Original Article

Detection of CMV DNA in intraocular fluid samples in patients clinically diagnosed with viral uveitis by molecular methods

Ayfer Yolcu[1], Nuran Karabulut[1], Sema Alacam[1], Mustafa Onel[1], Belgin Izgi[2], Ali Agacfidan[1]

From, [1]Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology, Division of Virology and Fundamental Immunology, Istanbul, Turkey. [2]Istanbul University, Istanbul Faculty of Medicine, Department of Ophtalmology, Istanbul, Turkey.

Correspondence to: Dr. Ayfer Yolcu, Istanbul University, Istanbul Faculty of Medicine Department of Medical Microbiology, Division of Virology and Fundamental Immunology, 34000 Istanbul, Turkey. Email: dr.ayfer.bakir@gmail.com

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ABSTRACT

Background: Latent Cytomegalovirus (CMV) infections may be reactive in consequence of immunosuppression. Recently, CMV-associated uveitis cases have also been reported in immunocompetent individuals. **Aim**: The aim of this study was to obtain epidemiological data by evaluating CMV DNA PCR results, clinical characteristics and risk factors of patients diagnosed with viral uveitis. **Methods:** Between the years 2011-2017, anterior chamber fluid samples of 35 patients with viral uveitis sent from the ophthalmology clinic to the Virology Laboratory were included in this study. CMV DNA real-time PCR results were evaluated. **Results:** CMV DNA positivity was detected in 9 (26%) of the 35 patients. CMV DNA positivity was highest (13%) in over 60 years (p=0.02). Acute recurrent uveitis in 44% (4/9) of patients, single eye involvement in 78% (7/9) and anterior uveitis in 44% (4/9) were found. **Conclusion:** In this study, CMV DNA positivity was detected in 26% of the patients with viral uveitis. Considering the presentations of a small number of cases related to CMV uveitis in our country, this study is important due to being the most comprehensive study in our country.

Key words: *Cytomegalovirus, CMV DNA, PCR, Uveitis*

Cytomegalovirus (CMV) is double-stranded DNA virus which belongs to the *Beta herpes virinae* subfamily and *Herpes viridae* family [1,2]. The worldwide prevalence of CMV infection is close to 60% in developed countries and 100% in developing countries [3, 4]. Following primary infection, CMV remaining latent in myeloid progenitor cells may cause anterior uveitis, which is the most common form of inflammation in the eye, by reactivation in patients receiving transplantation, AIDS, or immunosuppressive therapy [1-3]. Advanced age, diabetes mellitus, malignancy, autoimmune disease, inherited and / or acquired immunodeficiency disorders not associated with HIV are also risk factors. On the other hand, CMV anterior uveitis infection can also occur in immunocompetent patients.

Recently, case reports related to this issue have increased [4-6]. Epidemiology of infectious uveitis presents changes in geographical and climatic conditions, differences depending on the factors of the host [4-6]. Infectious uveitis accounts for 13% - 21% of uveitis cases in developed countries [7-9]. Infections are estimated to be mostly linked to herpes viruses and these viruses are among the important pathogens of the anterior and posterior uveitis. In developing countries, infectious etiology is responsible for 50% of uveitis. Tuberculosis, oncocerciasis, cysticercosis, leprosy and leptospirosis are thought to play a role especially in toxoplasmosis [15].

CMV can cause acute, recurrent, hypertensive or chronic anterior uveitis [16-18]. Acute uveitis is seen mostly in the ages between 20-50, and chronic uveitis in the age group of 40-70. Ocular involvement is often unilateral and seen in males [16, 17]. Laboratory tests are important because of the discrimination of infectious and non-infectious uveitis and the difficulties in identifying viral etiologic agents [19].

