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Seroprevalence and Molecular Detection of Human Parvovirus B19 in Beta Thalassemia Major Patients

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Abstract

Beta thalassemia major (BTM) is a genetic disorder that has been linked to an increased risk of contracting blood-borne viral infections, primarily due to the frequent blood transfusions required to manage the condition. One such virus that can be transmitted through blood is the Human Parvovirus B19 (B19V). The aim of this study was to investigate the frequency and molecular detection of B19V. This study included 60 blood donors as controls and 120 BTM patients. B19V was identified by serology, which measured B19-IgG and B19-IgM antibodies. Nested Polymerase Chain Reaction (nPCR) was employed to target the VP1/VP2 structural proteins. The results showed that B19V seropositivity represents 27.5% (33 out of 120) in BTM patients, and only 8 out of 60 subjects represents (13.3%) in the control group (P-value 0.078). Notably, male patients exhibited a significantly higher prevalence of B19-IgM and B19-IgG antibodies, with 32% and 24% of males testing positive, respectively, compared to female patients. Elevated levels of Aspartate and Alanine Transaminase were observed with values of 51.94 ± 50.09 and 46.81 ± 50.20 , respectively. Additionally, nPCR analysis detected B19V DNA in 4.16% (5 out of 120) of BTM patients, while no positive results were detected in the control group. Screening the blood and blood products for the virus in high-risk group can considerably reduce the prevalence. Preventive measures are required in such vulnerable population.

Keywords: Human Parvovirus, Beta Thalassemia Major, Seroprevalence, nPCR

الانتشار المصلي والكشف الجزيئي لفيروس البارفو بي 19 البشري في مرضى الثلاسيميا الكبرى نوع بيتا

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الخلاصة

الثلاسيميا الكبرى نوع بيتا (BTM) هو اضطراب وراثي مرتبط بزيادة خطر الإصابة بالعدوى الفيروسية المنقولة، ويرجع ذلك أساساً إلى عمليات نقل الدم المتكررة اللازمة لإدارة الحالة. أحد هذه الفيروسات التي يمكن أن تنتقل عن طريق الدم هو البارفو بي 19 البشري (B19V). الهدف من هذه الدراسة هو التحري عن التكرار والكشف الجزيئي عن B19V. شملت هذه الدراسة 60 متبرعاً بالدم كمجموعة ظابطه و 120 مريضاً من مرضى BTM. تم التعرف عن B19V باستخدام علم المصل، الذي قام بقياس الأجسام المضادة B19-IgG و B19-IgM. تم استخدام تفاعل البوليميراز المتسلسل المتداخل (nPCR) لاستهداف البروتينات الهيكلية VP1/VP2. أظهرت النتائج أن الأيجابية المصلية لـ B19V تمثل نسبة 27.5% (33 من 120) في مجموعة مرضى BTM، ويمثل 8 فقط من أصل 60 شخصاً تمثل نسبة 13.3% في المجموعة (P-value 0.078). والجدير بالذكر أن المرضى الذكور أظهروا انتشاراً أعلى بكثير للأجسام المضادة B19-IgG و B19-IgM، حيث كانت نسبة 32% و 24% من الذكور إيجابية للأختبار، على التوالي، مقارنة بالمرضى الإناث. كما لوحظت مستويات مرتفعة من الأسبارتات وألانين ترانساميناز بقيم 50.09 ± 51.94 و 50.20 ± 46.81 على التوالي. بالإضافة إلى ذلك، كشف تحليل nPCR عن الحمض النووي لفيروس B19V في 4.16% (5 من 120) من مرضى BTM، بينما لم يتم الكشف على أي نتائج إيجابية في المجموعة الظابطه. يمكن أن يؤدي فحص الدم ومنتجات الدم بحثاً عن الفيروس في المجموعة المعرضة للخطر إلى تقليل انتشار الفيروس بشكل كبير. هناك حاجة إلى تدابير وقائية في مثل هذه الفئة المعرضة للأصابة.

