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Association of Cytomegalovirus Seroprevalence and Blood Transfusion Among Patients Undergoing Chronic Hemodialysis

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Abstract

Introduction

Cytomegalovirus (CMV) belongs to the Herpes family of viruses is ubiquitous and endemic in most parts of the world. Infections caused by CMV viruses are common and generally asymptomatic. It infects around 50–90% of the adults, depending on the socioeconomic factors and other risk factors. CMV infection is a major concern in patients undergoing kidney transplantation, where repeated blood transfusion is an established risk factor for transmission.

Methods

This retrospective study analysed the data of 99

chronic kidney disease patients undergoing regular hemodialysis. We collected data such as basic demography, hemodialysis vintage, iron and erythropoietin-stimulating agent (ESA) therapies for anemia, and transfusion history and frequency for analysis. Blood analyses for the presence of CMV-specific immunoglobulin IgG and IgM were performed to study determine seroprevalence of CMV infection in hemodialysis population and investigate the association of CMV infection with history and frequency of blood transfusion; which is non-leucodepleted.

Results

Around 75% and 69% of the population were seropositive for CMV IgM and CMV IgG, respectively. There was a non-statistically significant association with history of blood transfusion; however, frequent blood transfusions showed a statistical significant association.

Conclusion

CMV infection is highly prevalent in chronic hemodialysis population. Repeated blood transfusion can be a major risk factor for viral transmission. However, other possible routes of infection should be investigated as many patients reported no history of blood transfusion. Optimizing medical treatment for anemia and implementation of strategies to limit blood transfusion is recommended. If blood transfusion is mandated, leucodepleted blood is favoured to reduce risk of CMV infection.

Keywords:

Cytomegalovirus, chronic kidney disease, leucocyte reduced blood, transfusion, seropositivity

Introduction

Cytomegalovirus (CMV) belongs to the Herpes family of viruses. It is ubiquitous and hence, endemic in most parts of the world. Infections caused by CMV viruses are common and generally asymptomatic. It infects around 50–90% of the adults, depending on the socioeconomic factors and other risk factors. Alternatively, its prevalence is lower in Europe, parts of North America, and Australia, and higher in Africa and Asia. CMV infections are a major risk factor for mortality in patients with acquired immunodeficiency syndrome (AIDS), and recipients of

organ transplants receiving immunosuppressive agents [1,2,3]. Viral proteins are expressed in both, the ear ly and late phases, and the genome consists of >200 potential reading frames that facilitate the production of effector proteins; however, merely a quarter of this is committed to replication. Most viral proteins have the capacity to modulate the cellular responses in the host. Among herpes viruses, CMV is the most versatile, because it produces the most of all genes that modify immune responses, both innate and adaptive responses in the host [4]. Patients with end stage renal disease (ESRD) undergoing hemodialysis, often have an impaired immune response that predisposes them to a greater risk of infection, including CMV infections. Infections could be a primary infection or due to reactivation of a latent virus or reinfection with viruses introduced during blood transfusion or organ transplant [3]. The high-risk group include patients with primary or secondary immunodeficiency, patients receiving blood transfusion or hemoproducts, and those who receive tissue or organ transplants [5,6].

Infections caused by CMV virus were associated with a high rate of morbidity and mortality in the past. However, with improved antiviral management and immunosuppressant therapies, the severity has reduced to a considerable extent. Nevertheless, despite the use of antiviral agents as prophylaxis for minimising the risk of infection, infection during the late prophylaxis period is a significant challenge in the management of infection in patients that have received tissue and organ transplants [7].

Kääriäinen et al. were the first to describe transfusion-transmitted CMV infections in 1966, and Tolpin et al. provided the molecular evidence for the same in 1985. CMV infections were reportedly a common problem even in patients after cardiac surgery, with seronegative patients turning seropositive and developing symptomatic infections [8]. Around the 1970s, it was felt that leucocytes in the blood sample intended for transfusion could potentially transmit CMV, activate the production of antibodies against human leucocyte antigen, and induce

immunosuppression. This initiated research on the role of allogeneic leucocytes in blood, and the effect of their exclusion from blood products [9]. This led to the use of blood products from seronegative donors, along with leucoreduction of blood products to minimize the risk of infection [7,8].

Post infection, the CMV resides in the mononuclear cells for life. Administration of immunosuppressive agents result in a loss of T-cell mediated control, thereby leading to the endogenous replication of CMV [8].

Repeated whole blood transfusions might predispose chronic ESRD patients on dialysis to a greater risk of CMV infection after tissue and organ transplant, which would require expensive chemoprophylaxis.

