



Research Article

Serological and Molecular Detection of Prevalence of Human Parvovirus (B19) in Beta
Thalassemia Major Patients in BaghdadNoor Thamir Al-Musawe^{1,2} , Maysaa Kadhim Al-Malkey^{3*} ¹Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq; ²Laboratory Division, Immamian Kadhman City Hospital, Alkarkh Health Directorate, Baghdad, Iraq; ³Tropical Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq

Received: 12 October 2024; Revised: 22 November 2024; Accepted: 17 December 2024

Abstract

Background: Beta thalassemia major (β -TM) is an inheritable condition with many complications, especially in children. The blood-borne viral infection was proposed as a risk factor due to the recurrent blood transfusion regimen (hemotherapy) as human parvovirus B19 (B19V). **Objective:** This study investigated the B19V seroprevalence, DNA presence, B19V viral load, and B19V genotypes in β -TM patients and blood donors. **Methods:** This is a cross-sectional study incorporating 180 subjects, segregated into three distinct groups each of 60 patients, namely control, β -TM, and β -TM infected with Hepatitis C Virus (HCV). For the B19V prevalence in the studied group, the ELISA technique and real-time PCR were used. The genotyping was followed by the resultant sequence. **Results:** Both B19V IgM and IgG antibody positivity rates are higher among β -TM patients compared to controls. The B19V IgM (35%) and B19V IgG (21.67%) antibodies positivity in β -TM patients compared to 23.3% and 18.33% positivity in the controls was significantly observed. The prevalence of B19V was (8.3%), and the viral copy number in β -TM patients ranged from $\geq 10^4$ – 10^6 copies/ml than in controls. The B19V genotype 1 subtype a was the only genotype according to the VP1-VP2 region (288 pb) in this study. **Conclusions:** The prevalence of B19V in patients may be higher than in controls. B19V screening in high-risk groups, such as blood donors, may considerably reduce the prevalence of B19V.

Keywords: Beta thalassemia major, Human parvovirus B19, Prevalence, Phylogenetic analysis.

الكشف المصلي والجزيئي عن انتشار فيروس بارفو البشري (B19) في مرضى بيتا ثلاسيميا الكبرى في بغداد

الخلاصة

الخلفية: بيتا ثلاسيميا الكبرى (β -TM) هي حالة وراثية لها العديد من المضاعفات، خاصة عند الأطفال. تم اقتراح العدوى الفيروسية المنقولة بالدم كعامل خطر بسبب نظام نقل الدم المتكرر (العلاج بالدم) مثل مثل فيروس البارفو بي 19 البشري. **الهدف:** بحثت هذه الدراسة في الانتشار المصلي لفيروس B19V، ووجود الحمض النووي، والحمل الفيروسي B19V، والأنماط الجينية B19V في مرضى β -TM والمتبرعين بالدم. **الطرائق:** هذه دراسة مقطعية تضم 180 شخصا، مقسمة إلى ثلاث مجموعات متميزة لكل منها 60 مريضا، وهي التحكم، β -TM، و β -TM المصابين بفيروس التهاب الكبد نوع سي. لدراسة انتشار B19V في مجموعات الدراسة، تم استخدام تقنية ELISA و Real-time PCR. تبعا للتنميط الجيني المتسلسل للناتج. **النتائج:** ان معدلات إيجابية الأجسام المضادة B19V IgM و IgG أعلى بين مرضى β -TM مقارنة بالضوابط. لوحظت إيجابية الأجسام المضادة B19V IgM (35%) و B19V IgG (21.67%) في مرضى β -TM مقارنة بإيجابية 23.3% و 18.33% في الضوابط. كان انتشار B19V (8.3%)، وتراوح عدد النسخة الفيروسية في مرضى β -TM من 10^4 – 10^6 نسخة/مل مقارنة بالضوابط. كان النوع الفرعي من النمط الجيني B19V 1a هو النمط الجيني الوحيد وفقا لمنطقة VP1-VP2 (288 pb) في هذه الدراسة. **الاستنتاجات:** قد يكون انتشار B19V في المرضى أعلى منه في الضوابط. قد يقل فحص B19V في الفئات المعرضة لخطر كبير، مثل المتبرعين بالدم، من انتشار B19V بشكل كبير.