1. Introduction

Beta thalassemia (BTM) is an inherited autosomal recessive disorder, due to reduction or absence of synthesis of beta globin chain of haemoglobin. In its homozygous state it leads to the transfusion dependent thalassemia major. BTM are generally on a hyper-transfusion regimen usually before the age of 2 years [1]. In Iraq, the thalassemia prevalence escalated between 2010 and 2015 registered 33.5 to 37.1 per 100,000 [2]. Over the past five years, the prevalence of thalassemia in Iraq has increased significantly, rising from 12,106 cases in 2018 to over 13,000 cases as of 2022 [3]. Both males and females are equally affected by BTM [4]. Several studies have focused on different risk factors and complications with BTM [5-7]. The transfusion-dependent thalassemia (TDT) patients mainly acquire transfusion-transmitted infections (TTIs), including viruses that infected the liver “hepatitis C virus HCV or hepatitis B virus (HBV)” [8]. Recent studies have suggested that other viruses, such as SEN virus, Epstein-Barr virus, and Human Parvovirus B19 (B19), may also affect patients with thalassemia-dependent transfusion (TDT) [9-11].

Human Parvovirus B19 (B19V) is a tiny naked icosahedral single stranded DNA virus (nearly 5.5 kb) belonging to genus *Erythrovirus* (*Parvoviridae* Family) [12]. Due to its strong affinity for erythroid progenitor cells, B19V can cause a transient red cell aplasia, which could be encountered not only in TM, but also with other inherited haemolytic anemias. This might eventually exacerbate anemia and temporarily stop bone marrow erythropoiesis, which is indicative of a transitory aplasia crisis [13]. In children with sickle-cell disease, B19V has been identified as the causative agent of severe aplastic crises, which can progress into a number of potentially fatal illnesses [14]. Patients, especially children with inherited hemolytic diseases like BTM are particularly vulnerable to B19V [15,16].

B19V infection typically lasts for short period, the virus can be detected in blood with 5 – 7 days. It was believed that B19V can be eradicated completely by the host immunity with the help of cellular and humoral immune response within a few weeks following acute infection. Recently, carrier for the B19V were reported in immunocompetent individuals such as blood

donors, despite the presence of specific IgG antibodies [17]. Nevertheless, the majority of hematological units across the globe do not routinely screen BTM patients or donors of blood for B19V. Therefore, this study aimed to investigate the prevalence of human parvovirus (B19V) among patients with BTM relies both on serological and molecular detection.

2. Materials and Methods

2.1 Patients and samples

This prospective case-control study involves 120 BTM patients who received treatment and blood transfusion at the Ibn Al Balady Children and Maternity Hospital in Baghdad, Iraq, between November 2023 and March 2024. The control group for this study consisted of sixty apparently healthy blood donors from the Iraqi National Center of Blood Transfusion in Baghdad, Iraq. All participants in this group tested negative for HCV, HBV, and HIV viruses. Blood samples were collected from all the patients and controls, 4 mL whole blood was collected in sterile EDTA- blood tubes and plasma were obtained by centrifugation of EDTA-blood tube at 1600g for 20 min, and then the separated plasma was aliquoted into three Eppendorf tubes and stored at -40°C until used.

The study protocol was approved by the Ethics Committee of the College of Science, University of Baghdad (CSEC/1223/0136) and in accordance with Iraqi Ministry of Health approval. All participants, or their parents/guardians, provided written informed consent prior to participation. The age of patients and control group less than 40 years were included in the study and the BTM were under recurrent blood transfusion. The individuals with chronic or autoimmune disorders were excluded from the study. The patients and control groups were investigated for age, gender, and biochemical parameters including, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Blood Urea Nitrogen (BUN), and Serum creatinine (Cr). All patients received regular blood transfusions.

2.2. Serological detection of human parvovirus B19

The Enzyme Linked Immunosorbent Assay (ELISA) kits from Demeditec Company, Germany were used to determine the levels of anti-parvovirus B19 antibodies (IgM and IgG Abs) in all plasma samples.

