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Molecular Diagnosis of the Cytomegalovirus (CMV) among Thalassemia Patients and its Relation to Different Clinical Factors

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Abstract : Thalassemia is a genetic blood disease with abnormal hemoglobin chains, leading to anemia. Regular blood transfusions are often required for treatment, but they carry the risk of transfusion-transmitted infections, including cytomegalovirus (CMV). Thalassemia patients are at increased risk of CMV-related complications due to frequent blood transfusions and potential iron overload. Real-time polymerase chain reaction (RT- PCR) has emerged as a powerful tool for detecting and quantifying CMV DNA in biological samples. A study involved 150 patients with thalassemia, and thalassemia major being the most common subtype. The median frequency of blood transfusions was once every 4 weeks, ranging from every 3 weeks to every 6 weeks. Most patients (84.0%) received chelation therapy, with deferoxamine being the most commonly used chelator. Liver involvement was a common finding, with 64 patients (42.7%) presenting with an enlarged liver. CMV DNA positivity was significantly higher in patients with thalassemia major (42.4%) than those with thalassemia intermedia (21.9%). The study highlights the importance of routine CMV screening and monitoring in thalassemia patients to prevent potential complications.

Keywords: Human cytomegalovirus (HCMV), HCMV DNA, Thalassemia, RT-PCR.

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Introduction

The thalassemia an inherited blood disorder that affects your body's ability to produce hemoglobin and healthy red blood cells, leading to different levels of anemia and other problems (1). Regular blood transfusions are often required as a life-long treatment for thalassemia major and intermedia to manage anemia and promote growth and development (2, 3). However, frequent blood transfusions carry the risk of transfusion-transmitted infections, including cytomegalovirus (CMV) (4, 5).

CMV is a ubiquitous herpesvirus that establishes a lifelong latent infection in the host following primary infection (6, 7). Although CMV infection is often asymptomatic in immunocompetent individuals, Immunocompromised patients, including those with HIV infection, organ transplant recipients, and those on immunosuppressive therapy, may have severe and sometimes fatal consequences as a result of it [8, 9].

Thalassemia patients, due to their frequent blood transfusions and potential iron overload, may also be at increased risk of CMV-related complications (10, 11, 12). Patients with thalassemia are affected by CMV infection in ways that go beyond the virus's acute symptoms. CMV has been implicated in organ damage, particularly in the liver and kidneys, in thalassemia patients (13, 14, 15). There is also growing evidence that CMV infection may negatively impact the effectiveness of chelation therapy, which is a crucial aspect of managing iron overload in thalassemia (16, 17). Therefore, To start proper treatment and avoid possible long-term problems, early identification and monitoring of CMV infection in thalassemia patients are crucial.

Real-time polymerase chain reaction (real-time PCR) has emerged as a powerful tool for detecting and quantifying CMV DNA in biological samples (18, 19). It offers high sensitivity and specificity, allowing for the rapid and accurate detection of low levels of viral DNA. The aim of this study, real-time PCR was used to find out how standard and heavy the CMV DNA was in thalassemia patients' blood and the link between the infection and different clinical factors.

Materials and Methods

Study population and sample collection

This study's cross-sectional design was conducted at the Center for Genetic Blood Diseases (Thalassemia) in Maysan Governorate, Iraq, from January 2023 to December 2023. The study included thalassemia major and intermedia patients who regularly received blood transfusions and chelation therapy. Patients with a history of solid organ or stem cell transplantation, those receiving immunosuppressive therapy for conditions other than thalassemia, and those with active infections requiring hospitalization at the time of sample collection were taken out of the study.

Clinical and demographic information, such as age, gender, transfusion frequency, chelation therapy, and iron overload assessment results (liver and spleen size, liver enzymes, and cardiac parameters), were collected from the participant's medical records. Ethylenediaminetetraacetic acid (EDTA) tubes were used to collect blood samples (2 mL) before blood transfusion and processed immediately for plasma separation. Plasma samples were aliquoted and kept cold (-80°C) until further examination.

