

Human parvovirus B19 sero-molecular prevalence in Madinah blood donors, Saudi Arabia

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ABSTRACT

The human parvovirus B19 is known to cause erythema infectiosum in children. The virus is transmitted through respiratory droplets, vertically from mother to fetus, via blood transfusion, and by transplantation of body organs. B19V may cause serious complications in pregnant women and patients with hemoglobinopathies, transient aplastic crisis, or immunodeficiency syndromes. The aim of this study is to estimate the seroprevalence of parvovirus B19 and investigate the presence of viremia by detecting viral DNA in acute infection cases in Madinah, Saudi Arabia. Three hundred blood donors were tested for anti-B19 IgG and IgM antibodies using ELISA. Then, IgM positive samples were tested for the presence of B19 DNA by PCR. A majority (66.67%, 200/300) of the tested samples were positive for anti-B19 IgG, while 1.67% was positive for both anti-B19 IgM and IgG. The prevalence of anti-B19 IgG was statistically different between the age groups of 18–25 years and 46–55 years ($p = 0.04$), while it was comparable between the age groups of 18–25, 26–35, and 36–45 years. Anti-B19 IgM prevalence was comparable between all age groups. No B19 DNA was detected in the tested IgM-positive samples. High prevalence of anti-B19 IgG was demonstrated in Madinah, and no viral DNA was detected in the anti-B19 IgM positive samples. Therefore, there is a negligible possibility of B19 transmission by transfusion of whole blood. However, screening of B19V should be performed for blood and blood products to be given to high-risk individuals such as pregnant women and patients with hemoglobinopathies, transient aplastic crisis, or immunodeficiency syndromes.

Keywords: Parvovirus B19, Blood Donors, Seroprevalence, viremia, ELISA

1. INTRODUCTION

The human parvovirus (B19V) is a member the Parvoviridae family classified under the genus Erythrovirus (Heegaard & Brown, 2002). There are three different genotypes of B19V. Genotype 1 is distributed worldwide, genotype 2 is present in Europe and the Americas, and genotype 3 seems to be predominant in Africa (Servant et al., 2002). B19V is a small, non-enveloped virus with a single-stranded DNA that is encapsidated by VP1 and VP2



structural proteins. Also, a single nonstructural protein (NS1) that plays roles in genome replication and cell apoptosis, and two small nonstructural proteins are encoded by the viral genome (7.5 kDa and 11 kDa) (Heegaard & Brown, 2002; Luo & Astell, 1993; St. Amand & Astell, 1993).

B19V is known to infect erythrocyte progenitor cells, in which the virus replicates and progeny viruses are formed, leading to cell apoptosis (Brown et al., 1993). B19V is transmitted via the respiratory tract, from mother to fetus, blood and its components transfusion and by bone marrow and organ transplantation (Servant-Delmas & Morinet, 2016). Infection with B19V elicits the production of B19V-specific antibodies (immunoglobulin M and immunoglobulin G). Immunoglobulin M (IgM) antibodies are produced within 10–14 days of infection and are usually detected in plasma for approximately five months. Immunoglobulin G (IgG) antibodies are produced after 15 days of infection and persist permanently (Corcoran & Doyle, 2004). It is well known that anti-B19V IgG prevalence increases with age: 2–21 % in children five years or younger, ~30–40 % in adolescents, ~40–60 % in adults, and more than 85 % in the elderly (Marano et al., 2015).

In children, infection with B19V is mainly asymptomatic but could lead to a disease known as infectious erythema or fifth disease, characterized by mild fever and rash (Heegaard & Brown, 2002). However, some virus infection symptoms are fatal. These symptoms depend on the individual's age, immune status, and, most significantly, their hematological condition. B19V infection in adults results in arthritis, especially in the large joints, that may persist for years (Qiu et al., 2017). In patients with a disease characterized by a high erythrocyte turnover rate, such as hemoglobinopathies, a transient aplastic crisis may result from B19V infection (Qiu et al., 2017). If a pregnant woman with no previous exposure to B19V is infected, the virus has the ability to get through the placenta and infect fetal red blood cells precursors, possibly leading to miscarriage in the first trimester or fetal hydrops and death in the second/third trimester (De Haan et al., 2008). Moreover, B19 infection of immunosuppressed patients could cause pure red cell aplasia, in which suppression of erythroid progenitor cell formation is permanent (Florea et al., 2007).

