

## Seroprevalence of Human Cytomegalovirus in Thalassemia Patients at Al-Muthanna province

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**Abstract**— Human cytomegalovirus (HCMV) infects 40–100% of the global population and is usually asymptomatic. However, it can cause severe disease in immunocompromised individuals, including thalassemia patients. This study aimed to detect HCMV infection by Enzyme-Linked Immunosorbent Assay (ELISA) among 50 thalassemia patients (31 males, 19 females) and 50 healthy blood donor controls in Al-Muthanna Governorate, Iraq, from November 2024 to April 2025. Results showed that 50% of thalassemia patients were infected with HCMV, compared to 14% in the control group. Among patients, infection was more frequent in males and most common in the 11–20-year age group (14 cases). Rural residents had a higher infection rate (32%) compared to urban ones (18%). The high prevalence among thalassemia patients may be associated with repeated blood transfusions. These findings emphasize the importance of routine HCMV screening and preventive measures for high-risk populations, particularly thalassemia patients.

**Keywords**—Human Cytomegalovirus, ELISA, IgG, IgM, Thalassemia Patients.

### I. INTRODUCTION

The human cytomegalovirus (HCMV) is a member of the Herpesvirales order, Herpesviridae family, and Betaherpesvirinae subfamily. Primates, including humans, are natural hosts. Human betaherpesvirus 5 (HCMV, human cytomegalovirus, HHV-5) is one of the 11 species in this genus that infects people [1]. Encased in an icosahedral nucleocapsid, the HCMV virus has a linear, double-stranded DNA genome that is roughly 235 kb in size. A 60% larger genome than HSV-1 can fit inside the beta structure capsid. With a diameter of 130 nm, the HCMV capsid's three-dimensional structure is notable for having 16 icosahedral lattices (162 capsomers, 12 pentamers, and 150 hexamers) joined by triplex structures [2]. Approximately 40–100% of people worldwide have HCMV. Because of the successful immune response, the majority of the infected population continues to be prevalent and symptomatic. Like other herpesviruses, Human Cytomegalovirus develops a lifelong latency after the primary infection has resolved. However, in immunocompromised people, a primary infection or viral reactivation can result in serious multiorgan disease. Because of the compromised immune response, a number of risk groups, including transplant recipients, patients in

critical care, patients with acquired immunodeficiency syndrome (AIDS), fetuses/newborns, and patients with thalassemia, are vulnerable to developing HCMV-mediated disease [3]. During infection, the human Cytomegalovirus (HCMV) alters the proteome, secretome, and interactome, among other aspects of the host-cell environment. For virions to replicate and be released, interactions between the host-host, host-virus, and virus-virus interactions required for productive replication and release of virions [4]. Infected maternal breast milk can spread HCMV infections during pregnancy or the postnatal period. Pregnancy's altered cytokine profile facilitates viral transmission from mother to fetus, leading to functional immune suppression. The virus can infect the growing fetus through the placenta during primary maternal infection, reactivated infection, or reinfection. Cytokine release, fetal and trans placental IgM and IgG release, and cytotoxic natural killer (NK) cell responses are the subsequent manifestations of a localized immune response. The fetal immune response is hampered once the virus enters the fetal compartment because the underdeveloped fetal CD4+ T-cells cannot appropriately multiply in response to the viral invasion [5]. In addition to the recently approved medications letermovir and maribavir, medications such as ganciclovir and foscarnet are frequently used to treat CMV infections and post-transplantation disease. However, as of right now, no CMV vaccine has received approval [6]. Pneumonia, hepatitis, retinitis, and nephritis are among the symptoms of HCMV disease that immunocompromised people, including organ transplant recipients and HIV patients, are susceptible to [7]. The main risk factors for HCMV transmission that can occur via a number of different routes including transfer of body fluids, such as saliva, urine, and placental cell transfer, or behaviors thought to be responsible for the transfer of fluids and infected cells such as breastfeeding, blood transfusion, transplantation, and sexual activity [8]. A quantitative defect in the synthesis of the beta globin chains of hemoglobin characterizes beta-thalassemia, a group of hereditary blood disorders that can cause a variety of phenotypes, from severe anemia to people who are clinically asymptomatic [9]. The two main effects on people with thalassemia are severe anemia and the body's bone marrow growing. These effects result in growth abnormalities, decreased physical



activity, brittle bones, and liver and spleen enlargement [10]. Blood transfusions have the potential to spread a variety of infectious agents, such as bacteria, viruses, and parasites [11]. More recently, it has been demonstrated that patients with thalassemia face significant challenges due to transfusion-related CMV infections [12].