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INTRODUCTION

Human Parvovirus B19 (B19V) is a tiny naked virus that has a single-stranded linear DNA genome of 5.6 kb [1]. In 1981, B19V productive infection occurred in human erythroid progenitor cells; hence, B19V was incriminated as the causative agent of severe aplastic crises in children with sickle-cell disease [2]. Viral transmission routes include respiratory droplets, vertical transmission from pregnant patients to fetus, and blood transfusion [3]. Although a self-limiting

disease occurs due to B19V infection, it is mostly in healthy subjects. But in patients with hereditary hemolytic anemias, it may result in many sequels, such as suppression of bone marrow, prompting a life-threatening decline in values of the hemoglobin [4]. Beta thalassemia major (β -TM) is characterized by inherited disorders in β -globin chain synthesis leading to chronic anemia due to RBC hemolysis and erythropoiesis dysfunction, which requires recurrent blood transfusion [5]. In Iraq, which belongs to the Eastern Mediterranean region, the β -TM is prevalent

[6]. The β -TM was registered with a prevalence of 35.7 per 100,000 in 2015 [7], and its prevalence over the last 5 years in Iraq has risen from 12106 cases in 2018 to 13390 cases in 2022 [8]. Some studies have proposed blood-borne viruses implicated in Iraqi patients with β -TM, including EBV [9], Hepatitis C virus [10,11], and SEN Virus [12]. Meanwhile, recent research was carried out to investigate the genetic polymorphisms at the BcL11A gene in Sudanese patients [13], and the transfusion-transmissible infection prevalence among blood donors in Saudi Arabian patients was also investigated [14]. So, this cross-section study was initiated to investigate B19V prevalence during hemotherapy in vulnerable patients suffering from β -TM among teenaged Iraqi patients.

METHODS

Study design and setting

This cross-section study included 60 patients with β -TM and another 60 β -TM patients infected by the hepatitis C virus (HCV) who were referred to the Ibn Al Balady Children and Maternity Hospital, Baghdad City, during the period extended from November 2023 to February 2024. Another 60 subjects who served as the control group were enlisted from healthy blood donors visiting the Iraqi National Center of Blood Transfusion, Baghdad city. The study design was shown in Figure 1.

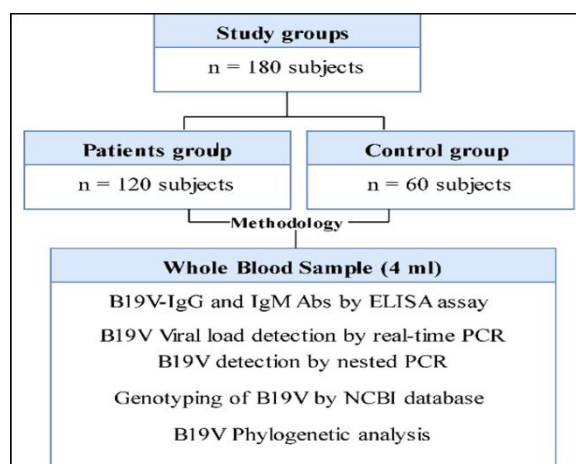


Figure 1: Schematic flowchart of the study design

Sample collection

Four mL of peripheral blood from the patients and control groups was drawn into a tube with EDTA, an anticoagulant, and then separated into two parts for plasma separation used for serological and molecular analysis.

Inclusion and exclusion criteria

The patient inclusion criteria included patients under 45 who were receiving regular blood transfusions and excluded patients older than 45. All patients suffering from autoimmune diseases and immunocompromised patients were also excluded.

Serological detection of human parvovirus B19

Whole blood specimens were collected in a sterile EDTA tube (3 mL) for plasma separation. All plasma samples were examined for the qualitative presence of anti-parvovirus B19 antibodies (IgM and IgG Abs) using enzyme-linked immunosorbent assay (ELISA) kits from Demeditec Company, Germany, using the Microplate system (wash, reader, and printer) from GloMax® Discover, USA.

