Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.09.004

Serological study of Human Parvovirus (B19) detected among Patients with Thalassemia

Ahmed Waleed Dheyab Alnassar[1]*, Mohammed Jasim Mohammed Shallal[2]

[1] Department of Microbiology, Medical Microbiology, College of Medicine, University of Thi-Qar

[2]Department of Microbiology, Medical Microbiology, Medical Virology, College of Medicine, University of Thi-Qar

***Corresponding author:** Ahmed Waleed Dheyab Alnassar, Department of Microbiology, Medical Microbiology, College of Medicine, University of Thi-Qar, Email: ahmed-w@utq.edu.iq

Submitted: 22 February 2023; Accepted: 19 March 2023; Published: 04 April 2023

ABSTRACT

Background: Parvovirus B19 is an icosahedral, single-strand DNA, non-enveloped virus. Its DNA genome has 5596 bases and is from the Parvoviridae family. Beta thalassemia, a hereditary illness, causes ruptured red blood cells and acute anemia due to aberrant haemoglobin synthesis.

Aim: Detect parvovirus (B19) in β-thalassemia major and study its association with demographic factors like sex, age, place of residence, etc. in specific patient groups.

Methods: From August 2022 to the end of February 2023. This study included the collection of serum samples for the detection of human parvovirus antigen in 60 patients with β-thalassemia major. The control group consisted of 30 individuals of different ages who did not have **beta-thalassemia**. All these serum samples are detected for parvovirus antigen by the ELISA method.

Results: The results of this study showed that the rate of detection of the presence of human parvovirus B19 in the group of patients with β-thalassemia major was not affected by most of the demographic factors. As there were no statistically significant differences between the study groups in terms of gender, age, in addition to COVID-19 infection, and vaccination against COVID-19. However, the rate of β-thalassemia major was significantly higher in rural areas than in urban areas (p = 0.040).

Keywords: *HPV: Human Parvovirus B19, β-thalassemia major*

INTRODUCTION

Human parvovirus B19 (B19V) infects the erythroid progenitors in the bone marrow. It is a small DNA virus that does not have an envelope. Despite its high erythroid tropism, studies have shown that it may persist in non-erythroid tissues (1). B19 can be transmitted in a variety of ways, including by the inhalation of infected particles, the transfusion of contaminated blood or blood products, the donation of organs, and vertical transmission from a mother to her unborn child (2).

Erythema infectiosum, also known as the 'fifth illness,' is a frequent symptom of B19V infection. Although B19V infection is typically mild, with symptoms including redness and fever that last for only a few days, it can have serious consequences for those who are particularly vulnerable (3). Fetal anemia, non-immune hydrops, and premature fetal mortality are all possible outcomes of B19V infection during pregnancy (4). B19V can cause severe anemia in immunosuppressed or people with red blood cell

disease. Hepatitis, nerve symptoms, and myocarditis were linked to B19V (5).The relationship between Parvovirus B19 and β-thalassemia major lies in the fact that erythroid progenitor cells that have been infected with Parvovirus B19 result in the inhibition of erythroid development or the death of erythroid cells via apoptosis (6,7). The pathogenic process may depend on the direct cytotoxic or apoptotic effects of viral proteins and may depend, in some cases, on the stimulation of the inflammatory response by viral proteins such as the NS1 protein or the phospholipase A (PLA) protein that is associated with VP1u; both of these proteins are associated with the virus (8). Parvovirus B19 is selective, but not exclusive, for erythroid progenitor cells. It shows selective tropism in the bone marrow for erythroid progenitor cells because of specific receptors like glycolipid globoside and a specific receptor binding the unique Nterminutensal domain of VP1 and functional internalization processes. In the erythroid lineage, viruses can only replicate and spread through the colony-forming unit-erythroid (CFU-E) and erythroblast stages of differentiation. This suggests that viral macromolecular synthesis is promoted by both lineage- and differentiation-specific factors (6,9). In infected erythroid progenitors, the virus is responsible for a complicated sequence of effects on the cellular environment. These effects include the production of a DNA damage response, the arrest of the cell cycle, and the induction of apoptosis. This cytotoxicity results in an interruption of the erythropoiesis process for a short period of time, which may or may not be followed by an aplasia of the erythroid cells (10,11).

PATIENTS AND METHODS

This study mainly targeted patients who were diagnosed with β-thalassemia major, which included 60 patients with thalassemia (30 males and 30 females), in addition to 30 individuals as a control group (15 males and 15 females). Excluded criteria: The included criteria in this study include a specific age group of patients and exclude patients age 70 or older. Also, all patients suffering from autoimmune diseases or chronic diseases are excluded. 3 ml of these blood samples are centrifuged at 4000 RPM for five minutes and then separated into two parts: 0.2 ml for virus detection were determined by Enzyme Linked Immuno-Sorbent Assay antigens according to the manufacturer's instructions (BT LAB Bioassay Technology Laboratory, China).