DNA extraction and real-time PCR for CMV DNA detection

The QIAamp DNA Mini Kit (Qiagen, Germany) was used to get DNA out of 200 µL of plasma, following the manufacturer's directions. The DNA was removed and eluted in 50 µL of elution water. It was then kept at -20°C until it was time for PCR analysis.

A real-time PCR kit (CMV PCR Kit, Sacace, Italy) was used to find and measure CMV DNA on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA). This kit is designed to find CMV's highly conserved glycoprotein B (gB) gene, ensuring the test is specific and accurate. The kit came with primers, probes, and a master mix. Nuclease-free water was added to make a final reaction amount of 25 µL, which had 5 µL of the extracted DNA template. For the first cycle, the protein was denatured at 95°C for 10 minutes. This was followed by 45 cycles of denaturing at 95°C for 15 seconds and

annealing/extension at 60°C for 1 minute.

A positive control (included in the kit) and a negative control (nuclease-free water) were used in every real-time PCR sample. A standard curve was generated using known concentrations of CMV DNA to quantify the CMV DNA load in the samples, and the results were expressed as international units (IU)/mL. Following the manufacturer's recommendations, a sample was considered positive for CMV DNA if the CMV DNA load exceeded 100 IU/mL.

Statistical analysis

The statistical analysis was done using IBM Corp.'s SPSS program (version 25.0, USA). The research participants' clinical and demographic information was compiled using descriptive statistics. Categorical data were shown as frequencies and percentages, whereas continuous variables were given as mean ± standard deviation or median and interquartile range. Where applicable, the chi-square test was used to compare categorical variables. The Mann-Whitney U or Kruskal-Wallis H tests were used to assess statistical differences between groups for continuous variables. Less than 0.05 was the threshold for statistical significance.

RESULTS AND DISCUSSION

Demographics and clinical characteristics

The research included 150 individuals with thalassemia in total. The patient's average age was 25.6 ± 7.8 years, and the study population comprised 82 females (54.7%) and 68 males (45.3%), as shown in Table 1. Thalassemia major was the most common subtype, with 118 patients (78.7%) having this disease. The median frequency of blood transfusions was once every 4 weeks, ranging from every 3 weeks to every 6 weeks. Most patients (84.0%) received chelation therapy, with deferoxamine being the most commonly used chelator (Table 1).

Table (1): Demographics and clinical characteristics of thalassemia patients

Demographic and Clinical Characteristics	n	%
Mean Age (± SD)	25.6 (± 7.8)	-
Female	82	54.7
Male	68	45.3
Thalassemia Major	118	78.7
Thalassemia Intermedia	32	21.3
Transfusion Frequency*		
Every 3 weeks	45	30.0
Every 4 weeks	58	38.7
Every 5 weeks	32	21.3
Every 6 weeks	15	10.0
Chelation Therapy		
Deferoxamine	92	61.3
Deferiprone	34	22.7
None	24	16.0

*Frequency of blood transfusions.

Liver involvement was a common finding, with 64 patients (42.7%) presenting with an enlarged liver. Elevated liver enzymes were observed in 47 patients (31.3%), and splenomegaly was present in 43

patients (28.7%). Cardiac complications, including cardiomyopathy and arrhythmias, were reported in 19 patients (12.7%) (Table 1).

Prevalence and load of CMV DNA in thalassemia patients

Out of the 150 plasma samples analyzed, 54 samples (36.0%) tested positive for CMV DNA by real-time PCR. The realtime PCR amplification curve of CMV was demonstrated in figure 1. The median CMV DNA load in the positive samples was 325 IU/mL, with an interquartile range of 215-560 IU/mL. There was a minimum of 120 IU/mL and 2850 IU/mL high in the CMV DNA load. The incidence of CMV DNA positive did not significantly vary between male and female individuals (p = 0.456).