Molecular detection of human parvovirus B19

Genomic DNA was isolated using a commercial extraction kit sourced from Geneaid Company in Taiwan. Then the real-time PCR protocol was used for quantitative detection of human parvovirus B19 using a commercial kit from Sacace Company, Italy. After DNA extraction from plasma, it was then amplified using real-time amplification with fluorescent reporter dye probes specific to Parvovirus B19 and Internal Control (IC) to determine the virus copy number for Parvovirus B19. The PCR thermal condition was applied: 1 cycle (95°C for 10 min), followed by five cycles for each (95°C for 10s, 60°C for 20s, and 72°C for 15s), and finally 40 cycles for each (95°C for 5s, 60°C for 20s *fluorescent signal detection*, and 72°C for 15s) using the rotor-type Real-Time PCR system (Mx3005P™, Agilent, USA). The nested PCR (nPCR) was carried out according to a previous study [15]. The PCR products were sent to the Macrogen® company to get the ABI files. By using the reference-based genotyping, the NCBI BLASTn analysis was conducted for the DNA sequence alignment of the VP1/VP2 locus of the analyzed samples. The BioEdit ClustalW alignment tool (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) was used for the B19V sequences, which were aligned, and the Mega ver. 7.0 neighbor-joining method with 1,000 bootstraps (<http://www.megasoftware.net>) to construct a phylogenetic tree. The genotype 1a reference sequences were taken from GenBank.

Ethical consideration

The College of Science, University of Baghdad Ethics Committee cleared the study protocol under number (CSEC/1223/0136) and by the Iraqi Ministry of Health approval (No. 62751 dated October 22, 2023). Written consent was given by all participants or by their legal guardians.

Statistical analysis

The data underwent coding and entry procedures using the statistical software SPSS version 26. Descriptive statistics, including mean, standard deviation, median, minimum, and maximum values for quantitative variables, as well as the number and percentage for qualitative values, were employed for data summarization. Numbers and percentages represented categorical variables, while the Kolmogorov-Smirnov test was used to assess the normal distribution of numeric variables. Statistical differences between groups were tested using the Chi-Square test for qualitative variables, the independent

sample test, and the ANOVA (analysis of variance) test for quantitative normally distributed variables. *p*-values less than or equal to 0.05 were considered statistically significant.

RESULTS

The patients' ages ranged from 5 to 45 years (18.6 ± 7.8 years). The median age was 15 years in patients with β -TM-ve for HCV, 26 years in patients with β -TM +ve

for HCV, and 24.5 years among the control group ($p < 0.01$). The sex distribution among the β -TM patients and controls revealed the predominance of males (60%) in the β -TM patients' group, 51.67% in β -TM patients positive for HCV, and 46.67% for controls ($p = 0.169$). Contrarily, the females were more predominant in the control group (53.3% versus 40% for β -TM patients and 48.3% for the β -TM patients +v HCV) as in Table 1. The prevalence of B19V IgM and IgG antibodies in different study groups was demonstrated in Table 2.

Table 1: Distribution of age and sex in different study groups

Demographic parameter	Thalassemia	Thalassemia with HCV	Control	<i>p</i> -value*
Age (year)	15	26	24.5	0.01
Sex				0.169
Male	36(60)	31(51.67)	28(46.67)	
Female	24(40)	29(48.33)	32(53.33)	

Values were expressed as number and percentage. *n*=60 in each group.* Chi square test.

Table 2: The prevalence of B19V IgM and IgG antibodies in different study groups

B19V prevalence	Thalassemia	Thalassemia with HCV	Control	<i>p</i> -value*
B19 IgM				0.0095
Positive	21(35)	12(20)	14(23.33)	
Negative	39(65)	48(80)	46(76.67)	
<i>p</i> -value†	0.0075	0.0001	0.0001	
B19 IgG				0.0001
Positive	13(21.67)	14(23.33)	11(18.33)	
Negative	47(78.33)	46(76.67)	49(81.67)	
<i>p</i> -value†	0.0001	0.0001		

Values were expressed as number and percentage. *n*=60 in each group.* Chi square test; † Fisher's exact test.

The B19V IgM Abs were positive in 35% of β -TM patients, while the β -TM patients infected by HCV recorded 20%, and 23.3% were positive in the controls ($p = 0.0095$). Meanwhile, 21.67% tested positive for B19V IgG Abs among the β -TM patients, 23.33% among the β -TM patients infected by HCV, and only 18.33% for the controls ($p = 0.0001$).