For the diagnosis of CMV uveitis, CMV serology may be useful to exclude viral etiology. Accompanying CMV IgM positivity in patients with CMV IgG positive indicates active systemic infection but does not prove ocular infection [16]. In large epidemiological studies on uveitis, 48% of the cases etiology is not found, but viruses are increasingly important in the anterior uveitis etiology with the developments in molecular diagnostic techniques [7]. CMV uveitis can be diagnosed by PCR detection of viral DNA in intraocular fluid samples or by Goldmann-Witmer analysis of anti-viral antibodies [19]. One of these tests is preferred depending on the immune status of the patient, the time of sampling, and the presence of acute or chronic uveitis. However, so there are studies suggesting that both tests should be done if possible [20].

In addition, identification of the "owl eye" morphology specific to CMV in infected cells using confocal microscopy is helpful for diagnosis [10]. PCR analysis of current intraocular fluid specimens is the most commonly used method to confirm the clinical diagnosis before the onset of treatment, when acute CMV uveitis is suspected [22]. It is very useful in terms of rapid detection and effective treatment of viral pathogen [11]. Systemic or local ganciclovir or both in combination or systemic valganciclovir may be used in the treatment of CMV uveitis. Since neutropenia is an important complication of valganciclovir therapy, valaciclovir is considered a promising option for preventing CMV relapses. The duration of treatment has not been known yet [10,12]. Therefore this study was designed to obtain epidemiological data by evaluating retrospectively PCR results of CMV DNA, clinical characteristics and risk factors of patients.

MATERIAL AND METHODS

Between the years 2011-2017, anterior chamber fluid samples of 35 patients diagnosed with viral uveitis sent from the ophthalmology clinic to the Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology, Virology Laboratory were included in this study. CMV DNA real-time PCR results were evaluated retrospectively. The study data were assessed using the patient files of the Clinic in Ophthalmology together with the laboratory patient files and the laboratory operating system. This present study was approved by Ethics Committee of Istanbul University, Istanbul Faculty of Medicine and adhered to the Declaration of Helsinki.

Viral DNA extraction and PCR analysis were performed using with AmpliPrep / COBAS TaqMan (CAP / CTM) 96 (Roche Diagnostics GmbH, Mannheim, Germany) for CMV DNA determination in anterior chamber fluid samples. The extracted viral nucleic acid product was transferred to the Cobas TaqMan 96 PCR unit automatically and amplified by PCR method with the AmpliPrep / Cobas CMV test (CAP / CPM) kit. In the software program, the test results of the studied samples were evaluated qualitatively.

Data analysis was performed by using SPSS 21 (SPSS Inc, Chicago, IL, USA) program. The normal distribution suitability of the variables was examined by visual methods (histogram and probability plots) and Kolmogorov-Smirnov test. Mean age between genders was compared using Student's t test, age groups and gender using CMV DNA positivity Fisher's test. p values below 0.05 were considered statistically significant.

RESULTS

The mean age of the 35 patients being included in the study and having ranging in age from 9 to 81 was 45.46 ± 15.56, 66% male (95% confidence interval [CI] 14-42) and 34% (95% CI 21-51) female patients. The mean age of male patients was 46.04 ± 14.62, while the mean age of female patients was 44.33 ± 17.84. There was no statistically significant difference between the genders in terms of age averages (p= 0.76). CMV DNA positivity was examined according to age groups and it was found to be higher than other age groups by 14% over 60 years old and statistically significant (p= 0.02). Distribution of patients diagnosed with CMV uveitis according to age groups and gender is showed in Figure 1.

CMV DNA was positively detected in 9 out of the 35

patients (26%), (95% CI 14-42), included in the study. The patients with CMV DNA detected in the anterior chamber fluid were 5 male and 4 female patients and their median age was 63 (range 9-78). There was no significant difference in CMV DNA positivity between gender (p= 0.69).

