RESEARCH ARTICLE

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Molecular and Serological Detection of Human Parvovirus B19 in a Sample of Iraqi Patients with Acute Lymphoid Leukemia in Relation to Hematological Parameters

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Abstract

Background: Acute Lymphoblastic Leukemia (ALL) is a common childhood blood cancer. Human Parvovirus B19 (PV-B19) mainly replicates in bone marrow erythroblasts and can persist long after initial infection. ALL patients frequently exhibit PV-B19 viremia, arising from either viral reactivation or coincidental infection amid acute leukemia. **Aims of the Study:** This study aimed to determine the prevalence PV-B19 infection in ALL patients, investigate its association with hematological parameters at presentation, and monitor these parameters and disease progression in relation to PV-B19 infection. **Patients and Methods:** This case-control study included 30 newly diagnosed acute lymphoblastic leukemia patients and 30 healthy blood donors as controls. Patients' data were collected through interviews or records. Plasma levels of anti-PV-B19 IgG and IgM antibodies were assessed by enzyme-linked immunosorbent assay (ELISA), and viral DNA quantification was performed using quantitative polymerase chain reaction (qPCR). **Results:** The IgG and IgM prevalence in patients was 36.67% and 3.33%, respectively, compared to 33.33% and 0% in controls. The molecular assay revealed 63.33% of patients were PV-B19 DNA positive, significantly higher than the 6.67% in controls. Patients had a significantly higher mean log10 viral load (5.17±1.39 copies/ml) than controls (2.64± 0.28 copies/ml) (P= 0.019). PV-B19 had no significant association with hematological parameters before chemotherapy; however, after chemotherapy, viral infection was significantly associated with reduced chemotherapy response and increased blood transfusion rate. **Conclusions:** PV-B19 infection seems to have more pronounce role in ALL after treatment initiation, where it associates with increased rate of blood transfusion and reduced response to chemotherapy. Therefore, PV-B19 should be considered in acute leukemia patients.

Keywords: Parvovirus- acute lymphoid leukemia- quantitative PCR

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Introduction

Human Parvovirus B19 (PV-B19) is a small, non-enveloped, single-stranded DNA virus in the Parvoviridae family and erythroparvovirus genus [1]. PV-B19 causes erythema infectiosum, transient aplastic crisis, arthropathy, non-immune hydrops fetalis in pregnant women, cardiomyopathy, and other tissue inflammations [2]. It can also induce persistent anemia in immunocompromised individuals [3]. Transmission primarily occurs via respiratory droplets, but has also been reported through blood transfusions, clotting factor concentrates, intravenous immunoglobulin infusion, and organ transplants [4].

The cellular receptor for PV-B19 in humans is the P antigen, which is found on mature erythrocytes, platelets, heart, liver, and lung tissues. The severity of PV-B19 infection is influenced by the age of the infected individual and their hematological and immunological status [5]. PV-B19 primarily infects human erythroid progenitor cells, leading to mild to severe hematological disorders [6]. Typically, individuals with malignant blood conditions are severely affected by the tropism of PV-B19 in erythroid progenitor cells [7]. PV-B19 is an important cause of anemia and cytopenia in patients with acute leukemia. PV-B19 infections are occasionally diagnosed in patients with leukemia and acute anemia during maintenance therapy [8, 9].

The diagnostic approach for PV-B19 involves detecting viral genomes or proteins and identifying anti-PV-B19 IgM and IgG [10]. The PV-B19 genome encodes the nonstructural protein NS1 and structural proteins VP1 and VP2 [11]. Approximately 50-80% of the human population is infected with PV-B19 during childhood, with anti-

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PV-B19 IgG antibodies reaching 70% in adults [12]. From 2000 to 2019, Iraq reported 8,570 childhood leukemia cases, averaging 429 annually. Leukemia constituted 32.96% of childhood cancers, with Acute Lymphoblastic Leukemia (ALL) being the most common, accounting for 33.56% [13]. Laboratory-confirmed infections increased the odds of ALL by 2.4-fold [14]. While some research has investigated the role of PV-B19 in ALL, few studies have focused on its role in preceding or triggering the disease [8, 15]. This study aims to determine the frequency and implications of PV-B19 infections in newly diagnosed Iraqi ALL patients before chemotherapy.