2.3 Molecular detection and sequencing of human parvovirus B19

Genomic viral DNA was extracted using a commercial extraction kit provided by Geneaid Company, based in Taiwan. Two DNA fragments of B19V in the overlapping area shared by the major (VP2) and minor (VP1) capsid protein genes were amplified using the nested PCR procedure, as detailed elsewhere [18]. In the first round, 398 bp of the target area were amplified using the outer primer sequences 5'-CAAAAGCATGTGGAGTGAGG-3' (nt 3187–3206) and 5'-CTACTAACATGCATAGGCGC-3' (nt 3584–3565). The 25 µl PCR reaction mixture was composed of 5.0 µl DNA template, 1.0 µl each of forward and reverse primers, 12.5 µl Promega master mix, and 5.5 µl nuclease-free water, which brought the total volume to 25 µl. In the second cycle, 288 bp of the initial product were amplified using inner primers (sequences 5'-CCCAGAGCACCATTATAAGG-3' (nt 3271–3290) and 5'-GTGCTGTCTAGTAACCTGTAC-3' (nt 3558–3539). One cycle at 94°C for five minutes as pre-amplification, then thirty cycles of 55°C for two minutes (first round) or 57°C for two minutes (second round) as annealing step, followed by thirty cycles of 72°C for three minutes as extension step, and a final extension at 72°C for five minutes were the thermal conditions for the nPCR protocol. A 2% agarose gel was used to visualize the amplified products. The amplified fragment was made up to 288 bp and was subsequently exposed to direct Sanger sequencing method to resolve the pattern of its genetic diversity for the analyzed human parvovirus isolates. By using the reference-based genotyping, the NCBI BLASTn analysis

was conducted for the DNA sequences alignment of the VP1/VP2 locus of the analyzed samples.

2.4 Statistical analysis

Using Statistical Package for Social Sciences (SPSS) version 25, a statistical program, data were coded and entered. Descriptive statistics, such as mean, standard deviation, median, minimum and maximum values for quantitative variables, and number and percentage for qualitative values, were used to summarize the data. Categorical variables were represented by numbers and percentages, while the Kolmogorov Smirnov test was used to assess the normal distribution of numeric variables. Statistical analysis between groups were tested using Chi Square test for qualitative variables, independent sample test and ANOVA (analysis of variance) test for quantitative normally distributed variables. P-values less than or equal to 0.05 were considered statistically significant.

3. Result and Discussion

3.1. Patients and control demographic parameters

The study included patients aged between 5 and 40 years, with a mean age of 18 ± 7.8 years. Among the BTM patients, 14 (12%) were under 10 years old. The BTM patients aged between 11 to 20 years were 39 (32%), and only 3 (5.0%) for the controls. Meanwhile, the BTM patients in the age group (> 20 years) were 67 (56%), for controls were 57 (95.0%) (P-value <0.001). Both the BTM patients and controls were comparable in mean age and sex distribution ($P > 0.05$). Regarding sex distribution, the males were more predominant among BTM patients 67 (56%) compared to the control group 32 (53%). The females were less predominant in patients with 53 (44%) compared to controls which were 28 (47%) (P value 0.075). In terms of B19V seropositivity status (IgM and IgG antibodies were tested positive), there were 33 (27.5%) positive among BTM patients, and only 8 of them (13.3%) are positive among the control group (P-value 0.078) (Table 1).

Table 1: Comparison of demographic parameters among study groups

Variables	BTM No. (%)	Control No. (%)	χ^2	P value
Age groups				
≤ 10 years	14 (12%)	0 (0%)	43.55	$<0.001^{**}$
11 – 20 years	39 (32%)	3 (5%)		
≥ 20 years	67 (56%)	57 (95%)		
Total	120 (100%)	60 (100%)		
Sex				
Male	67 (56%)	32 (53%)	3.17	0.075 NS
Female	53 (44%)	28 (47%)		
Total	120 (100%)	60 (100%)		
B19V IgG and IgM seropositivity				
Positive	33 (27.5%)	8 (13.3%)	5.09	0.078 NS
Negative	87 (72.5%)	52 (86.7%)		
Total	120 (100%)	60 (100%)		

BTM, Beta Thalassemia Major; B19V, Human parvovirus B19; χ^2 = Chi square Test; ** highly significant; NS, Non-Significant

The findings of this study align with various other local investigations conducted by numerous researchers concerning the demographics such as age and sex [9, 19, 20].

