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Immunological Detection of Epstein-Barr Virus Antigen and Evaluation of TNF-α and IL-10 in Patients with β-thalassemia Major

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ABSTRACT

Objective: *This study aims to detect Epstein–Barr virus antigens using the ELISA technique and to evaluate the immune status by measuring the levels of inflammatory cytokines, namely Tumor Necrosis Factor (TNF) and Interleukin-10 (IL-10).* ***Method:*** *This study was conducted on 150 patients previously diagnosed with major β-thalassemia by physicians at the hospital. Special kits were used to detect viral antigens, in addition to other kits used for measuring cytokine levels. Statistical analysis was also performed to evaluate the cytokine data.* ***Results:*** *The virus was detected in 82% of the total number of patients. The highest rate was recorded in patients aged between 11-20 years and females showed a higher infection rate compared to males. Regarding cytokines, there was a statistically significant increase in the levels of Interleukin-10 and Tumor Necrosis Factor in patients compared to the control group, p-values of 0.0048 and 0.0035, respectively.* ***Novelty:*** *The results revealed the presence of immune regulatory dysfunction in this group of patients, as they exhibited signs of both immune activation and suppression simultaneously. This underscores the importance of continuous immunological monitoring for this category of patients.*

INTRODUCTION

Epstein-Barr virus (EBV) is considered as one of the most widespread viruses across the globe [1]. EBV belongs to the family of Herpesviridae, gamma-herpesviruses is the subfamily to which it belongs [2,3]. EBV was first isolated in 1964 while researching Burkitt's lymphoma, it has been identified since with a variety of malignant tumors and immunity disorders [4]. 90% of earth's population were infected by it at a certain point in their lives. The primary infection is without any apparent symptoms, but in adults it may manifest as infectious mononucleosis [5,6].

This virus (EBV) can cause a latent infection after the initial one, this explains its existence in in B lymphocytes for long time [7]. In immune compromised patients the virus can re-activate itself causing serious complications as nasopharyngeal carcinoma, Burkitt's lymphoma, and Hodgkin's lymphoma, among other immune-related disorders such as autoimmune diseases [8]. so, diagnosis for the EBV infection is most important in battling these infections for the good of mankind [9].

IgM's and IgG's vs viral antigens is considered as the traditional serological method used to detect the virus in samples [10,11]. These tests primarily show past infections and are not effective in showing current active infections. Therefore, antigen detection tests relying on the ELISA technique are more accurate because they detect active viral proteins in blood serum [12].

Tumor Necrosis Factor (TNF) is also considered to be one of the most important pro-inflammatory cytokines [13], as it stimulates the immune

response against viruses by stimulating natural killer cells and cytotoxic T cells [14]. Although TNF, being so very important in enhancing immunity has extreme concentrations in the blood of patients with chronic diseases that result in negative impacts such as excessive inflammation or immune system dysregulation [15]. On the other hand, IL-10 is an anti-inflammatory cytokine of the immune system because its secretion by regulatory T cells and monocytes inhibits the cellular immune response, allowing the virus to lie dormant in the body [16]. Other studies have established that Epstein–Barr virus (EBV) is able to trigger the secretion of IL-10 to create a condition of immunosuppression, which facilitates its spread [17].

The study of these cytokines is particularly important in thalassemia patients, especially in β-thalassemia because they have been shown to have impaired immunity due to frequent blood transfusions as a result of defective synthesis of hemoglobin. This makes them susceptible to various infections, including Epstein–Barr virus (EBV) infection [18].Apart from the blood transfusion, there is a critical factor of iron overloading in the bloodstream that aids in immune suppression and creates a niche for viral proliferation [19].Subsequently, on the basis of this, the present study tried to detect Epstein–Barr viral antigens from the sera of patients with β-thalassemia using the ELISA technique together with estimating the level of tumor necrosis factor (TNF) and interleukin-10 (IL-10) in order to understand the immune response.

Ethical Considerations

This research was approved by the Ethics Committee of the College of Science at the University of Mosul, Iraq. Written informed consent was obtained from all participants and from the legal guardians of underage individuals after explaining the objectives of the study, the sample collection process, and the laboratory procedures that would be performed on their samples, document number 58910 (9/12/2025).

