SCREENING OF BETA-THALASSEMIA MAJOR PATIENTS BELOW 18 YEARS FOR ACUTE CYTOMEGALOVIRUS INFECTION BY IGM SEROLOGY IN MOSUL

Aws H. Al-Numan[1], Salih Abed Mohammed[2], Ghazwan Y. Al-Shamaa[3]
[1]Department of Pediatrics, College of Medicine, University of Mosul, Iraq - [2]Assistant professor, Tikrit University Medical College, Pediatric Department, Tikri, Iraq - [3]Pediatric Neurologist, Ibn-Sina Teaching Hospital, Mosul, Iraq

ABSTRACT

Introduction*: Patients with beta-thalassemia major have a weakened immune system, making them vulnerable to transfusion related infections like cytomegalovirus (CMV), which can have serious consequences, particularly for those undergoing stem cell transplantation. So, screening these Patients for CMV using IgM serology and determining the factors which enhance the acquisition of this virus are crucial.*

Materials and methods*: This cross-sectional study was performed on six hundred thirty-three thalassemia patients below 18 years in Mosul (North of Iraq). The fully automated immunoassay was used to detect the presence of IgM antibodies against cytomegalovirus as a mark to determine transfusion-transmitted active infection. In addition, six hundred forty-two healthy children and adolescent under 18 years were analyzed as a comparison group. The Chi-square test was used to ascertain statistical significance, while the odd ratio and likelihood ratio were used to evaluate the magnitude of the correlation between certain elements and the procuration of CMV infection.*

Results*: 12.79 per cent of patients with thalassemia were positive for active infection with cytomegalovirus compared to 4.36 per cent of the healthy non-thalassemia group. The key factors linked to an increase in infection rate are splenectomy, high ferritin, and repeated transfusions. No statistical significance has been established with respect to age, sex and residence in relation to CMV infection.*

Conclusion*: In comparison to other countries, there is a critical high incidence of cytomegalovirus infection among thalassemia patients; thus, steps to minimize this rate should be adopted.*

Keywords: *Thalassemia Major, Active infection, CMV- IgM, ferritin.*

DOI: 10.19193/0393-6384_2021_2_154

Received *March 15, 2020;* ***Accepted*** *February 20, 2021*

Introduction

Beta-thalassemia is among the world's commonest hereditary anemia disorders, with nearly 60000 seriously affected children born annually[(1)]. It is caused by defective production of beta-globin chains leading to ineffective erythropoiesis and accelerated erythrocyte degradation with resultant anemia and iron accumulation from a repeated blood transfusion[(2,3)]. Thalassemia is a major problem in Iraq primarily because equipment and medicines have not been available during many periods of war and turmoil.

There are more than11000 patients with thalassemia registered all over Iraq till 2016, beta-thalassemia major constitutes about 74% of them, Basra and Duhok governorates have the highest thalassemia prevalence of 45.5 and 45.1 for each 100 000 population respectively[(4-6)]. There are more than 600 patients with beta-thalassemia major receiving regular blood transfusion every month in Ibn al-atheer teaching hospital in Mosul city[(7)].

Patients with beta-thalassemia major are liable to many infections related to blood transfusions like hepatitis B, hepatitis C, HIV and non-infectious complications related to iron overload and

chelation therapy affecting endocrinal, cardiac, and skeletal systems as well as vision and hearing[8-11], such Infections are an important cause of morbidity and can be lethal in patients with thalassemia and hemoglobinopathies because of the associated affection of both cellular and humoral components of the immune system which is attributed to many causes related to the disease itself or its complications, primarily iron excess, but these infections vary worldwide based on the variations in the epidemiology of each infection and the socioeconomic status of each region, and the preventive and therapeutic approaches that are implemented[12-14]. In an Italian study done in different hospitals, infections in thalassemia were the second leading cause of death after heart failure[15]. Similar findings were reported in Greece[16], and in Taiwan[17], while infections are the primary cause of morbidity and mortality in Thailand's E-beta thalassemia patients[18].

Another important implicated mechanism of immune dysfunction is thought to be repeated transfusions, which are responsible for the continuous triggering of the immune system by allo-antigens and the transfer of immune depressing viruses, such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), and hepatitis C virus[12].