As the virus is known to infect erythrocyte progenitor cells, it is believed that the virus is highly persistent in the blood. In addition, because the virus cannot be inactivated by most inactivation methods and most infection cases are asymptomatic, there is a high risk of virus transmission through blood and its components to vulnerable recipients (Brown et al., 1993; Marano et al., 2015). Therefore, the only way to prevent spread of the virus via blood and its products is to prevent the transfusion of B19V. However, screening for B19V in healthy donors is not routinely done in most countries, although it has been recommended by the US Food and Drug Administration (FDA) to screen for B19V nucleic acid and discard blood units containing more than 104 IU/ml (Brown et al., 2001).

The aim of this work is to determine the B19V prevalence by detecting viral specific anti- IgM and IgG antibodies and viral DNA in serum from blood samples collected from blood donors at the Madinah Central blood bank. This study is the first to examine, to our knowledge, the parvovirus B19 prevalence in randomly selected samples from Madinah Central blood bank. In combination with other data from other centers in Saudi Arabia, these data will provide new insights into the epidemiology of B19V infection and may provide a basis for considering official guidelines regarding B19 testing in donated blood and blood products in Saudi Arabia.

2. MATERIALS AND METHODS

Study design

This is a cross-sectional study that was performed between April and July 2019, using blood donated by healthy Saudi male blood donors, aged 18–55 years, at Madinah central blood bank, Madinah, Saudi Arabia. All samples were negative for hepatitis C virus (HCV), human immunodeficiency virus (HIV), human T-cell lymphotropic virus (HTLV), hepatitis B virus (HBV), syphilis, and malaria.

Sample size

The minimum sample size was calculated by the following formula: $(n = z^2p(1-p)/e^2)$ as described previously (Pourhoseingholi et al., 2013) with $z = 1.96$ and $e = 0.05$. As the prevalence of B19V infection in Madinah blood donors is unknown, the prevalence (p) of 76.3% reported in a previous study in Makkah (Johargy, 2009) was used as a baseline. Thus, the minimum number required was 281; however, we selected 300.

Blood specimens

Whole Blood (~5 ml) from each participant was collected into EDTA tubes. Then, samples were centrifuged, and plasma was stored at -20°C until analysis. Unique study numbers was used to label the samples to anonymize the participants.

Indirect enzyme-linked immunosorbent assay (ELISA) detection of B19V antibodies

Anti-B19V IgM and IgG antibodies were detected using SERION ELISA classic Parvovirus B19 IgM and IgG kits (Virion \Serion, Würzburg, Germany) according to the manufacturer's instructions. Briefly, for the IgG assay, plasma samples were diluted 1 in 100 in dilution buffer, while for IgM assay, plasma samples were diluted 1 in 100 in diluted rheumatoid factor-absorbent. Then, 100 μ l from ready to use controls and each prepared sample was pipetted into appropriate wells and incubated for 1 hour at 37°C. Subsequently, 300 μ l of 1X washing solution was used to wash the plated four times and 100 μ l of IgG or IgM enzyme conjugate was loaded into the wells. Plates were then incubated at 37°C for 30 minutes. Plates were washed 4x with 300 μ l of 1X washing solution, then 100 μ l of the substrate was loaded into the plates, and they were incubated at 37°C for 30 minutes. Then, 100 μ l of stop solution was loaded into all wells, and sample absorbance was read at 405 nm wavelength using a spectrophotometer (ELX800, BioTek, Winooski, VT, USA).

