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Research Article

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Investigation of Parvovirus B19 infection in haemoglobinopathy patients in Damascus

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ABSTRACT

Parvovirus B19 infects especially thalassemia and sickle cell anemia patients .In these Patients, the production of precursors of red blood cells increases to compensate the dissolution of red blood cells. B19 infections lead to suppression of the erythrocytes formation and acute erythroblastopenia often called transient aplasia crisis which may be life-threatening. These patients usually become susceptible to dramatically viral infection and increased risk of transmission viral infection. This study included 200 patients with Haemoglobinopathy (101 male, 99 female). Specimens were collected randomly (100 Sickle cell anemia and 100 Thalassemia) from thalassemia center in Damascus-Syria between November 2014 and January 2015.B19V DNA were detected by NESTED PCR in sera.Of the 200 patients,18 (9%) were seropositive for B19DNA.This study showed that there was relationship between seropositive and age, but no relationship between B19 infection and gender .

Key words: Parvovirus B19, Sickle cell anemia, Thalassemia, Haemoglobinopathy Patients, DNA, Nested PCR

INTRODUCTION

Human parvovirus B19 (Parvovirus B19, B19V) is the smallest known viruses with virions in the range 18–26 nm in diameter ,nonenveloped, Single-stranded DNA (5.6 kb) virus with and icosahedral capsid .The Capsid of the virus consists of 60 protein molecules and is composed of major (VP2; 58 kD) By 95% and minor (VP1; 83 kD) by 5% as structural proteins .Parvovirus B19 belongs to the Erythrovirusgenus - Parvoviridae family. The virus is characterized by resistant to organic solvents, cold , stable in a wide range of pH changes and constant at 56 °C temperature for 60 minutes [1,2,3,4**]**.

The virus is common and widespread. The peak incidence rates happen in the late winter or early spring and Up to the epidemiological level every 3 to 4 years . The clinical manifestations of infection vary with the immunological and hematological status of the host .The Most commonly disease caused by a virus in children is fifth disease (erythema infectiosum) which generally occurs at the age of 4-7 years and even 10 years .Erythema infectiosum consists of two phases: a non-specific prodromal phase (Flu-like symptoms) Followed by exemplary Slapped Cheek Syndrome . In Haemoglobinopathy Patients (sickle cell anemia and thalassemia Patients), B19V infections cause transient aplasia crisis which can suppress erythropoiesis and induce acute erythroblastopenia. In addition to the lack of reticulocytes and deterioration of anemia Leading to life-threatening anemia . B19 infection may cause chronic anemia in the Immunodeficient Host [2,5,6].

The infection is mainly transmitted via aerosol or respiratory secretions where revealed the B19DNA in respiratory secretions in conjunction with blood Viremia , and through the derived blood products, which are often given by injection in asymptomatic stage. It can also be vertically transmitted from the pregnant to the fetus causing non-immune fetal hydrops (NIHF), spontaneous abortion, or intrauterine fetal death [5,7].

IgM antibodies reside 10 to 12 days postinfection, stay for two to three months from the initial viral infection and are directed against the capsid protein VP1 and VP2. In contrast, IgG antibodies directed against VP1 and VP2 appear after 2-3 weeks of infection and continue lifelong to give Permanent immunity [7,8].

The importance of DNA B19V detection is in diagnosis of cases which can not be revealed antibodies as aplastic anemia TAC (before the appearance of antibodies) and in Immunodeficient patients so the immune system fails to generate antibodies .Viral DNA can be detected by direct hybridization or PCR [2,7,11].

This study aimed to Investigate Parvovirus B19 Infection in Haemoglobinopathy Patients in Damascus and determine the relationship between B19 infection, age and gender .

EXPERIMENTAL SECTION

Study Subjects: 200 patients with Haemoglobinopathy (101 male ,99 female) . Specimens were collected randomly (100 Sickle cell anemia and 100 Thalassemia) diagnosed at thalassemia center in Damascus between November 2014 and January 2015 .Patients with immunological disorders were excluded .Samples were divided into three categories according to age.

Table (1) Samples according to age

Group	The number of patients in each category
1- <8 years	63
8- <16 year	76
= > 16 year	61

Sampling: Blood samples (5 ml) were collected and after centrifugation, serum was acquired and aliquots were stored at -80° C until testing.