CMV infection in thalassemic patients, especially those who had undergone splenectomy is serious, partly because of the immune- suppressive properties of the virus resulting in the possibility of acquiring serious opportunistic infections and the risk of developing serious CMV pneumonia with more than 50% fatality rate or other organs affections with the virus resulting in hepatitis, encephalitis, and retinitis particularly following stem cell transplantation, which is the ultimate cure for patients with beta-thalassemia[19-21].

Based on experiment utilizing patients and clinical specimens, the main source of Transfusion-transmitted (TT) CMV infections tends to be the peripheral white blood cells (WBCs), Transfusion-borne CMV infections occur frequently when the seropositive blood containing normal numbers of WBCs is transfused to patients at risk[22].

In this study, we aim to find out the prevalence of acute CMV infections by locating IgM antibodies in patients with beta-thalassemia major in Mosul city who are receiving multiple blood transfusions as part of their management plan and to determine the factors which increase the risk of acquiring this virus.

Material and methods

The academic ethics committee of the College of Medicine, University of Mosul, accepted this paper and informed consent was obtained from all participants. This paper adopted the ethical principles of the 1964 Helsinki Declaration and subsequent amendments

In this cross-sectional study, we enrolled 633 patients over a 6-month period between January and June 2018, all of them are under 18 years of age with B-thalassemia major attending Ibn Al-atheer hospital, the center of thalassemia in Mosul city (400 km North of Baghdad) in Iraq for regular blood transfusions. Besides, 642 non-thalassemic children visiting the outpatient clinic in the same hospital were also included in this study as a comparison group.

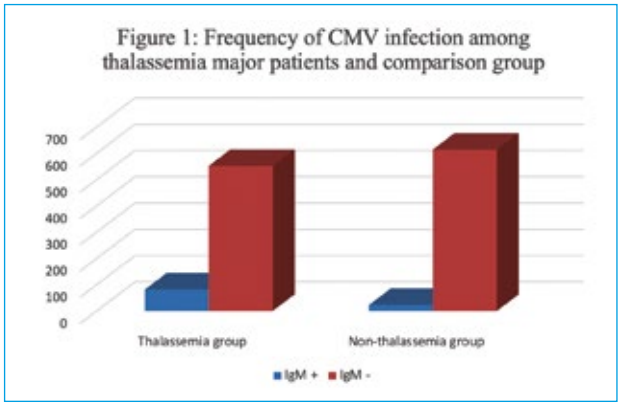
Detailed information including age, sex, residence, blood group, number of blood transfusions per year, serum ferritin, deferoxamine therapy and the presence of splenectomy were taken from each participant in the study.

Blood samples from both groups tested by Cobas e601 analyzer (Roche Diagnostic GmbH, Mannheim, Germany), using Elecsys® CMV IgM Immunoassay for the qualitative determination of IgM-antibodies against CMV. Samples reactive for CMV IgM antibodies indicate an acute, recent, or reactivated infection and it may remain raised for up to 1 year following the infection in up to 20% of the cases. The sensitivity and specificity for the detection of the CMV virus are 93% and 98.8% respectively[23,24].

Statistical analysis of our data carried out using SPSS (statistical package of social science) version 23, chi-square test used in the statistical analysis and the p value of less than 0.05 assumed statistically significant, odd ratio and likelihood ratio used to measure and quantify the strength of association between certain elements and the accusation of CMV.

Results and discussion

As shown in Figure 1 there is substantially higher IgM seroconversion in 81 (12.79%) out of 633 children, suggesting acute CMV infection in the main group of patients with thalassemia compared to seroconversion with only 28 (4.36%) out of 642 in the non-thalassemia community, the odd ratio is 4.0954 95 % CI (2.5214 to 6.6518) P<0.0001.

