

Parvovirus B19 seroprevalence in Turkish blood donors

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Background/aim: Parvovirus risk in blood transfusion has become a popular research topic since there are limited data on parvovirus seroprevalence in blood donors in Turkey. The aim of this study was to investigate parvovirus seroprevalence in blood donors in Turkey.

Materials and methods: Blood samples of 988 blood donors admitted to a university blood bank were obtained for parvovirus B19 IgM and IgG detection. The samples were analyzed using the ELISA method.

Results: IgM positivity of 3.92% and IgG positivity of 58.9% was detected in the blood samples. Parvovirus IgM positivity was found to be the highest in the age group of 41–50 years ($P = 0.045$) and IgG positivity was detected to be the highest in the age group of 31–40 years ($P < 0.001$). Parvovirus IgG positivity was significantly higher in women ($P = 0.041$). However, there was no difference regarding parvovirus IgM positivity in terms of sex ($P = 0.245$).

Conclusion: Although this study does not represent the whole country, it is still the largest investigation carried out on the topic in Turkey and the obtained results are generally similar to those of European countries. Therefore, it is thought that the results obtained from this study may be supportive for the first steps regarding plasma fractionation, which will soon begin in Turkey.

Key words: Transfusion medicine, ELISA, blood banking, parvovirus

1. Introduction

Human parvovirus B19 (PVB19) is a nonenveloped member of the family Parvoviridae. It typically infects erythroid precursor cells and has a cytotoxic effect on them. Therefore, a pause of erythropoiesis occurs, which is the most obvious feature of parvovirus infections. In many patients this transient pause of erythropoiesis does not cause a problem; however, in those with problems such as hemolytic anemia, severe aplastic anemia crisis might be seen (1,2).

The virus is generally spread by respiratory route, but blood transfusion is an alternative transmission route (3). PVB19 viremia occurs 1 week after the exposure and lasts at least 5 days. It reaches its highest level on the second day. Towards the end of the viremic period, immunoglobulin M (IgM) antibodies are detected. Generally, this detection is on days 10–14 of the infection. IgM positivity lasts until the 5th month. However, in some people, it may last longer. Immunoglobulin G (IgG) type antibodies become positive after 15 days and stay positive at higher titers for months. These antibodies can be analyzed using enzyme-linked

immunosorbent assay (ELISA), radioimmunoassay, and chemiluminescence or immunofluorescence methods (4). Although epidemiological features of the infection do not change, IgG positivity is found in 2%–21% of children at ages range between 1 and 5, in 30%–40% of adolescents, in 40%–60% of adults, and in more than 85% of the elderly population (5).

The main objective of professionals dealing with this issue is to ensure access to safe and effective blood components for patients who are in need of blood transfusion. Blood products obtained from plasma pools and PVB19 contamination are especially important for blood banking (5). Iatrogenic transmission is seen when a high level of viremia is present in the primary infection or if there is a virus load of more than 10^{12} geq/mL in early periods of acute infection in asymptomatic blood donors (6). However, in plasma products processed with solvent detergent technology, though HIV and hepatitis B and C are inactive, PVB19 is not affected because of its nonlipid enveloped structure. It is also resistant to heat and if the plasma is exposed to dry air at 80 °C for 72 h, there is no

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inactivation (5). PVB19 DNAs are detected in 50%–80% of nonviral inactivated factor VIII concentrates and 30%–50% of IX concentrates inactivated with the solvent/detergent method (7). However, there are limited data on patients having symptomatic infections transmitted by virus-containing blood components. Most cases of seroconversion are shown in asymptomatic receivers (8).

Infection does not occur if the PVB19 viral load of plasma-derived products is less than 10^3 – 10^4 geq/mL. The explanation for this might be the inadequacy of the amount causing an infection or the existence of neutralizing antibodies in the pool from which the plasma-derived products were obtained. Receiver factors also have an important role in the infection. PVB19 antibodies hinder reinfection and most adults have seropositivity for PVB19 (9,10). However, in the United States and Europe, there is permission for a maximum of 10^4 PVB19 viral load in plasma-derived blood products (11,12).

The prevention of parvovirus B19 transmission is especially important for patient groups with immune deficiency or hemolytic anemia. In these groups, a viral load of more than 10^7 presents a high risk (4,9).

To prevent the use of PVB19-contaminated blood components is the only way to prevent the transmission of this infection via blood.