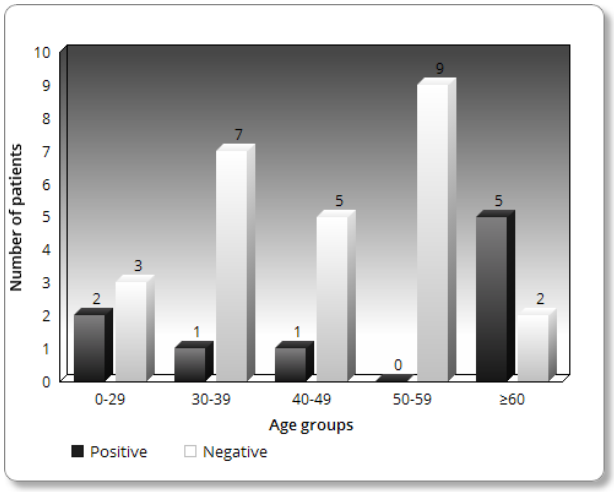

Figure 1. Distribution of patients diagnosed with CMV uveitis according to age groups and gender

Acute uveitis was found in 33% (3/9) of patients, acute recurrence in 44% (4/9) and chronic uveitis in 9% (1/9). In 44% (4/9) of cases anterior uveitis and 78% (7/9) of cases had unilateral involvement. Clinical findings, comorbid diseases and immunosuppressive drug use of CMV uveitis-diagnosed patients are shown in Table 1. Three of the nine patients with CMV DNA positive (one female, two male) did not have comorbid disease. The history of inhaled steroid use was detected for two patients due to their bronchial asthma. In one patient, ocular trauma was not detected except intraocular steroid injection. In patients, anti-HIV positivity was not detected.

DISCUSSION

Members of the herpes virus family that cause latent infections may show reactivation due to many risk factors. This explains the basic pathological mechanism in various diseases including uveitis [16]. In this study, the results of patients who detected CMV DNA positivity by PCR method in intraocular fluid sent to Virology Laboratory with viral uveitis diagnosis within six years period were examined. Epidemiological features of the disease were also evaluated with our laboratory findings.

Table 1 - Clinical findings, comorbid diseases and immunosuppressive drug use histories of patients with CMV uveitis

Patient/ Gender/Age	Course of uveitis	Laterality	Localization	Comorbidity	Trauma	İmmunsuppressive medication use
1/M/66	Chronic	Unilateral	Unknown	DM, NHL, CRD	None	None
2/F/44	Acute recurrence	Unilateral	Anterior	None	None	None
3/F/27	Acute recurrence	Unilateral	Anterior	Asthma, Behçet disease	None	Inhaled steroid
4/F/9	Unknown	Bilateral	Posterior	PIDD	None	None
5/M/38	Acute recurrence	Unilateral	Anterior	None	None	None
6/F/64	Acute	Unilateral	Posterior	Behçet disease ALL	None	Chemotherapy
7/M/78	Acute	Unilateral	Posterior	Asthma	IO steroid injection	Inhaled steroid
8/F/66	Acute recurrence	Unilateral	Anterior	None	None	None
9/F/63	Acute	Bilateral	Posterior	CLL,DM	None	Chemotherapy

DM: Diabetes mellitus; NHL: Non-Hodgkin lymphoma; CRD: Chronic renal disease; PIDD: Primary immune deficiency disease; ALL: Acute lymphoblastic leukemia; CLL: Chronic lymphocytic leukemia, IO: Intra ocular

CMV DNA positivity in intraocular fluid was found as 26% (9/35). CMV anterior uveitis cases are reported all over the world. When the CMV anterior uveits seroprevalance studies were examined, the rates of CMV DNA positivity were reported as 1%, 1.7%, 13.3% and 71.4% in a small number of studies reported in European countries [25-28]. In a study conducted in Turkey only four of the 51 patients diagnosed with viral uveitis, CMV

DNA was detected by PCR method [13].

Other studies were limited to a few case reports. In Asian countries, CMV positivity was reported in patients with uveitis between 1.68-12.7% and in the USA 0.8% [30-35]. The seroprevalence of CMV infection varies among continents in the world. It is about 69.1% to 98.6% in Asia, while it varies from 41.9% to 57% in Western countries [17]. More cases of CMV were reported in Asian countries than in Western countries. Thus, CMV can be attributed to differences in variable rates and geographical distribution of seroprevalence.

