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Molecular Detection Of Epstein-Barr Virus In Patients With Major B-Thalassemia In Iraq/Mosul City

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Abstract

Background *Transfusions of blood are among the most crucial elements.in establishing infection with EBV in Beta-thalassemia patients, as it renders their immune responses useless. This herpes family member (EBV) has a unique pathogenicity as it undergoes a latent period, after the initial infection, in which it resides within B cells waiting for immunity to drop so that it can cause reinfection.*

This research aims to detect this oncogenic virus in people with beta-thalassemia for multiple genders and ages using the polymerase chain reaction PCR ***Method*** *One hundred and fifty blood samples were collected from patients of different ages and genders from November/2024 to April/2025 in A specialized medical center in blood diseases and bone marrow transplantation – AlHadbaa Hospital. in Mosul/Iraq, each sample was diagnosed using polymerase chain reaction (PCR).* ***Results:*** *Positive results were 63.8% of all samples. The prevalence of EBV was as high as 34.6% in people between 11-20 years old, marking the highest percentage of all ages in this study. Females had 34.5% of the whole 63.8% infection percentage.* ***In conclusion,*** *we can estimate that the EBV virus is a common virus among those who suffer from beta thalassemia, and PCR is an efficient method and could be considered the most accurate to detect this virus.*

Keywords: *EBV, β-thalassemia, PCR*

INTRODUCTION

Thalassemia represents a significant global health concern. The term 'thalassemia' originates from the Greek word 'Thalassa', meaning sea, reflecting the disorder's prevalence in Mediterranean regions [1,2]. An estimated 5% of the global population carries the Alpha-thalassemia trait, in case of around 1.5% are carriers of beta-thalassemia [3]

Thalassemias comprise a group of inherited blood disorders transmitted in an autosomal recessive pattern, resulting from the reduction or absence of production of one or more of the globin chains that make up These globin chains combine to form hemoglobin (Hb) tetramers. Based on the specific globin chain that is deficient or defective, thalassemia is classified into two major types: alpha-thalassemia and beta-thalassemia [4]

Thalassemia is commonly found in various parts of the world, particularly throughout the Mediterranean basin, Africa, the Middle East, the Indian subcontinent, and Southeast Asia. It has become a major global health concern, with millions of individuals affected across different regions [5] The distribution of thalassemia is not uniform and tends to be more prevalent in areas where consanguineous (related) marriages are culturally accepted and frequent as in portions of Africa, the Middle East, and South Asia. These intra-family marriages increase the likelihood of inheriting thalassemia gene mutations, leading to a higher 7incidence within specific populations [6,7]. Roughly 5% of the global population carries the alpha-thalassemia gene, while beta-thalassemia is found in about 1.5% of people worldwide [3].

Alpha thalassemias occur more frequently. This inherited blood disorder results from reduced manufacturing of α-globin chains, creating Alpha and beta globin chains that are not balanced which contributes to a range of clinical manifestations [8,9,2]

β-thalassemia is a genetically diverse autosomal recessive anemia caused by decreased the lack of production of β-globin chains. Each year, around 68,000 kids worldwide possess from birth different forms of thalassemia syndromes. The condition is widespread, with an estimated 80 to 90 million carriers globally [9,10].

Beta thalassemia results from reduced or absent creation of chains of beta-globin, which causes an accumulation of excess alpha chains. The synthesis of beta-globin is regulated via a single gene on chromosome 11. This condition arises from over 200 known point mutations as well as, in rare cases, gene deletions. The amount of beta-globin produced can vary from nearly normal to completely lacking, resulting in different levels of imbalance between alpha and beta chains. Individuals with a defect in one

beta-globin gene, known as beta thalassemia trait (or minor), usually experience no symptoms but may show mild anemia and small red blood cells (microcytosis). When both genes are severely affected, the person develops beta thalassemia major, also called Cooley anemia [2].

EBV, or Epstein-Barr virus, was the first virus discovered to cause cancer in humans, widespread, affecting more than 90% of the global population. Since it is acknowledged as Classified as a Group 1 by the World Health Organization, an oncogenic virus, Epstein–Barr virus (EBV) is responsible for more than 200,000 cancer cases and approximately 1.8% of cancer-related deaths annually. EBV was first identified in 1964 by Yvonne Barr and Michael Epstein during their research on lymphoblastoid cell cultures derived from Burkitt lymphoma (BL) sufferers [11]. Additionally, EBV infection has been connected to various Autoimmune diseases, such as multiple sclerosis (MS) and systemic lupus erythematosus (SLE). EBV is an enveloped virus and it is Human herpesvirus is a member of the Gammaherpesvirinae subfamily of the Herpesviridae family. The virus possesses More than 80 viral proteins encoded by this linear double-stranded DNA genome, which is roughly 172 kilobase pairs (kbp) long as well as several noncoding RNAs [12,13].