Additionally, our findings come in line with an Iranian study by Arabzadeh *et al.*, (2017) [15]. According to a study by Abdul Sadah and Al-Marsome (2020) focused on the B19V detection among BTM patients, it was reported a 37% positive to the viral infection which disagree with this study findings [21]. The higher frequency of the B19V with increasing age can be attributed to cumulative exposure, the BTM patients are more likely to be exposed to the B19V at some point of them with increasing especially due to recurrent hemotherapy besides the immune system may underdoes changes with increased age, which may can affect the susceptibility to viral infections.

3.2. Serological detection of human parvovirus B19

Table 2 outlines the distribution of IgM and IgG antibody prevalence by age group and sex 41% (11 / 27) of the female participants and 59% (16 / 27) of the male participants tested positive for anti-B19 IgG Abs. Anti-B19 IgM antibody positivity was found in 64% of males (21 / 33) and 36% of females (12 / 33).

Table 2: Frequency of anti-B19 antibodies according to age and sex distribution among study groups

Age groups	Sex			Positive B19-IgG No. (%)			Positive B19-IgM No. (%)		
	Total	Male	Female	Male	Female	Total	Male	Female	Total
≤ 10 years	14	8	6	3 (37.5%)	0 (0%)	3	4 (50%)	0 (0%)	4
11 – 20 years	39	25	14	6 (24%)	1 (7%)	7	8 (32%)	2 (14%)	10
> 20 years	67	34	33	7 (20.5%)	10 (30%)	17	9 (26%)	10 (30%)	19
Total	120	67	53	16	11	27	21	12	33

Previous research on human parvovirus detection have indicated that B19V seroprevalence for IgG and IgM varied from 18.2–81% and 14.5–41.1%, respectively, among β -thalassemia patients [22]. In term of seropositivity, the current findings disagree with a study by Abdul Sadah and Al-Marsome (2020) who reported 21% IgG seropositivity [21]. A study by Alnassar and Shallal (2023) in Al-Muthana Governorate, reported the rate of 11.6% parvovirus antigen detection by ELISA assay in BTM patients this was less than what was stated in this study [9]. In Tunisia, a study conducted by Regaya *et al.*, (2007) showed that B19V IgG antibody positivity was 39.1% in patients with beta thalassemia [23]. The lower infection rate and IgG prevalence observed in this study may be due to differences in demographics and patient health status compared to other studies. Moreover, the timing of sample collection relative to the onset of infection can significantly impact the detection of B19V-IgG Abs which may explain the lower B19V-IgG Abs level observed. Lastly, variation in the methodologies used for B19V-IgG Abs, including differences in assay sensitivity and selectivity, could also contribute the discrepancies between this study findings and those of previous studies.

Males in the current study had a higher percentage of human parvovirus infection than female patients in the studied groups. The difference between study groups was significant which agree with Atbee *et al.*, (2020) study, who evaluated 30 BTM patients by seropositivity of IgG and IgM for parvovirus and reported 20% of male prevalence and 10% of female prevalence study in Basra [20]. The higher frequency of B19V in males may be due to different hormonal profiles which can influence the immune responses. The genetic differences between males and females may affect susceptibility to the viral infections.