Nucleic acid extraction and polymerase chain reaction

Viral genomic DNA extraction from 1 ml of plasma was performed using the Virus DNA/RNA Extraction Kit III (Geneaid, Taipei, Taiwan) following the manufacturer's instructions. Quality and quantity of DNA were determined using a NanoDrop 1000 UV-VIS Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). To detect B19V DNA, PCR was performed using the AMPLIRUN Parvovirus B19V DNA Control kit (Vircell, Granada, Spain). The kit contains purified B19V DNA plasmid (13,000 copies/ μ l) as a positive control and a primer mix (25 pmol/ μ l); PB19-F (5-AAGGATTCATGACTTTAGGTATAGCC-3') and PB19-R (5CCAGTTTTGTCTGTAACATCCTTAAC-3'). DNA amplifications were performed in 25 μ l total reaction volume: 5 μ l of each eluted viral DNA or positive control plasmid DNA, 2 μ l 10 μ M primers, 12.5 μ l of the 2X GoTaq Green Master Mix (Promega, Madison, WI, USA), and 5.5 μ l of nuclease-free water. The reactions were carried out on the Applied Biosystems Veriti 96-well Thermal Cycler (Thermo Fisher Scientific) for 2 minutes at 95°C, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 45 seconds at 72°C, followed by final extension of 10 minutes at 73°C. The amplified product (~638 bp) was analyzed on a 1.5% agarose gel.

Statistical analysis

Data were collected, tabulated, and statistical analysis was performed using GraphPad Prism 8.0 software (GraphPad Software Inc, San Diego, CA, United States). The frequencies of anti-B19V IgM and IgG among different donor age groups were compared using the Chi-square test. A p-value of less than 0.05 was considered significant.

3. RESULTS

The mean age of the 300 blood donors was 32.21 ± 11.08 , and the median was 32. The youngest and oldest participants were 18 and 55 years old, respectively (Table 1). Most of the participants (35%) were in the age range of 18–25 years; 81 (27%) were 26–35; 61 were (20.33%) were 36–45 and 53 (17.67%) were 46–55. The majority, 205/300 (66.67% and 1.67%; 95% CI, respectively), were seropositive for B19V IgG and IgM. All five donors who tested positive for B19V IgM positive were also positive for IgG (Table 1). Compared to the youngest age group, the 46–55 age groups showed a significantly higher rate of B19V IgG seropositivity ($p < 0.05$). However, there was insignificant difference in the rate of B19V IgG seropositivity in the 26–35 and 36–45 age groups compared to the youngest age group ($p > 0.05$).

Table 1 Frequency of Parvovirus B19 IgG by age

| Age (Years) | Total tested | Anti-B19 IgG positive | p-Value* |
|-------------|--------------|-----------------------|----------|
| 18–25 | 105 | 67 (63.81%) | -- |
| 26–35 | 81 | 48 (59.26%) | 0.52 |
| 36–45 | 61 | 43 (70.49%) | 0.38 |
| 46–55 | 53 | 42 (79.25%) | 0.04 |
| Total | 300 | 200 (66.67%) | |

*Significant at $p < 0.05$, representing the difference from the youngest age group.

The rate of IgM seropositivity was highest in the age group of 18–25 years (3.81%) and lowest at ≥ 51 years (Table 2). However, the frequency of B19V IgM seropositivity among all age groups compared to the youngest age group was not significant ($p > 0.05$).

Furthermore, All IgM positive samples were investigated for detection of B19V DNA by PCR and analyzed by agarose gel electrophoresis. No sample showed positive result for B19V DNA (Figure 1).

Table 2 Frequency of Parvovirus B19 IgM by age

| Age (Years) | Total tested | Anti-B19 IgM positive | p-Value* |
|-------------|--------------|-----------------------|----------|
| 18–25 | 105 | 4 (3.81%) | -- |
| 26–35 | 81 | 0 (0%) | 0.07 |
| 36–45 | 61 | 1 (1.64%) | 0.43 |
| 46–55 | 53 | 0 (0%) | 0.15 |
| Total | 300 | 5 (1.67%) | |

*Significant at $p < 0.05$, representing the difference from the youngest age group.