Assay:

- B19 DNA Isolation:

B19 DNA was extracted, using commercially a commercial GF-1 Viral Nucleic Acid

Extraction Kit (Vivantis, Malaysia) according to the manufacturer's instructions. Viral DNA which is template DNA in the PCR reaction was stored at -20° C.

- Nested PCR:

The reaction achieved using a thermal cycler biocycler TC-S and PCR Taq 2X Master Mix M0271S (Bio Labs, New England). The following primers (VBC Biotech, Vienna, Austria) were used in nested PCR ccording to a Durigon et al study in order to amplify a 103 bp fragment of the DNA of the NS1 region.

external Primers:P1 Forward (5` AATACACTGTGGTTTTATGGGCCG 3`) and P6 Reverse (5`CCATTGCTGGTTATAACCACAGGT 3`) and as internal Primers : P2 Forward (5`GAAAACTTTCCATTTAATGATGTAG 3`) and P5 Reverse (5` CTAAAATGGCTTTTGCAGCTTCTAC 3`) .

All reaction components were assembled in PCR tubes according to the manufacturer's instructions . External Primers (P6, P1) were used in the first round of the reaction .Then in the second round, internal Primers (P2 and P5) were used by using B19 DNA virus preamplifier in the first round (Amplicon) as Template DNA.The final products of PCR interaction were represented by electrophoresis method on agarose 2% gel within the electrophoresis device .

Statistical analysis: data were analyzed using Excel 2010 and SPSS 20 . A P-value of < 0.05 was used as the cut-off level for significance.

RESULTS AND DISCUSSION

Among the 200 patients tested, 18 (9%) were positive to B19 DNA in serum .

