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Research Article

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Molecular detection of Parvovirus B19 in a group of children in Damascus, Syria

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ABSTRACT

Parvovirus B19 infects children and adults, causing erythema infectiosum, polyarthritis, aplastic crisis and chronic anemia in patients with hematological or immunological disorders. Also it may lead to fetal hydrops or fetal death when infecting pregnant women. This study aims to detect the DNA of B19 virus ina group of children in Damascus, Syria. Specimens were collected randomly from 50 children (26 males and 24 females) aged one to 17 years. Specimens were obtained from Damascus Children Hospital between January 2014 and March 2014. B19V DNA were detected by polymerase chain reaction (PCR) in sera. Of the 50 children 7 (14%) were seropositive for B19 DNA. The difference in the presence of B19 DNA between genders was not statistically significant (p=0.605). This study showed an influence of geographic differences on transmission of parvovirus B19, and revealed that was no relationship between positivity and gender.

Keywords: Damascus, Syria, Parvovirus B19, DNA, Children, Nested PCR.

INTRODUCTION

Human parvovirus B19 was discovered by Cossart et al, while they were evaluating assays for hepatitis B virus in serum[1]. Parvoviruses are small, round viruses with a single-stranded DNA genome that lack a lipid envelope. Among the parvoviruses, B19 is the first known pathogenic human parvovirus [1]. The virus is mainly transmitted by personal contact via aerosol or respiratory secretions. Contaminated blood products, such as clotting factor concentrates, are also source of iatrogenic transmission [2]. B19 can be transmitted transplacentally from an infected mother to the fetus, leading to non-immune fetal hydrops (NIHF), spontaneous abortion, or intrauterine fetal death [3].

In children the most common clinical presentation of Parvovirus B19 infection is “fifth disease” or “erythema infectiosum”, an illness characterized by a non-specific prodromal phase, followed by the typical “slapped cheek” rash. Although joint symptoms are rare in children, they are more common in adults and generally in women. Joints become painful and swollen, and often symmetrically affect the wrists, knees and small joints of the hands [2,3].Infection in individuals with hemolytic disorders causes transient aplastic crisis (TAC). Persistent B19 infection in the immunocompromised host is manifested as pure red cell aplasia (PRCA) and chronic anemia. Although the infection is endemic, regional epidemics are reported preferentially during late winter and spring [2].

Parvoviruses B19 infection is common in childhood and adolescence, continues at a low rate throughout adult life, and by the time they are elderly, most people are seropositive [3]. After respiratory acquisition of B19V a massive viremia occurs with a viral load that can exceed 10^{13} copies of genome/mL [4]. IgM antibodies present10 to 12 days postinfection, coinciding with a peak in virus level [2].

IgG antibodies can be detected in serum 2-3 weeks after acquisition of infection and last for life, providing immunity against re-infection [5].B19 DNA-based assays are crucial for the diagnosis of B19 infection presenting as

transient aplastic crisis (before the antibody response) and in chronic infections in immunosuppressed individuals who fail to make an immune response [2].Viral DNA is usually detected by direct hybridization or PCR. A nested PCR assay is capable of accurately detecting all temporally and geographically diverse B19 isolates including V9 variant. It comprises two rounds of amplification [2,6].

The detection of parvovirus B19 infection in children, in Syria, has not been previously described. The aim, therefore, of this study was to determine the presence of parvovirus B19 DNA in a group of children in Damascus, Syria, to relate them to gender and to compare the results to those of other countries.

EXPERIMENTAL SECTION

Samples:

After an informed consent was obtained from the parents of each child, a total of 50 randomly selected paediatric patients' samples (male/female, 26/24) were collected from Damascus Children Hospital between January 2014andMarch 2014. Children with hematological or immunological disorders were excluded. A sample of 5 ml of blood was collected from each child. Sera were obtained, aliquoted into 3 Eppendorf tubes and stored at -80° C until testing.

Methods:

B19 DNA Isolation:

The serum samples were tested for the presence of B19 virus DNA, using a commercial GF-1 Viral Nucleic Acid Extraction Kit (Vivantis, Malaysia) to isolate viral genome from sera according to the manufacturer's instructions. The study was performed in Faculty of Pharmacy, Damascus University. Briefly, samples were lysed by adding Proteinase K and lysis buffer containing carrier RNA into each one. After homogeneously mixing by pulsed-vortexing, samples were incubated at 65° C for 10min, then absolute ethanol was added and mixed immediately and thoroughly.

Each sample was transferred into a column assembled in the collection tube, then centrifuged at5,000xg for 1 min. After the flow was discarded, the column was washed with 500µl of Wash Buffer 1 and centrifuged, then washed with 500µl of Wash Buffer 2 twice. The flow was discarded in each wash. DNA was eluted by adding Elution Buffer directly onto column membrane and Centrifuging after 2 minutes at 5,000 x g for 1 min. Viral DNA was stored at -20° C.