3.3. Serological detection of human parvovirus B19 and chemical parameters

There were 33 out of 120 (27.5%) of BTM patients were tested positive to anti B19 IgM with significant elevation in both AST and ALT levels (51.99 ± 5.09) and (46.18 ± 5.20), respectively among BTM patients tested B19 IgM Abs positive versus (67.85 ± 3.56) and (60.95 ± 3.18), respectively among BTM who tested B19 IgM Abs negative (Table 3). There was a significant difference between BUN and B19 IgM Abs positive versus negative among BTM patients (51.67 ± 1.97 vs 39.62 ± 2.48 ; $P = 0.0050$). Meanwhile; there were no significant elevation in the serum Cr level between BTM patients who tested B19 IgM positive and negative (1.99 ± 0.08 vs 1.62 ± 0.08 ; $P = 0.254$) (Table 3). The association between anti B19 IgG seropositivity in BTM and chemical parameters (AST, ALT, BUN, and Cr) were not tabulated, because there was no significant difference between them.

Table 3: Serological detection of B19V and chemical parameters in beta thalassemia major patients

Parameters	B19-IgM +ve (N=33) mean $\pm$ SD (95% CL)	B19-IgM -ve (N=87) mean $\pm$ SD (95% CL)	Control	Reference range	P value
AST (IU/l)	51.99 ± 5.09	67.85 ± 3.56	31.90 ± 1.8	15 – 40	0.010 **
ALT (IU/l)	46.18 ± 5.20	60.95 ± 3.18	32.80 ± 1.1 3	20 – 35	0.0268 *
BUN (mg/dl)	51.67 ± 1.97	39.62 ± 2.48	21.58 ± 1.95	5 – 20	0.0050 **
Creatinine (mg/dl)	1.99 ± 0.08	1.62 ± 0.08	1.13 ± 0.09	0.5 – 1.2	0.254 NS

AST: Aspartate Transaminase; ALT: Alanine Transaminase; BUN: Blood Urea Nitrogen, CL: Confidence limit; SD: Standard deviation, Disparity between mean values was analyzed using Student's t-test; *(P-value ≤ 0.05), ** (P-value ≤ 0.01).

The study findings regarding AST and ALT level increment are inconsistency with a local study by Hamed *et al.*, (2021) on children patients afflicted by BTM in Mosul city [6]. A study by Faiq *et al.*, (2021) in Sulaimania city claimed a high level of AST with 86.15%, and ALT with 27.7% among BTM patient [5]. Furthermore, the current findings agree with an Egyptian study by Al Ghwass *et al.*, (2016) in Fayoum [24]. BTM patients often require regular blood transfusion, leading to iron overload, this excess of the iron can accumulate in the liver causing live inflammation leading to elevation in live enzymes.

In a recent systemic review and meta-analysis study by Khandker *et al.*, (2023) that investigate the association between Glomerular Filtration Rate (GFR) and BTM, which were analysed 12 studies and found a negative correlation between GFR and BTM, the mean difference was (-16.94), suggesting that GFR tends to be lower in patients with BTM compared to healthy controls [25]. Tanous *et al.*, (2021), revealed in his study that renal injury in BTM patients treated with different iron-chelation therapy regimes were elevated including serum Cr [26]. In another local study by Hassan and Yasir (2020), investigated the correlation between HCV infection and serum Cr level pointed to the viral role in this matter in BTM [27].

3.4. Molecular detection and sequencing of human parvovirus B19

The Parvovirus B19 DNA was extracted then subjected to nested PCR to detect the B19V in BTM patients and controls. There were only 5 out of 120 (4.16%) samples were positive to parvovirus VP1/VP2 with PCR product size (288 bp) in BTM patients and non in the controls as in Figure 1. By using the reference-based genotyping, the NCBI BLASTn analysis was conducted for the DNA sequences alignment of the VP1/VP2 locus of the analyzed samples. The analysis revealed that the highest similarity between the sequenced samples and the

expected target, specifically the VP1/VP2 locus within human parvovirus genomic sequences indicated a 99% homology to the specified portions of the VP1/VP2 sequences with the reference strain under accession number JN211182.1 from Netherland deposited in National Center for Biotechnology Information (NCBI) data base. Precise genotyping analysis revealed that all five investigated isolates were classified as human parvovirus genotype 1a.