Chronic hemodialysis patients in Egypt often receive non-leucocyte reduced blood. This study aimed at detecting the seroprevalence of CMV antibodies, and establishing its association with history and frequency of transfusion of non-leucocyte reduced blood.

Materials and Methods

Patients

This retrospective study analysed the data of 99 chronic kidney disease patients undergoing hemodialysis thrice weekly at the Ain Shams University Hospital, Cairo, Egypt.

Sample Collection and Processing

We collected data such as basic patient demographics, hemodialysis vintage, iron and erythropoietin-stimulating agent (ESA) therapies for anemia, and blood transfusion history and frequency for analysis. Blood analyses for the presence of CMV-specific immunoglobulin (Ig) G and IgM were performed at the pathological laboratories at Cairo University. Around 3 mL of whole blood was collected in plain tubes, free of any anticoagulant such as ethylene diamine tetra-acetic acid (EDTA), labelled with the patient details and date of collection. The samples were kept at room temperature for 30–60 minutes for clotting. Thereafter, the samples were centrifuged, and 1 mL of serum was separated. Two aliquots of 0.5 mL each were prepared in order to avoid

repeated freezing and thawing of the same aliquot, which might alter results. The specimens were labelled and stored at -20°C until further analysis.

Serological testing

Specimens were thawed and allowed to equilibrate with the room temperature prior to analysis. Samples were analysed using commercial enzyme-linked immunosorbent assay (ELISA) kits Dr. G MED, USA (IgM) and Diasorin, Italy (IgG). Analyses were performed as per the instructions provided by the manufacturer. Absorbance was read on a photometer at 450 nm with an ELISA reader with 630 nm reference filter. The four-parameter mathematical method, Rodbard function was used for calculation of IgG levels in the linear range (10–1,000 IU/L).

Statistical Analysis

Continuous variables were presented as mean, standard deviation (SD), median, and ranges. Categorical data were presented as counts and percentages and analysed by the Pearson's Chi-square test. The SPSS software version 20was used for all analyses. Associations were considered significant if the p-value was less than 0.05.

Results

Demography and clinical characteristics

The data of 99 patients were analysed and the demographic data and patient details are presented in Table 1.

Table 1. Demogracy and clinical characteristics

Patients	N=99
Age (y)	
Median	62
Range (Min, Max)	28,79
Mean ±SD	58.86 ±10.95
Sex	
Male	69 (69.7)
Female	30 (30.3)
History or Iron Therapy	
Yes	55 (55.6)
No	44 (44.4)
History of Erythropoiesis Stimulating Agent Therapy	
Yes	88 (88.9)
No	11 (11.1)
Duration of Hemodialysis Treatments (months)	
Mean ±SD	54.3 ±49.96
Median	48.00
Range (Min, Max)	1,228
History of Past Blood Transfusion	
Yes	43 (43.4)
No	56 (56.6)
CMV-specific IgM	
Positive	75 (75.8)
Negative	24 (24.2)
CMV-specific IgG	
Positive	68 (68.7)
Negative	31 (31.3)
Figures in parentheses represent percentages	

Risk Factors and Seropositivity

History of past blood transfusion is a major risk factor in the prevalence of CMV infection. Around 57% of the population had no history of blood transfusion (Table 1). Among the various immunoglobulins, IgM is the first to be produced by the body against any infectious agent. Around 75% and 69% of the population were seropositive for CMV IgM and CMV IgG, respectively (Table 1).

Table 2 Association between risk factors and Cytomegalovirus IgM titre

Variable	Number of Patients	CMV IgM Positive	CMV IgM Negative	Significance
Blood Transfusion				
Yes	43	35	8	0.251
No	56	40	16	
Frequency of Blood Transfusion				
1	21	17	4	
2	12	12	0	
3	9	6	3	
4	16	12	4	0.006
5	10	5	5	
6	6	6	0	
10	40	30	10	
12	12	12	0	
20	20	20	0	

Abbreviations: CMV, Cytomegalovirus; Ig, immunoglobulin; ESA, erythropoietin-stimulating agent

Majority of the patients had received treatment with iron and ESA. The number of patients who were CMV-specific IgM positive was greater in this category. In contrast, most patients (56/99) did not undergo transfusion and a majority of these patients (40/56) tested positive for IgM. The frequency of blood transfusion varied widely with around 40% receiving 10 transfusions. However, none of these associations was statistically significant. Yet, IgM was detected in most of the patients who received transfusion at frequencies. This association was significant (p=0.006).

