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ORIGINAL ARTICLE

Cytomegalovirus infection in patients attending a tertiary care hospital – single center experience

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ABSTRACT

Background and Objectives: Human cytomegalovirus infection poses an important public health issue. This issue in India has not received enough attention. The majority of research workers have highlighted the seroprevalence of human cytomegalovirus. Hence this study was conducted to find out true magnitude of human cytomegalovirus disease.

Materials and Methods: Samples from 181 patients with suspected human cytomegalovirus disease were analyzed for human cytomegalovirus. DNA extraction was followed by real-time PCR. Human cytomegalovirus DNA-specific probes, fluorophore FAM™ and fluorophore JOE™ were utilized to detect human cytomegalovirus specific DNA and internal control at the same time. After completion of the assay, fluorescent growth curves were examined, and the response growth curves passing the threshold line in less than 36 cycles were deemed to be positive. All relevant clinical, demographic, and epidemiological information of the patients was also recorded.

Results: The most common clinical presentation was meningitis/meningoencephalitis. Out of the total samples, human cytomegalovirus infection was detected in 21% of the samples. Most positive samples were from infants (18.2%), followed by post-renal transplant cases (2.7%). Human cytomegalovirus was detected in urine samples (17.1%) followed by serum (3.8%). Four out of the 14 CSF samples were tested for other viruses as well, and they were positive for EBV (n=1, 7%), enterovirus (n=2, 14%), and varicella zoster virus (n=1, 7%).

Conclusion: PCR has a significant role in the detection of human cytomegalovirus disease at an early stage to avoid irreversible sequelae of late diagnosis.

Keywords: Human cytomegalovirus; Congenital; Post renal transplant; Real-time polymerase chain reaction

INTRODUCTION

Human cytomegalovirus (HCMV), the member of *Herpesviridae* family has the largest number of genes facilitating evasion from the innate and adaptive immunity of the host. HCMV, a widely spread virus, shows tropism to broad range of cells of any organ within its host, mostly due to alteration within the UL 128-131 gene locus. HCMV infection is commonly associated with salivary glands and lead to increased morbidity and mortality, especially in immunocompromised patients, most frequently newborns, post-transplant patients, and HIV-infected persons. Immunocompetent hosts remain asymp-

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tomatic during primary infections with this virus (1, 2). The frequency of congenital HCMV infection is 0.2-2.2 % of total HCMV infection and is the most prevalent intrauterine infection (3). It mostly presents itself through asymptomatic infections. Among symptomatic patients, neurological impairment and sensorineural deafness are seen in 90% of cases, followed by a poor quality of life and high fatality (4). Late symptoms may develop in asymptomatic children with progressive, irreversible sequelae (5). The global burden of HCMV infection varies, with rates soaring to 100% in developing countries (6). Croatia, United States, and France have reported prevalence rates of 74.4, 50.4 and 41.9% respectively (7). In India, the magnitude of this disease is still underrated and has not been well studied. Not much of the literature elucidates detection of HCMV by PCR, as the majority of studies emphasize the seroprevalence of the disease, demonstrating a high prevalence of HCMV -IgG antibodies in females of reproductive age group in most parts of India (8). As observed by Lone et al. in a study from this region, 16% of pregnant women studied were HCMV-specific IgM antibody positive (9). HCMV DNA detection distinguishes people with active HCMV disease from those who are at risk (10). Studies regarding burden of HCMV disease in high-risk groups have not been carried out in this region. This study is the first of its kind from this region to use real-time PCR for analyzing the magnitude of HCMV disease in this geographical area.

MATERIALS AND METHODS

Study aim and design. This four-year long hospital-based cross-sectional research was carried at the virology division of the Department of Microbiology of tertiary care super-speciality hospital in the year 2024, to analyze the burden of HCMV infection among patients attending this hospital with symptoms mimicking the disease. All methods were carried out by relevant guidelines and regulations.

Ethical approval. Institutional Ethical Committee approval was obtained before conducting the study under letter no. SIMS 131/IEC-SKIMS/2024-10.

Sample collection and processing. Urine, serum, and CSF samples were collected from symptomatic patients according to the clinical presentation and were processed immediately. The samples were fully anonymized and annotated by unique identification numbers and were stored at -70°C in case of delay before testing. All relevant clinical, demographic and epidemiological information was recorded from hospital information system.

DNA extraction and polymerase chain reaction. QIAamp® Mini Kit (QIAGEN) was used for DNA extraction followed by real-time PCR (RT-PCR) performed as per manufacturer's instructions using the Altona RealStar® CMV PCR Kit 1.0 on ABI Fast Dx Real time PCR machine (by Applied Biosystems). The quality of reagents was checked by using an internal control (IC) to identify possible inhibitors of PCR. HCMV DNA-specific probes designated as fluorophore FAM™ and fluorophore JOE™ for IC were utilized. The benefit of labelled probes is that they use distinctive dyes, making it possible to detect HCMV-specific DNA and IC at the same time. Furthermore, to validate the integrity of real-time PCR assay results, IC was analyzed for each patient sample, in addition to testing one replicate of positive and negative control in each batch. After completion of the assay, fluorescent growth curves were examined and the threshold was set appropriately in the exponential phase manually. When response growth curves passed the threshold line in less than 36 cycles and all of the controls satisfied the specified criteria, the specimen was deemed positive. The specimen was considered negative if growth curves did not cross the threshold line within 36 cycles and all controls met stated requirements. All categorical variables have been shown in terms of frequency and percentage. For categorical data, descriptive statistics included medians and interquartile range (IQR); for continuous data, counts and percentages were included. We contrasted patient demographic data with clinical features. Statistical significance was attained if the p-value was < 0.05. SPSS was used to analyze data.