Materials and Methods

Patients and control

This case-control study involved 30 newly diagnosed acute lymphoblastic leukemia (ALL) patients aged 2 to 50 years, recruited from three hospitals in Baghdad, Iraq: the Baghdad Center for Hematology in Medical City, Al Imamein Al Kadhimein Medical City, and the Central Teaching Hospital of Pediatrics. Conducted from November 2018 to February 2019, the study included 30 age-matched healthy individuals as controls, sourced from a blood donation center in Al-Imamein Al Kadhimein Medical City and leftover samples from private laboratories. ALL diagnosis was based on clinical features, complete blood counts, bone marrow biopsy, and a new diagnosis pre-chemotherapy. Exclusion criteria included secondary leukemia, hereditary hemolytic anemia, and relapsing acute leukemia. ALL patients were monitored for one month to evaluate hematological parameters such as hemoglobin (Hb), packed cell volume (PCV), white blood cells (WBCs), neutrophils, lymphocytes, blast cells, and blood transfusion frequency. The study had the Institutional Review Board (IRB) approval from Al-Nahrain University College of Medicine (No. 123, November 5, 2018).

Samples collections

Four milliliters of blood were collected from all patients and controls using sterile EDTA tubes. Plasma was isolated by centrifuging at 1,600 rpm for 20 minutes, then aliquoted and stored at -40°C for future serological and molecular assays.

Serological detection of PV-B19

Enzyme-linked immunosorbent assay (ELISA) kits (Demeditec, Germany) were employed for qualitative detection of anti-PV-B19 IgM and IgG antibodies in both patients and controls. Microtiter plates were coated with recombinant PV-B19 antigen (VP1 protein), and samples were diluted per the manufacturer's instructions. In the IgM procedure, samples were further incubated with IgG-RF-Sorbent to eliminate competitive inhibition from specific IgG and remove rheumatoid factors. Diluted plasma samples were pipetted into wells and incubated. Unbound samples were removed through washing, and horseradish peroxidase-conjugated antibodies were added to bind specifically to IgM for the anti-PV-B19 IgM ELISA kit and to IgG for the anti-PV-B19 IgG kit. After incubation, excess samples were washed away, and a substrate solution was added, turning blue if the sample was positive. The reaction was stopped by adding a stopping solution, turning the color yellow. The optical density (OD) of each well was measured to determine color intensity. Sample absorptions were compared with cutoff standard values. A sample was considered positive if its value was over 20% above the mean absorbance of the cutoff control and considered negative if it was more than 15% below.

Viral DNA extraction

Viral DNA was extracted from plasma samples with a DNA-Sorb-B kit (Ref. K-1-1/B) (Sacace, Italy) following the manufacturer's guidelines. The purity of the extracted DNA was assessed using a NanoDrop (Analytica Gena, UK), and DNA with a 260/280 absorption ratio of 1.7-1.9 was deemed acceptable.

Molecular detection of PV-B19

DNA was amplified using the Parvovirus B19 Real-TM Quant kit (Ref. V49-50FRT) (Sacace, Italy) for qualitative and quantitative detection of PV-B19 DNA in plasma. The kit includes fluorescent probes specific for PV-B19 (VP1) and an internal control (IC) to monitor amplification and detect reaction inhibition. The master mix was prepared, and the thermal cycler (Mic/Australia) was programmed per the manufacturer's guidelines. For qualitative detection, a sample is positive for PV-B19 if the Ct value in the JOE (Yellow)/HEX/Cy3 channel is ≤ 35. Samples with a $Ct \leq 27$ in the FAM (Green) channel and no fluorescence in the JOE (Yellow)/HEX/Cy3 channel are negative. For quantitative detection, a standard curve was created using data from known standards (QS1 and QS2), plotting Ct values on the Y-axis and concentration (copies/µl) on the X-axis. Results were interpreted by crossing the fluorescence curve with the threshold line using real-time PCR software, and PV-B19 DNA concentration was calculated accordingly.

PV-B19 DNA Copies/ml=(PV-B19 DNA copies/specimen)/(IC DNA copies/specimen) X 8.2 x 10^4 copies/ml

Statistical analysis

Statistical analyses were performed using SPSS and Microsoft Excel 2013. Data underwent normality testing. Categorical data are shown as counts and percentages, with associations described using Fisher's exact test and the chi-square test (χ^2-test). Numerical data are presented as mean ± standard deviation or median and range. The independent sample t-test or Mann Whitney U-test compared two groups with normal and non-normal distributions, respectively. The significance level (P value) was set at ≤ 0.05.

Results

Demographic, laboratory and clinical characteristics of study population

This case-control study encompassed 30 newly

diagnosed ALL patients and 30 age-matched healthy controls. The mean age of ALL patients was 16.6±13.41 years (range 2-50), compared to 22.17±15.29 years (range 4-50) in the control group. A majority of both ALL patients (63.33%) and controls (50%) were 20 years or younger. Females predominated among patients (53.33%) but constituted only (16.67%) of the control group, yielding a female-to-male ratio of 2.57:1. Both groups exhibited comparable frequencies of anti-parvovirus IgG and IgM antibodies. However, the molecular assay revealed a significantly higher number of positive cases in ALL patients (63.33%) compared to controls (6.67%) (P=0.001), Table 1. Among ALL patients, (73.33%) responded to chemotherapy regardless of PV-B19 detection, determined by blast cell counts, where a response was defined as less than 5% blasts in bone marrow aspirate without peripheral blood blasts. No response was indicated if bone marrow blasts exceeded 5% .

