World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-15, October 2022
ISSN: 2749-3644

MOLECULAR DETECTION OF PARVOVIRUS FROM SERA OF CHILDREN WITH RASH RED ILLNESS

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Article history:

Received: August 20th 2022
Accepted: September20th 2022
Published: October 26th 2022

Abstract:

Background:
A parvovirus infection is distinguished by a bright red rash on the cheeks. Human parvovirus B19 infection is a typical and contagious illness in children.

Methods:
From January 2022 to October 2022, serum levels will be tested in the hospitals of the Al-Muthanna province in Iraq. Patients with a suspected viral red rash illness will provide these samples. Serum samples will be used in the investigation for both IgM immune assays and parvovirus B19 molecular detection.

Results:
Out of 126 children who had parvovirus B19 rash fever illness. There was a significant difference according to the study's age, gender, and symptom parameters between the molecular detection of 20 positive cases and the immunological detection of 27 positive cases.

Conclusions:
The use of PCR techniques is essential to detect human parvovirus in the blood of infected children. The study show that parvovirus B19 infections are significantly more common in 4-5 year old children and that females are more likely than males.

Keywords: Rash, parvovirus, B19, rash fever illness, gender.

INTRODUCTION

As with all common viral infections, there are a variety of diseases that are linked to B19 infection. Children experience completely subclinical infections more frequently than adults do. A B19 infection may result in a completely non-specific illness with mild, transient fever and respiratory symptoms. The most prevalent clinical sign of infection is an erythematous rash, which can be diagnosed under a number of different headings depending on the type of rash and the circumstances surrounding its occurrence[19]. Viral infections are illnesses caused by a virus rather than bacteria or fungi. Skin rashes can be a symptom of many viral infections, particularly those that frequently affect toddlers and young children[20]. The Erythroparvovirus genus is related to the smallest human DNA virus, known as human B19V, which is a member of the Parvoviridae family[21].

The majority of maternity hospitals lack the B19V screening test, and common inactivation strategies eliminate enveloped viruses but have no effect on B19V due to its characteristics of greater resistance to external chemical and physical agents in addition to having such a small nonenveloped capsid. B19V can be transmitted in a variety of ways, including from mother to fetus and through blood or blood products [22].

Viral rashes can exhibit a wide range of characteristics. On lighter or darker skin, the majority, however, resemble blotchy red or purplish spots. These spots could appear overnight or over the course of several days. Additionally, they might be limited to one area or spread across several. Itchy or painful sensations can also be felt when touching viral rashes. The best way to determine whether a rash is viral is to look for any viral infection symptoms, such as fever, chills, and body

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aches[23]. According to the most recent research, children who experienced rash fever illnesses had a disease incidence that was significantly higher than average [22]. It is advised to screen patients using polymerase chain reaction (PCR) rather than immunoglobulin M (IgM) antibody-based serology when B19V infection is suspected[24].

This is the first report of B19 in the province of Muthanna because there haven't been any studies on human parvovirus infections. This study was carried out in order to ascertain whether B19V viral DNA was present in this high-risk population in Al-Muthanna Province and to look into any possible relationships between it and specific disease-related variables.

METHOD

Subjects:

A total of 126 samples will be tested during the period from Jan. 2022 to Oct. 2022. These samples will be obtained from suspected viral red rash illness patients. The study will be conducted on serum specimens for molecular detection of parvovirus B19 and for IgM immune assays of the virus infection. The study topic will be children under 5 years old visiting maternity and pediatric hospitals, consulting and special clinics in general hospitals in Al-Muthanna province, Iraq.

Real-Time PCR

Quantitative Real-Time PCR technique was conducted for detection of Parvovirus B19 based non-structure gene1 (NS1 gene). The DNA from serum samples were extracted by using gSYAN DNA extraction kit (Blood Protocol Procedure) Geneaid Biotech Ltd. Taiwan and The procedure was conducted as the manufacturer recommended. The purity and quality of extracted DNA were tested by using a Nano-drop one spectrophotometer. DNA amplification was monitored by using a lightcycler instrument and performed as company instructions. The designated primer in this study is shown in table (1).

Table (1): sequences of oligonucleotide primer of parvo virus B19 in this study.

Primers	Sequence (5'-3')	location
Parvovirus B19	F/AAACTATGGTAAACTGGTT	Non- structure gene1
	R/TGCTACATCATTAAATGGA	

Immune assay

The serum specimens in this study were analyzed for IgM assay (Sandwich Human ELISA Kit, US) of parvovirus B19 against viral proteins (VP) screening for the presence of IgM antibodies as described by Anderson J. *et al*. 1986[14].