Statistical Analysis

Statistical packages for the social sciences (SPSS) version 23 and Microsoft Office Excel 2010 were utilized throughout the data collection, summarization, analysis, and presentation processes. Quantitative (numerical) variables were first evaluated for normality distribution using the Kolmogorov-Smirnov test, and then accordingly normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation. Qualitative (categorical) variables were expressed as numbers and percentages, whereas quantitative (numerical) variables were expressed as numbers and percentages. (an index of dispersion). In this study, we utilized the following statistical tests: When the Chi-square test could not be relied upon, the Fischer exact test was utilized. The level of significance was determined to be attained when the P-value was equal to or below 0.05.

Ethical considerations

Participation in the study was subject to informed consent. All participants had beta-thalassemia major. This protocol was approved by the Board of the Research Ethics Committee of the Health Office in Al-Muthana Governorate, according to the ethical number (No. 866 on 1/8/2022). Informed consent was obtained from all patients and members of the control group. All samples taken from those patients are within the direct supervision of specialist physicians in hospitals.

RESULTS

The current study found that 11.7% of the human parvovirus B19-infected group consisted of thalassemia patients. Table 1 further shows that there was no statistically significant difference in the prevalence of certain viruses across diseases.

TABLE 1: The frequency distribution of results of HPV antigen ELISA detection according to study groups.

ELISA	Control group n = 30	Beta thalassemia major n = 60
Positive	0 (0.0 %)	7 (11.7 %)
Negative	30 (100.0 %)	53 (88.3 %)
p-value	Reference	0.090 F NS

F: Fischer exact test; n: number of cases; NS: not significant

Human parvovirus B19 infections were more common among males than females, according to the findings of the present study. The findings also showed that there was no statistically significant difference in the prevalence of viral infections between the sexes ($p > 0.05$), as shown in the results in Table 2.

TABLE 2: Antigen detection of human parvovirus B19 according to disease types and sex.

Group	Sex	Positive-HPV n (%)	Negative-HPV n (%)	Total n	p-value
β-thalassemia major	Male	5 (16.7 %)	25 (83.3 %)	30	0.424 F NS
	Female	2 (6.7 %)	28 (93.3 %)	30	

F: Fischer exact test; n: number of cases; NS: not significant

Beta thalassemia major rates were much higher in rural regions compared to urban areas, and there was a statistically significant link between residency and the disease. (Table 3).

TABLE 3: Antigen detection of human parvovirus B19 according to disease types and residence.

Group	Residence	Positive-HPV n (%)	Negative-HPV n (%)	Total n	p-value
β-thalassemia major	Urban	0 (0.0 %)	22 (100.0 %)	22	0.040 F *
	Rural	7 (18.4 %)	31 (81.6 %)	38	

F: Fischer exact test's;n: number of cases; *: significant at $p \leq 0.05$

The findings of the present study indicated the largest number of patients in the examined group infected with human parvovirus B19 were patients with non-Covid-19 infection. The findings also detected a non-significant difference in the distribution of viral infection according to COVID-19 infection at a p-value < 0.05, as shown in table 4.

TABLE 4: Antigen detection of human parvovirus B19 according to COVID-19 infection

Disease type	Covid-19 infection	Positive-HPV n (%)	Negative-HPV n (%)	Total n	p-value
β-thalassemia major	Positive	0 (0.0 %)	2 (100.0 %)	2	1.000 F NS
	Negative	7 (12.1 %)	51 (87.9 %)	58	

F: Fischer exact test; n: number of cases; NS: not significant

According to the findings of the present research, human parvovirus B19 infection was most common among those who had not received the COVID-19 vaccine, whereas infection was least common among those who had received the vaccine. Table 5 displays the findings, which likewise revealed no statistically significant difference in the prevalence of viral infection by COVID-19 vaccination status

TABLE 5: Antigen detection of human parvovirus B19 according to COVID-19 vaccination.

Disease type	Covid-19 vaccine	Positive-HPV n (%)	Negative-HPV n (%)	Total n	p-value
Beta thalassemia major	Positive	0 (0.0 %)	1 (100.0 %)	1	1.000 F NS
	Negative	7 (11.9 %)	52 (88.1 %)	59	

F: Fischer exact test; n: number of cases; NS: not significant

Table 6 shows the rate of HPV antigen detection by ELISA based on age. There was no significant difference in mean age between patients with beta thalassemia major who had positive HPV antigen detection and those who did not have positive HPV antigen detection.

TABLE 6: Rate of antigen detection of HPV according to ELISA with respect to age.