Viral load among patients and controls

The molecular assay (qPCR) results were deemed the gold standard for viral positivity, with viral load calculated in copies/ml using a specific formula. Due to the high viral load, these values were transformed into the log10 formula to normalize data distribution. ALL patients exhibited the highest mean log10 viral load (5.17±1.39 copies/ml), significantly different (P=0.019) from the controls (2.64±0.28 copies/ml).

Sensitivity and specificity of IgG in PV-B19 detection

Using qPCR as the gold standard, the IgG-dependent ELISA technique's sensitivity and specificity for detecting PV-B19 infection in ALL patients were 78.95% and 63.64%, respectively (Table 2).

Table 1. Laboratory and Clinical Characteristics of Study Population

Variables	ALL Patients (n=30)	Controls (n=30)
	Frequency %	frequency (%)
Age, years		
≤ 20	19 (63.33%)	15 (50%)
21-40	9 (30%)	11 (36.67%)
≥41	2 (6.67%)	4 (13.33%)
Sex		
Male	14 (46.67%)	25 (83.33%)
Female	16 (53.33%)	5 (16.67%)
Anti-parvovirus IgM		
Negative	29 (96.67%)	30 (100)
Positive	1 (3.33%)	0 (0%)
Anti-parvovirus IgG		
Negative	19 (63.33%)	20 (66.67%)
Positive	11 (36.67%)	10 (33.33%)
Molecular detection		
Negative	11 (36.67%)	28 (93.33%)
Positive	19 (63.33%)	2 (6.67%)
Response to chemotherapy		
No	8 (26.67%)	----------
Yes	22 (73.33%)	

Table 2. The Sensitivity & Specificity of Anti-PV-B19 IgG for Detecting PV-B19 in ALL Patients, Using qPCR as the Gold Standard

PV-B19 DNA	Anti-PV-B19 IgG		Total	Sensitivity	Specificity
	Positive	Negative			
Positive	6	13	19	78.95%	63.64%
Negative	5	6	11		
Total	11	19	30		

Comparison of hematological indices and blood transfusion before and after chemotherapy in ALL patients

Before chemotherapy, there was a slight but non-significant decline in Hb concentration and PCV%. The most notable change was a significant drop in total WBC count, from a median of 10.75×10^3/ml before chemotherapy to 1.75×10^3/ml after chemotherapy (P=0.001). Neutrophil and leukocyte percentages remained similar with no significant differences. Notably, blood transfusions were significantly more frequent after chemotherapy than before (P=0.001), with a mean of 3.3±0.95 compared to 1.1±0.55 (see Table 3).

Association of PV-B19 infection with hematological indices in ALL patients

Before chemotherapy

Patients without viral infection exhibited higher Hb (8.15±1.91 g/dl) and PCV (26±2.98%) compared to those with viral infection (7.74±2.9 g/dl and 24.9±3.67%,

Table 3. Comparison of Hematological Indices and Blood Transfusion before and after Chemotherapy in ALL Patients

Variables	Before chemotherapy (n=30)	After chemotherapy (n=30)	p-value
Hb, (g/dl)			
Mean±SD	7.89±2.55	8.81±1.82	0.204†
Median (range)	8.05 (6.1-13.2)	8.6 (6.2-9.5)	
PCV%			
Mean±SD	24.0±3.35	27.32±2.84	0.108†
Median (range)	24.9 (9.3-37.9)	25.4(17.28.7)	
Total WBC ($\times10^3$/ml)			
Mean±SD	25.69±34.3	1.93±0.87	<0.001‡
Median (range)	10.75 (2.2-141)	1.75 (0.2-3.5)	
Neutrophil%			
Mean±SD	24.94±19.91	28.16±23.95	0.779‡
Median (range)	21.3 (11.3=79.5)	21.3 (9.8-96.9)	
Lymphocyte%			
Mean±SD	61.37±24.58	63.13±25.0	0.657‡
Median (range)	64.2 (12.3-96.9)	70.5 (8.8-90.5)	
Blood transfusion requirement (Unit)			
Mean±SD	1.1±0.55	3.3±0.95	<0.001†
Median (range)	1.0 (0-2)	3.0 (2-5)	