RESEARCH METHOD

A total of 150 blood samples were collected from 150 patients previously diagnosed with β-thalassemia by specialized physicians. These samples were obtained from Al-Hadbaa Specialized Hospital for Hematology and Bone Marrow Transplantation in Mosul, Iraq, which serves as the main referral center for thalassemia diagnosis and treatment in the city. The sample collection took place between December 2024 and April 2025. In addition, 20 blood samples from healthy individuals were included as a control group for the immunological cytokine measurements.

Five milliliters of venous blood were collected from each patient in plain tubes without anticoagulant. Serum was separated from the blood components by centrifugation at 3000 rpm for 10 minutes. The obtained serum samples were then stored at −20°C until further analysis.

The ELISA technique was used to detect Epstein-Barr virus (EBV) antigens present in serum samples, following the manufacturer's instructions provided with the

commercial kit (Human Epstein-Barr Virus (EBV)ELISA Kit Catalogue Number: SL2574Hu) belongs to Sun Long Biotech Co., LTD company made in China. The assay is based on the sandwich ELISA method, in which specific antibodies are pre-coated onto the wells of the microplate. Serum samples were added, followed by the enzyme-conjugated detection reagent to bind the captured antigens. The final step involved measuring the absorbance at 450 nm using an ELISA reader.

The level of Tumor Necrosis Factor-alpha (TNF-α) in serum was also measured by the ELISA technique. The standard curve was run using the standard solutions of the kit (Human Tumor Necrosis Factor α (TNF-α) ELISA Kit), Catalogue Number: SL1761Hu is a product of Sun Long Biotech Co., LTD company made in China, and the results were calculated by reading the absorbance at 450 nm using an ELISA reader.

In addition to measuring Tumor Necrosis Factor-alpha, the level of Interleukin-10 (IL-10) was also measured using the ELISA technique. A commercial kit (Human Interleukin 10 (IL-10) ELISA Kit Catalogue Number: SL0967Hu, from Sun Long Biotech Co., LTD company in China was used with the standard solutions provided by the company. The result was interpreted by measuring the absorbance at 450 nm using an ELISA reader.

Statistical analysis was made using SPSS software version 26. The data means and standard deviations were calculated. To compare the patient group and the control group, the unequal variance T-test (Welch's t-test) was applied. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Results

The presence of Epstein–Barr virus (EBV) antigens was investigated in 150 patients diagnosed with major β-thalassemia using the ELISA technique. The results revealed that 124 patients (82.6%) tested positive for EBV viral antigens. The highest positivity rate was recorded in the age group of 11–20 years, accounting for 34.7% of the total cases. Additionally, the prevalence was higher among females (34.5%) compared to males (29.3%).

Table 1. Prevalence of EBV according to different age/year groups in beta-thalassemia major patients by using the ELISA technique.

Age groups (years)	Number	positive	Percentage%
1-10	42	36	24
11-20	72	58	38.76
21-30	36	30	20
Total	150	124	82.6