Group	Age	Positive	Negative	p-value
Beta thalassemia major	Mean ±SD	10.90 ±5.46	11.60 ±5.92	0.769 I NS
	Range	1.3 -16	1 -27	

I: Independent samples t-test; n: number of cases; NS: not significant

DISCUSSION

The results of the current study are indicated to point to the following sub-proportions: In this study, the ELISA method was used to detect viral antigen rather than IgG or IgM in 11.67% of β-thalassemia major. This is because the detection of human parvovirus B19 as a viral antigen has a higher specificity than the detection of IgM or IgG directed against the virus. According to the previous studies focused on the detection of parvovirus in humans, it was found that among the β-thalassemia patients, the B19V seroprevalence for IgG and IgM ranged from 18.2–81% and 14.5–41.1%, respectively (12). Therefore, the rate of 11.67% in β-thalassemia patients in this study was lower than that reported in the previous studies. However, the difference in the methodology of detection of the virus should be taken into consideration, and according to (13), the antigen method requires a high titer of the virus for the correct detection of the virus in the blood, so it can be said that it is less sensitive than ordinary serological methods that are based on detecting IgG or IgM.

In one Iraqi study, the rate of parvovirus B19 in association with β-thalassemia major was 21.3 % according to an IgG ELISA test (14). In another Iraqi study, the rate of parvovirus B19 in association with β-thalassemia major was 1.7 for IgM and 30.8 % for IgG (15). Again, it should be noted that in these Iraqi studies, the method of viral detection was based on IgM and/or IgG detection, whereas in this study, the viral specific antigen was detected.

In the present study, the proportion of human parvovirus infection in males was higher than what was reported in females in study groups, including β-thalassemia major, but from a statistical point of view, the difference was not significant.

According to the study of (16), Prevalence of HPV infection was greater in males with β-thalassemia major compared to females, but this variation was not statistically significant. Thus, it

is agreed with (16), who evaluated the evidence of the virus in 90 patients with β-thalassemia major using the ELISA method for IgG and IgM. Also in line with this observation, an Iraqi study on the serological prevalence rate of HPV in β-thalassemia major patients described no significant variation in the proportion of positive viral infection between males and females (17). In addition (18), also described that there is no significant difference in the frequency distribution of HPV infection, detected serologically for IgG and IgM, between males and females, giving another support to the results of this current study.

The incidence of transmission in patients with β-thalassemia major was greater and more restricted in rural regions than in patients from urban areas. In accordance with our study, (19)and(20) stated that there was no significant relationship between residence location and prevalence of serum parvovirus B19. However, the significantly higher rate of HPV infection in rural areas among children with β-thalassemia major in the current study may be explained by the fact that the poor health facilities and lack of education in rural environments will produce a less tolerant immune status, which will facilitate infection with HPV.

The present research found no statistically significant correlation between HPV and COVID-19 transmission rates among all participants. This point of the recent study research can be considered novel because, to our best knowledge, no previous research has dealt with the association between HPV infection and COVID-19 in the general population or in a particular subset of them, including β-thalassemia major. The lack of significant association in this study can be explained by the fact that although immune suppression might be associated with COVID-19, it will not be sufficient to increase susceptibility to HPV infection. In addition, no previous article has evaluated the role of the COVID-19 vaccine in relation to HPV infection, and in our opinion, the vaccine status would not affect the overall immune state, so that no susceptibility or prevention can occur with respect to HPV.

According to age, it is observed that there is no significant difference in mean age between cases with positive detection and cases with negative detection of HPV antigen in all cases, in patients with β-thalassemia major. With respect to β-thalassemia major, previous studies by (17,18)and (16) found no significant difference in mean age in patients with beta thalassemia in positive cases contrasted to negative cases. This finding is supportive to our results which can be explained in that in β-thalassemia major the exposure to the virus is not variable with age and that changes in immunity does not increases rate of infection with HPV with increasing age.

CONCLUSION

The conclusions of the study are that most demographic factors did not have a significant impact on the rate of detection of human parvovirus B19 in patients with beta-thalassemia. The study found no significant differences in the detection rate of human parvovirus B19 among patients in terms of gender, age, COVID-19 infection, or vaccination against COVID-19. However, the study did find a statistically significant difference in the incidence of beta thalassemia major between rural and urban areas, with a higher incidence in rural areas.

These findings suggest that healthcare providers should be aware of the potential risk of human parvovirus B19 in patients with beta-thalassemia regardless of their demographic characteristics. Additionally, public health authorities should consider targeted interventions to improve access to care and support for patients with beta-thalassemia, particularly those living in rural areas where the incidence of beta-thalassemia major may be higher. Further research is needed to understand the factors contributing to the higher incidence of beta thalassemia major in rural areas and to develop strategies to address this disparity.

