Original Article

Detection of Human Parvovirus (B19) in Beta Thalassemia Major Patients

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Abstract

Background: Beta-thalassemia major (β-TM) is inheritable condition with many complications especially in children. The blood-borne viral infection was proposed as a risk factor due to recurrent blood transfusion regimen (hemotherapy). **Objectives:** This study aimed to investigate Human parvovirus B19 (PVB19) prevalence in β-TM patients by serological and molecular means. **Materials and Methods:** This is a cross-sectional study incorporated 180 subjects, segregated into three distinct groups, namely, control (n = 60), β-TM (n = 60), and β-TM infected with hepatitis C Virus (HCV) (n = 60). The enzyme-linked immunosorbent assay for qualification detection of PVB19 was employed, and then real-time detection of PVB19 was done for revealing viral copy number in different groups, alongside other risk factors were explored. **Results:** Both PVB19 IgM and IgG antibodies positivity rates are higher among β-TM patients compared to controls, the PVB19 IgM (35%) and PVB19 IgG (21.67%) positivity in β-TM patients compared to 23.3% and 18.33% positivity in the controls was significantly observed. The mean of PVB19 copy number interestingly higher in control (21.58 ± 1.95) compared to β-TM patients infected with HCV (4.75 ± 1.58). Moreover, serum ferritin showed a significant increase in β-TM patients with HCV (4283.22 ± 351.92) compared to control (28.55 ± 1.06). **Conclusion:** Both PVB19 IgM and IgG Abs positivity rates are higher significantly among β-TM patients compared to controls. Although, the highest mean PVB19 copy number among controls, this finding was not significant. Nevertheless, screening high-risk groups including blood donors for PVB19 may considerably reduce the prevalence of PVB19.

Keywords: Beta-thalassemia major, detection, human parvovirus B19, prevalence

Introduction

Thalassemia is characterized by inherited disorders in β-globin chain synthesis leading chronic anemia due to red blood cells hemolysis and erythropoiesis dysfunction, the beta-thalassemia major (β-TM) is the most common form. The disorder is prevalent in the Eastern Mediterranean region, including Iraq.[1] Patients with β-TM are generally on a multiple blood transfusion routine usually before the age of 2 years.[2] β-TM is the most common type of hereditary anemia registered in Iraq with a prevalence of 35.7 per 100,000 in 2015.[3] The thalassemia prevalence over the past 5 years in Iraq has been raised from 12,106 cases in 2018 to 13,390 cases in 2022.[4] While some studies have proposed blood born viruses implicated on Iraqi patients with β-TM, including Epstein-Barr virus,[5] hepatitis C virus (HCV),[6,7] Sendai Virus,[8] and human parvovirus B19 (PVB19).[9,10]

PVB19 is a tiny naked virus with a nearly 23–26 nm diameter which has a single-stranded linear DNA genome of 5.6 kb.[11] During the screening of hepatitis B virus (HBV), the sample number containing the PVB19 in panel B and no.19.[12] Human erythroid progenitor cells are where PVB19 productive infection occur, so PVB19 was implicated as the etiologic agent of severe aplastic crises in children with sickle-cell disease in 1981.[13]

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Viral transmission routes including through respiratory droplets, vertical transmission from pregnant patients to fetus, and blood, infection with PVB19 during pregnancy is an important cause of intrauterine fetal death, stillbirth, and non-immune hydrops fetalis.[14] Although, PVB19 infection mainly causes self-limiting conditions in healthy subjects. Nevertheless, infection by PVB19 may occur in patients with inherited hemolytic anemia's causing different outcomes. High-titer replication may result in bone marrow suppression, triggering a life-threatening drop in hemoglobin values (transient aplastic crisis).[15]

This prompts crucial inquiries about the safety of blood transfusion and the potential risk of PVB19 during hemotherapy to vulnerable patients suffering from β-TM. At present, there is no agreement regarding the implementation of a PVB19 screening initiative for blood donations utilized in hemotherapy for β-TM patients. We initiated this study to investigate the prevalence of PVB19 among β-TM at a main thalassemia center in Baghdad. Moreover, different potential risk factors such as age and gender hematological parameters were explored.

Materials and Methods

Patients and control groups

This study was cross-sectional study included of (60) patients with β-TM and another (60) β-TM patients infected by HCV 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 served as control group were enlisted from healthy blood donors visiting the Iraqi National Center of Blood Transfusion, Baghdad city.

Inclusion and exclusion criteria

The patient's inclusion criteria included patients aged under 45 patients whom under regular blood transfusions and exclude patients aged older than 45 years. Furthermore, all patients suffering from autoimmune diseases and immunocompromised patients were excluded from the study. The study variables included demographic data, hematological parameters, and serum Ferritin level.