The aim of the present study is to (i) to determine the seroprevalence of Human Cytomegalovirus (HCMV) among thalassemia patients in AL-Muthanna Province through the detection of CMV-specific antibodies, (ii) To provide baseline epidemiological data that may help in improving blood transfusion safety and infection control strategies for thalassemia patients.

## II. MATERIALS AND METHODS

### A. Study population and sample collection .

Blood serum samples were taken from 100 person (50 patients and 50 healthy blood donors) who visited the Women and Children's Teaching Hospital in Samawah, between November 2024 and April 2025 .The age of the thalassemia patients ranged from 1 to 30 years whereas the control group ranged from 18 to 50 years . The blood samples were collected in gel tubes and subjected to centrifugation at 2000 rpm for 10min.The serum was separated, labeled, and stored at -20°C. All Samples were subjected for HCMV antibodies estimation using ELISA techniques.

### B. Detection of human CMV IgM and IgG antibodies by ELISA.

Commercial ELISA kits (ABIA, Germany) were used to analyze sera samples for anti-CMV IgM and IgG antibodies in accordance with the manufacturer's instructions. In short, positive and negative controls were added to microplate wells along with diluted serum samples (1:100) and incubated for 30 minutes at 37 °C. Following washing, the enzyme conjugate was added, and it was once more incubated for 30 minutes at 37 °C. TMB substrate was added to wells after they had been cleaned, and they were then left to incubate for 20 minutes at room temperature in the dark. With a reference wavelength of 620–680 nm, absorbance was measured at 450 nm after the reaction was stopped with a stop solution. The kit protocol was followed in the interpretation of the results

## III. RESULTS

### A. Detecting of Human Cytomegalovirus by ELISA in thalassemia patients and control group .

Table 1 presents the ELISA results showing that among 50 thalassemia patients, 25 (50%) were positive for HCMV IgG antibodies and 7 (14%) were positive for HCMV IgM antibodies. In contrast, only 7 (14%) of the 50 healthy blood donor control samples were positive for IgG and 1 (2%) for IgM. These findings indicate a markedly higher prevalence of both previous (IgG) and recent (IgM) HCMV infections among thalassemia patients compared to the control group. Fig. 1 shows the incidence rates of Human Cytomegalovirus infection in thalassemia patients and the control group

TABLE 1. The frequency of HCMV (IgG and IgM) antibodies in thalassemia patients and controls.

Test Type	Patients				Control			
	Negative	%	Positive	%	Negative	%	Positive	%
IgG	25	50	25	50	43	86	7	14
IgM	43	86	7	14	49	98	1	2

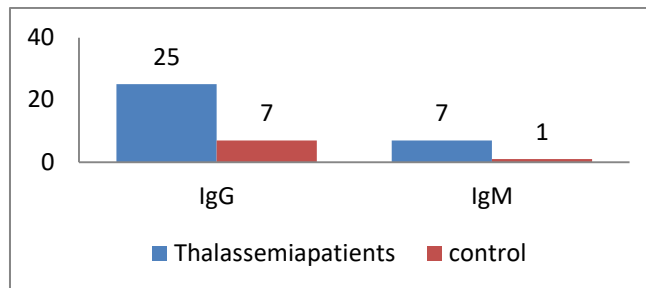


Fig.1. Positive sample of human Cytomegalovirus (IgG,IgM) according to detection of ELISA kit test

