Prevalence of HCMV in Indian Scenario

S. Shukla, *P. Singh, P. Tidke, V. Bhatia, S. Dutt

Onquest Laboratories Pvt. Ltd, 3-Factory Road, Adj. Safdarjung Hospital, New Delhi 110029. India.

ABSTRACT

A cytomegalovirus (HCMV) infection is one of the leading cause of morbidity in immunocompromised patients especially in solid organ transplant recipients (SOTRs). HCMV is found throughout all geographic locations and socioeconomic groups but it is more widespread in developing countries and in areas of low socioeconomic conditions. CMV is one of the most successful human pathogen, it can be transmitted vertically or horizontally and it is also known as Human herpes virus 5 (HHV-5). The endeavour of this study was to ascertain the prevalence of CMV in developing countries like India. Over a period of two years (October 2012- October 2014), 912 clinical specimens were received from various hospitals and clinics from diverse regions of India for screening of CMV as a part of routine testing. Among 912 specimens, only 900 specimens were tested and 12 specimens were not included in the study because these were rejected due to inappropriate specimen condition. Real time PCR assay was used for viral load estimation. On the basis of result of HCMV viral load detection of all 900 samples, we found 443 (49 %) CMV positive patients, out of those positive specimens, male positivity was 45.21% and female positivity was 59.72%. We found that the prevalence of cytomegalovirus was significantly higher in females as compared to males. Age wise correlation was also conducted. The highest age-specific cytomegalovirus prevalence i.e. 76.67 % was found in the age group of 21-30 followed by > or =60.

Keywords: Human cytomegalovirus (HCMV), human herpes virus 5 (HHV-5), real-time PCR, solid organ transplant recipients (SOTRs), viral load

INTRODUCTION

Congenital CMV infection is one of the foremost infectious cause of various disabilities and mental disorders in children [1]. This study provides valuable information that can be used to examine the incidence of infection in the community. This study was carried out to measure the prevalence of HCMV with suspected clinical specimens.

Human cytomegalovirus is a species of virus that belongs to the viral family known as Herpesviridae or herpesviruses. It is classically shortened as HCMV and is also known as Human herpesvirus 5 (HHV-5) and is associated with cancer and worsening of the immune system due to poor treatment choice [2]. Human Cytomegalovirus (HCMV) is the most frequent reason of congenital infections affecting 0.5% to 2% of all live births in the developed countries. HCMV is a common virus that can infect anyone. Human Cytomegalovirus (HCMV) has appeared as a major cause of morbidity and mortality in children and immunocompromised adults [3]. It is believed that humans are the only reservoir for HCMV and transmission occurs from person to person by direct or indirect, close or intimate contacts. The virus sheds in semen, saliva, urine and upto some extent in other body fluids. Besides contact with seropositive mothers (through genital secretions, breast milk etc.) blood transfusion and organ transplantation are other common modes of postnatal spread of HCMV [4, 5]. Once infected with HCMV, body retains the virus for lifetime. However, HCMV generally remains inactive if person have strong immune system. People with weak immune systems have a higher risk of becoming ill from HCMV. Most people do not know that they have HCMV because it rarely shows symptoms. However, HCMV is a cause of
concern in solid organ transplant recipients (SOTRs), pregnant women and immunocompromised men & women. There is no treatment for HCMV but few drugs are available to treat newborns and people with weak immune systems. It is an ubiquitous virus, the seroprevalence of which reportedly varies between 30 to 100% in different countries [6, 7]. HCMV positivity rates are higher in females, older people and those of immunocompromised patients like organ transplant recipients or HIV infected. Among general population worldwide seroprevalence in adults varies from 40 to 90% [8, 9]. Serological tests, antigenemia tests, culture methods and molecular methods are applied for the diagnosis of HCMV infection. In addition to CMV positivity study we also studied the gender and age wise correlation of CMV positivity. The present study was conducted with the objective to scrutinize the prevalence of CMV infection in developing countries like India, so that the prevention, prognosis and management can be made easy. Such viability studies have been very few in India.

**MATERIALS & METHODOLOGY**

**Clinical Specimens:** Over a period of two years (October 2012- October 2014), 912 clinical specimens were received from various hospitals and clinics from assorted regions of India for screening of HCMV as a part of routine testing. Total 912 samples were received from suspected HCMV patients for the detection of HCMV viral load along with specifically designed data sheets from patients visiting/attending various secondary and tertiary collection centers located in different cities/towns of India. Knowledge approval has been taken in written from each participated patient including demographic characteristics, age, and estimated time of infection. Out of 912 specimens, only 900 specimens were tested and 12 specimens were not included in the study due to inappropriate sample condition. The total numbers of males were 656 (72.88%) and females were 244 (27.11%). Different types of specimens like EDTA plasma, CSF, BAL fluid & urine were tested for the screening of HCMV infection. The collected whole blood EDTA specimens were centrifuged at 2000 x g for 15 minutes for separation of plasma and the separated plasma was stored at -20°C.

