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Original Article

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Seroprevalence of cytomegalovirus infection in the patients with chronic lymphocytic leukemia

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Abstract

***Background & Aims*:** Cytomegalovirus (CMV) is a member of herpesvirus family with high prevalence in the normal population, which can lead to life-threatening latent infection. This virus has potential oncogenicity and can be reactivated in terms of immune suppression. This study was conducted to investigate the prevalence of CMV in the patients with chronic lymphocytic leukemia (CLL).

***Materials & Methods*:** To determine CMV prevalence, serum samples were collected from CLL patients after establishment of diagnosis by oncologist in Imam Khomeini Hospital, Urmia, Iran as well as from age-matched healthy subjects as control group. A total of 65 study participants, 33 males and 32 females, were included in this study in two groups, study group (n=31) and control group (n=34). All serum samples were tested for CMV-specific IgG and IgM antibodies using the enzyme-linked immunosorbent assay (ELISA) method. Data were analyzed by SPSS version 16.0. Student's t-test and Chi-square test were used for comparison of variables and between the categorical variables, respectively. The statistical significance considered as $P < 0.05$.

***Results*:** The prevalence of CMV infection in all participants was 84.6%. The prevalence of CMV current infection (IgM positive) and previous infection (IgG positive only) were respectively 29.03% and 70.97% in the patient group and 97.05% and 2.95% in the control group.

***Conclusion*:** Based on our results, previous exposure to CMV was significantly higher in the CLL patients compared with the control group. Good treatment and care strategies for the CLL patients can play an important role in preventing the reactivation of latent viral infections. Further studies and longtime monitoring should be done to know the factors affecting the reactivation of latent CMV infection and other latent viral infections in the CLL patients.

***Keywords*:** Chronic Lymphocytic Leukemia, Cytomegalovirus, Iran, Seroprevalence

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Introduction

Human cytomegalovirus (CMV) is an opportunistic, ubiquitous DNA virus belonging to herpesvirus family, carried by up to 90% of the adult population through the worlds. Primary CMV infection is usually asymptomatic, except rare mononucleosis-like syndrome, and can lead to a permanent lifelong latent infection (1). Both cellular and humoral immunity against CMV, especially virus-specific T cells develop and controls viral reactivation (3). CMV-specific neutralizing IgA, IgG, and IgM antibodies inhibit cell-to-cell spread of the virus, its reactivation from latency and viral dissemination (4). In virus-specific cellular immunity, pp65 and immediate-early protein directed CD4+ and CD8+ T lymphocytes have a key role in the CMV infection control. CMV-specific immune response may be conserved even in the patients with progressive disease. However, there is a possibility of reactivation and tumorigenicity in conditions of immune system suppression as well as in chronic CMV infection.

CMV as a pathogen can lead to life-threatening infection in the patients with cancers (2). Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in ages over 60 years (67–72). There are several new, oral, and targeted therapies in addition to chemotherapy and immunotherapy in the treatment of CLL. CLL treatment has been developed by various novel monoclonal antibodies and cytostatic agents. This progress has resulted in higher response rates and more profound levels of immunosuppression in compared with previously utilized treatments (6). Thus, the patients experience some degree of immunosuppression induced by leukemia or CLL therapeutic agents. Moreover, because of old age of most of them, the patients may show some degree of immune senescence, which favors reactivation of viral infection.

It has been known from previous studies of CLL that infectious complications are a main reason of mortality and morbidity. Physicians of these patients should have a full understanding of the host situation predisposing to infection related to the disease process and its treatment (5). CMV infection may be reactivated in immune suppression situations in CLL treatment course and led to symptomatic infection. CMV can be identified from examinations on serum of leukemia subjects that are susceptible to CMV infection early after transplantation (7). Therefore, identification and treatment of CMV infections in CLL can play an important role in the survival and longevity of the patients. In this study, we examined the seroprevalence of CMV in the CLL patients in comparison with healthy controls.

Materials & Methods

Participants:

This case-control study was performed on CLL patients referred by oncologist to Imam Khomeini Hospital, Urmia (Iran). A total of 65 study participants, 33 males and 32 females, were included in this study in two groups, study group (n=31) and control group (n=34). The study protocol was reviewed and approved by the Ethics Committee of Urmia University of Medical Sciences, Iran. Sampling for CLL patients was carried out through simple non-random consecutive sampling. Healthy control individuals were randomly chosen from healthy age-matched without any diagnostic chronic diseases. Informed consents were obtained from both patients and controls.