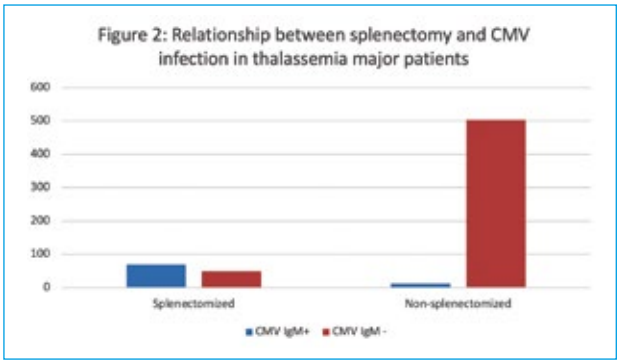


Many categorical variables, including age, sex, residence, ferritin level, condition of the spleen, and the number of blood transfusions per year have been tested with regard to Cytomegalovirus IgM seroconversion in thalassemia major patients as seen in table 1 in this study.

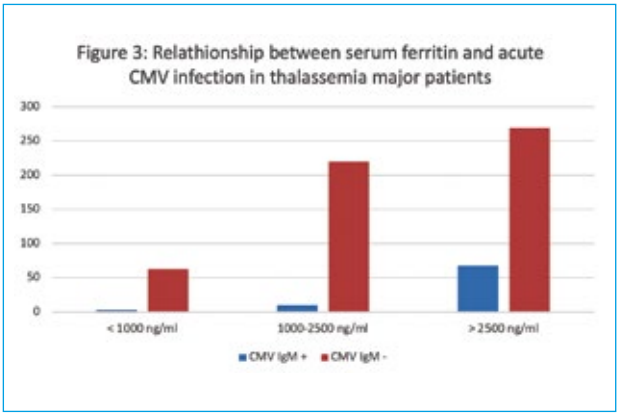
			CMV IgM Status	
		Frequency	IgM +	IgM -
Ferritin level	<1000 ng/ml	66	3	63
	1000-2500ng/ml	230	10	220
	>2500 ng/ml	337	68	269
Age group	<5 years	82	5	77
	5-10 years	191	27	164
	11-18 years	360	49	311
Transfusions/year	<8	119	9	110
	8-12	219	21	198
	>12	295	51	244
Residence	urban	423	53	370
	rural	210	28	182
Spleen status	splenectomized	118	69	49
	Non-splenectomized	515	12	503
Sex	male	322	39	283
	female	311	42	269

Table 1: Frequency distribution of categorical independent variables in thalassemia major patients.

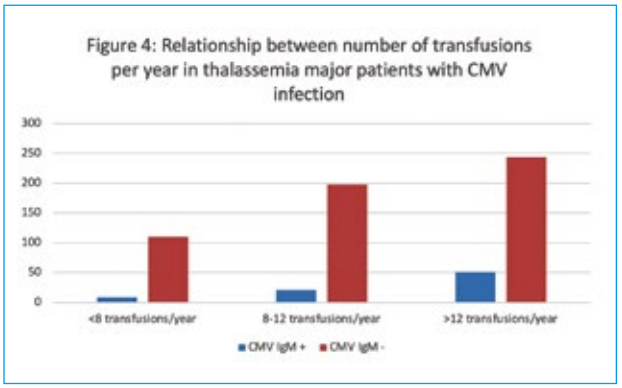
Splenectomy seems to be the most important statistically significant variable in predisposing thalassemia major patients for CMV infection, odd ratio 59.02 95% CI: 29.9155 to 116.4617 P < 0.0001 as in figure 2.



In addition, high serum ferritin in thalassemia major patients is associated with increased risk of acquiring CMV infection P<0.0001, and the likelihood ratio is 38.633 as illustrated in figure 3.



The increasing frequency of blood transfusion is also associated with the higher infection rate with CMV in thalassemia major patients, P<.006 and the likelihood ratio is 10.421, this is demonstrated in figure 4.



Other factors, such as age, sex and residence, were all statistically insignificant in terms of increased risk of cytomegalovirus infection in patients with major thalassemia, with p values of 0.148, 0.6001 and 0.7756, respectively.

Cytomegalovirus infection is an important transfusion-transmitted disease that could affect the quality of life in immunocompromised children like those with beta-thalassemia major, it may lead to serious infections following bone marrow transplant and even transplant failure due to reactivation of the latent virus in the white blood cells[25].

The main result of this study is the finding of a higher rate of active Cytomegalovirus infection in beta-thalassemia major patients in Iraq / Mosul than that in healthy people, which appears to be consistent with what is found in Greece[19], Iran[26], Egypt[27], and Italy[28], although in our study the rate is 12.79%,

which is much higher than the 4% in Ramadan et al paper in Egypt[27], 2% in Duran Canatan paper in Antalya Turkey[29], and 0.42% in Muhammad Rafiq et al in Lahore Pakistan[30].