2. Materials and methods

2.1. Study design

Nine hundred and eighty-eight blood samples were taken from individuals admitted to a university blood bank between 2012 and 2014. They were eligible for blood donation (based on blood donor database registers, blood donor questionnaires, and whole blood count results). Blood samples were centrifuged and separated serums were kept at -80 °C until study time. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The blood samples were taken from the blood donors in accordance with Gazi University Medical Faculty Ethics Committee Approval Document No. 184.

2.2. Detection of parvovirus B19 IgM and IgG antibodies

For parvovirus B19 IgM and IgG detection, two different commercial ELISA kits were employed. The NovaLisa parvovirus B19 IgM and IgG ELISA kits (NovaTec Immundiagnostica GmbH, Germany) were used for PVB19 screening and PVB19 IgM-positive samples and 90 randomized IgG-positive samples were confirmed via DRG parvovirus B19 IgM and IgG ELISA kit (DRG Instruments GmbH, Germany). The serum samples were diluted with sample dilution buffer at a ratio of 1:100 for

DRG IgG and NovaLisa IgM and IgG tests. For DRG IgM assay, the serum samples were diluted at a ratio of 1:50, then diluted again with an IgG-RF-Sorbent solution within the kit at a ratio of 1:1. The assays were performed according to the manufacturer's instructions. At the end of the study, optical densities (ODs) of the wells in the microplate were measured in 450/620 nm wavelength (Plate Reader, DAS, Italy) and the results were evaluated.

2.3. Evaluation of the results

According to validation measurements of the assay in the NovaLisa Parvovirus B19 IgM and IgG ELISA kits, the test is considered to be operated properly if the substrate blank well is less than 0.100, the negative control is less than 0.200, the cut-off value is between 0.150 and 1.300, and the positive control is higher than the cut-off value. The mean of the two cut-off values is taken. If OD values of the samples are 10% and higher than the cut-off value, the result is considered as positive, and if they are lower, the result is considered to be negative. The sensitivity and specificity of the kit were indicated as 95%.

According to the DRG parvovirus B19 IgM and IgG ELISA kits, the test is considered to be performed properly if the substrate blank well is less than 0.100, the negative control is less than 0.200, the cut-off value is between 0.350 and 0.850, and the positive control is between 0.650 and 3.000. The mean of the two cut-off values was taken. If OD values of the cut-off value were 20% and higher than the cut-off value, the result was positive. If they were 15% and lower, it was negative. The instruction manual of the kit declared sensitivity and specificity as 100%.

2.4. Statistical analysis

SPSS 15 (SPSS Inc., Chicago IL, USA) was used for the evaluation of the data obtained from the study and to form tables. The data were presented in tables as numbers and percentages. The differences between parvovirus IgM and parvovirus IgG positivity regarding both sex and age groups were analyzed using the Pearson chi-square test. Finally, the significance level was $P < 0.05$ in all statistical analysis.

3. Results

The results obtained at the end of the study and their distribution concerning age and sex are presented in Tables 1 and 2.

There was a significant difference between both parvovirus IgM ($\chi^2 = 8.038$; $P = 0.045$) and IgG positivity rates ($\chi^2 = 69.003$; $P < 0.001$) in terms of age. Parvovirus IgM positivity was at its highest rate in the age group of 41–50 years and lowest in the 18–30 age group. On the other hand, the parvovirus IgG positivity rate was highest at 31–40 years and lowest at ≥ 51 years. Although there was no significant difference between parvovirus IgM positivity in terms of sex ($\chi^2 = 1.354$; $P = 0.245$), parvovirus

Table 1. Distribution of parvovirus IgM and IgG positivity according to age groups.

Age groups	Blood donors number	IgM positivity number	IgM positivity percent	IgG positivity number	IgG positivity percent
18–30	336	8	2.38	202	60.3
31–40	280	14	5	200	71.7
41–50	154	11	7.14	100	64.7
51–65	218	6	2.75	80	36.6
Total	988	39	3.9	582	58.9

Table 2. Distribution of blood donors according to age groups and sex.

Blood donors age groups	Male	Female	Total
18–30	268	68	336
31–40	222	58	280
41–50	104	50	154
51–65	189	29	218
Total	783	205	988

IgG positivity rates were significantly different ($\chi^2 = 4.192$; $P = 0.041$). Finally, the parvovirus IgG positivity rate was significantly higher in women (65.40%) than men (57.20%).

