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Seroprevalence of Cytomegalovirus Antibodies among COVID-19 Patients

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One of the biggest infectious diseases for humans in modern history is the COVID-19 pandemic. The disease mechanisms of human viral infections have been modified by virus-virus interaction. This study was carried out to detect the seroprevalence of CMV in COVID-19 patients. A total of 105 cases (58 females and 47 males) with an age range of 17-65 years (mean age 39.52±11.02 years) were included in this study. HCMV-IgG and IgM were determined in subjects' cases. Sixty-five out of 105 (62%) were positive for SARS-CoV-2, while 40 (38%) had negative SARS-CoV-2. Forty-seven (72.3%) and 11 (16.9%) cases out of 65 SARS-CoV-2 positive patients were positive for CMV-IgG and IgM antibodies, respectively. No significant differences were noted between females and males in terms of seroprevalence rates. A high rate of positive CMV-IgG was observed among 40-64 years COVID-19 patients. It is concluded that the seroprevalence of CMV antibodies amongst COVID-19 cases is high in relation to findings in cases without SARSCoV-2.

Keywords: COVID-19; Cytomegalovirus; CMV-IgG; CMV- IgM; SARS-CoV-2.

COVID-19 is instigated by severe acute respiratory syndrome coronavirus-2. In older adults, the outbreak of this disease tends to cause the greatest mortality and morbidity (Kadambari *et al*., 2020). This disease has a wide range of symptoms including asymptomatic infection, mild upper respiratory system disease, and serious viral pneumonia with a high mortality rate acute respiratory syndrome (Zhu *et al*., 2020). It is suspected that n-CoV originates in wildlife when bat transfers the virus into a secondary host that, by its nature, transmits the virus to humans through direct contact in the Wuhan market (Hui *et al*., 2020). The typical symptoms of COVID-19 are fever, malaise, dry cough, respiratory distress, and shortness of breath, whereas the loss of taste and olfactory perceptions is documented in several cases (Yang, 2020; Ibrahim *et al*., 2020). According to its genetic makeup, SARS-CoV-2 is a member of Betacoronaviruses, such as the MERS HCoVs and SARS viruses (Ibrahim *et al*., 2020; Chan *et al*., 2015).

Up till now, seven different strains of human coronaviruses (HCoVs) have been documented, comprising the NL63 and 229E strains of HCoVs (Alphacorona viruses), and the HKU1, OC43, MERS, SARS, and SARS-CoV-2 HCoVs (Betacoronaviruses). The most well-known and violent strains of coronaviruses are the SARS and MERS HCoVs, each causing approximately

800 deaths. The mortality rates for SARS and MERS HCoV are 10% and 36%, respectively, according to the WHO (WHO, 2016; Elfiky *et al*., 2017; Hui *et al*., 2020).

Human cytomegalovirus (HCMV) is one of the Herpes subfamilies (beta herpes) and is present worldwide in different geographic areas. Often considered to be frequently transmitted to a developing fetus is this viral infection (Forbes, 1989). DNA genome of HCMV is complex and encodes Functional proteins, noncoding RNA, and small peptides (Krishna *et al*., 2019). HCMV remains for the lifespan of the host after primary infection, the persistence is supported by latency within the host by integrating into the host cell genome or by maintaining replication with a low level by functioning immune response (Riou *et al*., 2016). Reactivation rarely occurs unless the immune system is suppressed. This weakness may be temporary or permanent and is commonly observed in stressed patients, such as organ transplantation, ionizing radiation, pregnancy and viral co-infection (Griffiths and Emery, 1997). CMV pneumonia usually includes respiratory failure and diffuses x-rays of the lung. The diagnosis is carried out with serological, molecular analysis, and histologic results on the pulmonary biopsy. Regarding the management of serious disease in immunocompetent patients like CMV pneumonia, there are no formal guidelines for treatment (Celina *et al*., 2018).

MATERIAL AND METHODS

Study population

One hundred and five cases were registered in the present study. The 105 cases included 47 males and 58 females, with age range 17-65 means of (39.52±11.02) years. All blood samples were collected from different hospitals (Manshyet El-Bakri, General Ain Shams, Banha, and Shebeen El-Kom hospitals). Cases were subdivided into two groups; the patient group consists of positive cases for SARS-CoV-2 (n= 65) and the control group (n=40) consists of negative cases for SARS-CoV-2, HBV and HCV antibodies. The infected cases (n=65) had already been diagnosed as COVID-19 patients using clinical and laboratory investigation. Consent forms that include (name, age, gender, history of blood transfusion) were obtained from each subject before sampling. The age, gender, and antibodies against HCMV (IgG, IgM) were assessed and recorded for two groups (patient and control).

Serological analysis of HCMV

The enzyme-linked immunosorbent assay (ELISA) technique was used to determine HCMV-IgM and HCMV-IgG antibodies by using commercially available CMV-IgM and IgG Kits (BioCheck, Foster City, CA, USA). Tests were performed according to the manufacturer's instructions and results of HCMV IgG and IgM were presented as optical density (O.D) units.

Statistical analysis

SPSS software package version 23.0 (Armonk, NY: IBM Corp) was used for data management and data analysis. The statistical significance of difference was considered when $p \leq 0.05$.

RESULTS

Total HCMV antibodies responses

In the patient group: 47 out of 65 (72.3%) were positive for HCMV-IgG antibodies, while 18 (27.7%) were negative for HCMV- IgG. Among the Control group, 13 out of 40 (32.5%) had detectable HCMV-IgG antibodies, while 27 (67.5%) cases were negative for HCMV-IgM. Generally, the HCMV-IgG antibody rate in both group was higher than HCMV-IgM (table.1).

