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ORIGINAL ARTICLE

The Role of Human Parvovirus B19 in the Pediatric Patients with Pancytopenia?

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SUMMARY

Background: **Parvoviruses are small DNA viruses causing erythema infectiosum, which is known as the fifth disease. The aim of this study was to investigate the presence of Parvovirus B19 DNA by Real-Time-PCR retrospectively in clinical samples of children diagnosed as acute leukemia and aplastic anemia when investigating the cause of pancytopenia and to investigate its relationship with the clinical manifestations.**

Methods: **The study samples were collected between March 2014 and March 2018 in Gazi University, Faculty of Medicine, Department of Pediatric Hematology. Sixty pediatric patients; 37 males and 23 females, were included in the study. Nucleic acid isolation was performed by using MagNA-Pure Compact Nucleic Acid Isolation Kit (Roche, Germany). Extracted DNA was studied with LightCycler® 2.0 using the Real-Time PCR method and LightCycler® Parvovirus B19 Quantification Kit (Roche, Germany), and the results were evaluated quantitatively. Parvovirus B19 DNA detection interval of the kit was 10^1 - 10^6 copies/mL.**

Results: **Sixty serum samples were investigated and 8.3% (5/60) Parvovirus B19 DNA positivity was determined. Of the five patients with Parvovirus B19 DNA positivity, three had acute lymphoblastic leukemia and two were diagnosed as aplastic anemia. Regarding viral load; 2/5, 1/5, 1/5, and 1/5 of the samples had a viral load of 10^2, 10^3, 10^4, and 10^5 copies/mL, respectively. Parvovirus B19 DNA positivity was detected in samples from March (2/5), April (2/5), and August (1/5).**

Conclusions: **Patients with acute leukemia and aplastic anemia in childhood using immunosuppressive drugs, blood, and blood products during chemotherapy, encounter Parvovirus B19 infections in the follow-up period and are diagnosed by serological and molecular methods. As a result of the study, we suggest that the detection of Parvovirus B19 DNA by Real-Time PCR method in children being admitted with pancytopenia and diagnosed as acute leukemia and aplastic anemia is useful in the follow-up and treatment.**

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KEY WORDS

Parvovirus B19, real-time polymerase chain reaction (Real-Time PCR), pediatric patient, acute lymphoblastic leukemia (ALL), aplastic anemia

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INTRODUCTION

Parvoviruses are small, non-enveloped, linear, single-stranded DNA viruses. Human Parvovirus B19 (PVB-19) was first described in 1975 by Cossart et al. [1] and was known as the one and only member of the Parvoviridae family until Human Bocavirus as a respiratory tract pathogen and Human Bufavirus as a gastroenteritis pathogen were described. PVB19 is the causative agent of erythema infectiosum, which is commonly known as the fifth disease and has the characteristic appearance of 'slapped cheek' [2,3].

Parvovirus B19 can also cause severe anemia. PVB19 is characterized by a tropism to rapidly proliferating erythroid progenitor cells. These progenitor lines are found in the bone marrow, umbilical cord blood, fetal liver, peripheral blood, and erythroid leukemic cell lines. PVB19 is thought to cause anemia by infecting erythrocyte precursor cells in the bone marrow and forming lytic reactions in these cells, and also by stimulating the cellular mechanism that induces apoptosis [4,5].

Parvovirus B19 is resistant to inactivation methods such as filtration, treatment with solvent and detergent, since it is a relatively small non-enveloped virus. For this reason, PVB19 can be transmitted by blood and blood products in patients with severe anemia, and the presence of PVB19 has been shown in several studies during the preparation process of blood and blood products [6-9]. PVB19 infection should be considered when anemia develops in leukemic patients who receive immunosuppressive therapy and in patients who have hematological malignancies and need regular transfusion of blood and blood products. Also reactivation of the virus may occur due to immunosuppressive therapy in these patients [10]. However, there are limited number of studies detecting the presence of PVB19 when investigating the cause of pancytopenia in pediatric patients diagnosed with acute leukemia and aplastic anemia in our country [11,12].