RESULTS

A total of 181 samples were received (CSF=79, serum=37, urine=65) from suspected cases of HCMV disease which included 96 males and 85 females. The average age of the cases was 24.5 years IQR (3-32

years). The majority of patients presented meningitis/meningoencephalitis followed by symptoms of HCMV disease in post-renal transplant patients (Table 1). Of all the samples, the highest percentage of HCMV positivity was found in urine followed by serum. New-borns and post-renal transplant patients with symptoms of HCMV disease presented the highest positivity (Table 2). CSF samples from fourteen symptomatic patients (with presentation of viral meningitis) were also tested for Epstein-Barr Virus (EBV), enterovirus and varicella zoster virus (VZV) out of which one was positive for EBV; two for enterovirus and one for varicella zoster virus VZV (Table 1). The geographical distribution of patients is depicted in Fig. 1.

DISCUSSION

Even though HCMV is highly prevalent worldwide, epidemiological surveillance for this virus is still disregarded (11). It is critical to increase knowledge about the spread of HCMV in India in order to prevent infection and disease and provide clinical care for immunocompromised patients, particularly post-transplant patients, newborns and women of childbearing age group and pregnant (12). The primary causes of increased HCMV disease have previously been identified as being low educational attainment, unsanitary living conditions, cultural influences, and large family sizes (13). Twenty-one percent of the research population has HCMV dis-

Table 1. Demographic and clinical characteristics of hospitalized patients suspected of HCMV disease (N=181)

Median age (years)		**24.5**			
Male: Female (infection)		**2:1**			
Patient distribution (N)		**RT-PCR for HCMV DNA Positive N (%)**	**RT-PCR for HCMV DNA Negative N (%)**	**Other viruses detected on PCR**	**Total N (%)**
Age group (years):	<1	33.0 (18.2)	29.0 (16)	0.0	62 (34.25)
	1-20	02 (1.1)	23 (12.7)	03 (21.4) *	25 (13.8)
	21-40	3 (1.6)	40 (22)	0.0	43 (23.7)
	41-60	0	18 (17.1)	0	18 (9.9)
	>60	0	33 (18.2)	01 (7.1) **	33 (18.2)
Clinical presentation	Meningitis / Meningoencephalitis	1 (0.6)	60 (33.1)	03 (21.4) *	61 (33.7)
	Post renal transplant	05 (2.7)	21 (11.6)	0	26 (14.4)
	Acute renal injury	02 (1.1)	11 (6.0)	01 (7.1) **	13 (7.2)
	Pneumonitis	04 (2.2)	02 (1.1)	0	06 (3.3)
	Sepsis with encephalopathy	04 (2.2)	10 (5.5)	0	14 (7.7)
	Fever	10 (5.5)	15 (8.2)	0	25 (13.8)
	Cerebrovascular accident	01 (0.6)	08 (4.4)	0	09 (5)
	Signs of Congenital CMV syndrome	10 (5.5)	14 (7.7)	0	24 (13.3)
	Leukemia	01 (0.6)	02 (1.1)	0	03 (1.6)
	Total	38 (21)	143 (79)	4/14 (28.5)	181 (100)

* Two Enteroviruses; One varicella zoster virus.

** One Epstein Barr virus

Table 2. HCMV Positivity in different samples

Sample distribution (N)		**RT-PCR CMV DNA positive Frequency N (%)**	**RT-PCR CMV DNA negative Frequency N (%)**	**Total samples Frequency N (%)**
Sample distribution	CSF	0	79 (43.6)	79 (43.6)
	Serum	07 (3.8)	30 (16.6)	37 (20.4)
	Urine	31 (17.1)	34 (17.8)	65 (36)
	Total	38 (21)	143 (79)	181 (100)