†, Student t-test; ‡, Mann Whitney U-test

Table 4. Association of PV-B19 with Hematological Indices in ALL Patients before Chemotherapy

Variables	With viral infection (n=19)	Without viral infection (n=11)	p-value
Hb (g/dl)			
Mean±SD	7.74±2.9	8.15±1.91	0.141†
Median (range)	7.2(5.1-13.2)	8.2 (5.9-11.7)	
PCV%			
Mean±SD	24.9±3.67	26.0±2.98	0.134†
Median (range)	24.5 (12.3-37.9)	24.8 (15.5-33.9)	
Total WBC (×103/ml)			
Mean±SD	21.9±1.05	32.16±36.71	0.518‡
Median (range)	4.8 (22-141)	25.9 (26-114.4)	
Neutrophil%			
Mean±SD	29.84±22.45	16.49±10.9	0.127‡
Median (range)	28.0 (11.7±79.5)	14.3 (13.2-32.2)	
Lymphocyte%			
Mean±SD	56.921±25.23	69.05±22.44	0.168‡
Median (range)	55.7 (12.3-93.9)	73.8 (15.15-96.5)	
Blood transfusion requirement (Unit)			
Mean±SD	1.05±0.62	1.18±0.4	0.543†
Median (range)	1.0 (0-1)	1.0 (1-2)	

†, Student t-test; ‡, Mann Whitney U-test

respectively), but the differences were not significant (P= 0.141 and 0.134, respectively). Leukocytosis and lymphocytosis were not statistically significant in ALL patients with viral infection (P= 0.518 and 0.168, respectively). No significant difference was observed in blood transfusions between the two groups (P= 0.543) (Table 4).

Table 5. Association of Parvovirus B19 with Hematological Indices and Blood Transfusion in ALL Patients after Chemotherapy

Variables	With viral infection (n=19)	Without viral infection (n=11)	p-value
Hb (g/dl)			
Mean±SD	7.73±0.7	7.95±1.1	0.488†
Median (range)	7.7 (5.8-9.2)	7.6 (6.9-9.5)	
PCV%			
Mean±SD	24.26±2.9	24.43±2.8	0.871†
Median (range)	24.6 (17-28)	23.6 (21.2-28.7)	
Total WBC (× 103/ml)			
Mean±SD	1.84±0.87	2.09±0.89	0.863‡
Median (range)	1.7 (0.5-3.5)	2.0 (0.2-3.1)	
Neutrophil%			
Mean±SD	30.44±26.0	24.2±20.5	0.533‡
Median (range)	23.8 (2.8-95.9)	15.0 (4.2-55.7)	
Lymphocyte%			
Mean±SD	63.54±24.07	62.42±27.71	0.376‡
Median (range)	70.8 (2.8-90.2)	66.3 (17.4-90.5)	
Blood transfusion requirement (Unit)			
Mean±SD	3.58±0.96	2.82±0.75	0.032†
Median (range)	4.0 (2-5)	3.0 (2-4)	
Response to chemotherapy			
No	6(31.58%)	2(18.18%)	0.029††
Yes	13(68.42%)	9(81.82%)	

†, Student t-test; ‡, Mann Whitney U-test; ††, Chi-square

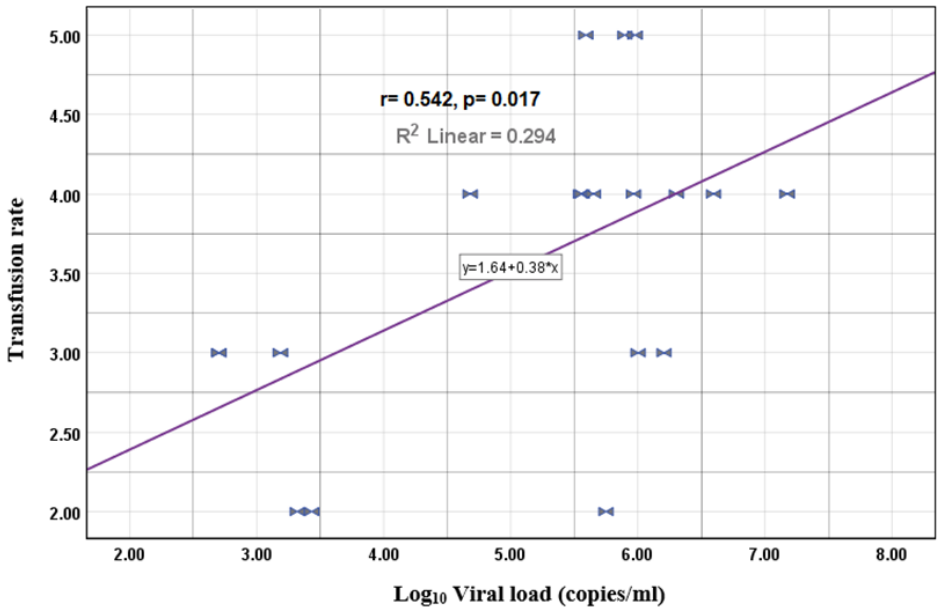

Figure 1. Correlation between log10 Viral Load of PV-B19 and Blood Transfusion in ALL Patients