Figure 1: PCR products of human parvovirus (B19) on 2% gel electrophoresis. Lane M: DNA marker (100-1500 bp), lanes 1 – 3, 14, and 19: positive result for amplification (288 bp), lane 4 – 13, 15 – 18, and 20: negative result for amplification.

Pichon *et al.*, (2019) evaluated the performance of optimized NS1-VP1u for detecting and sequencing of HPV-19 in clinical samples and claimed (100%) sensitivity and specificity [28]. Another study conducted in South India by Seetha *et al.*, (2021) reported a (7.2%) of human parvovirus detected by nested PCR in Kerala State [29]. The results of study are agreed with Atbee *et al.*, (2020) who reported (4.8%) of BTM patients in Basra city were detected by molecular methods using the nested PCR [20]. However, the current finding disagrees with Al nasser and Shallal, (2023) who reported a (1.7%) of BTM patients in Al-Muthana province [30].

Conclusion

In this study, acute human parvovirus B19 infection was detected serologically revealing high rate, suggesting a potential association between BTM and B19V. The patients are under regular blood transfusion therapy which may explains the high rates of seropositivity. However, molecular diagnostic testing uncovered relatively low detection rates among patients diagnosed with BTM. Further research with a large sample size is needed to explore this association. There are currently no policies or procedures in place for B19V screening blood donors. Implementing preventive measures, such as screening given blood, is necessary to lower the blood-borne virus prevalence in this susceptible group.

Limitation of the study

The study was carried in single center in Baghdad, the patients sample size was not large, other risk factors such as Iron overload, hepcidin, and ferritin serum levels were not explored. The B19V viral load and gene expression by real time PCR may be more sensitive method for the viral prevalence in vulnerable group.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

- [1] H. A. Hamamy, and N. A. Al-Allawi, "Epidemiological profile of common haemoglobinopathies in Arab countries," *Journal of Community Genetics*, vol 4, no. 2, pp. 147–167, Apr. 2013, doi: 10.1007/s12687-012-0127-8.
- [2] K. A. Kadhim, K. H. Baldawi, and F. H. Lami, "Prevalence, Incidence, Trend, and Complications of Thalassemia in Iraq," *Hemoglobin*, vol. 41, no. 3, 2017, doi: 10.1080/03630269.2017.1354877.
- [3] R. K. Lafta, "Burden of Thalassemia in Iraq," *Public Health Open Access*, vol. 7, no. 1, pp. 1–7, 2023, doi: 10.23880/phoa-16000242.