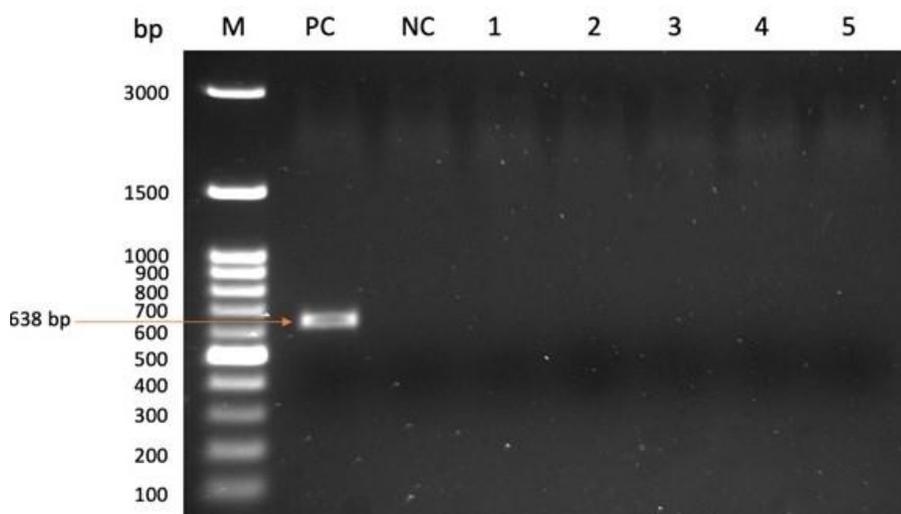


Figure 1 Analysis of PCR products of B19V DNA by 1.5% agarose gel electrophoresis. M: Marker, PC: positive control, NC: negative control. Lanes 1–5: PCR products of donor samples.

4. DISCUSSION

B19V is a known causative agent of a range of illnesses and morbidity in humans, especially pregnant women, immunocompromised patients, and patients with hematopathologies. B19V mainly spreads via the respiratory tract but can also be transmitted by blood and its components transfusion (Parsyan & Candotti, 2007). The frequency of transmission of blood-borne viruses has declined because of advances in transfusion medicine, such as the use of nucleic acid amplification technologies (NAT). The use of NAT has shortened the infection window period and enhances the blood and its components safety. Although B19V NAT screening in blood is not practiced worldwide, several countries have implemented the procedure and its derivatives by screening plasma pools using PCR (Grabarczyk et al., 2012; Sakata et al., 2013; Schmidt et al., 2007). However, there still are chances for transmission of some viruses that are not routinely tested in donated blood or blood derivatives, such as B19V, even when inactivation was performed. Several previous studies have reported transfusion of B19V contaminated blood or its components (Dos Santos Bezerra et al., 2021; Juhl & Hennig, 2018; Nagaharu et al., 2017; Satake et al., 2011). Screening of B19V in blood donors in Saudi Arabia has yet to be implemented, although it is known that this virus could cause severe complications.

So far, only one study has investigated the frequency of anti-B19V antibodies among blood donors in Makkah region, Saudi Arabia (Johargy, 2009). However, our study evaluated both the sero- and molecular prevalence of B19V. In our study, 66.7% and 1.67% of participants were tested for positive anti-B19V IgG and IgM, respectively. This IgG prevalence is lower than the results reported in Makkah, Saudi Arabia, in which 76% of the donor samples tested positive (Johargy, 2009) as well as in Egyptian, Belgian and Italian donors (80.20%, 74%, and 79.1%, respectively) (Hasanain et al., 2021; Letalef et al., 1997; Manaresi et al., 2004). However, our IgG results are comparable with results reported in studies from Qatar, Sudan, and South Africa (62.7%, 63.3%, and 62.2%, respectively) (Abdelrahman et al., 2021; Francois et al., 2019; Hassan Omer et al., 2017). In contrast, our IgG is higher than in results

reported for blood donors in Iran (27.4% and 60.5%) (Karimnia et al., 2020), India (39.97%) (Kumar et al., 2013), and Turkey (58.9% and 42.7%) (Göral et al., 2018; Uskudar Guclu et al., 2020).