The B19V-IgM, IgG Abs, and B19V DNA frequencies related to age were also assessed in each age group. The B19V genome was found in 33 out of 120 (27.5%) patients and 8 out of 60 (13.3%) controls ($p = 0.078$) (Figure 2). The patients were allocated into three age groups, including ≤ 10 , 11-20, and > 20 years old, to explore the association between age and B19V prevalence in patients who were categorized into three age groups, including ≤ 10 , 11-20, and > 20 years old. The results of this investigation demonstrated that the patient's group had a greater B19 viral load than the control group. Five patients had $\geq 10^4$, another four had 10^5 , and only one had 10^6 copies/ml of B19 DNA. Besides, three participants in the control group had 10^3 , and two had 10^5 copies/ml ($p = 0.058$) (Figure 3).

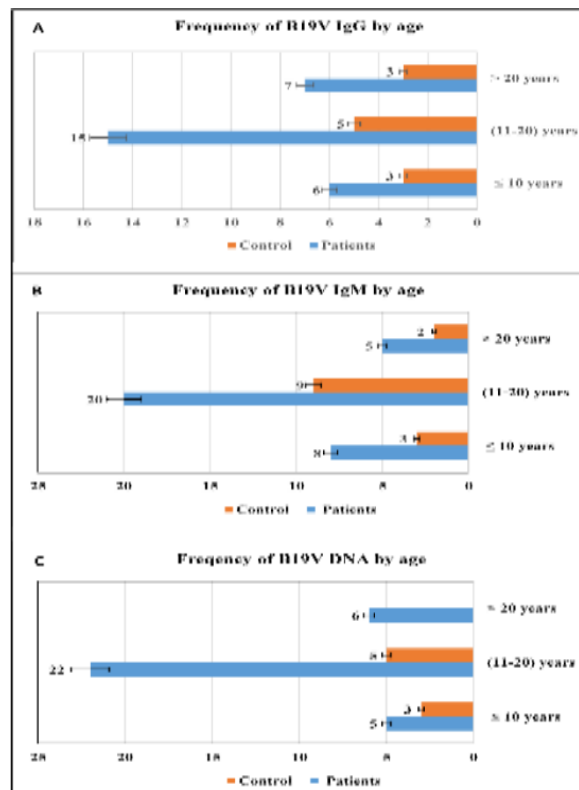


Figure 2: The Frequency in beta-thalassemia major patients: (A) B19V-IgG antibodies by age group, (B) B19V-IgM antibodies by age groups, (C) B19V DNA by age groups.

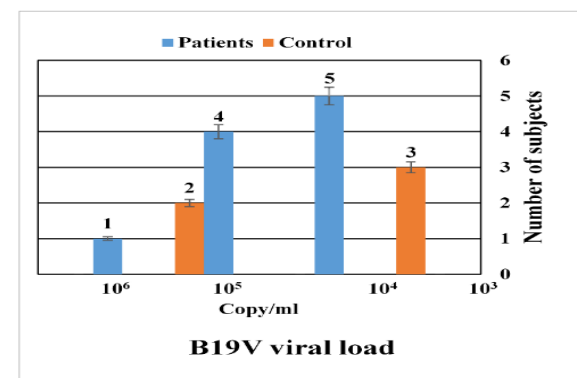


Figure 3: The distribution of age and B19V Copy number between different study groups.

The Parvovirus B19 DNA was extracted and then subjected to PCR to detect the B19V in β TM patients and controls. There were only 8 out of 120 (6.6%) who were positive for parvovirus VP1/VP2 with PCR

product size (288 bp) in β TM patients and none in the controls (Figure 4).