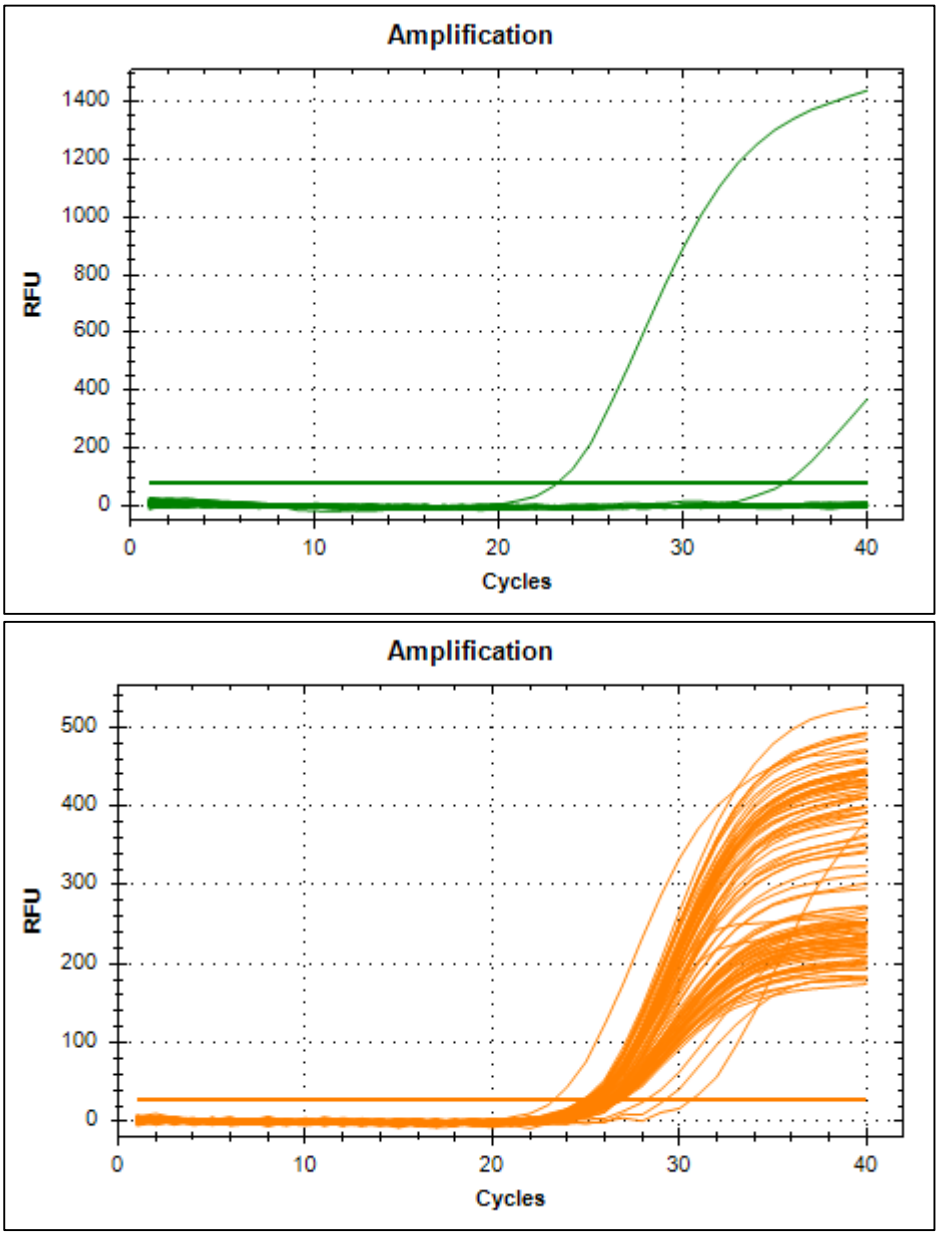

Figure (1): Real-time PCR amplification curve of Cytomegalovirus.

Table (2): Presents the full findings

CMV DNA Positivity and Load	n	%	Median Load (IU/mL)
CMV DNA Positive	54	36.0	325
CMV DNA Negative	96	64.0	

Range 120 – 2850

Thalassemia patients face unique challenges due to their underlying disease and the frequent blood transfusions they require. CMV infection is a significant concern, and our study highlights its prevalence and impact in this population. We detected CMV DNA in over one-third of the blood samples analyzed using real-time PCR. This prevalence is higher than reported in some previous studies (20, 21) but aligns with findings from other regions (22, 23, 24). Geographical differences, transfusion practices, and diagnostic sensitivity may contribute to the variation in prevalence rates.