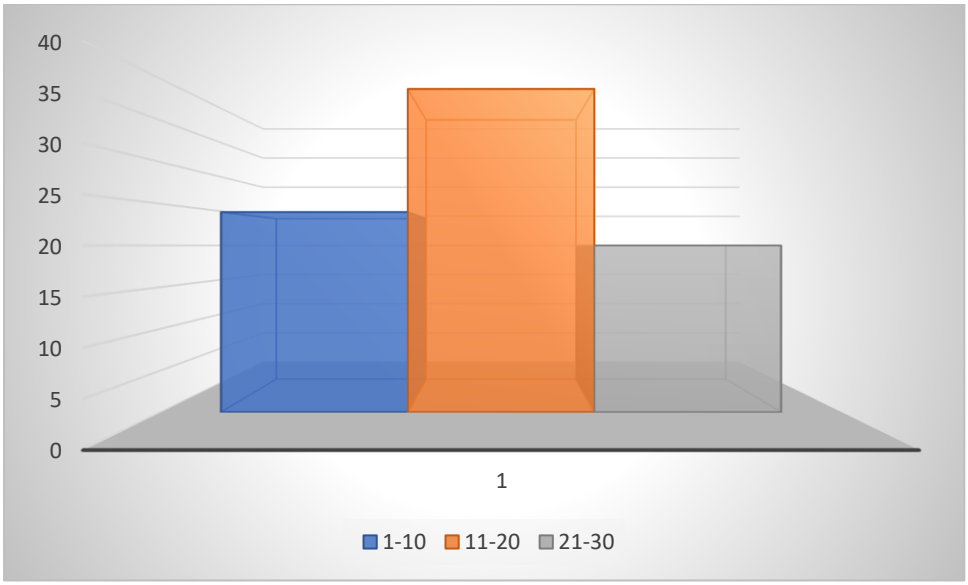

Figure 1. Prevalence of EBV according to different age/year groups in beta-thalassemia major patients by using the ELISA technique.

Table 2. Prevalence of EBV according to different sex groups in beta-thalassemia major patients by using the ELISA technique.

Sex groups	Number	Positive	Percentage%
Male	57	48	32
Female	93	76	50.6
Total	150	124	82.6

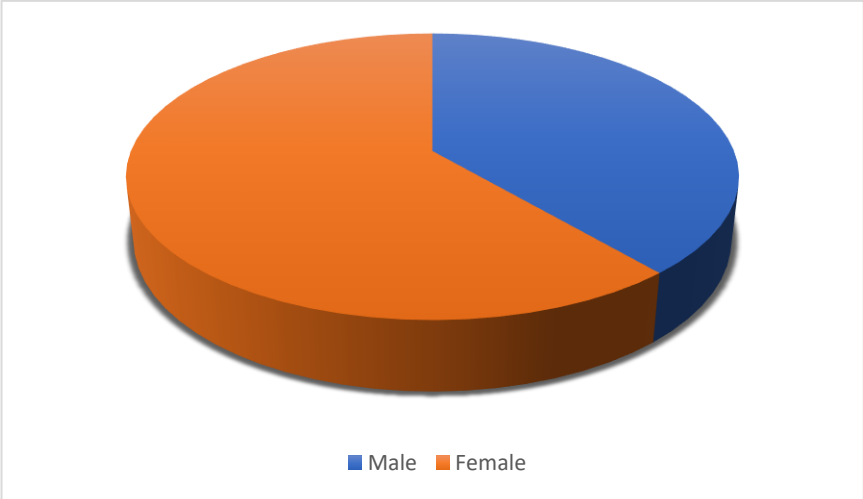

Figure 2. Prevalence of EBV according to different sex groups in beta-thalassemia major patients by using the ELISA technique.

From an immunological perspective, the levels of the cytokines Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-10 (IL-10) were measured in 130 patients using the ELISA method. These results were compared with a control group consisting of 20 healthy individuals.

The mean concentration of TNF-α in β-thalassemia patients was 118.8 ± 30.4 pg/mL, compared to 87.1 ± 137.7 pg/mL in the control group, with a statistically significant difference (p = 0.0035).

Table 3. The mean concentrations and standard deviation of TNF-α in thalassemia patients and controls.

Groups	Mean	Standard deviation	Number
Patient	118.3	215.64	130
Control	20.3	6.52	20