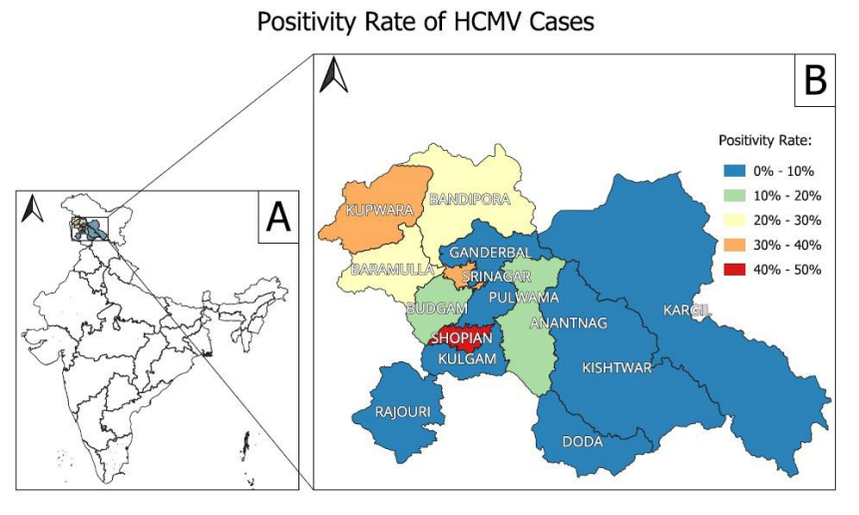

Fig. 1. Demographic distribution of HCMV disease in Kashmir (North India).

ease, which is lower than the findings from countries of both developed and developing world (6, 7). This may be attributed to the fact that real-time PCR was used for the detection of HCMV disease in symptomatic patients in contrast to other studies which used serological methods to check the burden of HCMV infection. Despite being the first study of its kind to detect HCMV disease by utilizing the modality of real-time PCR and study center being tertiary care referral hospital, receiving patients from all parts of the union territory (Fig. 1), multicentric studies, are much needed in the future. Although HCMV is widespread around the world, it is more prevalent in developing nations (14). It was observed that HCMV infection was more common in male (28%) than in female (12%) patients. Some studies have revealed that men are more susceptible to sexual transmission. Also, post organ transplant male patients show higher rates (1). Most of the HCMV infected patients were infants, which suggests a high rate of congenital transmission.

The results of this study are in accordance with observations of research work from developing countries where HCMV disease occurs early in life among both immunocompetent and immunocompromised individuals. In a study conducted by Lauro et al., the prevalence of HCMV congenital infection was modest at 1.2% in neonates under study (15). World over and in India, unlike the present study, most of the studies have focused on seroprevalence of HCMV in the population and have observed it to vary greatly with different epidemiological factors (8, 9, 16). Up to 70% of cases of vertical virus transmission occur in the third trimester, with 20-30% during first and second trimesters following primary HCMV infection (17). Among infants with congenital infections, 90% are asymptomatic in the neonatal period up to two years of life, and 5-17% experience signs of sensorineural hearing loss, chorioretinitis, or neurologic impairments. However, in the present study the most common presentation was jaundice followed by pneumonitis. An infant with pneumonitis, among the total patients studied had fatal outcome. Most of the studies report symptoms in 10% of the infected newborns, death in 20%, and 90% of the survivors experience serious aftereffects (18). Over 90% of newborns who survive HCMV illness experience late consequences, and mortality rates may reach 30% (19). Infants with recurrent congenital HCMV infections are usually asymptomatic and develop long-term sequelae (20). There are few studies on the frequency of birth abnormalities in India, and it is unknown how frequently infections recur. Nevertheless, initial infections continue to be a major source of congenital illnesses, with high rates of morbidity (21).

A second major group of patients with HCMV disease observed in the present study was post-renal transplant patients. It is well known that as people age, HCMV reactivates from its dormant state in leukocytes. Multiple new strains are also produced by the recombination of existing strains. Both processes may be responsible for HCMV disease in transplant recipients from India, where the seroprevalence is high. A prior study found that latent HCMV infections are present in 60-90% of post-renal transplant

patients, but only 20-60% of these patients develop HCMV disease (22). When no HCMV prophylaxis is administered, the total incidence of HCMV disease within the first three months post-transplant is 60% and 25%, respectively, making HCMV the most common cause of viral infectious disease in the early stages after renal transplantation (23). Among HCMV positive samples, highest positivity was found in urine more significantly for infants followed by serum for post renal transplant patients.

Real time PCR was used in this study targeting significant genes in the highly preserved regions of virus as well as several additional genes that are targets for HCMV detection. Additionally, sample deterioration in case of delay post sample collection do not pose a problem with PCR testing as compared to other modalities to check HCMV disease. Moreover, the modality was compatible to extract DNA from different type of samples, similar to the observations of other studies. Hence, suggesting vast range of samples can be used for testing, including sterile body fluids and products like whole blood, plasma, WBC's, CSF, BAL or even tissue biopsy and urine (24).

CONCLUSION

Detection of HCMV DNA by utilizing the real-time PCR in present study detected HCMV instantly, helping in timely initiation of treatment with an aim to reduce the devastating effects of HCMV. Moreover, its compatibility with wider range of samples without any concern of sample deterioration in case of any delay is an additional boon of the modality. Burden of HCMV disease in this geographical region is comparable to the results of earlier research carried out in low-income areas, where majority of infected were infants. A substantial rate of disease in transplant patients and renal failure cases was also observed in this study. Notably, a national policy to screen and test suspected cases, reduce the probability of viral transmission, identify late symptoms and sequelae, and monitor the patients is one of the most critical strategies needed.

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