A significant difference ($p = 0.04$) was detected in anti-B19V IgG prevalence between the 18–25 group (63.81%) and 46–55 group (79.25%) but not between other age groups and the youngest age group. These results are consistent with previous studies that stated that the prevalence of anti-B19V IgG is associated and increases with age, reaching more than 80% in people older than 61 years (Parsyan & Candotti, 2007). However, the recruitment of a small sample size in our study could be the cause of the insignificant difference in anti-B19V IgG prevalence between two age groups (26–35 and 36–45 years) compared to the youngest age group (18–25 years), similar to what was reported in previous studies (Abdelrahman et al., 2021; Hassan Omer et al., 2017; Sunday A. U, 2013). This comparable anti-B19V IgG frequency among different age groups, with the 26–35 age group exhibiting the lowest seropositivity rate (59.26%), is similar to results from Turkey (Uskudar Guclu et al., 2020) and Qatar (Abdelrahman et al., 2021).

Regarding the prevalence of anti-B19V IgM in blood donors in Saudi Arabia, this is the first investigation of IgM seropositivity. In this study, anti-B19V IgM seropositivity was detected in five samples (1.66%). This IgM prevalence is lower than results reported in Egyptian, Turkish, and Congolese blood donors (6.2%, 3.9%, and 5.3%, respectively) (Chabo Byaene et al., 2020; Göral et al., 2018; Hasanain et al., 2021). Nevertheless, our IgM result is comparable with results reported in studies from Qatar and Iran, 2.1% and 2.6%, respectively (Abdelrahman et al., 2021; Zadsar et al., 2018). In contrast, our IgM is higher than the results reported in blood donors in Turkey and the USA (0.2% and 1%) (Doyle et al., 2000; Uskudar Guclu et al., 2020). All anti-B19V IgM positive samples were also positive for anti-B19V IgG. However, no B19V DNA was detected in all IgM positive samples. This result is similar to what was reported in a study from Iran that showed no B19V DNA viremia in blood donors (Karimnia et al., 2020). Viral specific IgG usually becomes detectable in immunocompetent individuals at week three post-infection and lasts for years. In contrast, IgM appears within two weeks and declines within months. Our results indicate that most of this study's participants had previous exposure to B19V and thus developed a humoral response against the virus.

5. CONCLUSION

In conclusion, our study investigated the sero-molecular epidemiology of B19V in Saudi Blood donors. It showed high anti-B19V IgG prevalence among blood donors in Madinah (66.76%), which increased with age. Also, low IgM seroprevalence was detected in five positive samples (1.67%), which were also positive for IgG but negative for viral DNA. These results are comparable with what was previously reported locally, regionally, and globally. Our results show the low risk of B19V transmission via blood or its components transfusion. The development of antibody response against B19V results in the production of antibodies that neutralize the virus and subsequently prevent infection. Blood from donors with a persistent high anti-B19 IgG antibody titer could be considered safe as a single unit. Therefore, there is no need for the health authorities to implement screening tests for B19V in all volunteer blood donors. However, screening for blood-derived products is still recommended to protect vulnerable people such as patients with hemoglobinopathies, transient aplastic crisis, and immunodeficiencies and pregnant women.

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Conflicts of interests

The author declares that there are no conflicts of interests.

Ethical consideration

The study was approved by the Ethics Committee of the College of Applied Medical Sciences (ethical approval code: MLT-2019-02) at Taibah University, Madinah, Saudi Arabia, and written informed consent was collected from participants before their recruitment.

Data and materials availability

All data associated with this study are present in the paper.

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