4. Discussion

Due to advances in transfusion medicine, blood-borne viral infection frequency has decreased recently. This was achieved using certain strategies such as blood donor selection, development of screening methods for the viral organism, and widespread use of viral inactivation methods concurrently. The use of nucleic acid amplification technologies has also shortened the window period and contributed to blood transfusion safety. PVB19 may lead to serious clinical complications in pregnant women, children with hematological problems, and patients with immune deficiencies. If parvovirus is studied using different methods to analyze the blood of donors, one parvovirus case in 20,000 to 50,000 donations can be detected. In epidemic periods, this can be as high as one case in 260 blood donors (13).

In Turkey, there are limited data on parvovirus epidemiology. Türk Dağı et al. investigated IgG frequency in a study carried out in Konya in 2010. Their study group included blood donors 18–60 years old and children admitted to the hospital for various reasons. In 631 blood donors from the adult group, PVB19 IgG positivity was 36% (14). In 2012 Motor et al. analyzed parvovirus B19 seroprevalence in adults with essential hypertension in

Hatay. In the 45 individuals who formed the healthy control group of the study, IgM positivity was 15.6% and IgG positivity was 31% (15). Moreover, Erden et al. evaluated the relation between Hashimoto thyroiditis and PVB19 infection in 2013. In healthy participants making up the control group in this study, the IgM positivity was 5.7% and the IgG positivity was 22.9% (16). Motor et al. analyzed the role of parvovirus in patients with ankylosing spondylitis in 2014. In the control group of this study, IgM positivity was 4.3% and IgG positivity was 30% (17). On the other hand, an IgM positivity of 3.9% and an IgG positivity of 58.9% were determined in our study. In all the studies mentioned above, the results showed significant variations. These variations may have occurred because all the data except one set came from control groups; there may also be seasonal and/or geographical differences, there are variations in the age distributions among the groups, and, finally, the study groups in other studies were not large enough.

The frequency of IgG antibodies against parvovirus B19 infection increases with age and has geographical differences. In Chile in 2007, for example, the frequency in blood donors (18–64 years old) was 54.8% (18), which is in accordance with our results. In Poland, the first peak for parvovirus infection was at a preschool age and by the age of 40 the rate was as high as 80% (19). Furthermore, 40% of women of child-bearing age were reported to be at risk of parvovirus B19 infection, especially during epidemic years. In Australia, seroprevalence for parvovirus B19 was 51% at ages of 10–19 and 78% at ages of >50 (20). In England and Wales, seropositivity was 50%–60% in young adults, but 85% after the age of 70 (21). A study done in blood donors in 2004 in Italy revealed that the seropositivity rate was 77% between the ages of 18 and 27, and the rate was 88.5% between the ages of 48 and 57 (22). However, the highest seropositivity rate was found between the ages of 31 and 40 in our study. In contrast with the literature, it was observed that there is a significant decrease in the seropositivity rate especially after 50 years.

Seropositivity rates of parvovirus infection indicate geographical differences. In Far East Asian countries

seropositivity is lower than in the western world. For example, in Thailand, this rate was 10.94% in young adults in 2003 (23). In another Far East country, Malaysia, this rate for the same age group was 32% in 2002 (24). In a similar study carried out in 1998 in Spain, IgG antibodies were positive in 64.7% of blood donors, but IgM antibodies were not found in any participants (25). While the seropositivity rate of blood donors in Belgium was 74%, that of blood donors in Tunisia was 65% in 1997. This difference might originate from geographical differences between north and south (26).

The methods that decrease parvovirus transmission in transfusion medicine are still under investigation. Among these methods being discussed are the nucleic acid amplification test (NAT), in which screening is done in a single case or in a small number of pools, and techniques that are applied in viral inactivation and detergent solvent methods such as multiple steps of superheating (3 days at 80 °C), pasteurization, and nanofiltration (27). Screening

of all blood donation with NAT-based algorithms is performed in Germany, Austria, Poland, and Japan (28,29). Not using plasma with higher than a certain amount of viral load (10^5 IU/mL) decreases the viral load in plasma pools and also diminishes the seroconversion or transmission rate of symptomatic diseases. However, this may destroy much of the plasma, so the maintenance of the system should be guaranteed (5). However, low transmission risk with transfusion brings the question of availability, especially in low endemic countries (30).

The largest study that shows the parvovirus B19 seroprevalence in blood donors in Turkey was performed in this research and the ratios changing with age and sex were also presented.

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