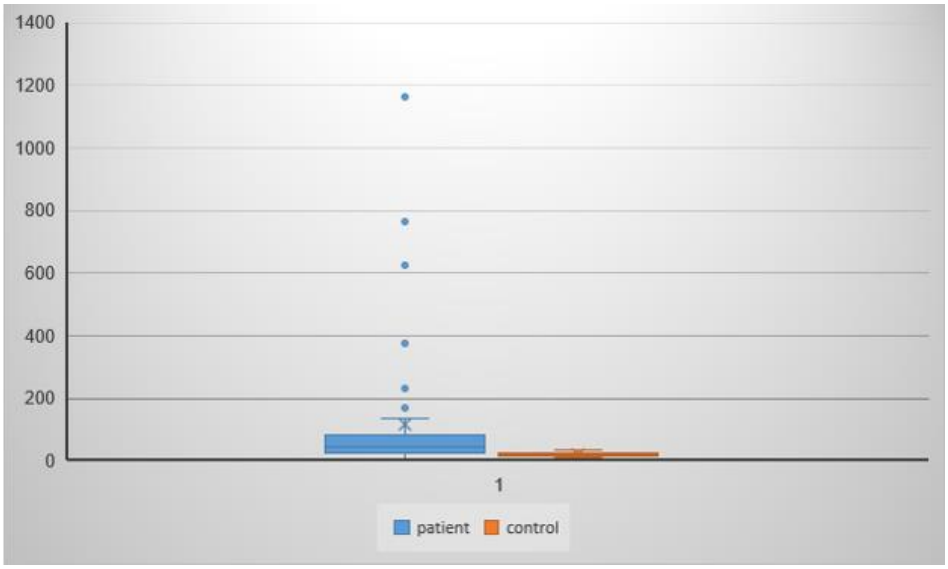

Figure 3. Box plot showing the distribution of IL-10 concentrations in thalassemia patients and the control group.

Similarly, the mean concentration of IL-10 in patients was 138.5 ± 10.2 pg/mL, compared to 30.2 ± 16.3 pg/mL in the control group, and this difference was also statistically significant (p = 0.0048).

Table 4. The mean concentrations and standard deviation of IL-10 in thalassemia patients and controls.

Groups	Mean pg/mL	Standard deviation	Number
Patients	31.56	27.93	130
Control	19.24	3.77	20

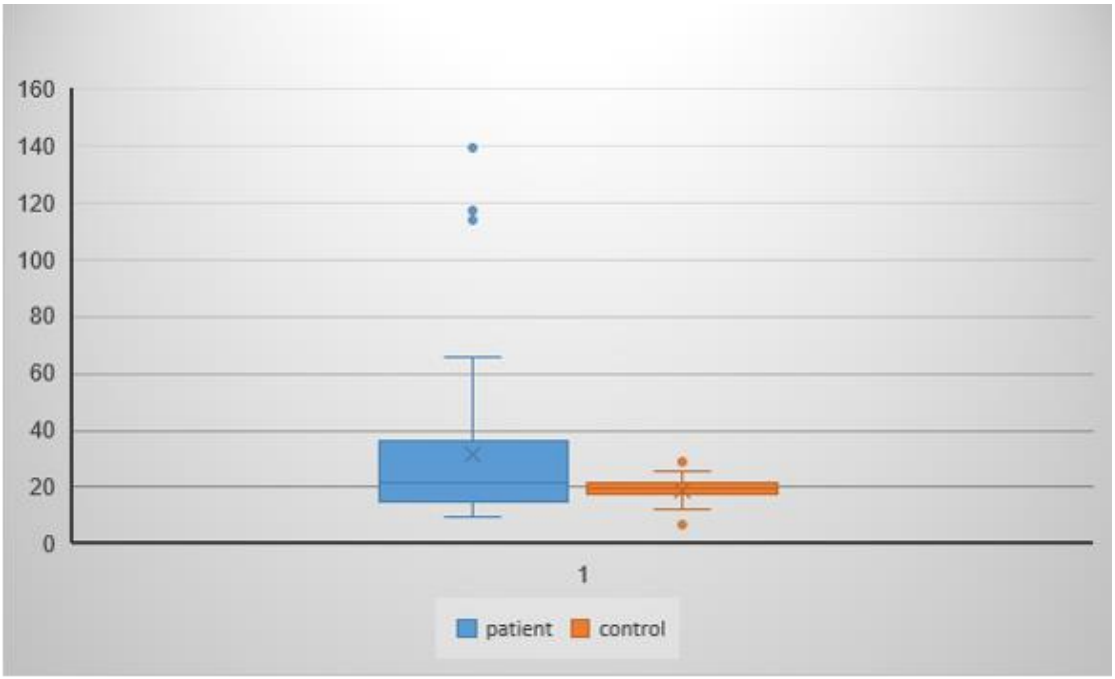

Figure 4. Box plot showing the distribution of IL-10 concentrations in thalassemia patients and the control group.