EBV is capable of infecting various cell types but primarily targets both epithelial cells and B cells. Its entry mechanism is highly complex, allowing the virus to infect different host cells efficiently. EBV encodes up to 13 glycoproteins, with eleven present on the viral envelope. Successful viral entry requires the coordinated action of multiple glycoproteins. Among these, the fusing of the core proteins gH/gL and gB are essential for the entrance of viruses across all herpesviruses, whereas more glycoproteins contribute variably to determining viral tropism, infection outcome, and the entry process itself [12].

EBV infections are common among patients who receive multiple blood transfusions, including those with beta-thalassemia [14,15].
Saliva is the primary route for Epstein–Barr virus (EBV) transmission. To maintain long-term persistence, EBV effectively replicates entre mucosal B-lymphocytes and oropharyngeal epithelial cells. Although EBV primarily targets B-lymphocytes and epithelial cells., it is also capable of infecting natural killer (NK) cells, follicular dendritic cells, and T-lymphocytes [16]. Additionally, the virus can spread through organ transplantation and blood transfusions. Elderly people with impaired immune systems are especially susceptible to the severe consequences of infection with EBV [17].
EBV infection typically occurs during early childhood as well as is generally moderate and self-restricting. It is the primary cause of infectious mononucleosis and the first symptoms of it include fever, swollen and tender lymph nodes, and pharyngitis. Furthermore, case reports and epidemiological studies have suggested a link between EBV infection and various conditions, including the development of several autoimmune diseases, such as rheumatoid arthritis (RA), multiple sclerosis (MS), systemic lupus erythematosus (SLE), and others, has been connected to lymphoproliferative disorders, vitamin D deficiency, chronic fatigue syndrome, thyroid disorders, and Epstein-Barr virus (EBV) infection. [11]

Aim of the study

The aim of this study is to detect the prevalence Epstein-Barr virus (EBV) in beta-thalassemia patients in Mosul / Iraq, using polymerase chain reaction (PCR) as a molecular diagnostic method.

MATERIAL AND METHODS

The study enrolled one hundred and fifty patients who had been diagnosed and confirmed histopathologically with Beta-thalassemia Major, who were under regular blood transfusion in A specialized medical center in blood diseases and bone marrow transplantation – AlHadbaa Hospital in Mosul/ Iraq.
Five milliliters of blood were collected from each patient in a gel tube, serum was taken by centrifugation at 3000 rpm for 5 min, and DNA extraction was applied to it according to the procedure of the AddPrep Viral Nucleic Acid Extraction kit, REF10034, manufactured in Korea.
Amplifying DNA extraction by the PCR (The technology of polymerase chain reaction). The viral gene that was used in amplifying is EBNA1(BKRF1), it is of F: GTCATCATCATCCGGGTCTC, R: TTCGGGTTGGAACCTCCTTG sequence, and the amplicon size was 270 bp [18].
12.5 µl GoTaq® G2 Green Master Mix was added, 8 µl of extracted DNA, 1 µl of forward primer (F), 1 µl of reverse primer (R), and 2.5 µl of nuclease-free water. The optimum conditions were used for the

PCR run for primers; initial denaturation temperature was 94°C for 5 min, and cycle number was 40, The denaturation step was performed at 94°C, followed by annealing at 63°C for 45 seconds. The initial extension occurred at 72°C for 45 seconds, with a final extension at 72°C for 5 minutes [19].
After PCR amplification, 6 µl of the PCR products (amplicons) were either subjected to electrophoresis immediately on a 2% agarose gel using a UV transilluminator apparatus to see bands [20,21].
Ethical approval This case control study was conducted by the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with the patient's verbal and analytical approval before the sample was taken. The study protocols, the subject information, and the consent were reviewed and approved by a local ethics committee according to document number 58910 (in 9/12/2025) to get this approval.