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 kits from Demeditec Company, Germany, using microplate system (wash, reader, and printer) from GloMax® Discover, USA.

Molecular detection of human parvovirus B19

Genomic deoxyribonucleic acid (DNA) was isolated using a commercial extraction kit sourced from Geneaid Company in Taiwan. Then the real-time polymerase chain reaction (PCR) protocol was used for quantitative detection of Parvovirus B19 using commercial kit from Sacace Company, Italy. After DNA extraction from plasma, then amplified using real-time amplification with fluorescent reporter dye probes specific for parvovirus B19 and internal control to determine virus copy number for parvovirus B19 was followed. The PCR thermal condition was applied: one cycle (95°C for 10 min), followed by five cycles for each (95°C for 10 s, 60°C for 20 s, and 72°C for 15s), and finally 40 cycles for each (95°C for 5 s, 60°C for 20 s *fluorescent signal detection*, and 72°C for 15 s) using rotor type real-time PCR system (Mx3005P™, Agilent, USA).

Statistical analysis

The data underwent coding and entry procedures using the statistical software SPSS version 25. Descriptive statistics including mean, standard deviation, median, minimum and maximum values for quantitative variables, the number and percentage for qualitative values were employed for data summarization. Categorical variables were represented by numbers and percentages, 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, independent sample test, and analysis of variance test for quantitative normally distributed variables. *P* value less than or equal to 0.05 was considered statistically significant.

Ethical consideration

The study protocol was approved by the College of Science, University of Baghdad Ethics Committee (CSEC/1223/0136 dated December 16, 2023) and in accordance with Iraqi Ministry of Health approval (No. 62751 dated October 22, 2023). A written consent was given by all participants or by their legal guardians.

Results

The β-TM patients (both HCV negative and HCV positive) ages ranged from 5 to 45 (mean, 18 ± 7.8 years), the median of age was 15 years in patients with β-TM −ve for HCV, the median of age was 26 years in patients with β-TM +ve for HCV, and the median of age was 24.5 years among the control group ($P < 0.01$). There was significant association between HCV −ve thalassemia patients' group and HCV +ve thalassemia patients' group ($P < 0.001$), also significant association between HCV −ve thalassemia patients' group and control group ($P < 0.001$). Meanwhile, there was no significant association between HCV +ve thalassemia patients' group and control group ($P = 0.059$) as shown in Figure 1.

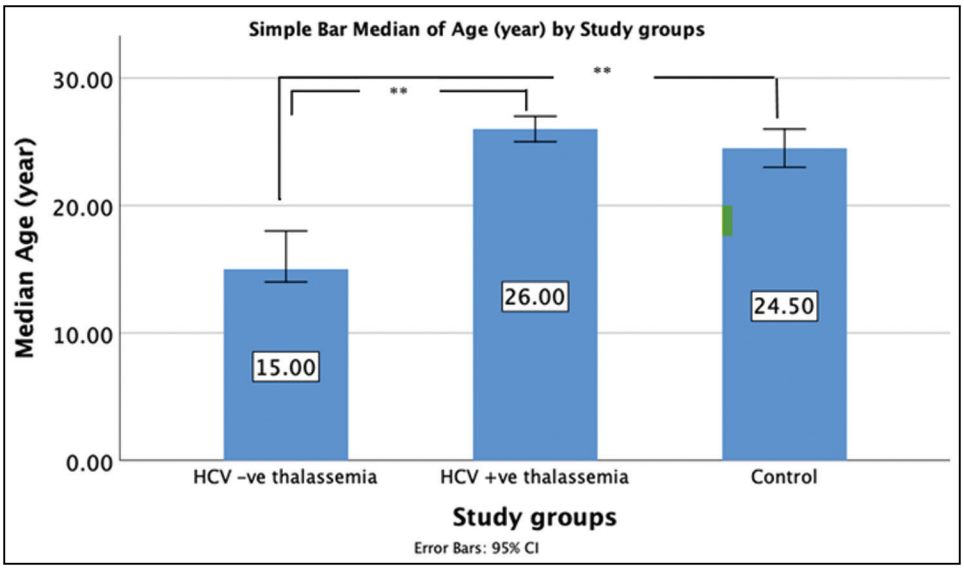

Figure 1: The median of age (year) in different study group

Table 1: Distribution of gender in different study groups

Demographic parameter		Thalassemia (No = 60)	Thalassemia with hepatitis C virus (No = 60)	Control (No = 60)	*P* value
Gender	Male	36 (60.00%)	31 (51.67%)	28 (46.67%)	0.169 NS
	Female	24 (40.00%)	29 (48.33%)	32 (53.33%)	
	P value	0.0498*	0.896 NS	0.641 NS	

*($P \leq 0.05$), NS: non-significant

Table 2: The prevalence of PVB19 IgM and IgG antibodies in different study groups