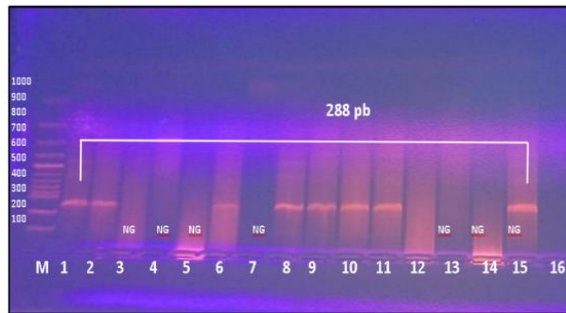


Figure 4: PCR products of human parvovirus (B19) on gel electrophoresis. Lane M: DNA marker (100-1000 bp), lanes 1–2, 6, 8–11, and 15: positive result for amplification (288 bp), lanes 3–5, 7, 12–14, and 16: negative result for amplification.

The PCR-positive samples were eight and subjected to the sequence to detect a 288 bp fragment of the VP1-VP2 region. The genotyping was done with reference sequences retrieved from the GenBank database to construct a phylogenetic tree. Only the eight PCR-positive samples were eligible for genotyping and deposition in NCBI GenBank. The B19V isolates had 100% homology with genotype 1a sequences from the Netherlands in the GenBank (acc. no. JN211139.1) (Figure 5).

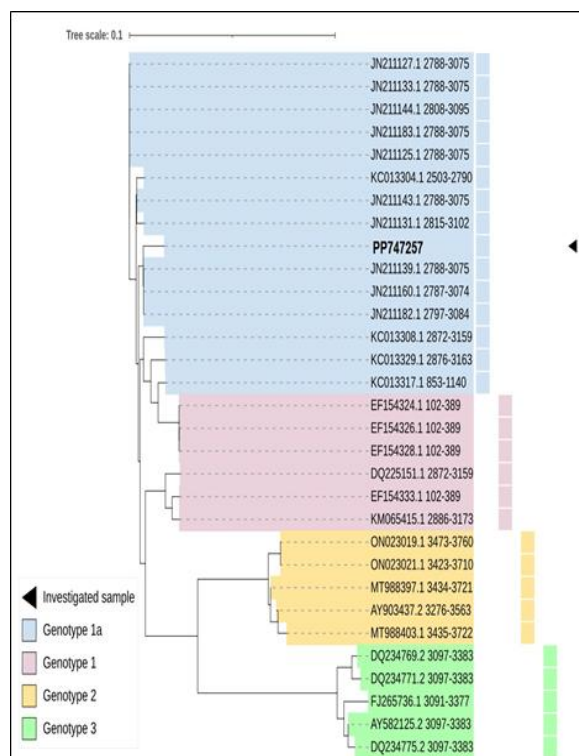


Figure 5: Phylogenetic tree of B19V isolated from Iraqi thalassemia patients compared to GenBank reference sequences based on VP1-VP2 region (288 bp). Genotype 1a was dominant in the Iraqi isolated strain (Black triangle D1 deposited in NCBI under acc no. PP747257.1).

DISCUSSION

This study aimed to evaluate the B19V prevalence and genotyping in studied groups (patients with β -TM and blood donors). The age of patients was a risk factor in adults, especially those β -TM patients infected with