Table 3 Association between risk factors, therapies, and Cytomegalovirus IgG titre

Variable	Number of Patients	CMV IgG Positive	CMV IgG Negative	Significance
Blood Transfusion				
Yes	43	31	12	0.522
No	56	37	19	
Frequency of Blood Transfusion				
1	21	14	7	
2	12	12	0	
3	9	9	0	
4	16	8	8	0.000
5	10	0	10	
6	6	6	0	
10	40	30	10	
12	12	12	0	
20	20	20	0	

Abbreviations: CMV, Cytomegalovirus; Ig, immunoglobulin; ESA, erythropoietin-stimulating agent

CMV-specific IgG was detected in most of the patients who received treatment with iron and ESA therapies, and blood transfusion. However, none of these associations were statistically significant. IgG was detected in most of the patients who received transfusion at frequencies. This association was significant ($p<0.005$).

Discussion

Anemia of chronic kidney disease (CKD) is a multifactorial process resulting from relative erythropoietin hormone deficiency, uremia induced inhibitors of erythropoiesis, shortened erythrocyte survival, and disordered iron homeostasis. [10]. Management of anemia entails the administration of iron and ESA therapies. Generally, young patients receive ESA. Although these agents lower transfusion probabilities, the risk of transfusion persists [11]. Transfusion of whole blood initiates a vicious cycle of CMV transmission, often leading to a seroconversion that would mandate use of prophylactic antiviral agents for transplant recipients as CMV infection increases the manifestation of age-related changes in T-cells of patients on immunosuppressive therapy [12].

Evidence of significant past CMV infection in patients than in controls has been reported by Hardiman et al [13]. The incidence of CMV infections in patients receiving whole blood transfusion was a topic of significant research for over two decades, and led to the observation that leucocytes were a strong cause of infection transmission [8]. Minimizing the incidence of the disease were explored using filtration and leucocyte reduction. The absence of CMV in such samples was validated by testing polymerase chain reactions (PCR) [14].

Leucocyte reduction is a technological advancement in the past decade. Currently, it is an established technology for selected transfusion recipients across North America, Europe, Asia, and Australia. In recent times, the health authorities in several European nations and Canada have made this process mandatory for all transfusion patients, with a view towards minimizing febrile transfusion reactions, HLA alloantibody formation,

and transmission of CMV [15]. It is noteworthy that historically, the terms leucoreduction and leukodepletion have been used interchangeably. Whereas leucoreduction implies the removal of leukocytes by a gross removal method, leukodepletion refers to the removal of these white cells with the help of certain filters and devices. However, leukoreduction is associated with a lower risk of only some clinical conditions. Nevertheless, it is justified because of the reduced rate of non-hemolytic febrile transfusion reactions, HLA alloimmunization, and platelet refractoriness observed in patients who have received multiple transfusions, and transmission of leucotropic viruses. It is noteworthy that such an exercise would be an expensive affair [16]. It remains to be seen if developing countries can translate this into reality soon.

Recently, Roback et al. reported that CMV DNA is rarely detected in healthy seropositive blood donors [17]. Similar to the Seperhrvand et al survey of CMV among hemodialysis patients in Iran, the results of this study suggest that although the prevalence is high, there is little correlation between the antibody titres and other covariates [18].

In the present study, it has been demonstrated that although there's no significant association merely with history of blood transfusion, frequent transfusion of non leucoreduced blood (which is the standard practice in Egypt), would put chronic hemodialysis patients at risk of CMV seroconversion. This would be particularly important for dialysis patients who are candidates to receive a kidney transplant. Hence, cost-effectiveness of transfusing leucodepleted blood to chronic hemodialysis patients (especially transplant candidates) should be investigated, given the high cost of chemoprophylaxis and therapy with expensive antiviral agents.

In this study, patients were seropositive even if they did not receive a single transfusion; which suggests that other routes of infection do play a role in viral transmission. Cross

contamination and inadequate infection control practices at dialysis units may play a role. However, the lack of any conclusive evidence from this study may be attributed to the small sample size, and the limitation that CMV DNA was not tested. Therefore, a follow-up study that includes a large population is warranted for confirmation of these observations.

Conclusion

The current study aimed at detecting the seroprevalence of CMV in chronic hemodialysis patients as well as to the possible association between CMV infections and blood transfusion history and frequency. Study results demonstrated that CMV infection was highly prevalent, and that repeated blood transfusion can be a major risk factor for viral transmission. However, other possible routes of infection should be investigated as many patients reported no history of blood transfusion. Optimizing medical treatment for anemia and implementation of strategies to limit blood transfusion would be recommended. If blood transfusion is mandated, leucodepleted blood would be favoured to reduce risk of CMV infection, especially for transplant candidates.

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