RESULTS

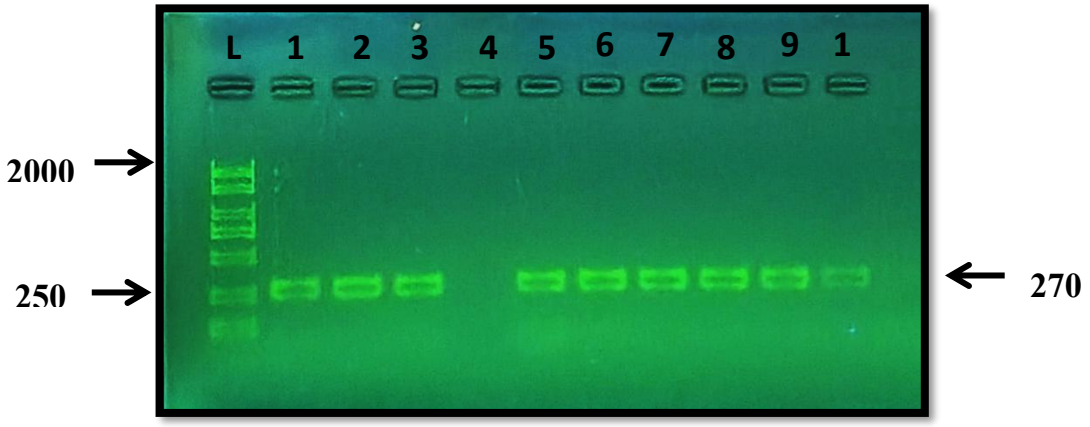

Figure1: Detection of EBV for EBNA1 gene with (270 bp), Line (L) Ladder, lines (1-3), (5-10) represent positive result of EBV for gene EBNA1, line (4) Negative. Electrophoresis conditions are 5v /cm^2.

From 150 samples, we obtained 96 positives, and according to Table 1, the distribution of β-thalassemia major patients was 14.6% in (1-10) years 34.6% in (11-20) years, and 14.6% in (21-30) years.
The results showed that the highest percentage recorded was 52 positive samples (34.6%) in (11-20 years.

Ages/years	Number	positive	Percentage%
1-10	42	22	14.6%
11-20	72	52	34.6%
21-30	36	22	14.6%
Total	150	96	63.8%

Table 1: Prevalence of EBV according to different age/year groups in beta-thalassemia major patients.

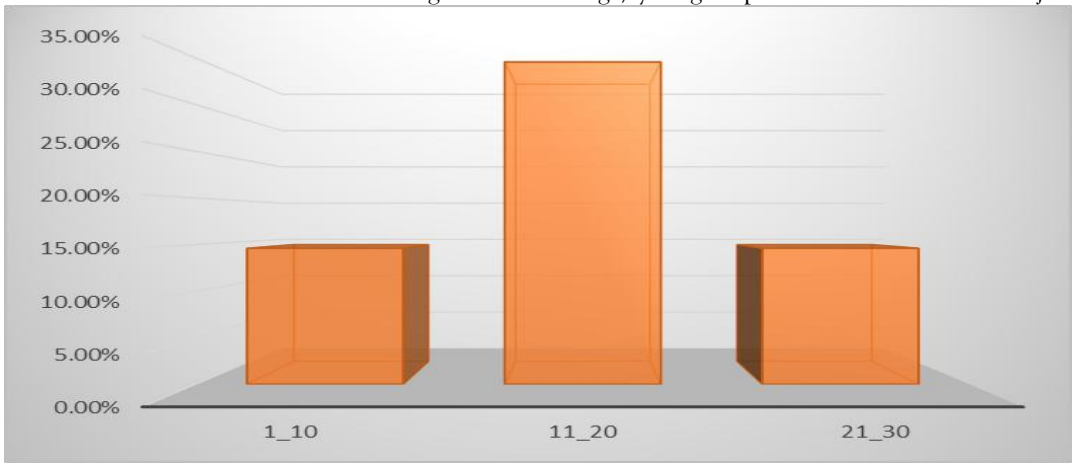

Figure 2: Prevalence of EBV according to different age/year groups in beta-thalassemia major patients by using the PCR technique

According to Table 2, the distribution of β-thalassemia major patients based on sex was 29.3% in males and 34.5% in females.

Sex	Number	positive	Percentage %
Male	57	44	29.3%
Female	93	52	34.5%
Total	150	96	63.8%