After chemotherapy

Most hematological indices were similar between patients with and without viral infection, showing no significant differences. However, patients with viral infection required more blood transfusions (3.58±0.96) compared to those without (2.82±0.75), which was statistically significant (P=0.032). The response to chemotherapy also differed significantly with the detection of PV-B19 (P=0.029) (Table 5).

Correlation between Blood transfusion and Viral Load:

Pearson's correlation test indicated a moderate positive correlation between log10 viral load and blood transfusion times (r = 0.542, P = 0.017), as depicted in Figure 1.

Discussion

This study examined the role of PV-B19 in patients with ALL. The findings showed that 3.33%, 36.6%, and 63.33% of leukemia patients tested positive for anti-PV-B19 IgM, IgG, and viral DNA, respectively, compared to 0%, 33.33%, and 6.67% in healthy controls (Table 1). Similarly, a local study by [16] investigated 45 ALL patients and 45 healthy controls using ELISA and qPCR, revealing higher PV-B19 prevalence among patients. The seropositivity rates for IgM and IgG were 15.6% and 40% in patients, compared to 4.4% and 13% in controls, with viral DNA detected in 13.3% of patients and 0% of controls. Another studies [17, 18] in apparently healthy children aged 1-15 years, found anti-PV-B19 IgG and PV-B19 DNA in 10% and 2%, respectively. In Egypt, Soliman et al. conducted a study involving 39 ALL patients undergoing chemotherapy and 30 age-matched healthy controls. Serum IgG was detected in 66.67% of patients and 60% of controls, with no IgM detected in either group. Nested PCR revealed viral DNA in 28.2% of patients and none of the controls [19]. In Saudi Arabia, another study found that 37.5% of ALL patients during maintenance chemotherapy tested positive for IgG, with 25% of patients and none of the controls testing positive for IgM and conventional PCR detected viral DNA in 20% of patients but not in controls [20].

Studies consistently show that PV-B19 incidence is significantly higher in leukemia patients compared to healthy controls, despite differing rates of seropositivity or DNA detection. This discrepancy can be attributed to factors such as geographical virus distribution, socioeconomic standards, blood transfusion frequency, nosocomial infection risk, viral detection methods, sample size variation, and patient immunosuppression levels [2]. In general, three main explanations for the high PV-B19 rate in acute leukemia contexts are: reactivation of latent virus due to immunosuppression, coincidental acquisition during cytotoxic treatment or blood transfusion, and acquisition before leukemia onset suggesting the virus may contribute to leukemia initiation [21, 22].

Anti- PV-B19 IgG sensitivity and specificity in ALL patients are 78.95% and 63.64%, respectively, as shown in Table 2. Only one ALL case tested positive for IgM, indicating the unreliability of serological tests for detecting parvovirus PV-B19 in these patients. This aligns with studies reporting no IgM levels in leukemia patients [19]. Other studies have shown IgM seropositivity rates as high as 31.1% [23] and 26% [24]. IgG appears 16 days post-infection and persists for years or a lifetime [7], suggesting past or current reactivation rather than active infection [25].

The elevation in Hb level and PCV%, and reduction in WBC after chemotherapy are anticipated in acute leukemia patients since chemotherapy targets tumor cells and partially restores normal hematopoiesis. A cause-effect relationship between these indices and blood transfusion is likely. Chemotherapy, especially during induction, is cytotoxic to bone marrow, exacerbating anemia and necessitating blood transfusion, which then

elevates Hb and PCV levels. Thus, the increase in blood transfusion time, rather than chemotherapy, is likely the primary cause of increased Hb and PCV% [26]. However, most patients in this study were receiving maintenance therapy during the second blood sampling. Maintenance therapy is generally less cytotoxic than induction therapy, allowing for nearly normal hematopoiesis, as shown in Table (3). Prior to treatment, no significant differences in hematological indices were observed between individuals with viral infections and those without (Table 4). This suggests that ALL has a greater impact on anemia than viral infection. Acute leukemia is known to be associated with anemia and may cause pancytopenia [27] due to the extensive infiltration of malignant cells in the bone marrow, reducing normal blood cell production, including red blood cells [28]. Notably, this study found a significant correlation between PV-B19 infection and the need for blood transfusions in ALL patients post-chemotherapy, while the virus did not affect anemia. It is plausible that frequent transfusions prevent anemia in PV-B19-infected individuals, aligning with Azzay et al.'s findings [29] in 80 children with hematological disorders, including ALL, where blood transfusion frequency was higher in infected patients. This outcome is corroborated by a previous study [30], although at least one study did not find such an association [31].