Statistical analysis

All data in this study were analyzed using Microsoft Office Excel 2010 and SPSS version 23. Any difference in mean was checked by an independent t-test. The significance difference was considered at a P-value of <0.05 using the Chi-square test. (Daniel 2009).

The results

The current study included 126 children with suspected rash fever illness of parvovirus B19. The molecular detection of virus by using SYBR Green qPCR method was 20 positive cases; figure (1) explained the positive samples were showed threshold cycle (t_c) numbers ranged (17-25).

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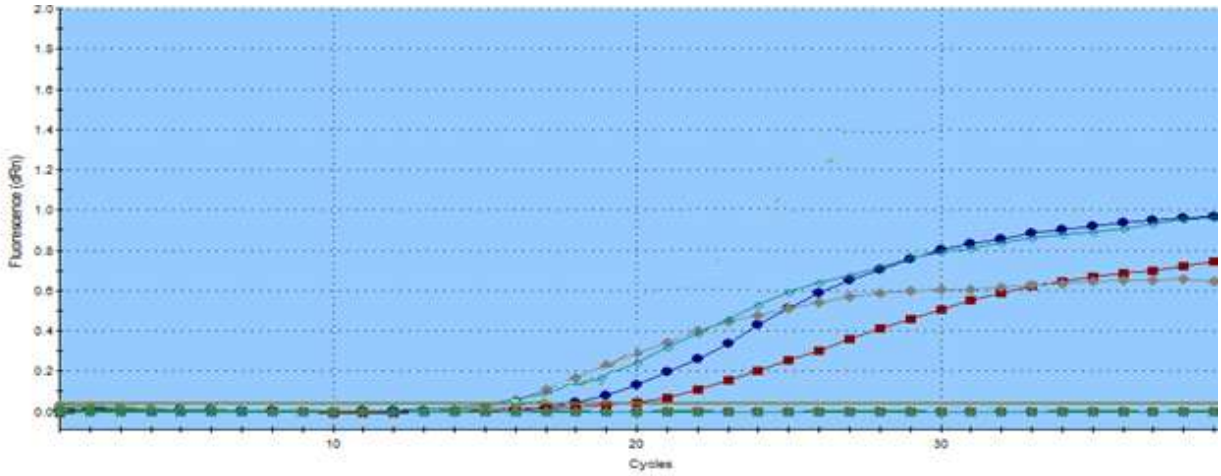

Figure (1): Amplification curves of SYBR Green PCR of positive and negative cases of present study.

Also immunological assay investigation for same subjects was 27 positive cases using IgM antibodies detection. Table (2) shown positive cases investigation either molecular or immunological detection according to different area of Al-Muthanna province or the most positive cases of virus isolation were in Maternity and pediatric hospitals.

Table (2): Molecular detection of B19V and immune detection of IgM antibodies in children associated with rash red illnesses in Al-Muthanna province/Iraq

area	Suspect patients investigated	Molecular positive case detection (%)	Immunological positive case detection (%)
Maternity and pediatric hospitals	61	11(18)	14(22.9)
Specific clinics	15	3(20)	5(33.3)
Private clinics	18	1(5.5)	2(11.1)
General hospitals	32	5(15.6)	6(18.7)
total	126	20(15.9)	27(21.4)

The frequency age distribution of children less than 5 years was shown in table (3). The children with 37-60 months were the most detect for B19V, 11 positive cases by qPCR methods and 13 positive cases by IgM ELISA technique while the results were lowest in children aged 6-12 months.

Table (3): Positive cases detection of B19V Al-Muthanna province according to age parameter.

Age groups	Molecular detection of B19V		Immunological detection of B19V	
	Positive (%)	Negative	Positive (%)	Negative
6-12 month	1(5)	38	3(11.1)	30
13-24 month	3(15)	22	5(18.5)	24
25-36 month	5(25)	15	6(22.2)	14
37-60 month	11(55)	31	13(48.1)	31
total	20	106	27	99

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Volume-15, October 2022
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Table (4): Positive cases detection of B19V in Al-Muthanna province according to gender parameter.

Positive cases Gender	Molecular detection of B19V			Immunological detection of B19V		
	positive	Percent %	*P value*	positive	Percent%	*P value*
females	12	60	>.05	14	51.9	>.05
males	8	40		13	48.1	
total	20	15.5		27	20.9	

The comparison between males and females according to frequency distribution of viral infections is shown in table (4). The results of table 4 pointed there was no statically significant difference (>.05) in both gender with molecular and immunological detection of study.