Table 2: Prevalence of EBV according to sex in beta-thalassemia major patients

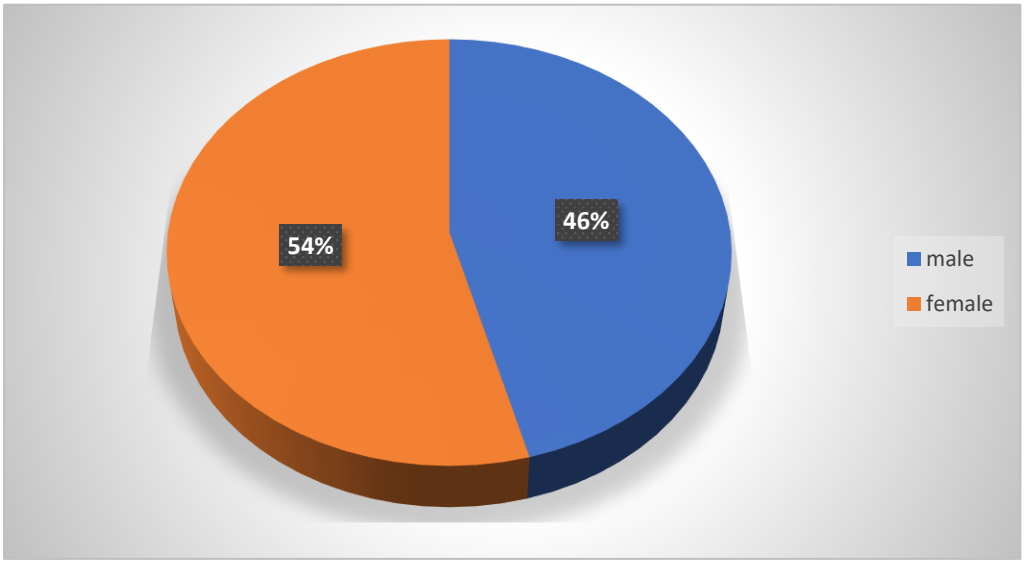

Figure 3: Prevalence of EBV according to different sex groups in beta-thalassemia major patients by using the PCR technique

DISCUSSION

This study indicated that Epstein–Barr virus (EBV) infection was generally more common in females than in males. Among females, the infection rate was 34.5%, while in males it was 29.3%, particularly among those aged between 11 and 20 years. These differences were attributed to social factors. On the other hand, Li et al. (2021) found that hormones such as estrogen were believed to influence the immune response to EBV, especially in B cells. Other studies explained this by suggesting that estrogen could have affected the gene expression of EBV during its latent phase. This hormone might have made females more susceptible to infection. Another study such as Chien et al. (2025) showed that infection developed more in females due to differences in immune response, as females produced higher levels of antibodies against the virus compared to males [22,23].

From a social perspective, and according to (Winter et al. 2020) delayed exposure to EBV tended to occur more in older individuals than in younger ones. As a result, the 11–20 age group became more vulnerable. One study showed a rise in EBV infection among girls aged 10 to 14. These factors significantly influenced the spread of the virus in this age group [24,25].

As for patients with beta-thalassemia, they face a higher risk of viral infections, mainly because of frequent blood transfusions. These repeated procedures raise the chance of being exposed to blood-borne viruses like EBV. Additionally, many thalassemia patients already have weakened immune systems, due to chronic inflammation, iron overload, or the genetic disorder itself, which makes fighting off viruses even harder. This means managing viral infections in these patients requires close medical follow-up and care [26].

EBV, like other herpesviruses, can stay in the body for years in a latent state, especially in B cells. Understanding how it remains hidden and later reactivates is key to grasping its long-term effects and complications [26].

Early and consistent transfusions are critical for managing β-thalassemia major, especially when paired with proper iron chelation. Still, unsafe transfusions can expose patients to serious infections such as HCV, HBV, and HIV, especially as the total number of transfused blood units increases [27].

EBV poses more danger in immunocompromised patients like those with β-thalassemia. In these cases, it can lead to more severe problems, including cancers like Hodgkin lymphoma, due to the weakened immune system. Chronic EBV infections can trigger long-term immune activation, which worsens the patient's ability to control the virus and increases complications [28].

On another level, the inflammation observed in thalassemia patients gave insight into how the immune system functioned. The elevated response of cytokines such as Interleukin-6 and Tumor Necrosis Factor not only indicated the presence of anemia but also possibly encouraged the reactivation of latent viruses, such as the Epstein–Barr virus. [29].

Preventing these complications did not only involve maintaining appropriate iron levels and avoiding anemia, but also included vaccination programs, screening of blood donors, and the use of advanced diagnostic techniques to control infection rates. Moreover, the educational and cultural background of patients supported a reduction in the risk of such viral infections. [30].

Limitations

- Small sample size limited to a single city (Mosul), which may not be representative of the broader population.
- Lack of longitudinal follow-up to assess EBV persistence or reactivation.
- No serological confirmation of EBV infection, only PCR was used.
- The immune status of patients was not evaluated, which could influence viral detection.

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Conflict of interest

Others declare no conflict of interest

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