PV-B19 infection and blood transfusions exhibit a reciprocal relationship, with the virus transmitted through frequent transfusions [11]. The pooled prevalence of B19V DNA, anti-B19V IgM, and anti-B19V IgG among blood donors was 0.4%, 2.2%, and 50.1%, respectively [32]. Notably, patients with PV-B19 infection face a higher risk of developing aplastic anemia and may require more transfusions than virus-free individuals. Evidence and prior studies indicate that PV-B19 infection increases the need for transfusions [33], as well as, most research reports low seroprevalence among blood donors and the infection rate declines with age, suggesting low infection rates among adult blood donors [8, 33]. In this study, the regression equation supports this, attributing about 30% of transfusions to viral infection (Figure 1), considering transfusions as the dependent variable and log10 viral load as the independent variable.

This study revealed a significant inverse relationship between PV-B19 and treatment response (Table 5), consistent with previous reports of treatment failures in Acute Myeloid Leukemia patients with PV-B19 infection [34]. For leukemia patients, assessing viral infection status is essential for optimal therapy [35]. PV-B19 can cause persistent infection post-acute phase, potentially remaining in host tissue for years or a lifetime [36, 37]. In this study, a peripheral blast percentage below 5% post-induction therapy was deemed a successful response. Despite this criterion, most patients responded, while a minority did not. The significantly higher number of unresponsive patients in the virus-infected group lacks a clear explanation. Current literature provides no evidence that the virus interferes with chemotherapeutic drugs. However, this finding supports the virus's role in the pathogenesis of acute leukemia. It is plausible that the virus contributes to the malignant transformation of bone marrow, leading to the continuous production of newly transformed blood cells despite chemotherapy [7, 38]. These data collectively suggest that PV-B19 may not affect hematological indices or blood transfusion rates in ALL patients before chemotherapy. However, post-treatment, PV-B19 infection may be associated with increased blood transfusions and treatment resistance in ALL patients.

Author Contribution Statement

Rahema conducted the laboratory work and drafted the paper as part of his MSc thesis. Dr. Al-Shuwaikh designed, interpreted, critical review and supervised the study. Dr. Tameemi assisted with sample collection and clinical aspects. All authors read, arranged, and approved the final manuscript.

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Ethical approval

This study received approval from the Institutional Review Board (IRB) of Al-Nahrain University College of Medicine (No. 123, November 5, 2018).

Registering Authority

This study is part of Mohammed A. Rahema's MSc thesis, endorsed by the Medical College of Al-Nahrain University, supervised by Prof. Dr. Arwa M. Al-Shuwaikh and Prof. Dr. Waseem F. Al Tameemi.

Conflicts of interest

There were no conflicts of interest

References

1. Holterhus M, Hennies M, Hillmann H, Thorer H, Rossig C, Burkhardt B, et al. Parvovirus b19 infection in pediatric allogeneic hematopoietic cell transplantation - single-center experience and review. Transpl Infect Dis. 2023;25(2):e14028. https://doi.org/10.1111/tid.14028.
2. Rahema MA, Al-Shuwaikh AM, Al-Tameemi WF. Role of Human Parvovirus B19 in persistence anemia in a sample of Iraqi patients with acute myeloid leukemia. Biochem Cell Arch. 2020;20(2):5165-5172.
3. Pakkyara A, Jha A, Al Salmi I, Siddiqi WA, Al Rahbi N, Kurkulasurya AP, Mohsin J. Persistent anemia in a kidney transplant recipient with parvovirus B19 infection. Saudi J Kidney Dis Transpl. 2017;28(6):1447-1450. https://doi.org/10.4103/1319-2442.220846.
4. Ege Akdi Ö, Aykaç K, Cengiz AB, Özsürekçi Y. Parvovirus B19 infection in children: Is it more severe than expected?. Turk J Pediatr. 2024;66:625-629. https://doi.org/10.24953/turkjpediatr.2024.4895
5. Agrawal M, Paul RT, Pamu P, Avmr N. Parvovirus b19 induced transient aplastic crisis in an immunocompetent child. Turk Patoloji Derg. 2015;31(2):158-60. https://doi.org/10.5146/tjpath.2014.01263
6. Luo Y, Qiu J. Human parvovirus B19: a mechanistic