- [4] M. S. Hossain, E. Raheem, T. A. Sultana, S. Ferdous, N. Nahar, S. Islam, M. Arifuzzaman, M. Abdur Razzaque, R. Alam, S. Aziz, H. Khatun, A. Rahim and M. Morshed, "Thalassemias in South Asia: clinical lessons learnt from Bangladesh," *Orphanet Journal of Rare Diseases*, vol. 12, no. 1. 2017. doi: 10.1186/s13023-017-0643-z.
- [5] A. B. Faiq, S. O. Hamabor, and M. A. Hama Salih, "Assessment of Liver, Thyroid Gland and Growth Hormone Functions in Beta Thalassemia Major," *Iraqi Journal of Science*, vol. 63, no. 4, pp. 1413–1422, 2022, doi: 10.24996/ij.s.2022.63.4.2.
- [6] O. M. Hamed, R. A. Al-Taii, and M. H. Jankeer, "Biochemical and genetic study in blood of β -thalassaemia children in Mosul city, Iraq," *Iraqi Journal of Science*, vol. 62, no. 8, pp. 2501–2508, 2021, doi: 10.24996/ij.s.2021.62.8.2.
- [7] M. S. Majeed, "Evaluation of some Biochemical and Endocrine Profiles in transfusion-dependent Iraqi major β -thalassemia patients," *Iraqi Journal of Science*, vol. 58, no. 2A, pp. 639–645, 2017.
- [8] C. Borgna-Pignatti M. C. Garani, G. L. Forni, M. D. Cappellini, E. Cassinerio, C. Fidone, V. Spadola, A. Maggio, G. Restivo Pantalone, A. Piga, and F. Longo, "Hepatocellular carcinoma in thalassaemia: An update of the Italian Registry," *British Journal of Haematology*, vol. 167, no. 1, 2014, doi:10.1111/bjh.13009.
- [9] A. W. D. Alnassar and M. J. M. Shallal, "Serological study of Human Parvovirus (B19) detected among Patients with Thalassemia," *Journal of Population Therapeutics and Clinical Pharmacology*, vol. 30, no. 9, 2023, doi: 10.47750/jptcp.2023.30.09.004.
- [10] M. T. S. Al-Ouqaili, Y. H. Majeed, and S. K. Al-Ani, "SEN virus genotype h distribution in β -thalassemic patients and in healthy donors in Iraq: Molecular and physiological study," *PLoS Neglected Tropical Diseases*, vol. 14, no. 6, pp. 1–16, 2020, doi: 10.1371/journal.pntd.0007880.
- [11] H. K. Fanos and A. H. Mohammed, "Human Parvovirus B19 In Patients with Beta Thalassemia major: A Case Study," *Biochemical and Cellular Archives*, vol. 21, no. 1, 2021.
- [12] B. Kaufmann, A. A. Simpson, and M. G. Rossmann, "The structure of human parvovirus B19," *Proceedings of the National Academy of Sciences*, vol. 101, no. 32, 2004, doi:10.1073/pnas.0402992101.
- [13] L. Singhal, B. Mishra, A. Trehan, N. Varma, R. Marwaha, and R. K. Ratho, "Parvovirus B19 infection in pediatric patients with hematological disorders," *Journal of Global Infectious Diseases*, vol. 5, no. 3. 2013, doi:10.4103/0974-777X.116881.
- [14] G. R. Serjeant, K. Mason, J. M. Topley, B. E. Serjeant, J. R. Pattison, S. E. Jones, and R. Mohamed, "Outbreak of aplastic crises in sickle cell anaemia associated with parvovirus-like agent," *The Lancet*, vol. 318, no. 8247, pp. 595-597, 1981, doi: 10.1016/S0140-6736(81)92739-2.