Experimental Analysis:

After assessment of the inclusion criteria by oncologist and interviewing with subjects and collecting demographic data, blood samples were drawn from all individuals. After centrifugation of blood samples, serum samples were isolated and stored in −70°C.

In order to investigate CMV IgG and IgM antibodies in serum samples of the CLL patients and healthy subjects, an enzyme linked immunosorbent assay method (ELISA) (EUROIMMUN, Lübeck, Germany) was used.

Based on serological examination, each series of results were classified as follows:

- CMV seronegative, which both CMV-IgM and CMV-IgG was negative.
- CMV seropositive which CMV-IgG was positive and CMV-IgM was negative.

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- Primary CMV infection, which CMV-IgM was positive and CMV-IgG was negative.
- Reactivated CMV infection which both CMV-IgM and CMV-IgG was positive.

Statistical analyses:

Data were analyzed by SPSS software [version 16.0 (SPSS Inc., Chicago, IL., USA)]. Data are expressed as mean ± standard deviation (SD), and categorical variables are defined via relative frequencies.

The Student's *t*-test was applied for comparisons of variables. The Chi-square test was used for comparisons between the categorical variables. The statistical significance considered as $P < 0.05$.

Results

Figure 1. shows CMV Antibodies, IgG and IgM in the CLL patients and controls.

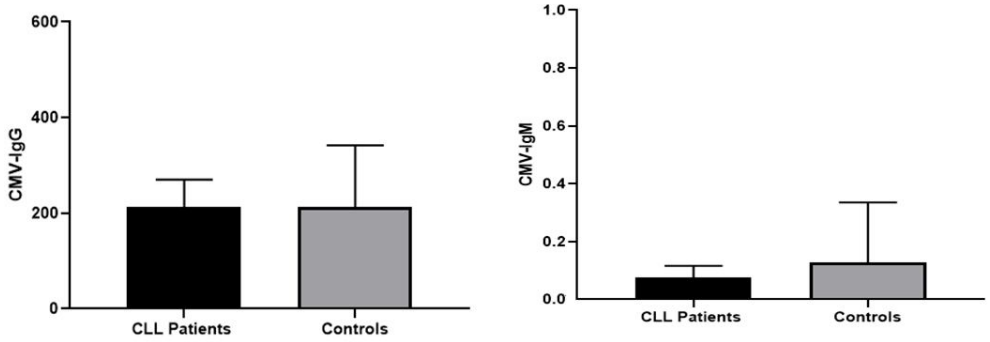

Fig. 1. CMV Antibodies, IgG and IgM. Qualitative detection of IgG and IgM antibodies to CMV in the CLL patients and controls to show the following: no exposure to CMV or current or previous infection with CMV.

CMV–IgG (Result & Interpretation)		CMV-IgM (Result & Interpretation)	
< 10	Negative	<0.9	Negative
≥10	Positive	≥ 1.1	Positive
		0.9-1.1	Borderline result

Table 1. CMV IgM and IgG results as determined by ELISA for the patients and control groups consecutive CMV positive serum samples.

	N	no exposure to CMV	current infection	previous infection	Gender
Control group	34	0 (0%)	1 (2.95%)	33 (97.05%)	Female;17(50%) Male; 17(50%)
CLL patients group	31	0 (0%)	9 (29.03)	22 (70.97)	Female;15(48.38%) Male; 16(51.62%)

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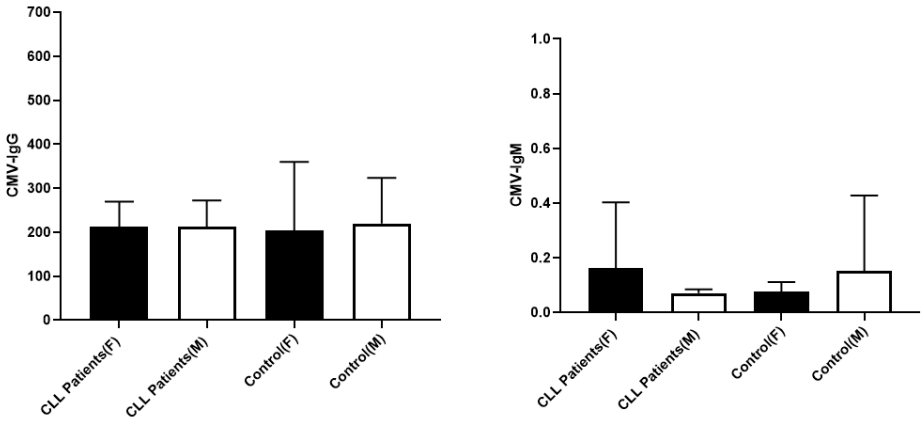

Fig. 2. Seroprevalence of CMV Antibodies (IgM and IgG) among female and male in the CLL patients and control groups.