### B. Sex distribution of thalassemia patients infected with HCMV

According thalassemia patients showing that 50% were caused by HCMV. Table 2 demonstrates of HCMV-positive in thalassemia patients based on sex. The study found that from 25 thalassemia patients infected with Human Cytomegalovirus, the percentage of male 16 (32 %) was higher than female 9 (18%). The difference between male and female was highly significant ( $p \leq 0.001$ )

TABLE 2. Sex distribution of thalassemia patient infected with HCMV

Gender	Patients				Control			
	Negative	%	Positive	%	Negative	%	Positive	%
Male	15	30	16	32	42	84	7	14
Female	10	20	9	18	1	2	0	0

Tableted Chi-square = 6.63, df = 1, Calculated Chi-square = 10.32,  $P \leq 0.001$

### C. Distribution of Thalassemia Patients with HCMV According to Living Style

The distribution of thalassemia patients infected with Human cytomegalovirus according to the living style was explained in Table 3. The highest number of patients 16 (32%) lived in rural area. However, the lowest number of patients 9(18%) lived in urban area. The differences between them were highly significant ( $P \leq 0.001$ ).

TABLE 3. Distribution of thalassemia patients with HCMV According to living style

Type	Seropositivity	Site	
		Rural	Urban
Patient	Negative	13	12
	%	26	24
	Positive	16	9
	%	32	18
Control	Negative	29	14
	%	58	28
	Positive	6	1
	%	12	2

Tableted Chi-square = 6.63, df = 1, Calculated Chi-square = 8.62,  $P \leq 0.001$

### D. Distribution of Thalassemia Patients Infected with Human Cytomegalovirus by Age Groups

Table 4 showed the number and percentage of Thalassemia patients infected with Human cytomegalovirus according to age groups. The maximum number 14(28%)

was in age group (11-20) year. Whereas the minimum number 0(0%) was in age group (31-40) (41-50) year. The difference among age groups was highly significant.

TABLE 4. Distribution of Thalassemia Patients Infected with Human Cytomegalovirus by Age Groups.

Age	Patients				Control			
	Negative	%	positive	%	Negative	%	Positive	%
1-10	7	14	7	14	0	0	0	0
11-20	13	26	14	28	8	16	3	6
21-30	4	8	4	8	14	28	1	2
31-40	1	2	0	0	15	30	2	4
41-50	0	0	0	0	6	12	1	2

Tableted Chi-square = 15.09, df = 1, Calculated Chi-square = 23.89, P ≤ 0.001.

#### IV. DISCUSSION

This study was performed to evaluate the prevalence of Human Cytomegalovirus antibody among thalassemia patient in Al Muthanna Governate, and the association of HCMV and different factors including sex, age, and type of residence area (urban or rural).

According to the ELISA results, the prevalence of HCMV infection is significantly higher in thalassemia patients than in healthy blood donors. In particular, 14% of thalassemia patients tested positive for IgM antibodies, indicating a recent or ongoing infection, whereas 50% tested positive for IgG antibodies, indicating prior exposure. IgG (14%) and IgM (2%), on the other hand, were significantly lower in the control group, indicating either little exposure or infection. According to these results, patients with thalassemia are much more likely to contract HCMV, most likely because of their frequent need for blood transfusions, which raises the risk of viral transmission. Local studies conducted in Iraq have reported similar findings such as, Abbas and Zaman [13] showed that HCMV seropositivity rates were significantly higher in thalassemia patients in Kirkuk (IgG 76.0% and IgM 7.0%) than in blood donors (IgG 37.77% and IgM 3.88%). The findings that thalassemia patients are more susceptible to HCMV infection than healthy controls are further supported by Saif et al [14] in Najaf, who reported a high incidence of HCMV among this patient group. While Alsayab et al [15] found 123 children and adolescent; 50 normal and 73 were patients with  $\beta$ -thalassemia patients on regular blood transfusion. A blood sample was taken from each individual, controls and pre-transfusion patients, and submitted to serological (ELISA) and molecular (real time PCR) study. The prevalence of CMV IgG specific antibody was 100% in both patients and control while no one of them showed reaction to CMV IgM specific antibody. We recognize that the control group's sample size was comparatively small, which may have led to an underestimation of the HCMV prevalence among healthy blood donors. A higher infection rate consistent with the general population might be obtained by testing a larger cohort