Serological and molecular methods are used in the diagnosis of Parvovirus B19 infection. IgM antibodies can be detected in the serum approximately 10 days after the transmission of parvovirus B19 before the onset of symptoms and IgG antibodies are detected approximately one week after IgM and indicate previous infection. However, inadequate or delayed immune response of immunosuppressive patients makes the serologic diagnosis of Parvovirus B19 infection difficult, and the diagnosis is made by detecting the changes in the patient's clinical chart and typical changes of bone marrow. In real-time polymerase chain reaction (Real-Time PCR), a very small amount (10 copies/mL) of PVB19 DNA can be detected. PVB19 DNA positivity were found in the samples with negative IgM and IgG by Real-Time PCR method and comparing with serological analysis, Real-Time PCR method was found to be faster concluding that it is the most sensitive and specific method to be used in early diagnosis of PVB19 infection [13-14].

Considering blood transfusion and drug therapy in patients with immunosuppression and hematological malignancies, PVB19 infection poses a high risk. Due to the fact that even small quantities of PVB19 DNA can be detected by Real-Time PCR method, the use of Real-Time PCR method in early diagnosis and treatment of these patients is gaining importance.

The aim of this study was to investigate the presence of Parvovirus B19 DNA by Real Time-PCR retrospectively in clinical samples of children diagnosed as acute leukemia and aplastic anemia when investigating the cause of pancytopenia and to investigate its relationship with the clinical manifestations.

MATERIALS AND METHODS

Sample collection

Sixty serum samples obtained from Gazi University, Faculty of Medicine, Pediatric Hematology Clinic between March 2014 and March 2018 were investigated for Parvovirus B19 DNA with Real Time-PCR. Serum samples were separated after centrifugation of blood samples at 3,000 rpm for 5 minutes and stored at -80°C until study.

Nucleic acid isolation

Nucleic acid isolation from clinical samples was performed by using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche, Germany) in MagNA Pure Compact Instrument (Roche, Germany). Viral DNA was obtained according to the manufacturer's protocol. The Extracted DNAs were stored at -80°C until amplification.

Viral DNA amplification

Amplification was performed by Real-time polymerase chain reaction method in LightCycler® 2.0 (Roche Applied Science, Germany) using Light Mix® Kit Parvovirus B19 (TIB Molbiol GmbH, Germany), which contains primers amplifying 184 bp of the ORF2 region of the PVB19 genome. The limit of detection of the kit was 10 copies/mL. Six standards, each with a different quantitation (10^1 - 10^6 copies/mL) of PVB19 DNA and one negative control were used for PVB19 analysis. The amplification curves of the standards were evaluated with 'absolute quantification' and 'melting curve' analysis in 640 channels of LightCycler® 2.0 (Roche Applied Science, Germany) device and the results were quantified by PVB19 positivity.

No peak was observed in the curves of the negative controls used in the analysis. The evaluation of the negative results and the accuracy of the analysis were provided by internal control in 705 channels of LightCycler® 2.0 (Roche Applied Science, Germany) device by evaluating 'absolute quantification' and 'melting curve' analysis.

Statistics

Statistical analysis was performed using SPSS 20.0 (SPSS Inc. USA) programme. Data were evaluated by using Mann-Whitney *U* and Chi-Square (X^2) tests and $p < 0.05$ was considered statistically significant.

RESULTS

Thirty seven male (61.6%) and 23 female (38.4%) patients were included in the study. The numbers and age ranges of pediatric patients included in the study are shown in Table 1.

Serum samples were analyzed by Real-Time PCR method. Of the clinical samples of patients included in the study, 63% (38/60) had acute leukemia (acute lymphoblastic leukemia (n: 33), acute myeloid leukemia (n: 5) and 37% (22/60) had anemia (aplastic anemia (n: 10), infectious anemia (n: 12).