Our study designed to disclose any factor that could be responsible for increased infection with the cytomegalovirus in thalassemia patients, splenectomy seems to be one of the most significant factors, and this result is in line with the findings of the meta-analysis done by George Dimitrios Liatsos about post-splenectomy primary CMV infection[31], which can be explained by the immunological dysfunction in both humoral and cellular immunity following splenectomy[31]. The next important element in our study, which is linked to a higher infection rate with CMV is elevated ferritin blood level as a sign of iron overload in beta-thalassemia major patients, this result is supported by Dimitrios Farmakis et al work which clarifies the important rule of iron and its binding proteins in regulating the immune system, and the effect of iron excess on encouraging the growth of infectious microorganisms[12].

Another major cause for the elevated prevalence of acute CMV infection, according to our study is repeated transfusions, especially if it is more than 12 / year, this outcome is supported by Hasan et al systematic review[25], the possible explanations for this are the lack of both CMV screening and leukocyte reduction technologies for donated blood.

No substantial difference in the prevalence of CMV-IgM seropositivity among male and female patients in this research, and this agreeing with the finding of Choobineh et al in Iran[32], so sex hormones don't affect the susceptibility for CMV infection. In our research, the prevalence of CMV-IgM seropositivity outside of Mosul City (rural areas) does not vary from that within the city (urban area), this means that overcrowding and air pollution does not play any role in disease spread

There was no difference in the prevalence of CMV-IgM seropositivity among thalassemia in various age groups, this finding disagrees with what is found by Choobineh et al in Iran and Germens et al in Athens, Greece[32,33].

Conclusion

The present study demonstrates that there is a critical, widespread CMV infection in patients with beta-thalassemia major related to the disease itself and its complications like iron overload, defective immunity, splenectomy, and to the repeated transfusion of unscreened unfiltered blood, so boosting immunity, providing effective chelation therapy and effective CMV screening and leukocyte reduction techniques in blood banks are crucial.

References

1) Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis 2010; 5: 11.
2) Cao A, Galanello R. Beta-thalassemia. Genet Med 2010; 12: 61-76.
3) Cohen AR, Glimm E, Porter JB. Effect of transfusional iron intake on response to chelation therapy in beta-thalassemia major. Blood 2008; 111: 583-7.
4) Kadhim KA, Baldawi KH, Lami FH. Prevalence, Incidence, Trend, and Complications of Thalassemia in Iraq. Hemoglobin 2017; 41: 164-168.
5) Hassan MK, Taha JY, Al-Naama LM, Widad NM, Jasim SN. Frequency of haemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency in Basra. East Mediterr Health J 2003; 9: 45-54.
6) Al-Allawi NA, Jubrael JM, Hughson M. Molecular characterization of beta-thalassemia in the Dohuk region of Iraq. Hemoglobin 2006; 30: 479-86.
7) Al-Nuaimi MA, AL-Hially YA, AL-Hafidh N. β thalassemia major patients' profile in Ninevah governorate-Iraq. Tikrit Medical Journal 2012; 18: 250-60
8) Cunningham MJ, Macklin EA, Neufeld EJ, Cohen AR; Thalassemia Clinical Research Network. Complications of beta-thalassemia major in North America. Blood 2004; 104: 34-9.
9) Haidar R, Musallam KM, Taher AT. Bone disease and skeletal complications in patients with β thalassemia major. Bone 2011; 48: 425-32.
10) Aydinok Y, Darcan S, Polat A, Kavakli K, Nigli G, et al. Endocrine complications in patients with beta-thalassemia major. J Trop Pediatr 2002; 48: 50-4.
11) Abdulzahra MS, Al-Hakeim HK, Ridha MM. Study of the effect of iron overload on the function of endocrine glands in male thalassemia patients. Asian J Transfus Sci 2011; 5: 127-31.
12) Farmakis D, Giakoumis A, Polymeropoulos E, Aessopos A. Pathogenetic aspects of immune deficiency associated with beta-thalassemia. Med Sci Monit 2003; 9: 19-22.
13) Weiss G. Iron and immunity: a double-edged sword. Eur J Clin Invest 2002; 1: 70-8.
14) Walker EM Jr, Walker SM. Effects of iron overload on the immune system. Ann Clin Lab Sci 2000; 30: 354-65.
15) Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004; 89: 1187-93.
16) Ladis V, Chouliaras G, Berdousi H, Kanavakis E, Kattamis C. Longitudinal study of survival and causes of death in patients with thalassemia major in Greece. Ann N Y Acad Sci 2005; 1054: 445-50.
17) Chern JP, Su S, Lin KH, Chang SH, Lu MY, et al. Survival, mortality, and complications in patients with beta-thalassemia major in northern Taiwan. Pediatr Blood