- [15] S. A. M. Arabzadeh, F. Alizadeh, A. Tavakoli, H. Mollaei, F. Bokharaei-Salim, G. Karimi, M. Farahmand, H. S. Mortazavi, and S. H. Monavari, "Human parvovirus B19 in patients with beta thalassemia major from Tehran, Iran," *Blood Research*, vol. 52, no. 1, pp. 50, 2017, doi: 10.5045/br.2017.52.1.50.
- [16] A. M. Yates, J. S. Hankins, N. A. Mortier, B. Aygun, and R. E. Ware, "Simultaneous acute splenic sequestration and transient aplastic crisis in children with sickle cell disease," *Pediatric blood & cancer*, vol. 53, no. 3, 2009, doi: 10.1002/pbc.22035.
- [17] J.J. Lefrère, A. Servant-Delmas, D. Candotti, M. Mariotti, I. Thomas, Y. Brossard, F. Lefrere, R. Girot, J. P. Allain, and S. Laperche, "Persistent B19 infection in immunocompetent individuals: implications for transfusion safety," *Blood*, vol. 106, no. 8, pp. 2890-2895, 2005.
- [18] P. Jain, A. Jain, S. Prakash, D.N. Khan, D.D. Singh, A. Kumar, N. R. Moulik, and T. Chandra, "Prevalence and genotypic characterization of human parvovirus B19 in children with hemato-oncological disorders in North India," *Journal of Medical Virology*, vol. 87, no. 2, pp. 303–309, Feb. 2015, doi: 10.1002/jmv.24028.
- [19] H. M. Majeed, "Detection of Parvovirus B19 Infection in Thalassemic Patients in Tikrit City, Serological Study," *Medico-Legal Update*, vol. 20, no. 1, pp. 924–930, 2020, doi: 10.37506/v20/i1/2020/mlu/194339.
- [20] M. A. K. A. Atbee, H. A. M. A. Hmudi, and S. K. A. A. Salait, "Molecular detection of human parvovirus B19 in patients with hemoglobinopathies in Basrah Province-Iraq," *International Journal of Pharmaceutical Research*, vol. 12, no. 2, 2020, doi: 10.31838/ijpr/2020.12.02.305.
- [21] R. R. Abdul Sadah and H. D. Al-Marsome, "Detection of Parvovirus B19 in B-thalassemia Major Patients by Serological and Molecular Method," *Medico-Legal Update*, vol. 20, no.4, pp. 2187–2191, 2020.
- [22] S. Soltani, A. Zakeri, A. Tabibzadeh, M. Zandi, E Ershadi, S. Akhavan Rezayat, S. Khaseb, A. M. Zakeri, M. Ashtar Nakhaei, S. Afzali, and A. Farahani, "A literature review on the parvovirus B19 infection in sickle cell anemia and β -thalassemia patients," *Tropical Medicine and Health*, vol. 48, no. 1, pp. 1–8, 2020. doi: 10.1186/s41182-020-00284-x.
- [23] F. Regaya, L. Oussaief, M. Bejaoui, M. Karoui, M. Zili, and R. Khelifa, "Parvovirus B19 infection in Tunisian patients with sickle-cell anemia and acute erythroblastopenia.," *BMC Infectious Diseases*, vol. 7, pp. 123, 2007, doi: 10.1186/1471-2334-7-123.
- [24] M. E. Al Ghwass, S. M. El Shafei, W. S. Mohamed, and B. S. Mohamed, "Seroprevalence of parvovirus B19 infection in patients with beta thalassemia major in Fayoum University Hospital," *Egyptian Pediatric Association Gazette*, vol. 64, no. 3, pp. 126–130, 2016, doi: 10.1016/j.epag.2016.06.002.
- [25] S. S. Khandker, N. Jannat, D. Sarkar, A.H. Pranto, I. A. Hoque, J. Zaman, M. N. Uddin, and E., "Association between Glomerular Filtration Rate and β -Thalassemia Major: A Systematic Review and Meta-Analysis," *Thalassemia Reports*, vol. 13, no. 3, pp. 195–205, 2023, doi: 10.3390/thalassrep13030018.
- [26] O. Tanous, Y. Azulay, R. Halevy, T. Dujovny, N. Swartz, R. Colodner, A. Koren, and C. Levin, "Renal function in β -thalassemia major patients treated with two different iron-chelation regimes," *BMC Nephrology*, vol. 22, no. 1, pp. 1–11, 2021, doi: 10.1186/s12882-021-02630-5.
- [27] S. Y. Hassan and S. J. Yasir, "Correlation between HCV infection and Creatinine Level in Thalassemia Patients," *International Journal of Drug Delivery Technology*, vol. 10, no. 1., pp. 164–169, 2020. doi: 10.25258/ijddt.10.1.29.
- [28] M. Pichon, C. Labois, V. Tardy-Guidollet, D. Mallet, J. S. Casalegno, G. Billaud, B. Lina, P. Gaucherand, and Y. Mekki, "Optimized nested PCR enhances biological diagnosis and phylogenetic analysis of human parvovirus B19 infections," *Archives of Virology*, vol. 164, no. 11, pp. 2775–81, 2019, doi: 10.1007/s00705-019-04368-w.
- [29] D. Seetha, H. R. Pillai, S. R. C. Nori, S. G. Kalpathodi, V. P. Thulasi, and R. R. Nair, "Molecular-genetic characterization of human parvovirus B19 prevalent in Kerala State, India," *Virology Journal*, vol. 18, no. 1, pp. 96, 2021, doi: 10.1186/s12985-021-01569-1.
- [30] A. W. D. Alnassar and A. M. J. M Shallal, "Detection of Human Parvovirus (B19) among patients with Cancer, Thalassemia, and Viral Hepatitis," Ph.D. Thesis. 2023.