Factors associated with CMV DNA positivity

The association between CMV DNA positivity and various clinical parameters was analyzed (Table 3). CMV DNA positivity was significantly higher in patients with thalassemia major (42.4%) compared to those with thalassemia intermedia (21.9%) ($p = 0.021$). Patients with an enlarged liver were more likely to test positive for CMV DNA (46.9%) compared to those with a normal-sized liver (26.7%) ($p = 0.012$). A significant association was also found between CMV DNA positivity and elevated liver enzymes ($p = 0.009$). However, no significant associations were observed between CMV DNA positivity and age, frequency of blood transfusions, splenomegaly, or cardiac complications.

Table (3): The association between CMV DNA positivity and various clinical parameters of thalassemia patients

Factor	CMV DNA Positive	CMV DNA Negative	p-value
Thalassemia Major	50 (42.4%)	68 (57.6%)	0.021*
Thalassemia Intermedia	7 (21.9%)	25 (78.1%)	
Enlarged Liver	30 (46.9%)	34 (26.7%)	0.012*
Normal-Sized Liver	24 (26.7%)	68 (73.3%)	
Elevated Liver Enzymes	35 (46.7%)	12 (16.0%)	0.009*
Normal Liver Enzymes	19 (25.3%)	81 (74.7%)	

*Statistically significant ($p < 0.05$).

Ths study also revealed significant associations between CMV infection and specific clinical parameters in thalassemia patients. CMV DNA positivity was higher in patients with thalassemia major than those with intermedia. This finding is consistent with previous research (25), suggesting that increased blood transfusions in thalassemia-significant patients may contribute to a higher risk of CMV exposure.

Correlation between CMV DNA load and clinical parameters

The relationship between CMV DNA load and different clinical factors

was analyzed using the Spearman correlation value (Table 4). Researchers discovered a weak positive relationship (rho = 0.287, p = 0.001) between CMV DNA load and age. Older patients are more likely to have higher CMV DNA loads. The results show a moderately positive relationship between CMV DNA load and liver size (rho = 0.412, p < 0.001). People with more enormous livers tend to have higher CMV DNA loads. Notably, no significant links were discovered between the amount of CMV DNA and other clinical factors, such as the number of blood transfers, the size of the liver, or the parameters of the heart.

Table (4): The relationship between CMV DNA load and different clinical factors using the Spearman correlation value of thalassemia patients

Clinical Parameter	Spearman's rho	p-value
Age	0.287	0.001*
Liver Size	0.412	<0.001*
Spleen Size	0.126	0.148
Frequency of Blood Transfusions	0.089	0.285
Cardiac Parameters	-0.045	0.594

*Statistically significant (p < 0.05).

One of the most notable findings of our study was the association between CMV infection and liver involvement in thalassemia patients. We observed a significantly higher prevalence of CMV DNA positivity in patients with an enlarged liver and elevated liver enzymes. These results support the hypothesis that CMV infection may contribute to liver damage in thalassemia patients (26, 27). The pathogenesis of CMV-related liver disease in thalassemia is not fully understood. Still, it is believed that CMV-induced inflammation and direct cytopathic effects on hepatocytes may play a role (28).

It was also found a weak positive correlation between CMV DNA load and age, indicating that older patients tend to have higher CMV DNA loads. This finding aligns with previous research (29) and may be explained by the cumulative effect of repeated CMV reactivations and a gradual decline in immune function with age (30).

Furthermore, our study identified a moderate positive correlation between CMV DNA load and liver size, suggesting that patients with larger liver sizes tend to have higher CMV DNA loads. This finding reinforces the link between CMV infection and liver involvement in thalassemia patients. Further research is warranted to explore this correlation in more detail.

Conclusion

This study underscores the significant prevalence of DNA from CMV in blood samples of individuals with thalassemia and its association with thalassemia major and liver involvement. The detection of CMV DNA by RT-PCR allows for the timely detection and treatment of CMV infection, which is crucial for preventing potential complications. The findings highlight the importance of routine CMV screening and monitoring in thalassemia patients. Future studies should focus on the clinical implications of CMV infection and the impact of antiviral therapy on patient outcomes.

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