Based on our results, previous exposure to CMV was significantly higher in the CLL patients compared with the control group ($P<0.05$). As seen in the figures and table, there are no significant differences in IgM and IgG seroprevalence among female and male in the patients' group ($p>0.05$). Likewise no significant difference in IgM and IgG seropositivity was presented among female and male in the control group ($p>0.05$).

Discussion

Viral infections are considered as serious threats to immunosuppressed patients such as solid tumors and leukemias during chemotherapy, transplant recipients under immunosuppressive treatments, and stem cell transplants from preconditioning time to full recovery. In recent years, major advances in therapeutic strategies along with rapid viral diagnostic tests have led to marked improvement in the management of immunosuppressed conditions (8). Immunosuppression favors latent reactivation of viral infections, which are the main cause of viral infections rather than acquisition of primary infection. Infections caused by herpes viruses, including cytomegalovirus and Epstein-Barr virus as well as respiratory viruses and hepatitis B virus are frequently associated with high morbidity and mortality in the immunocompromised hosts (9). There is an increasing concern about drug-resistant CMV infections, which can lead to health problems, particularly in immunosuppressed conditions and older people (3).

In this study we evaluate serum CMV IgG and IgM in the CLL patients and healthy control subjects. Based on our findings there was no significant differences in CMV IgG and IgM seroprevalence in the CLL patients in comparison with healthy controls. Likewise, no significant difference was observed among women and men in both studied groups. Also, the mean age among both groups showed no significant differences. These results can indicate a good treatment strategy and management of the patients, although long-term continuous monitoring may be necessary to achieve such a result.

It has been shown that CMV seropositive patients had significantly worse overall survival than CMV negative patients in univariate analysis (3). In the study by parry et al., there was no evidence that CMV impacts on the clinical outcome of the patients with B-CLL. Also in another related study, the CLL patients have preserved CMV-specific antibody responses despite progressive decay of total IgG and IgG subclasses. CMV-specific IgG levels are frequently boosted in contrast to that of other herpesviruses, indicative of a higher rate of CMV reactivation and antigen presentation. In contrast to the reactivity of multiple

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different CLL-rAbs with pUL32, boosts of humoral immunity are triggered apparently by other CMV antigens than pUL32, like glycoprotein B (4).

In another study, the seroprevalence of anti-CMV IgG was 96.4% (95% CI: 95.23-97.50) and IgM anti-CMV was 2.3% in the blood samples of donors used in serological screening during a one-year study (95% CI: 1.39 - 3.20) (10). The overall prevalence of CMV infection in this study was 91.3%. The highest rates were found in the patients suffering from platelet disorders (94.5%), anemia (93.3%), or leukemia (91%). Another study showed that CMV infection was prevalent in the patients with hematological diseases from the Brazilian western Amazon (11).

In a study by Al-kaabi et al., CMV IgG was positive in 84 control subjects (87.5%) and 45 hematological malignancies (93.8%) without any statistically significant association with blood transfusion history (*P value* > 0.05). Whereas CMV IgM positive was observed in 2 controls (2.1%) and 10 patients (20.8%) with a statistically significant correlation (*P value* <0.05) with exposure to blood products. The high prevalence of CMV infection in the patients with a history of blood transfusion or blood products also shows the necessity of using leuco-depleting filters or CMV negative blood products (12).

In conclusion, to prevent or reduce the risk of cytomegalovirus (CMV) infection in immunosuppressed patients, preventive measures by limiting exposure to sources of CMV and continuous monitoring for reactivation of latent infection are necessary. It seems that the main source of cytomegalovirus infection in immunosuppressed patients is blood products. Therefore, it is necessary to use highly sensitive screening tests on blood products and use virus transmission reducing tools during the injection of blood products, such as leuco-depleting filters. The high prevalence of CMV seropositive blood products is the main obstacle for creating a list of CMV seronegative products in the blood bank. However, focused screening on a specific group of seronegative blood donors would be the most efficient way to achieve this goal. According to the proper treatment management of the CLL patients, it seems that observing the precautions mentioned about blood products (if a blood product is needed) can help in maintaining stable conditions of these patients. Further studies and longtime monitoring should be done to know the factors affecting the reactivation of latent CMV infection and other latent viral infections in the CLL patients. More comprehensive studies with more study groups and population is recommended.

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Conflict of interest

The authors have no conflict of interest in this study.

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