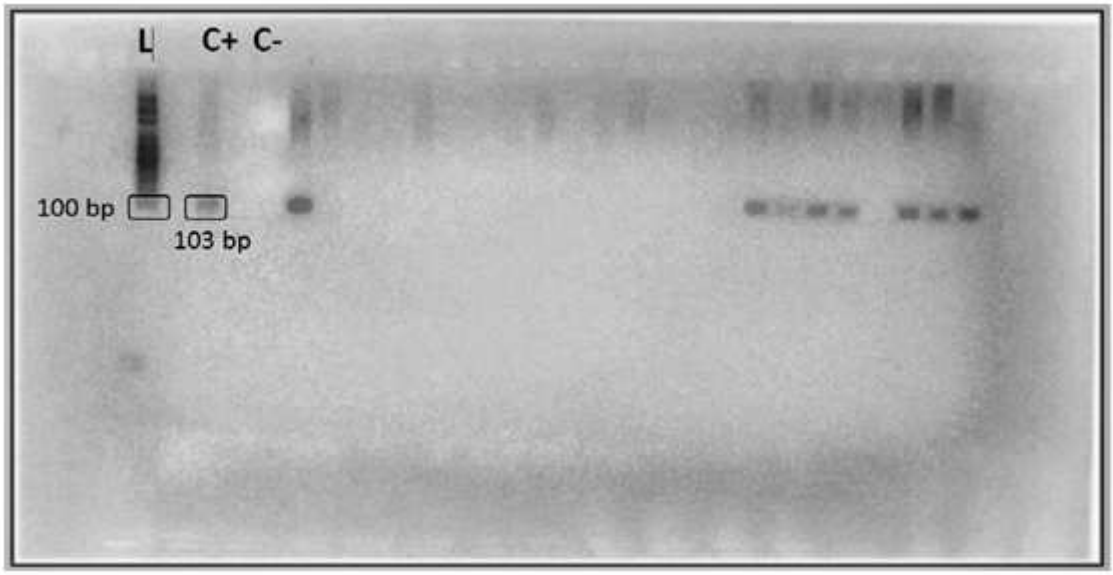

Figure (1): Nested PCR of amplified gene NS1 fragment in B19V genome

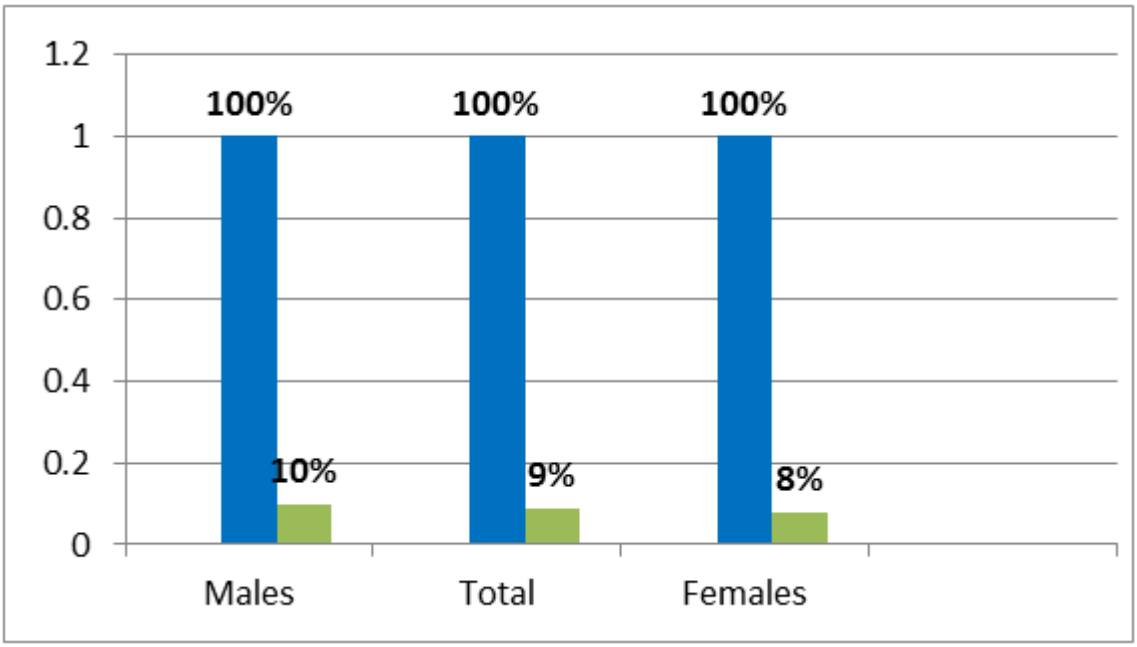

Figure (2): B19 DNA positive rates between genders

The number of positive results in infected males was (8:101) (10%) and (10:99) (8%) in infected females (Figure 2).There was no difference in positivity rates between males and females (p=0.67) .This result is compatible with all previous studies conducted in Saudi Arabia, Tunisia , Brazil , Jamaica and New York which indicating no difference in DNA positive rates between genders [1,4,5,9,10].

The study demonstrated that the difference in the positivity rate of Parvovirus B19 DNA between the first category (1- <8 years) and the second category (8- <16 year) was statistically significant (p= 0.038) (table 2) , which could be explained by the high number of transmitted blood units at these ages(8- <16 year).In addition to the infection is mainly transmitted via aerosol or respiratory secretions therefore the incidence of infection increases between the school students .

Table (2) : the results of the investigation of B19 DNA virus according to age groups:

Group	The number of patients in each category	positive samples	Percentage
1- <8 years	63	3	5%
8- <16 year	76	12	12%
= > 16 year	61	3	4.9%

In this study, the presence of Parvovirus B19 Infection at Haemoglobinopathy Patients in Damascus is up to 9%. Our findings were higher than the 2.89% reported in Saudi Arabia ,the 4%reported in Thailand and the 4.4% reported in Tunisia. However, It was lower than 17.2% reported in Brazil ,37% reported in Jamaica [1,4,5,10]. This difference in rates could be explained by the difference in the geographical area , method sensitivity (because NESTED PCR is more sensitive than other methods) and the difference between number of taked samples in comparison with some other studies . The difference might also belong to the difference in age groups in this study Compared to previous studies.

CONCLUSION

This study has detected for the first time the presence of Parvovirus B19 Infection at Haemoglobinopathy Patients in Damascus - Syria (up to 9%) and proved that no significant effect of gender .However, there is relationship between B19V infection and age as the infection increases at Haemoglobinopathy Patients among 8 - < 16 year (the school students) .

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