Discussion

This study revealed that the active infection with Epstein–Barr virus (EBV) reached a rate of 82.6%, which is considered high for this group of patients. This can be attributed to their repeated exposure to blood transfusions, whose transmission risk of blood-borne viruses is increased, especially in the context of chronic immune suppression [20,21]. Additionally, aggregation of great amounts of iron in the blood causes weakness to the immune system leading to reactivation of latent viruses, or even new infections by the virus [22,23]. Our study concurs with findings of a previous study, which demonstrated that blood recipients had a high rate of EBV infection, with a seroprevalence of 96.4% [20].

The age group of 11–20 years showed the highest percentage of EBV positivity, maybe due to high exposure during adolescence or higher susceptibility during this period. The seropositivity was also significantly high in females, which may be due to higher or more prolonged immune responses. This finding is consistent with Al-Khafaji and others, who reported a high seroprevalence of EBV among transfusion-dependent individuals [24].

This study agrees with the study done by Haghpanah and others in showing an elevated concentration of TNF- α in the blood of thalassemia patients, these elevated concentrations are due to the fact that these patients receive immune stimulations continuously as a form of repeated blood transfusions and iron

accumulation. Even thought, the high concentrations of TNF-α levels among patients can be attributed to infections, such as, viral infections [25].

The enhanced detection of EBV and B19V antigens in female patients in this study may be caused by gender-specific immune factors. Females have more efficient humoral

and cellular immunity, and this may result in higher persistence of antigens or higher detectability on serological tests. This has been observed in other studies in transfusion-dependent patients [24,26].

Physiologically, TNF-α is one of the key pro-inflammatory mediators of several immune pathways. Its chronic increase can directly lead to the suppression of hematopoiesis and thus result in systemic complications in thalassemia patients [27].

Our findings are in agreement with those of Balouchi et al., who reported significantly greater baseline levels of IL-10 in serum from β-thalassemia major patients compared to healthy controls [28].

Interleukin-10 (IL-10) is an anti-inflammatory regulatory cytokine secreted by several types of immune cells, including regulatory T cells (Tregs), B lymphocytes, and macrophages. Its primary function is to avert excessive immune stimulation through the inhibition of pro-inflammatory cytokine synthesis, including TNF-α and IL-6, and by downregulating antigen presentation as well as macrophage-mediated inflammatory responses [29].

The elevated levels of IL-10 observed in the current study might be a regulatory response whereby the immune system attempts to suppress tissue damage associated with chronic inflammation. However, persistently high IL-10 levels may also reflect a state of secondary immunosuppression, one of the factors underlying the increased susceptibility of thalassemia patients to infections particularly viral infections [30].

CONCLUSION

Fundamental Finding : This study revealed that active infection with Epstein–Barr virus (EBV) reached 82.6% in β-thalassemia major patients. The highest positivity occurred in the 11–20 age group, with females showing higher rates than males. There was a statistically significant increase in Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-10 (IL-10) levels compared to controls ($p = 0.0035$ and $p = 0.0048$, respectively). Findings indicate immune regulatory dysfunction, with simultaneous immune activation and suppression. **Implication :** The high EBV prevalence suggests that β-thalassemia major patients are at elevated risk due to repeated transfusions and iron overload, which weaken immunity and facilitate viral persistence. Elevated TNF-α reflects a chronic pro-inflammatory state that may suppress hematopoiesis, while high IL-10 levels suggest secondary immunosuppression. This dual immune profile may explain their increased susceptibility to opportunistic infections and highlights the need for targeted monitoring and intervention strategies. **Limitation :** The study was limited to a single specialized hospital, restricting generalizability. Its cross-sectional design provides only a snapshot of infection and immune status without causal inference or follow-up data. A small control group may reduce the statistical robustness of cytokine comparisons. The exclusion of other viral markers limited the broader understanding of immune status. **Future Research :** Further multicenter and longitudinal studies should explore EBV dynamics over time, the impact of transfusion frequency, and interactions with other transfusion-transmissible viruses. Research should examine gender-specific

immune responses and employ genomic or proteomic approaches to clarify mechanisms underlying the observed immune dysregulation, guiding better preventive and therapeutic strategies.