PVB19 DNA positivity was detected in 8.3% (5/60) of the clinical samples studied. Of the five patients with Parvovirus B19 DNA positivity, three were acute lymphoblastic leukemia and two were diagnosed as aplastic anemia.

Regarding the age and gender of the patients with parvovirus B19 DNA positivity; two and one of the patients diagnosed with ALL were male and female respectively, and they were 15, 7 and 5 years old; two patients diagnosed with aplastic anemia were male and they were 5 and 8 years old.

Of the patients included in the study, 9% (3/33) of pediatric patients diagnosed with ALL and 20% (2/10) of pediatric patients diagnosed with aplastic anemia were positive for PVB19 DNA (Table 2).

PVB19 DNA positivity was higher in patients diagnosed with aplastic anemia than in ALL patients, but no statistically significant difference was found ($p > 0.05$). The quantitation of DNA detected in the parvovirus B19 DNA positive samples and the distribution according to the underlying diseases are shown in Table 2. Regarding the relationship between the quantitation of PVB19 DNA and underlying diseases; PVB19 DNA quantitation was higher in patients diagnosed with ALL than the patients diagnosed with aplastic anemia, but there was no statistically significant difference ($p > 0.05$).

Regarding seasonal distribution; two of the eight samples in March, two of the seven samples in April and one of the eleven samples in August were positive for PVB19 DNA. PVB19 DNA positivity was not detected in the samples from other months.

DISCUSSION

The presence of Parvovirus B19 DNA was investigated retrospectively in sixty children who were diagnosed with acute leukemia and aplastic anemia in a pediatric hematology clinic in this study. In our study, due to the low number of patients, the percentage values may not reflect the truth but PVB19 infection is also related to pancytopenia it is thus conceivable. PVB19 DNA positivity was found to be 8.3% in these patients and three and two of the five patients were diagnosed as acute lymphoblastic leukemia and aplastic anemia respectively.

Parvovirus B19 infection is most prevalent among school-age children between 5 - 15 years. PVB19 seroprevalence is 10% of cases occuring among children < 5 years old, 70% of cases occur in children aged 5 to 15 years [11,12,15]. In our study, four of the five patients with PVB19 DNA positivity were between the ages of 5 - 15 years as the cases reported in the literature.

In our study, 9% PVB19 DNA positivity was detected in all children diagnosed with ALL. In a study among acute and chronic leukemia patients in our country, PVB19 DNA positivity was reported to be 10% similar to our study [16].

Jitschin et al. [17] studied PVB19 infection in children with hematologic or oncological diseases and found that PVB19 DNA positivity was 16% in children diagnosed with ALL. Lindblom et al. [18] found PVB19 DNA positivity as 15% (18/117) in 117 children with acute lymphoblastic leukemia. Heegard et al. [19] reported a case of newly diagnosed ALL in a pediatric patient during the follow up period of PVB19 infection and reported that PVB19 could be one of the factors that triggered ALL onset. Although the relationship between ALL and PVB19 has not been fully elucidated, it is stated that PVB19 has been reported to induce leukemia by suppressing erythroid series by the help of inducing tropism to erythroid progenitor cells and leading to abnormal proliferation in immune cells by the help of increasing GM-CSF in patients diagnosed with ALL [5]. These studies and our study point out that PVB19 infection should be kept in mind in the presence of cytopenia during first diagnosis of ALL in pediatric patients and PVB19 DNA should be investigated with Real-Time PCR method [16-19].

In our study, 20% of PVB19 DNA positivity was detected in the pediatric patient group diagnosed as aplastic anemia. Similarly, Gupta et al. [20] reported PVB19 DNA positivity as 27% among 66 pediatric acquired aplastic anemia patients. El-Mahallawy et al. [21], investigated the relationship between PVB19 positivity in leukemic patients with anemia and non-anemia, and found that PVB19 DNA positivity was 22% and 5.9% in leukemic patients with anemia and non-anemia respectively and they concluded that PVB19 DNA infection causes anemia during acute infection.