Nested PCR:

The primers (VBC Biotech, Vienna, Austria) P1 (5′-AATACACTGTGGTTTTATGGGCCG-3′) and P6 (5′-CCATTGCTGGTTATAACCACAGGT-3′) as external primers, and the primers P2(5′-AATGAAAACTTTCCATTTAATGATGTAG-3′) and P5 (5′-CTAAAATGGCTTTTGCAGCTTCTAC-3′) as internal primers were used in nested PCR to amplify a 103 bp fragment of the DNA of the NS1 region [7].

The reaction achieved using PCR Taq 2X Master Mix M0271S (Bio Labs, New England). All reaction components were assembled in PCR tubes on ice as shown in Table 1,and mixed gently. Primers for the first and second round were P1, P6 and P2, P5 respectively.

Table 1. Reaction components according to the manufacturer's instructions

Component	50 µl Reaction	Final Concentration
20 µM Forward Primer	1 µl	0.4 µM
20 µM Reverse Primer	1 µl	0.4 µM
Quick-Load Taq 2X Master Mix	25 µl	1 X
Template DNA	5 µl	
Nuclease-Free Water	to 50 µl	

PCR tubes were transferred from ice to a PCR machine (BOECO, Germany)with the block preheated to 94°C and thermocycling was started with conditions shown in Table 2.

Table 2. Thermocycling conditions for both PCR rounds

STEP		TEMPERATURE	TIME
	Initial Denaturation	94° C	7 min
40 Cycles	Denaturation	94° C	30 sec
	Annealing	55° C	30 sec
	Extension	72° C	30 sec
	Final Extension	72° C	2 min

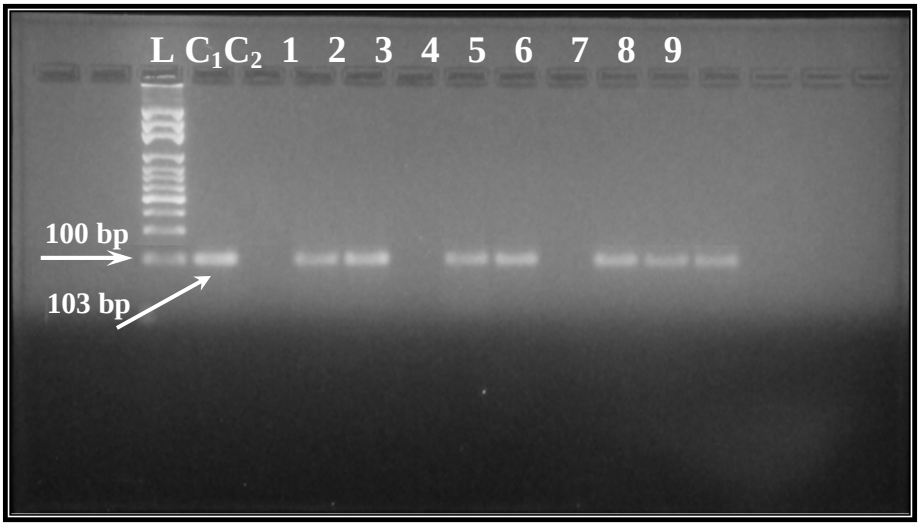
The first round product was the template in the second round. Final products were represented using gel electrophoresis method. DNA molecules were moved through Agarose 2% gel. Bands were viewed under UV light.

Statistical analyses

To analyze the data, the statistical package for social sciences (SPSS) was used. A p-value of <0.05 was used as the cut-off level for significance.

RESULTS AND DISCUSSION

Among the 50 children tested, 7 (14%) were positive to B19 DNA in serum (bands are shown in Figure 1), which mean they had acute infection. The remaining 33 (66%) were negative and therefore susceptible to parvovirusB19 infection.



L well contains DNA ladder start from 100bp, C1 contains positive control, C2 contains negative control, Other wells contain samples, and the arrow indicates to a result of amplification reaction band (103 bp)

Figure 1. Nested PCR products of amplified gene NS1 fragment in B19 genome on Agarose 2% gel staind with Ethidium Promide

The overall positive rate of B19 DNA for males and females was 11.54% (3/26) and16.67% (4/24) respectively. There was no difference in positivity rates between males and females (p=0.605).

The investigation of parvovirus B19 infection in children in Syria has not been described previously. In our study parvovirus B19 DNA was found in 14%.Our findings were higher than the 2.7% reported in Kingdom of Saudi Arabia [8], and the2.5% reported in Hong Kong[9]. This difference in rates could be explained by method sensitivity, because those studies use specific B19 IgM antibodies assay to detect acute infection. However, It was lower than the 39.5% among children in Lagos, Nigeria [10], which could be a result of more exposure to the B19V in that country due to the difference in geographical environment from ours.

Our study demonstrated that the difference in the positivity rate of parvovirus B19 DNA between males and females was not statistically significant. This goes in line with the data from the children population in Kingdom of Saudi Arabia, Nigeria, Australia, England and Wales indicating no difference in DNA positive rates between genders[8,10,11,12].

CONCLUSION

This study has identified for the first time the presence of parvovirus B19 DNA in the children population in Damascus, and has indicated an influence of geographic differences on transmission of parvovirus B19.Also has found that no significant effect of gender.

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