overview of infection and DNA replication. Future Virol. 2015;10(2):155-167. https://doi.org/10.2217/fvl.14.103.
7. da Costa AC, Bendit I, de Oliveira ACS, Kallas EG, Sabino EC, Sanabani SS. Investigation of human parvovirus B19 occurrence and genetic variability in different leukaemia entities. Clin Microbiol Infect. 2013;19(1):E31-E43. https://doi.org/10.1111/1469-0691.12058.
8. Kishore J, Sen M, Kumar A, Kumar A. A pilot study on parvovirus B19 infection in paediatric haematological malignancies. Indian J Med Res. 2011;133(4):407-13.
9. Fritch Lilla SA, Burgett SE, McGann KA, Wechsler DS. Persistent and Prolonged Parvovirus B19 Viremia in a Pediatric Patient With Acute Lymphoblastic Leukemia. J Pediatric Infect Dis Soc. 2015;4(3):e38-40. https://doi.org/10.1093/jpids/piu112.
10. Gallinella G. Parvovirus B19 achievements and challenges. ISRN Virology. 2013;2013:33.
11. Juhl D, Hennig H. Parvovirus B19: What Is the Relevance in Transfusion Medicine? Front Med (Lausanne). 2018;5:4. https://doi.org/10.3389/fmed.2018.00004.
12. Algwaiz G, Alharbi A, Alsehaim K, Alahmari A, El Fakih R, Aljurf M. Hematologic Manifestations of Parvovirus B19 Infection. Hematology/Oncology and Stem Cell Therapy. 2023;16(4):316-322. https://doi.org/10.56875/2589-0646.1031
13. AL-Hashimi MMY. Incidence of Childhood Leukemia in Iraq, 2000-2019. Asian Pac J Cancer Prev. 2021;22(11):3663-3670. https://doi.org/10.31557/APJCP.2021.22.11.3663
14. Hwee J, Tait C, Sung L, Kwong JC, Sutradhar R, Pole JD. A systematic review and meta-analysis of the association between childhood infections and the risk of childhood acute lymphoblastic leukaemia. Br J Cancer. 2018;118(1):127-137. https://doi.org/10.1038/bjc.2017.360.
15. Ibrahem WN, Hasony HJ, Hassan JG. Human parvovirus B19 in childhood acute lymphoblastic leukaemia in Basrah. J Pak Med Assoc. 2014;64(1):9-12.
16. Abdullah IM, Abdullah SF, Al-Jadiry MF. Serological and molecular detection of parvovirus b19 in children with acute lymphoblastic leukemia. J Fac Med Baghdad. 2014;56(3):318-21.
17. Khudair EA, Al-Shuwaikh AM, Abdoun DS. Detection of Human Parvovirus B19 IgG in a group of Iraqi children with newly onset type 1 diabetes mellitus. Iraqi JMS. 2024;22(2):212-216. https://doi.org/10.22578/IJMS.22.2.4
18. Khudair EA, Al-Shuwaikh AM, Abdoun DS. Detection of Human Parvovirus B19 IgG and DNA in a group of Iraqi children with newly onset type 1 diabetes mellitus. Afr J Biol Sci. 2024;6(6):11-22. https://doi.org/10.33472/AFJBS.6.6.2024.11-22.
19. Soliman Oel-S, Abd El-Aal Hegazi Hasan M, El-Ashry R, Zaghloul MH, Kora B. Parvovirus B19 infection in pediatric oncology patients: diagnostic value of clinical and serologic parameters compared with nested PCR. J Pediatr Hematol Oncol. 2009;31(3):173-6. https://doi.org/10.1097/MPH.0b013e3181983b2d.
20. Zaki Mel S, Hassan SA, Seleim T, Lateef RA. Parvovirus B19 infection in children with a variety of hematological disorders. Hematology. 2006;11(4):261-6. https://doi.org/10.1080/10245330600841089.
21. Kerr JR, Mattey DL. The role of parvovirus B19 and the immune response in the pathogenesis of acute leukemia. Rev Med Virol. 2015;25(3):133-55. https://doi.org/10.1002/rmv.1830.
22. Cangerana Pereira FA, Mirra AP, Dias de Oliveira Latorre MD, de Assunção JV. Environmental risk factors and acute lymphoblastic leukaemia in childhood. Revista Ciencias de la Salud. 2017;15(1):129-44.
23. Zaki ME, Ashray RE. Clinical and hematological study for Parvovirus b19 infection in children with acute leukemia. Int J Lab Hematol. 2010;32(2):159-66. https://doi.org/10.1111/j.1751-553X.2009.01150.x.