REFERENCES

[1] Y. Wong, M. T. Meehan, S. R. Burrows, D. L. Doolan, and J. J. Miles, "Estimating the global burden of Epstein–Barr virus-related cancers," *J. Cancer Res. Clin. Oncol.*, vol. 148, no. 1, pp. 31–46, 2022.

[2] A. Riaz, K. Murtaz-ul-Hasan, and N. Akhtar, "Recent understanding of the classification and life cycle of herpesviruses: a review," *Sci. Lett.*, vol. 5, pp. 195–207, 2017.

[3] M. F. Haddad, A. A. Al Taie, and B. A. Abdullah, "Relation between Epstein–Barr virus (EBV) with some biochemical variables in high-risk aborted women in Mosul city, Iraq," *J. Appl. Nat. Sci.*, vol. 15, no. 4, pp. 1332–1338, 2023.

[4] M. A. Epstein, G. Henle, B. G. Achong, and Y. M. Barr, "Morphological and biological studies on a virus in cultured lymphoblasts from Burkitt's lymphoma," *J. Exp. Med.*, vol. 121, no. 5, pp. 761–770, 1965.

[5] H. S. Abdulkareem and A. A. AlTaie, "Detection of Epstein–Barr virus (EBV) among thyroid disorders patients using enzyme-linked immunoassay (ELISA) and indirect immunofluorescence assay (IFA) in Mosul city, Iraq," *Malays. J. Microbiol.*, vol. 20, no. 6, pp. 253–257, 2024.

[6] S. K. Dunmire, P. S. Verghese, and H. H. Balfour Jr., "Primary Epstein–Barr virus infection," *J. Clin. Virol.*, vol. 102, pp. 84–92, 2018.

[7] M. Kalla and W. Hammerschmidt, "Human B cells on their route to latent infection – early but transient expression of lytic genes of Epstein–Barr virus," *Eur. J. Cell Biol.*, vol. 91, no. 1, pp. 65–69, 2012.

[8] B. Zhang, Y. Zong, J. He, B. Zhong, and S. Lin, "Primary nasopharyngeal non-Hodgkin lymphoma and its relationship with Epstein–Barr virus infection," *Chin. Med. J.*, vol. 116, no. 6, pp. 913–917, 2003.

[9] D. J. Moss, S. R. Burrows, S. L. Silins, I. Misko, and R. Khanna, "The immunology of Epstein–Barr virus infection," *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, vol. 356, no. 1408, pp. 475–488, 2001.

[10] T. Shi, L. Huang, L. Luo, Q. Yu, and J. Tian, "Diagnostic value of serological and molecular biological tests for infectious mononucleosis by EBV in different age stages and course of the disease," *J. Med. Virol.*, vol. 93, no. 6, pp. 3824–3834, 2021.

[11] A. A. Al Taie, M. A. Ibraheem, and K. B. Fadhil, "Epstein–Barr virus infection in Thalassemia patients related to blood group in Mosul, Iraq," *Ann. Trop. Med. Public Health*, vol. 22, p. 136, 2019.

[12] S. Tischer et al., "Evaluation of suitable target antigens and immunoassays for high-accuracy immune monitoring of cytomegalovirus and Epstein–Barr virus-specific T cells as targets of interest in immunotherapeutic approaches," *J. Immunol. Methods*, vol. 408, pp. 101–113, 2014.

[13] R. F. Souza, M. A. F. Caetano, H. I. R. Magalhães, and P. Castelucci, "Study of tumor necrosis factor receptor in the inflammatory bowel disease," *World J. Gastroenterol.*, vol. 29, no. 18, pp. 2733–2746, 2023.

[14] N. M. Gálvez, K. Bohmwald, G. A. Pacheco, C. A. Andrade, L. J. Carreño, and A. M. Kalergis, "Type I natural killer T cells as key regulators of the immune response to infectious diseases," *Clin. Microbiol. Rev.*, vol. 34, no. 2, pp. 10–1128, 2021.

[15] G. Van Loo and M. J. Bertrand, "Death by TNF: a road to inflammation," *Nat. Rev. Immunol.*, vol. 23, no. 5, pp. 289–303, 2023.

