR has higher diagnostic value in comparison with serology and can be helpful in reaching to definite results. Infact, the superiority of the present study is simultaneously conductance of serological and PCR test, which can dramatically enhance the diagnostic power.

This study however suffers some limitation. This study was a cross sectional and non-perspective, therefore we were not able to estimate the disease emergency, and we did not follow the pregnant women to term to know the fetal outcome. Further studies that can resolve the existing limitations could be helpful.

From this study it may be recommended to conduct further studies among pregnant women to detect the prevalence rate of Parvovirus B19 DNA and related predisposing risk factors using recent laboratory techniques.

5. Conclusion

The frequency rate of Parvovirus B19 DNA seropositivity was high (65%) among the pregnant women investigated. The association of Parvovirus B19 DNA seropositivity with gestational age and history of blood transfusion was statistically significant.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare that they have no conflict of interest to disclose.

Statement of ethical approval

The study protocol was reviewed and approved by the Research Ethics committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Authors Contribution

Conceptualization: Nneka, R. Agbakoba, Investigation: Eucharia A. Dilibe:
Methodology: Eucharia A. Dilibe, Samuel I. Ogenyi,
Supervision: Nneka R. Agbakoba, written- original draft: Eucharia A. Dilibe,
Written review and editing: Ifeoma G. Nwafuluaku, Eucharia A. Dilibe, Chidozie V. Udeogu, Samuel I. Ogenyi, Nneka R. Agbakoba.
All authors read and approved the final manuscript.

Abbreviation

NAUTH: Nnamdi Azikiwe University Teaching Hospital Nnewi.
PCR: Polymerase Chain Reaction
B19 V: Parvovirus B19
IgM: Immunoglobulim M.
IgG: Immunoglobulin G
DNA: Deoxyribonucleic Acid
Ss DNA: Single stranded DNA virus
VP1: Viral Protein I
VP2: Viral Protein 2
BM: Bone Marrow
NHF: Non- immune hydro fetalis
S/D: Solvent/detergent
NAT: Nucleic Acid Testing
HPV: Human Parvovirus B19 virus
NAT: Nucleic Acid amplification Technology
CDC: Center for disease control
EDTA: Ethylene diamine tetracetic acid
RPM: Revolution per minute
PBS: Phosphate buffered saline
bp: base pair
NC: Negative control
PC: Positive control
TBE: Tris-boratec EDTA Buffer
F_1: forward primer
R_1: Riverse primer

Epi-info: Epidemiology information

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Abdullahi, N., Adekola, H., A., Emeribe, A.U., Ahmed, Yunusa, T., Zakari., M.M., (2020). Association of Haemoglobin Electrophoretic Phenotype among pregnant women attending Abuja Tertiary hospital, Nigeria, Mijgoums, 14(3)33-39. URL:http://mij.goums.ac.ir/article-1-1290-en-html.

[2] Ahga, N., Kawthar, A., Zakarea, A., (2020). 'Seroepidermiology of human Parvovirus B19 among pregnant women in Erbil, Iraq". Medicinal Journal of Babylon, Volume 17 no.1 , Jan.Mar 2020.

[3] Bijit, B., Narendra, N. N., Keya, B., Aparajita, D., Rivu, B., Bobby, P. (2022). Transfusion-Transmitted Infectious, Its risk factors and impact on quality of life: An epidemiological study among B-thalassemia major children SAsian Journal of Transfusion Science Volume 16(1):99-105.

[4] Bouraddane, M., Ward, K., Zouhair, S. (2021).Parvovirus B19 and pregnant -women. A Bibliographic Reviews. Open Journal of Obstetrics and Gynaecology, 11, 1543-1564 doi.10.4236/ojog.2021.1111/45.

[5] Dovile, K., Virginia, P., (2022). Parvovirus (B19) Infection during pregnancy: Possible effect on the course of pregnancy and rare fetal outcomes. A case Report and Literature Review. Medicina Journal, 58 (5):664., https://doi.org/10.3390/medicina.58050664.

[6] Hadout, S., Youssouf, N., Wajih, O., Jalal, M., Lamrissi, A., Fichtali, K., Bouhya, S. (2022). Congenital Hydrocephalus Secondary to Parvovirus Infection (About one case). Medical and Clinical Case Reports. 2(1):1-3.

[7] Hegaard, H. K, Rom, A. L., Christensen, K. B., Broberg L., Hogh, S., Christiansen, C. H., Nathan, N.O, de Wolff, M.C., Damm, P. (2021). Lifestyle Habits among pregnant women in Denmark during the first COVID-19 Lockdown compound with Historical period-A Hospital Based Cross sectional study. International Journal of Environmental Research and Public Health, 18,7128. https://doi.org/10.3390/ijerph18137128.

[8] Karimnia, A., Elikaei, A., Shariff, Z (2020). Human Parvovirus B19 Infection among blood donors in North-East of Iran. Archives of Infectious, Diseases, 15(4) Doi:10.5812/archcid.97856.

[9] Lindahl, J.P, Barlinn, R., Abrahgmsen., I.W, Spetaler, S., Medtvedt, K., Jensen, T., (2022). Case Report Pure Red Cell, Aplasia caused by Refractory Parvovirus B19 Infection After Pancreas transplantation Alone. Frontiers in Medicine Journal 9.849783.doi,10.3389/fmed.2022.8497-83

[10] Ma., J., Sun., W.,Tang, L., Yang, D., (2022). Case Report and Literature Review: Behcet's Disease with a novel TFPI Gene Mutation. Frontier in Medicinal Journal. 9.873600.doi.10.3389/fmed.2022.873600.