24. El-Mahallawy HA, Mansour T, El-Din SE, Hafez M, Abd-el-Latif S. Parvovirus B19 infection as a cause of anemia in pediatric acute lymphoblastic leukemia patients during maintenance chemotherapy. J Pediatr Hematol Oncol. 2004;26(7):403-6. https://doi.org/10.1097/00043426-200407000-00001.
25. Wargo JA, Reuben A, Cooper ZA, Oh KS, Sullivan RJ. Immune Effects of Chemotherapy, Radiation, and Targeted Therapy and Opportunities for Combination With Immunotherapy. Semin Oncol. 2015;42(4):601-16. https://doi.org/10.1053/j.seminoncol.2015.05.007.
26. DeZern AE, Williams K, Zahurak M, Hand W, Stephens RS, King KE, et al. Red blood cell transfusion triggers in acute leukemia: a randomized pilot study. Transfusion. 2016;56(7):1750-7. https://doi.org/10.1111/trf.13658.
27. Jalaeikhoo H, Kashfi SMH, Azimzadeh P, Narimani A, Gouhari Moghadam K, Rajaienejad M, et al. Acute Myeloid Leukemia as the Main Cause of Pancytopenia in Iranian Population. Iran J Pathol. 2017;12(3):265-271.
28. Chiarini F, Lonetti A, Evangelisti C, Buontempo F, Orsini E, Evangelisti C, et al. Advances in understanding the acute lymphoblastic leukemia bone marrow microenvironment: From biology to therapeutic targeting. Biochim Biophys Acta. 2016;1863(3):449-463. https://doi.org/10.1016/j.bbamcr.2015.08.015.
29. Azzazy EA, Shaheen AA, Mousaad AA, Abdel Salam MM, Ibrahim RA. The prevalence of human parvovirus B19 infection in children with a variety of hematological disorders. Egyptian J Hematol. 2013;38(3):115-121. https://doi.org/10.7123/01.EJH.0000430749.56529.5a
30. Lindblom A, Heyman M, Gustafsson I, Norbeck O, Kaldensjö T, Vernby A, et al. Parvovirus B19 infection in children with acute lymphoblastic leukemia is associated with cytopenia resulting in prolonged interruptions of chemotherapy. Clin Infect Dis. 2008;46(4):528-36. https://doi.org/10.1086/526522.
31. Kuo SH, Lin LI, Chang CJ, Liu YR, Lin KS, Cheng AL. Increased risk of parvovirus B19 infection in young adult cancer patients receiving multiple courses of chemotherapy. J Clin Microbiol. 2002;40(11):3909-12. https://doi.org/10.1128/JCM.40.11.3909-3912.2002.
32. Farahmand M, Tavakoli A, Ghorbani S, Monavari SH, Kiani SJ, Minaeian S. Molecular and serological markers of human parvovirus B19 infection in blood donors: A systematic review and meta-analysis. Asian J Transfus Sci.2021;15(2):212-222. https://doi.org/10.4103/ajts.ajts_185_20
33. Kumar S, Gupta RM, Sen S, Sarkar RS, Philip J, Kotwal A, Sumathi SH. Seroprevalence of human parvovirus B19 in healthy blood donors. Med J Armed Forces India. 2013;69(3):268-72. https://doi.org/10.1016/j.mjafi.2012.11.009.
34. Tüfekçi Ö, Yilmaz Bengoa Ş, Erdem M, Ören H. Induction Failure in Acute Leukemia or Parvovirus B19 Infection? J Pediatr Hematol Oncol. 2017;39(7):565. https://doi.org/10.1097/MPH.0000000000000866.
35. Al-Shuwaikh AM, Hanna DB, Al-Obaidi AB, Jasim GA. Molecular Detection of Epstein - Barr virus and Human Herpesvirus-6 in a Sample of Iraqi Patients with Acute Leukemia. Int J Res Pharm Sci. 2020;11(2):2234-2240
36. Norja P, Hokynar K, Aaltonen LM, Chen R, Ranki A, Partio EK, et al. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue.

Proc Natl Acad Sci U S A. 2006;103(19):7450-3. https://doi.org/10.1073/pnas.0602259103.
37. Söderlund M, von Essen R, Haapasaari J, Kiistala U, Kiviluoto O, Hedman K. Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy. Lancet. 1997;349(9058):1063-5. https://doi.org/10.1016/S0140-6736(96)09110-6.
38. Vasconcelos GM, Christensen BC, Houseman EA, Xiao J, Marsit CJ, Wiencke JK, et al. History of Parvovirus B19 infection is associated with a DNA methylation signature in childhood acute lymphoblastic leukemia. Epigenetics. 2011;6(12):1436-43. https://doi.org/10.4161/epi.6.12.18464.