[16] H. Islam, H. Neudorf, A. L. Mui, and J. P. Little, "Interpreting 'anti-inflammatory' cytokine responses to exercise: focus on interleukin-10," *J. Physiol.*, vol. 599, no. 23, pp. 5163–5177, 2021.

[17] R. Incrocci, M. McCormack, and M. Swanson-Mungerson, "Epstein–Barr virus LMP2A increases IL-10 production in mitogen-stimulated primary B-cells and B-cell lymphomas," *J. Gen. Virol.*, vol. 94, no. 5, pp. 1127–1133, 2013.

[18] A. Bazi, I. Shahramian, H. Yaghoobi, M. Naderi, and H. Azizi, "The role of immune system in thalassemia major: a narrative review," *J. Pediatr. Rev.*, vol. 6, no. 2, pp. 29–36, 2018.

[19] E. M. Walker and S. M. Walker, "Effects of iron overload on the immune system," *Ann. Clin. Lab. Sci.*, vol. 30, no. 4, pp. 354–365, 2000.

[20] R. A. Kathem, A. H. Kareem, and N. T. Altaee, "The frequency of Epstein–Barr virus among hemodialysis patients," *Int. J. Virol.*, vol. 15, no. 2, pp. 85–91, 2019.

[21] E. A. Ihsan, L. M. Mohammed, and W. M. Ali, "Prevalence of Epstein–Barr virus among hemodialysis patients in Kirkuk city," *NTU J. Pure Sci.*, vol. 2, no. 4, pp. 17–22, 2023.

[22] S. Ni, Y. Yuan, Y. Kuang, and X. Li, "Iron metabolism and immune regulation," *Front. Immunol.*, vol. 13, p. 816282, 2022.

[23] S. Puntarulo, "Iron, oxidative stress and human health," *Mol. Aspects Med.*, vol. 26, no. 4–5, pp. 299–312, 2005.

[24] B.-N. Ding, Y.-L. Wu, Y.-Y. Zhang, and Y.-G. Li, "Association between Epstein–Barr virus infection and serum positivity rate of anti-nuclear antibodies in Chongqing, China: A cross-sectional observational study," *Medicine (Baltimore)*, vol. 103, no. 32, p. e39233, 2024.

[25] S. Haghpanah et al., "Cytokine levels in patients with β-thalassemia major and healthy individuals: A systematic review and meta-analysis," *Clin. Lab.*, vol. 68, no. 11, pp. 1201–1210, 2022.

[26] G. Ceccarelli et al., "Reassessing the risk of severe parvovirus B19 infection in the immunocompetent population: A call for vigilance in the wake of resurgence," *Viruses*, vol. 16, no. 9, p. 1352, 2024. [Online]. Available: https://www.mdpi.com/1999-4915/16/9/1352. [Accessed: Jul. 4, 2025].

[27] D. I. Tanyong, P. Panichob, W. Kheansaard, and S. Fucharoen, "Effect of tumor necrosis factor-alpha on erythropoietin- and erythropoietin receptor-induced erythroid progenitor cell proliferation in β-thalassemia/hemoglobin E patients," *Turk. J. Hematol.*, vol. 32, no. 4, pp. 304–310, 2015.

[28] S. Balouchi, M. Gharagozloo, N. Esmaeil, B. Moayedi, and E. Nafiseh, "Serum levels of TGF-β, IL-10, IL-17, and IL-23 cytokines in β-thalassemia major patients: The impact of silymarin therapy," *Immunopharmacol. Immunotoxicol.*, vol. 36, no. 4, pp. 271–274, 2014.

[29] V. Carlini et al., "The multifaceted nature of IL-10: Regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions," *Front. Immunol.*, vol. 14, p. 1161067, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1161067/full. [Accessed: Jul. 4, 2025].

[30] A. Gluba-Brzóźka, B. Franczyk, M. Rysz-Górzyńska, R. Rokicki, M. Koziarska-Rościszewska, and J. Rysz, "Pathomechanisms of immunological disturbances in β-thalassemia," *Int. J. Mol. Sci.*, vol. 22, no. 18, p. 9677, 2021.

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