Cancer. 2007; 48: 550-4.
18) Wanachiwanawin W. Infections in E-beta thalassemia. J Pediatr Hematol Oncol 2000; 22: 581-7.
19) Germenis A, Politis C. Thalassemic patients are at high risk for transfusion-transmitted cytomegalovirus infections. Acta Haematol 1989; 82: 57-60.
20) Ljungman P, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. Hematol Oncol Clin North Am. 2011; 25: 151-69.
21) Boeckh M, Ljungman P. How we treat cytomegalovirus in hematopoietic cell transplant recipients. Blood 2009; 113: 5711-9.
22) Vamvakas EC. Is white blood cell reduction equivalent to antibody screening in preventing transmission of cytomegalovirus by transfusion? A review of the literature and meta-analysis. Transfus Med Rev 2005; 19: 181-99.
23) Taylor GH. Cytomegalovirus. Am Fam Physician 2003; 67: 519-24.
24) Van Zuylen WJ, Hamilton ST, Naing Z, Hall B, Shand A, et al. Congenital cytomegalovirus infection: Clinical presentation, epidemiology, diagnosis and prevention. Obstet Med 2014; 7: 140-6.
25) Al Mana H, Yassine HM, Younes NN, Al-Mohannadi A, Al-Sadeq DW, et al. The Current Status of Cytomegalovirus (CMV) Prevalence in the MENA Region: A Systematic Review. Pathogens. 2019; 8: 213.
26) Moghimi M, Doosti M, Vahedian-Ardakani HA, Talebi A, Akhavan-Ghalibaf M, et al. Serological Study on Cytomegalovirus and Toxoplasma Gondii in Thalassemia Major Patients of Yazd, Iran. Iran J Ped Hematol Oncol 2015; 5: 149-54.
27) Mahmoud RA, El-Mazary AA, Khodeary A. Seroprevalence of Hepatitis C, Hepatitis B, Cytomegalovirus, and Human Immunodeficiency Viruses in Multitransfused Thalassemic Children in Upper Egypt. Adv Hematol 2016; 2016: 9032627.
28) Giardini C, Lucarelli G. Bone marrow transplantation for beta-thalassemia. Hematol Oncol Clin North Am 1999; 13: 1059-64.
29) Canatan D. The Thalassemia center of Antalya State Hospital: 15 years of experience (1994 to 2008). J Pediatr Hematol Oncol 2013; 35: 24-7.
30) Khan MR, Anwar S, Faizan ME, Nosheen S. The burden of transfusion related infections on thalassemia major children. Pak J Med Health Sci 2017; 11: 882-6.
31) Liatsos GD. The immunity features and defects against primary cytomegalovirus infection post-splenectomy indicate an immunocompromised status: A PRISMA-compliant meta-analysis. Medicine (Baltimore) 2019;98: e17698.
32) Choobineh H, Alizadeh SH, Yazdi MS, Vaezzadeh F, Dargahi H, et al. Serological evaluation of major beta thalassemia patients below15 for cytomegalovirus infection in Iran. Research J. Biol. Sci. 2009; 2: 584-9.
33) Germenis A, Politis C. Latent cytomegalovirus (CMV) infections affect immunoglobulin levels of patients with thalassemia major. Haematologica 1990; 75: 42-5.

Corresponding Author:
Aws H. Al-Numan
Mbchb Fibms Mrcpch
Department of Pediatrics, College of Medicine, University of Mosul-Iraq
Email: awshazimah72@uomosul.edu.iq
(Iraq)