As PVB19 specifically affects immature erythrocyte cells, the production of mature erythrocytes stops and finally can lead to aplastic anemia [4,5,15]. Although it is known that PVB19 infections cause aplastic anemia, the number of studies related to aplastic crisis and aplastic anemia due to PVB19 infection is limited. Kurtoglu et al. [22] reported a case of PVB19 infection manifested by aplastic crisis and emphasized that

Table 1. Evaluation of the numbers and age ranges of pediatric patients included in the study.

	Number of patients	Median (min - max)	Mean ± SD
Female	**23**	**8.0 (1 - 18)**	**8.4 ± 5.2**
Male	**37**	**6.0 (4 months - 19)**	**7.4 ± 5.2**
Total	**60**	**6.0 (4 months - 19)**	**8.0 ± 5.2**

SD - standard deviation.

Table 2. The quantitation of DNA detected in PVB19 DNA positive samples and their clinical relationship.

Department	Diagnosis		Serum (copy/mL)	p-value	PVB19 DNA positivity	p-value
Pediatric hematology	**Acute lymphoblastic leukemia**	**Patient 1 ***	**10^2**	**0.30**	**9% (3/33)**	**0.34**
		Patient 2	**10^5**			
		Patient 3	**10^6**			
	Aplastic anemia	**Patient 4**	**10^2**		**20% (2/10)**	
		Patient 5	**10^3**			

*** - During the follow-up period of ALL, this patient was treated with bone marrow transplantation and transfusion of blood products.**

PVB19 infection should be investigated in anemic or immunosuppressive patients presenting with cytopenia. In another study, a child who presented with fever and pancytopenia in our country has been reported to develop hemophagocytic syndrome due to PVB19 [23].

Parvovirus B19 infection might also be seen with persistence of the virus and the transmission of the virus by transfusion of blood from blood donors [4,6]. Gumustekin et al. [16] reported that 7.7% of healthy blood donors were positive for PVB19 DNA. Plantz et al. [24] investigated the presence of PVB19 DNA in patients with hematological malignancy using blood products and positivity of PVB19 DNA was 1% (21/2,123) among blood products, whereas in patients treated with these blood products, PVB19 DNA positivity was 12%, but no symptoms were observed in patients. As a result of this study, Planz et al. [24] reported that, because of having PVB19 transmission risk, patients receiving immunosuppressive therapy, bone marrow transplantation or blood and blood products donation for the treatment of leukemia, aplastic anemia should be investigated for PVB19 transmission regularly.

In our study, finding a patient PVB19 DNA positive (low titer, 10^2 copy/mL) who was treated with bone marrow transplantation and blood products donation during acute lymphoblastic leukemia, made us think donation originated PVB19 transmission is a risk in this group of patients. We suggest that transmission of PVB-19 infection with blood and blood products in these patients must be kept in mind.

In our study, PVB19 DNA positive samples were found in March, April and August. Parvovirus B19 infections are seen sporadically throughout the year, but it is known that these infections are more prevalent in winter and spring [15].

As a result; PVB19 infection is frequently seen among children having transfusion therapy for hematological malignancy and anemia which may be due to many factors such as immunosuppressive therapy and seasonal appearance of the virus. For this reason, the use of Real-Time PCR method for PVB19 DNA presence is essential for early diagnosis, treatment and follow-up of patients in this group. In addition, not only the presence of PVB19 DNA, but also the amount of viral load can be detected by Real-Time PCR method providing the clinician great convenience in following up the patient.

This study suggests that PVB19 DNA should be investigated by Real-Time PCR in patients with acute lymphoblastic leukemia and aplastic anemia. Investigation of PVB19 infection, as one of the causes of unexpected cytopenia, would be useful in the following up the patients and the management of these diseases.

Declaration of Interest:

There are no conflicts of interest in connection with this paper, and it is not under consideration for publication elsewhere. The final form of the manuscript has been seen and approved by all authors**.**

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