[11] Mattola, S., Aho, V, Bustamante-Jaramillo, L.F., Pizzioli. E., Kann, M., & Vihinen-Ranta, M (2022). Nuclear entry and Parvovirus. Molecular Microbiology, Early online. https://doi.org/10-1111/mm14974.

[12] Maxime, P., Clement, L., Ve´ronique, T., Delphine, M., Jean-Sebastien, C., Genevieve, B., Bruno, L., Pascal, G., Yahia, M., (2022). Optimized nested PCR enhances biological diagnosis and phylogenetic analysis of human Parvovirus B19 Infections. Archives of Virology Journal. https://doi.org/10.1007/s00705-2022-04368-w.

[13] Mengyi, Z., Yuhui, S. I., Yujla,, L.I., Zhan, G.A., Lan, Y.U., Mei, H., Jianhua, W.A.N., Weilan, H.E, Wei, M., Jie, C., C, Jingyu, Z, Yijing, Y., Yanli., G.,Qiulei, Z., Yang, H., Limin, E., Shan, H., Miao, H.E., Zhengxin, F. (2022). Metagnomics of potential emerging infectious pathogens identification in blood donors with a molecular epidemiology investigation of Human Parvovirus B19: A multi-center study in China. Doi:https://doi.org/0.21203rs.3.rs-1575896/VI.

[14] Orth, H.M., Fuchs, A, Lubke, N,, Jensen, B.O,, Luedde, T., (2022). Recurrent Parvovirus B19 virema resulting in two episodes of hemophagocytic lymphohistocytosis, virology Journal 19 (1): 107. https://doi.org/10.1186/s12985.022-01841-y.

[15] Pengfei, Q., Doudou, Z.,Shaonong, D.(2022). Risk assessment for Birth Defects in offspring of Chinese pregnant women. International Journal of Environmental Research and Public Health, 19 (14):8584.

[16] Pietruszka,. K.S., Magdalena. S., Agnieszka., S., (2022). Parvovirus B19 infection during pregnancy" Current issues in Medical Sciences, Volume 35 (1) PP 41-46. http://doi.org/10.2478/cipms-2022-0008.

[17]   Prasad, D. S., Anson. G., (2021). Amplican Sequencing of primate erythroParvovirus B19V from Clinical Samples. doi:10.175041protocols.10.bx23pgn [Crossref]

[18]   Quihang, Z., Jiaxin, C., Pei-Song, C., Jiaxin, C., Dingbang., C., Han, X., King, C., Huiyu, F., Li, F. (2022). Multi system involvement induced by Human Parvovirus Infection in a North-Immunosupressed Adult: A case Report, Frontier in   Medicine   Journal(Laysanne).2022AP,12:9:808205.doi:10.3389/fmed.2022.808205PMID:35492345, PMCID:PMC9039281.

[19]   Reza , T., Fatemeh, F., Masomeh., N. (2002). Molecular evaluation of human Parvovirus B19 infection and associated risk factors Among Pregnant Women in Bushehr Province, The Americian Journal of Tropical Medicine and Hygiene, Volume 106 (5):1539-1546. Doi:https//doi.org/100.4269/ajtmh 21-1088

[20]   Salah-Eldin, M., Ali., I., Wafa. I., (2018). Molecular Detection of Human Parvovirus B19 among pregnant women attending Saudi Maternity Teaching Hospital (Khartoun). African Journal of Medical Sciences.3(2):1-7era, Mse.info.

[21]   Sedighen., J., Ali, F., Gholami, Za, R.D., Shirin, F., Sedighen, S., Leza, R., Noorossadat, S. Sepidc, N., Abbas, B., (2022). The pathogenic aspects of human Parvovirus B19 NSI Protein in Chrronic and inflammatory Diseases. Hindawi Interdisciplinary   perspectives   on   infectious,   volume   2022,   Article   ID   1639990,   9   pages https://doi.org/10.1155/202211639990.

[22]   Marwan, M., (2015). Seroprevalence of Parvovirus B19 among Pregnant Women in Diamaseus Syria. International Journal of Pharmaceutical Science Review and Research, 30 (2).227-229.

[23]   Tagwa, H.A., Nusaiba, E.M., Jala, S.K., Mustafa. E.Y., Alkhair A.A. (2022). Detection of Parvovirus B19 DNA in Pregnant Sudanese Women attending the military hospital using Nested PCR technique. Research Square Journal. Doi: https://doi.org/10.21203/rs.3.rs-1203245/v1.

[24]   Verteramo, R., Santi. E., Ravennati., F., Scutiero, G., Greco, P., Morano, D., (2022). Ultrasound findings of fetal infections.   Current   Knowledge.   Reproductive   Medicine   2022,   3,201-221. https://doi.org/10.3390/reprodmed3030016.

[25]   Zinab M.M., Asmaa, H.H., Abdul-Razak, S.H., Hasan. (2022). Seroprevalence of Parvovirus B19 Infection and its Attributable factors among Blood Donors in Diyala Governorate, Iraq. HIV Nursing Journal 22(2):2880-2884.

[26]   Dean, A.G., Garner, T.G., Sangama S., Sunki, G.G. Friedman, R., Lantinga, M. (2013) EPI info 2013, A database and statistics program for public Health professional. For use on window 7, 8, NT and 2013 computers, 2013 Division of Public Health Surveillance and Informatics. Epidermiology Program Office, MSK 74. Centers for Diseases Control and Prevention (CDC) Atlanta Georgia 30341-3717.

[27]   Anon, http:/primers. Ut.ee. Accessed January 18, 2017).