B19 antibodies prevalence		Thalassemia (No = 60)	Thalassemia with hepatitis C virus (No = 60)	Control (No = 60)	*P* value
B19 IgM	Positive	21 (35.00%)	12 (20.00%)	14 (23.33%)	0.0095**
	Negative	39 (65.00%)	48 (80.00%)	46 (76.67%)	
	P value	0.0075**	0.0001**	0.0001**	
B19 IgG	Positive	13 (21.67%)	14 (23.33%)	11 (18.33%)	0.0001**
	Negative	47 (78.33%)	46 (76.67%)	49 (81.67%)	
	P value	0.0001**	0.0001**		

**$P \leq 0.01$

The gender distribution among the β-TM patients and controls revealed the predominance of males 60% in β-TM patients' group, and 51.67% in β-TM patients +ve for HCV, and only 46.67% for controls; ($P = 0.169$). Contrary, the females were more predominate in the control group (53.3% *vs.* 40% for β-TM patients and 48.3% for the β-TM patients +v HCV) as shown in Table 1.

The prevalence of PVB19 IgM and IgG antibodies in different study groups is demonstrated in Table 2. The PVB19 IgM Abs were positive in (35%) of β-TM patients, the β-TM patients with infected by HCV recorded (20%), and (23.3%) were positive in the controls ($P = 0095$). Meanwhile, there were (21.67%) tested positive for PVB19 IgG Abs among the β-TM patients, (23.33%) among β-TM patients infected by HCV, and only (18.33%) for the controls ($P = 0001$) as shown in Table 2.

The distribution of PVB19 copy number among and age among the different study groups is illustrated in Table 3. The age mean of β-TM patients was (16.03 ± 0.78 years), the age means of β-TM patients with HCV (24.20 ± 0.71 years); ($P = 0.0001$). The age mean for controls was

Table 3: The distribution of age and PVB19 copy number between different study groups

Group	Age (year) Mean ± SE	PVB19 copy number Mean ± SE
Thalassemia	16.03 ± 0.78[b]	3.58 ± 1.41
Thalassemia with hepatitis C virus	24.20 ± 0.71[a]	4.75 ± 1.58
Control	24.63 ± 0.33[a]	21.58 ± 1.95
LSD value	1.784**	3.722 NS
P value	0.0001	0.243

LSD = least significant difference

Means having different letters in same column are differed significantly; ** $P \leq 0.01$; reference of PVB19 copy number = 2×10^3

Table 4: Platelets and serum ferritin level in different study groups

Group	Hematological parameters Mean ± SE	
	PLT ($10^3/\mu L$)	Ferritin (ng/mL)
Thalassemia	336.71 ± 15.47	4189.73 ± 357.93[a]
Thalassemia with hepatitis C virus	308.65 ± 21.42	4283.22 ± 351.92[a]
Control	301.06 ± 14.75	28.55 ± 1.06[b]
LSD value	48.773 NS	808.83**
P value	0.317	0.0001

LSD = least significant difference

Means having different letters in same column differed significantly, ** $P \leq 0.01$, NS: non-significant

24.63 ± 0.33 years. The mean of PVB19 copy number in β-TM patients was 3.58 ± 1.41, meanwhile in β-TM patients with HCV was 4.75 ± 1.58 and was 21.58 ± 1.95 for controls; ($P = 0.234$).

The hematological parameters including platelets and serum ferritin among different study groups are demonstrated in Table 4. The platelets showed no significant difference among the study groups. Meanwhile, serum ferritin showed a significant increase in β-TM patients with HCV (4283.22 ± 351.92) compared to control (28.55 ± 1.06).

Discussion

β-TM is a life-threatening condition with many complications which may lead to death especially in children. The blood-borne viral infection was proposed as a risk factor due to recurrent blood transfusion regimen (hemotherapy). In the current study, 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 a local studies by Alnassar and Shallal[9] in Al-Muthana Governorate, Hussein *et al.*[16] in Diyala Province, and Al-Sharifi *et al.*[17] in Babylon Province regarding the age, in which that the β-TM patients are more prone to viral infection increased with age due to recurrent hemotherapy. Meanwhile, the gender was not a risk factor, both males and females may be afflicted by β-TM disease regardless their HCV status, this finding comes compatible with a studies by Lafta[4], Alnassar and Shallal[9], Hussein *et al.*,[16] and Al-Sharifi *et al.*[17]