**DNA ISOLATION & QUANTITATION**

One hundred microliter of eluted DNA was mixed with 20 μl of protease and 200 μl of lysis buffer and the mixture was incubated at 56°C for 10 minutes and then processed by using a QIAamp Blood mini-kit (Qiagen, Valencia, Calif.). The DNA absorbed to the QIAamp spin column was eluted with 50 μl of AE buffer and then subjected to PCR. Quantitative PCR was performed on Rotor-Gene Q Real Time PCR system by using Amplisens® HCMV screen/monitor – FRT PCR kit. Ten microliter of template was added in 15 microlitre of master mix to prepare 25 μl of reaction mixture. PCR Profile was as follows: Initial denaturation for 15 minutes at 95°C, followed by 40 cycles of denaturation at 95°C for 5 seconds, annealing at 60°C for 20 seconds and extension at 72°C for 15 seconds.

**RESULTS**

**Prevalence of Cytomegalovirus by Sex**

Among tested population the prevalence of HCMV was 49.0%. HCMV DNA was detected in the total of 441 patients. In the entire population females (59.42%) were more likely to be HCMV DNA positive than males (45.12%). HCMV DNA was detected in the 145 out of 244 (59.42%) females and in 296 out of 656 (45.12%) males. The significant difference has been observed in the positivity rate of males and females. (Table 1 and Figure 1).

**Prevalence of Cytomegalovirus in Indian population by age groups:**

Among different age groups the maximum positivity (76.67%) was found in the age group of 21 -30. Highest HCMV DNA positivity was observed in the age group of 21-30 (76.67 %) followed by the age group of > or = 60 (60 %). The frequency of infection in other age groups are shown as 44.80 % in 41-50, 44.06 % in 31-40, 42.59 % in 0-10 and 36.80 % in 11-20. The prevalence of cytomegalovirus in different age groups is shown in (Table 2 and Figure 2).
Table 1: Prevalence of Cytomegalovirus by Gender (Males and females)

<table>
<thead>
<tr>
<th></th>
<th>Total No. of samples N= 900</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (656)</td>
</tr>
<tr>
<td>Positives</td>
<td>296</td>
</tr>
<tr>
<td>Negatives</td>
<td>360</td>
</tr>
<tr>
<td>% positivity</td>
<td>45.12</td>
</tr>
</tbody>
</table>

Figure 1: Prevalence of Cytomegalovirus by Gender (Males and females)

Table 2: Prevalence of Cytomegalovirus by age groups from 0 to > 60 years old

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>No. of Specimens</th>
<th>Positives</th>
<th>Negatives</th>
<th>% Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>54</td>
<td>23</td>
<td>31</td>
<td>42.59</td>
</tr>
<tr>
<td>11-20</td>
<td>152</td>
<td>56</td>
<td>96</td>
<td>36.80</td>
</tr>
<tr>
<td>21-30</td>
<td>263</td>
<td>202</td>
<td>61</td>
<td>76.67</td>
</tr>
<tr>
<td>31-40</td>
<td>160</td>
<td>71</td>
<td>89</td>
<td>44.06</td>
</tr>
<tr>
<td>41-50</td>
<td>102</td>
<td>46</td>
<td>56</td>
<td>44.8</td>
</tr>
<tr>
<td>51-60</td>
<td>114</td>
<td>34</td>
<td>80</td>
<td>29.41</td>
</tr>
<tr>
<td>&gt;60</td>
<td>55</td>
<td>33</td>
<td>22</td>
<td>60</td>
</tr>
</tbody>
</table>

Figure 2: Prevalence of Cytomegalovirus by age groups from 0 to > 60 years old

DISCUSSION
HCMV is a severe pathogen mainly for immunocompromised individuals, although significant differences in the seroepidemiology exist between and within countries. Detection of HCMV disease at an early stage of infection ensures effective treatment. In India, a coarse estimation of the frequency of new CMV infections is essential for understanding and preventing viral transmission. In adults, HCMV transmission commonly occurs via infected newborns and children, in particular those below 30 months old, actively excrete the virus in their saliva and urine. Thus, a hypothesis to explain the higher female HCMV DNA prevalence would be that women have more contacts with children and have more prospects for HCMV infection during pregnancy, delivery and menstruation. In different parts of India, serological surveys have shown 80-90% prevalence of HCMV IgG antibodies in women of childbearing age [10-12]. In present study the Real Time PCR was used for the detection of HCMV DNA. We found significant difference in the prevalence of cytomegalovirus in males and females. Present study established that the prevalence of cytomegalovirus infection was significantly higher in females (59.42%) as compared to males (45.12%). Among all age groups the minimum frequency of infection (29.41%) was found in the patients of age group 51-60, whereas the patients of 21-30 age group has the highest positivity (76.67%).

CONCLUSION

Each year, thousands of CMV infections occur in pregnant women & immunosuppressed patients in developing and developed countries, putting numerous unborn babies & patients at risk of infection. Based on the above study our results showed higher positivity of HCMV infection in females as compared to males. Among females, the higher HCMV infection rate may be due to more contact with toddlers or even a high frequency and duration of breast feeding among current adolescents compared with other parts of the world. If our observations for India reflect national trends, a large proportion people of India will be susceptible to a primary HCMV infection. Prevalence and knowledge of risk factors for HCMV infection will help with prevention strategies and can be exploited for vaccine design by targeting appropriate ages.

ACKNOWLEDGEMENT

The authors are thankfull to Laboratory technical staffs of Oncquest Laboratories Ltd, New Delhi, India for helping in this study.

REFERENCES