Human Cytomegalovirus (HCMV) infection was more common in male thalassemia patients in this study (32%) than in female patients (18%). These results align with earlier local research. Abbas and Zaman [13] reported that male thalassemia patients in Kirkuk exhibited significantly higher HCMV seropositivity compared to female. Similarly, Al-Hamadany et al.[12] found that, HCMV infection was more prevalent in male thalassemia patients than in females. Both studies corroborate the findings of the

present study that male thalassemia patients are more vulnerable to HCMV infection than female patients are, most likely because of immunological differences and frequent blood transfusions. However, previous research from Yazd, Iran Moghimi et al [16] showed that IgM antibodies against HCMV were higher in female thalassemia patients, indicating that females may occasionally have higher rates of active infection. This disparity emphasizes how sex-related HCMV susceptibility may differ by population and location, possibly due to immunological, environmental, or genetic factors.

Our results demonstrated that the prevalence of Human Cytomegalovirus (HCMV) infection among thalassemia patients living in rural areas (32%) was almost twice as high as that in urban areas (18%). This difference may be explained by several factors, including weaker healthcare infrastructure in rural regions, less consistent screening of blood products, and higher household density, which facilitates interpersonal transmission. Although thalassemia patients are consistently at high risk of CMV infection due to repeated transfusion, only a few studies have specifically compared rural and urban populations. Al-Numan and Saleh [17] conducted a study in Mosul and reported no significant variation in CMV infection between rural and urban thalassemia patients. Similarly, the Kirkuk study by Abbas and Zaman [13] confirmed higher CMV prevalence among thalassemia patients compared with blood donors but did not evaluate residence as a variable. An Iranian study in Yazd Moghimi et al [16] reported very high CMV IgG seropositivity (>95%) among transfused thalassemia patients, without stratification by rural or urban residence. Taken together, these findings indicate that the impact of residence may vary depending on the local context. Our results therefore add novel evidence by demonstrating a statistically significant rural predominance, highlighting the need for improved blood safety protocols and health education in rural communities. In addition, the results overall agreed with Steer and Al-Mahdawi [18], that found revealed that the prevalence of HCMV IgM was 1.6% in the study participants of urban population and 2.0% in the study participants of rural population. Socioeconomic factors and lower health awareness in rural communities may account for the higher prevalence in rural compared to urban settings. Furthermore, the virus may spread horizontally through close contact and saliva in rural households due to larger family sizes and crowding.

The present study showed that the age group 11–20 years had the highest prevalence of HCMV infection among thalassemia patients. Germenis and Politis reported a higher prevalence of CMV antibodies among thalassemia patients aged 5–39 years compared with healthy controls, which includes the 11–20 years group highlighted in our study [19]. This supports our findings, as patients in this age range are more likely to have undergone repeated transfusions over time, leading to cumulative exposure to CMV and increased susceptibility due to iron overload and immune dysfunction. Similarly, a study from Iran demonstrated a very high seroprevalence of CMV IgG (95.9%) among thalassemia patients younger than 18 years [16], further confirming that the risk of CMV infection. In contrast, the study in Najaf [14] reported the highest prevalence in children aged 4–7 years, while the Basrah study [15] found

the 5–10 years age group to be most affected. This variation may be due to differences in sample sizes, the age at which blood transfusions are initiated, and regional differences in healthcare practices and environmental conditions. The cumulative exposure to blood transfusions over time is responsible for the higher seroprevalence seen in the age group (11–20 years). Compared to younger children, older patients have received a larger total volume of blood units, greatly raising their risk of coming into contact with contaminated blood.

#### V. CONCLUSION

This study demonstrates a higher prevalence of HCMV infection among thalassemia patients compared to healthy blood donors, largely due to frequent blood transfusions as a key risk factor. The findings also reveal increased infection rates among males; patients aged 11–20 years and, rural residents reflecting sociodemographic and clinical vulnerabilities. These results emphasize the need for enhanced blood screening, routine CMV monitoring, and targeted preventive strategies for high-risk groups

#### CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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