Sugita et al [22] found that sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PCR results for infectious ocular diseases were 91.3%, 98.8%, 98.5%, 92.4% respectively and reported that PCR is a reliable method for excluding infectious agents. Kharel et al [36] reported sensitivity, specificity, PPD and NPD values of CMV PCR as 100%, 71%, 15.4% and 100%, respectively. In other studies, it has been reported that the sensitivity of PCR method is 80.9-90% and the specificity is 95-100% for HSV, VZV and CMV [37, 38]. When the PCR method is used for ocular fluids, it is reported that the false positive rate is very low. Whereas false positive results may be due to contamination, false negative results might result from polymorphism, sample impairment or sample taken during the acute phase of the disease. Positive PCR results confirm the diagnosis of infection, but negative PCR results do not exclude viral infections [7, 39].

In this study, median age of patients with CMV DNA positivity in intraocular fluid was 63. In other studies conducted in this regard, in a study involving Belgium and the Netherlands, the mean age of CMV DNA-positive patients was 38.57±12.11, 55.2±18.6 in Spain, and 47.5± 14.8 in Korea [3, 28, 40]. The mean age in this study was found to be higher when compared to the results of other studies, besides found to be consistent with the literature. In this study, when the distributions according to age groups were examined, the intraocular CMV DNA positivity was found to be higher than the other groups with 14% over 60 years old and statistically significant (p= 0.02). In a study evaluating the distribution of CMV uveitis by age groups in Vietnam, it was found to be 6% between the age of 21-40 years, 4.1% between the age of 41-60 years and 3.7% the age of over 60 years and there was no found significant difference between the age groups in terms of the frequency of CMV uveitis [33]. CMV uveitis has been reported to be seen at all ages. Nevertheless, acute recurrent anterior uveitis, the most commonly described clinical manifestation of CMV uveitis, is reported to be more frequent in the third and fifth decades of life [10]. In this study, CMV uveitis was found to be more common in male patients. However, the difference was not statistically significant. In other studies, similar to this study, is generally reported male predominance [3, 28, 30, 35]. There are also studies reporting that CMV uveitis is seen more frequently in female patients, but these studies are quite limited [33]. The cause of the more frequent occurrence in male patients is unknown.

CMV has been reported to be an anterior uveitis, with the most common form of inflammation in the eye [5-7]. However, in this study, only anterior uveitis due to CMV was detected in 44% of the patients. We think that these results can be caused by the fact that the smaller sample size compared to other studies. In this study, four patients were diagnosed with posterior uveitis. These four patients also had comorbid disease supporting immunosuppression (Table 1). Contrary to isolated anterior uveitis, CMV-associated retinitis has been reported to occur in immunocompromised individuals [37]. In this study, comorbid disease was not detected in only three of nine patients with CMV DNA positive in the intraocular fluid. Concomitant comorbid disease in one of the cases was non-Hodgkin lymphoma (NHL). In the literature, there are only one case reports of CMV retinitis receiving chemotherapy after NHL diagnosis [41, 42]. In a study involving 18 patients with CMV-positive, posterior uveitis and panuveitis, comorbid diseases such as immunosuppressive drug use (hematologic malignancies, systemic autoimmune diseases and organ transplantation), NHL, primer immunodeficiency, diabetes mellitus were reported in patients [43].

In this study, similar comorbid diseases were detected in the patients with CMV uveitis. Although CMV uveitis is a defined disease in the immunocompromised host, the number of anterior uveitis cases in immunocompetent patients is increasing steadily [6, 34]. This increase can be explained by the development of diagnostic techniques and the increased use of highly sensitive molecular methods [21]. A very few case reports of CMV uveitis in

immunocompetent patients in our country are reported [29].

CONCLUSION

CMV DNA positivity was found in 26% of patients diagnosed as having viral uveitis in this study. In Turkey, when considering the limited number of case reports, it is the most comprehensive study of the CMV uveitis. These case reports do not provide sufficient data for the epidemiology of CMV uveitis infection. We believe that our study will lead to similar studies.

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