Table (5): Positive cases detection of B19V in Al-Muthanna province according to location parameter.

Positive cases residence	Molecular detection of B19V			Immunological detection of B19V		
	positive	percent	*P value*	positive	percent	*P value*
center	4	20	<.05	8	29.6	<.05
periphery	16	80		19	70.4	
total	20	15.5		27	20.9	

The parvovirus B19 in present study appears to be more prevalence in periphery areas (80%) of Al-Muthanna province than central areas (20%) with significant different (<.05) as shown in table (5).

Table (6): The clinical sign and symptoms distribution according to the results of positive molecular detection of B19V.

Characteristic	Total *n* (%)	Molecular detection of B19V		*p*
		Positive *n*(20)	Negative *n* (106)	
Sign and symptom				
fever	58(46.4)	20(100)	38(35.8)	<.05
Muscle aches	80(64)	18(90)	62(58.5)	
headache	95(76)	11(55)	84(79.2)	
Rash	60(48)	8(40)	52(49.1)	

The overall findings indicate that the most frequent clinical symptoms of B19V infections were fever (100%) and Muscle aches (90%) than other symptoms frequency with headache (55%) or Rash (40%) with statically significant difference (<.05), table (6).

DISCUSSION

Little is known about the epidemiology studies of human parvovirus B19 in Al-Muthanna province, Iraq, and this report appears to be first sight to screen B19V as an etiological cause of rash red illnesses in Al-Muthanna province through our screening, molecular detection and immunological detection with anti-IgM antibodies for B19V from suspected clinical samples. In our study, positive parvovirus B19 infection cases were shown among 20 subjects 0f 129 suspected of children less than 5 years when tested by cyber green qPCR method. There are many reports about detection this virus in the pediatric subjects [1, 2].

The most molecular and immunological detect of the virus were in maternity hospitals of the province or maternity wards of general hospitals, Dowell, *et al.*, (1995) explained the most B19V infections in pediatric wars due to nosocomial outbreaks a source for transmission of pediatric parvovirus [3] or by B19V infected maternity wards staff [4, 5] . Sero-prevalence of the virus were 27 positive subjects of 129 suspect patients defined by sera anti IgM, Pillay D. *et al.,* (1992) reported the immunological detection of pediatric B19V due to immunocompromised status in the patients[6].

In terms of age, the most infections of human B19V in this study were 55% and 48.1% for up 4 years children,

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Volume-15, October 2022
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respectively, Barash J. *et al.*, (2002), reported the mean age of the children with B19V was 5.21 years, and 22 of total infected children were under four year[7]. Infection with parvovirus B19 has been reported from every continent and has spread worldwide. Numerous studies have highlighted the higher prevalence of this condition than is typically believed, and their seroprevalence varies with age and location[8]. There is also the fact that most studies were likely conducted during a time when there was no B19V epidemic[9, 10]. The present report recorded 60% of positive B19V cases were females while the other findings were males (40 %) and The result of present study revealed the prevalence of B19v was estimated as 54% And this outcome is better than that of earlier research done by Vyse*. et al.,* (2007) [12] .
In the Southern region of Brazil, blood donors' parvo virus B19 DNA positivity rates were 1.9% and 53.9%, respectively, compared to the findings of studies conducted in other nations [13]. Therefore, it is increasingly advised for B19V diagnosis to combine quantitative molecular PCR and serology to distinguish between recent and earlier infections[15, 16].

The current study suggests that the Al-Muthanna province's outlying areas have a high prevalence of B19V in children. Salimi *et al.,* (2008) disagreed with this outcome[17]. According to the current findings, there was a significant ($p<0.05$) difference between the frequency distribution of Muscle aches and fever in infected children with positive cyber green qPCR results and other B19V symptoms. Overall, the results of this study indicate that fever and Muscle aches were the most frequent clinical signs of human parvovirus B19 infection, this supports Smith-Whitley *et al.*, (2004) explanation, which held that most B19V-infected children complained of pain or fever while only a small number sought symptoms of anemia, such as pallor or headache [18, 11].

CONCLUSION

According to the results of this study, Parvovirus B19 infections are significantly more common in 4-5 year old children, and females are more likely than males to contract the virus when they have a rash or fever. The use of PCR techniques is essential to detect human parvovirus in the blood of infected children. To accurately diagnose parvovirus B19 in plasma, sensitive nucleic acid testing techniques like cyber green qPCR are advised in conjunction with IgM ELISA.

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