HCV compared to β -TM patients; this finding was in line with local studies by Alnassar and Shallal (2023) in Al-Muthana Governorate and Hussein *et al.* (2021) in Diyala Province regarding the age, in which the β -TM patients are more prone to viral infection increased with age due to recurrent hemotherapy [16,17]. The age of β -TM patients was 16.03 ± 0.78 years, which is significantly different from the age means of β -TM patients with HCV and controls, agreeing with a study by Sadulla *et al.* (2020) carried out in Duhok Thalassemia Center, Iraqi Kurdistan, concluding that patients with β -TM in Iraq have a lesser median age, and the β -TM patients are in the necessity of more accurate methods to be addressed for blood donor screening [11]. Meanwhile, sex was not a risk factor; both males and females may be afflicted by β -TM disease regardless of their HCV status; this finding is compatible with a study by Lafta (2023) [8], Alnassar and Shallal (2023) [16], and Hussein *et al.* (2021) [17]. The serological studies suggest that over fifty percent of individuals contract the infection during childhood, with even higher elevation in the prevalence rates noted in children within certain tropical regions, including the Middle East region [18]. The findings of this study are consistent with several other investigations locally conducted by many researchers regarding the prevalence of B19V detected by serological methods. According to a study by Abdul Sadah and Al-Marsome (2020) in Baghdad, which focused on the detection of B19V (anti-IgG Abs) among β -TM patients, it was reported that 37% of patients were positive, which agrees with this study's findings [19]. Meanwhile, a study by Alnassar and Shallal (2023) in Al-Muthana Governorate reported only 11.7% of β -TM patients were positive for B19V using the B19V viral antigen detection method [16]. A study by Majeed (2020) in Tikrit City investigated 130 thalassemia patients in the age range of 20–40 years and 50 controls. It was reported that 11.5% were positive for B19V IgM Abs in the thalassemia patients' group; only 38.5% were positive for B19V IgG Abs in thalassemia patients and 4% in controls [20]. Moreover, the findings of this study regarding the control's positivity are in line with a study by Mohammed *et al.* (2022) in Diyala Governorate, who investigated the B19V positivity among the blood donors using B19V IgG Abs and reported that 33% of blood donors in the age range of 40–49 years were positive for B19V [21]. Another study by Abdelrahman *et al.* (2021) in Qatar investigated the seroprevalence of B19V (anti-B19V IgM and IgG); the study reported that 60% (561/930 tested samples) of blood donors were positive for anti-B19V IgG, while only 2% (20/930) were anti-B19V IgM positive [18]. The B19V can be readily transmitted through blood transfusion and with plasma-derived product therapy. Additionally, its viral load in such products varies between 2×10^1 and 1.3×10^3 copies/ml [22]. The mean of the B19V copy number was notably increased (21.58 ± 1.95) for controls compared to patients' groups. The current study finding is not consistent with a systematic review and meta-analysis study by Farahmand *et al.* (2021) regarding the prevalence of

the virus genome in blood donors, which was less than (1%) [23], but closely related to what was reported in an Iranian study by Zadsar *et al.* (2018); the titers of B19 DNA in (0.8%) of blood donors were more than 10^6 IU/mL (high-level B19 viremia), concluding that B19V testing for plasma-derived products seems important in Iranian donors [24]. The findings of this study align with a recent study by Seetha *et al.* (2021); the B19V prevalence rate was 7.2% (33 out of 456) among positive cases identified by nested PCR. These cases were subsequently subjected to validation through real-time PCR and Sanger sequencing, revealing that only three samples had viral loads below the detectable limit for real-time PCR confirmation [25]. A study by Keshavarz *et al.* (2022) investigated the seroprevalence of B19V, the DNA presence, as well as genotypes circulating in patients with hemophilia; the study reported that the cases registered 3% were anti-B19V IgM positive. There was a 47% anti-B19V IgG positive, and lastly, the detection of B19V DNA was 16%. The controls registered 38% as anti-B19V IgG positive. The B19V DNA was positive in 5% [26]. This study revealed that genotype 1a was registered in β -TM Iraqi patients, which is compatible with many local studies [9,16,19]. When it comes to regional countries, the only B19V circulating genotype in the Iranian study reported by Keshavarz *et al.* (2022) was genotype 1a in Mashhad City [26], which is an analogue to another study in Iran among children infected with measles [27]. In South India, Seetha *et al.* (2021) reported that all positive samples of B19V belonged to genotype 1a [25].

Study limitations

The study was carried out in a single center in Baghdad, and the patients' sample size was not large enough. The study did not investigate the prevalence of B19V according to the NS1-VP1u region (926 bp) by nPCR.

Conclusion

This study reports a high prevalence rate of active infection of B19V (35%) in β -TM patients compared to controls. Despite screening protocols used to clear blood and blood products from viral infections (HBV, HCV, and HIV), until now, there have been no guidelines or protocols for B19V screening during blood donations, which B19V may considerably reduce the prevalence rate of B19V. Preventive measures such as future vaccination against B19V infection must be implemented to reduce the prevalence of this blood-borne virus in this vulnerable population.

ACKNOWLEDGMENTS

The authors thank all medical staff of Ibn Al Balady Children and Maternity Hospital for their cooperation. The thanks are extended to all patients who agreed to donate their blood despite their affliction through lifelong illness. They also appreciate the blood donation from the controls.

Conflict of interests

No conflict of interest was declared by the authors.

Funding source

The authors did not receive any source of funds.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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