The serological studies suggests that over 50% of individuals contract infection during childhood, with even higher prevalence rates noted among children with sickly cell anemia in certain tropical regions, including middle east region.[18] The findings of this study are consistent with several other investigations locally conducted by many researchers regarding the prevalence of PVB19 detected by serological methods, according to a study by Abdul Sadah and Al-Marsome[19] in Baghdad focused on the detection of PVB19 (anti-IgG Abs) among β-TM patients it was reported a 37% of patients were positive which agrees with this study findings. Meanwhile, a study by Alnassar and Shallal[9] in Al-Muthana Governorate reported only 11.7% of β-TM patients were positive to PVB19 using viral antigen detection method. A study by Majeed[20] in Tikrit City investigated 130 thalassemia patients with age ranged (20–40 years) and 50 controls reported (11.5%) were positive for PVB19 IgM Abs in thalassemia patients' group only, (38.5%) were positive for PVB19 IgG Abs in thalassemia patients and (4%) in controls. Moreover, the findings of this study regarding the control's positivity are in line with a study by Mohammed *et al.*[21] in Diyala Governorate who investigated the PVB19 positivity among the blood donors using PVB19 IgG Abs and reported a (33%) of blood donors with age range (40–49 years) were positive to PVB19. Another study by Abdelrahman *et al.*[18] in Qatar investigated the seroprevalence of PVB19 (anti-PVB19 IgM and IgG), the study reported that a 60% (561/930 tested samples) of blood donors were positive to anti-PVB19 IgG, while only 2% (20/930) were anti-PVB19 IgM positive. In another study by Saud and Majid[22] in Babylon Province reported that, there is no significant difference in immunoglobulin level (IgG, IgM, and IgA) between splenectomized and non-splenectomized β-TM patients and control groups. Moreover, there is significant increase in mean frequency of infection/year for patients with β-TM in comparison to control, specifically, splenectomized patients are having significant increase in frequency of infection in comparison to non-splenectomized.

The PVB19 can be readily transmitted through blood transfusion and therapy with plasma-derived products. In addition, its viral load in such products varies between 2×10^1 and 1.3×10^3. The mean of PVB19 copy number was notable increased (21.58 ± 1.95) for controls compared to patients' groups.[23] The current study finding is not consistent with a systemic review and meta-analysis study by Farahmand *et al.*[24] regarding the prevalence of the virus genome in blood donors which was less than (1%), but closely related in what reported in an Iranian study by Zadsar *et al.*[25], the titers of B19 DNA in (0.8%)

of blood donors were more than 10^6 IU/mL (high level B19 viremia), concluded into PVB19 testing for plasma-derived products seems important in Iranian donors. The findings of this study align with those of Seeth *et al*. (2021), a PVB19 prevalence rate of 7.2% (33 out of 456) among positive cases identified by nested PCR. These cases 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.[26] Regarding the age mean 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 agrees with a study by Sadulla *et al.* (2020) carried in Duhok Thalassemia Center, Iraqi Kurdistan concluded to, patients with β-TM in Iraq have a lower median age, and the β-TM patients are in the need to introduce more sensitive methods for blood donor screening.[7]

While some Iraqi studies have focused on the iron chelation therapy in β-TM patients,[27] meanwhile Hasan and Saud (2023) in Basarh Governorate investigated Zinc and Copper in transfusion-dependent thalassemia (TDT) patients on different iron chelators reported that serum iron, ferritin, and zinc levels were significantly higher among patients with TDT, while the hemoglobin level was significantly lower than that in the healthy population ($P < 0.05$).[28] In another study explore the health-related quality of life in Iraqi Kurd patients including age and serum ferritin being identified as independent predictors.[29] A study by Sadullah *et al*.[7] assessed the complications and challenges facing the management of β-TM patients in Kurdistan region including serum ferritin level, iron overload, and HCV infection, meanwhile. The current study also investigates serum ferritin and platelets levels as hematological parameters in β-TM patients, there was a slight increment in mean level of platelets (336.71 ± 15.47) among the β-TM patients which in agreement with a study by Urio *et al.*[30], in which there was no association between PVB19 with platelets counts. Moreover, the mean of serum ferritin level in this study recorded (4189.73 ± 357.93) which come in agreement with Sadullah *et al.*[7] study, and another study by Al-Zuhairy *et al.*[31] also reported that β-TM patients had significantly higher serum ferritin which comes in accordance with this study findings.

Limitation of the study

The study was carried in single center in Baghdad, the patients sample size was not large enough, other risk factors such as iron overload, hepcidin serum levels were not explored.

Conclusion

In this study, both PVB19 IgM and IgG Abs positivity rates are higher significantly among β-TM patients compared to controls. Although, the highest mean PVB19 copy number among controls, this finding was not significant. Nevertheless, screening high-risk groups including blood donors for PVB19 may considerably reduce the prevalence of PVB19. Until now, there have been no guidelines or protocols for HPV-B19 screening during blood donations. Preventive measures such as screening of donated blood must be implemented to reduce the prevalence of this blood-borne virus in this vulnerable population.

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Conflicts of interest

There are no conflicts of interest.

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