ACKNOWLEDGMENTS

We would like to express our sincere gratitude to all participants who agreed to participate in this study. We thank the staff of the hospitals in each of Thi-Qar Governorate and Al-Muthana

Governorate (in Iraq) who provided assistance in recruiting participants and collecting data. We also appreciate the help of the research ethics committee in approving this study protocol (No. 866 on 1/8/2022) and (NO. 509 on 21/7/2022). We acknowledge the valuable contributions of the specialist physicians who supervised the collection of samples from the patients. Also, we thank all the members and staff of the Thalassemia Center in both Thi Qar and Muthana governorates who contributed their time, effort, and expertise to the successful completion of this study. Finally, we express our thanks and gratitude to the staff of the Graduate Studies Division of the College of Medicine – University of Thi-Qar for their great cooperation in overcoming obstacles throughout the research period.

REFERENCES

1. Xu, Man, et al. Prevalence, cell tropism, and clinical impact of human parvovirus persistence in adenomatous, cancerous, inflamed, and healthy intestinal mucosa. Frontiers in Microbiology. 2022; 13(5):1–13.
2. Yousuf, Amjad. Human parvovirus B19 sero-molecular prevalence in Madinah blood donors, Saudi Arabia. Medical Science. 2022; 25(113):1551-1557.
3. Juhl, David; Hennig, Holger. Parvovirus B19: what is the relevance in transfusion medicine? Frontiers in Medicine. 2018, 5(4): 1-10.
4. Maisonneuve, Emeline, et al. Fetal brain injury associated with parvovirus B19 congenital infection requiring intrauterine transfusion. Fetal Diagnosis and Therapy. 2019; 46(1):1–11.
5. Teixeira, Ana Beatriz Gomes. O espectro das manifestações clínicas da infecção por Parvovírus B19. 2017. PhD Thesis.
6. Reggiani, Alessandro. A genome editing approach to the study of Parvovirus B19. 2022.
7. Sim, Jun Yi, et al. Human parvovirus B19 infection in patients with or without underlying diseases. Journal of Microbiology, Immunology and Infection. 2019; 52(4): 534-541.
8. Verdonschot, Job, et al. Relevance of cardiac parvovirus B19 in myocarditis and dilated cardiomyopathy: review of the literature. European journal of heart failure. 2016; 18(12): 1430-1441.
9. Manaresi, Elisabetta; Gallinella, Giorgio. Advances in the development of antiviral strategies against parvovirus B19. Viruses. 2019; 11(7):2:1-21.
10. Xu, Peng, et al. Parvovirus B19 NS1 protein induces cell cycle arrest at G2-phase by activating the ATR-CDC25C-CDK1 pathway. PLoS pathogens. 2017; 13(3):1–28.
11. Manaresi, Elisabetta; Gallinella, Giorgio. Advances in the development of antiviral strategies against parvovirus B19. Viruses. 2019; 11(7):659.
12. Soltani, Saber, et al. A literature review on the parvovirus B19 infection in sickle cell anemia and β-thalassemia patients. Tropical Medicine and Health. 2020; 48(1): 1-8.
13. Jawetz, Melnick, Adelberg's. Parvoviruses. In: MEDICAL MICROBIOLOGY. 28th edition. 2019. p. 460.
14. Sadah, Rand Rasid Abdul; AL-MARSOME, Huda Dhaher. Detection of Parvovirus B19 in B-thalassemia Major Patients by Serological and Molecular Method. Medico-Legal Update.2020; 20(4):2187-2191.
15. Fanoos HK. The Role of Parvovirus B19 in Thalassemia Patients in Thi- Qar Province. Thi-Qar; 2020.
16. Kishore, Janak; Srivastava, Manisha; Choudhury, Nabajyoti. Serological study on parvovirus B19 infection in multitransfused thalassemia major patients and its transmission through donor units. Asian journal of transfusion science.2011; 5(2): 140.
17. Majeed, Hala Mohamed. Detection of Parvovirus B19 Infection in Thalasemic Patients in Tikrit City, Serological Study. Medico-legal Update. 2020; 20(1): 287-291.
18. Adnan H T, Seroprevalence of Human Parvovirus B19 Infection among Thalassemic Children in Babylon Center of Hereditary Blood Disorders. Medical Journal of Babylon .2013; 10(2):491-496.
19. Vilibic-Cavlek, Tatjana, et al. Parvovirus B19 in Croatia: A Large-Scale Seroprevalence Study. Medicina. 2021; 57(11): 1279.
20. Habibzadeh, Shahram, et al. The prevalence of parvovirus B19 infection among pregnant women of Ardabil in 2013. Iranian journal of microbiology.2016; 8(3): 214.