HCMV antibodies response in both genders of study groups

No gender preference was noted 21/65 male, 26/65 female were positive for CMV-IgG, while 4/65 males and 7/65 females were positive for CMV-IgM in the patient group. Also, there was no significant variation noted between male and females in CM antibodies response in the control group (Table 2).

HCMV antibodies responses in different ages for the study groups

The data presented in Table 3 clearly demonstrated a decrease in CMV-IgG response by decreasing the age range, where IgG response increased in elderly patients. There is an opposite trend toward CMV-IgM rates, where IgM response in young cases of the control group was higher than that in older cases. Also, the results displayed in Table 3 showed CMV-IgG antibody in control

group, whose age ranged 41:65 years, had the highest response.

DISCUSSION

Cytomegaloviruses (CMVs) are the omnipresent β-herpes viruses that affect 70-90% of the global human population. In developing nations, CMV infection is more prevalent. In general, infections with HCMV are regulated by the immune system effectively, but without the ultimate clearance of the virus (Roberts *et al*., 2010; Wikby *et al*., 2002).

CMV reactivation occurs when the immune system is weak and down-regulated, such as stress associated with illness and treatment with pharmaceutical products, or during persistent activation of the immune system, such as co-infection with other viruses or inflammatory diseases (Reeves and Sinclair, 2008; Griffiths *et al*., 2015). In 2002, CMV was identified by Wikby and colleagues as part of the 'immune risk phenotype' linked with increased mortality in elderly (Lindau *et al*., 2019). In various studies, the impact of CMV sero-status on vaccine responses has also been investigated in older people with contradictory results (Frasca and Blomberg, 2016; Van den Berg *et al*., 2019).

The clinical complications of infection with CMV contain several distinctive manifestations and some of these would indicate that, in particular, this virus could have a significant effect on the SARS infection's clinical outcome (Kathleen *et al*., 2020). In this regard, any such correlation could be seen either in the degree of viral replication of SARS-CoV-2 or in the nature of the succeeding immune response (Moss, 2020). Increased reactivation of CMV due to secondary impact of acute inflammation must also be taken into consideration (Chiche *et al*., 2009).

In the present study, we investigate the seroprevalence of HCMV in Egyptian COVID-19 patients. The displayed results showed that the percentage of seropositive CMV-IgG was

Table 1. HCMV-IgG and IgM antibodies rates in Patient group and Control group

	HCMV-IgG			HCMV-IgM		
	Positive N (%)	Negative N (%)	Total	Positive N (%)	Negative N (%)	Total
Patient group	47 (72.3%)	18 (27.7%)	65	11 (16.9%)	54 (83.1%)	65
Control group	13 (32.5%)	27 (67.5%)	40	7 (17.5%)	33 (82.5%)	40

Table 2. HCMV-IgG and IgM antibodies rates in both genders of study groups

	HCMV-IgG			HCMV-IgM		
	Male N (%)	Female N (%)	Total	Male N (%)	Female N (%)	Total
Patient group	21 (44.7%)	26 (55.3%)	47	4 (36.4%)	7 (63.6%)	11
Control group	5 (38.5)	8 (61.5%)	13	2 (28.6%)	5 (71.4%)	7

Table 3.

Age range	Patient group (n=65)		Control group (n=40)	
	HCMV-IgG	HCMV-IgM	HCMV-IgG	HCMV-IgM
17-25	4 (8.5%)	3 (27.2%)	3 (23.1%)	5 (71.4%)
26-40	16 (34%)	4 (36.4%)	3 (23.1%)	1 (14.3%)
41-65	27 (57.5%)	4 (36.4%)	7 (53.8%)	1 (14.3%)
Total	47/65 (72.3)	11/65 (16.7%)	13/40 (32.5%)	7/40 (17.5%)

significantly higher (P>0.01) in the patient group (COVID-19 patients) than those in the Control group (negative SARS-CoV-2). Also, CMV-IgM was detected in 17% of COVID-19 patients (78% of them had positive IgG antibodies) compared with 7.6% of the control group with negative CMV-IgG. The results of this study showed the seropositivity of CMV-IgG, and IgM was higher in elderly patients than young COVID-19 patients. Our findings were in agreement with other studies concluded, the correlation between CMV seropositivity and prevalent frailty in older people (Wang *et al*., 2010).

Also, our findings showed insignificant variation between male and female COVID-19 patients with positive HCMV antibodies. These data is similar to other studies concluded that, there is no significant difference in CMV seroprevalence by gender (Gutie´ rrez-Salinas *et al*., 2008; Ahmed *et al*., 2016).

This study showed an interaction between CMV prevalence and COVID-19, so future studies should be carried out to determine how SARS-CoV-2 viral load can be changed by CMV, and how CMV modulates the SARS-CoV-2-specific immune response.

Titre of CMV-specific antibodies are increasing after subclinical viral reactivation that occurs during stress, inflammation, or ongoing disease (Bennett *et al*., 2002). Reactivation of Cytomegalovirus has been correlated with the mortality rate of critically ill patients (Osawa and Singh 2009). Most elderly patients with COVID-19 are seropositive to CMV at the onset of disease development (Limaye *et al*., 2017; Zhu *et al*., 2019).

CONCLUSION

New treatment strategies to limit the possible CMV inflammatory role may be established. By understanding the significance of CMV reactivation on the immune response of severs COVID-19 patients.

CMV reactivation can act as a risk factor for clinical studies after infection with SARS-CoV-2, and this may be important regarding epidemiological control and clinical management plan optimization.

There is no certain basis for this; Most of the infection with SARS-CoV-2 is associated with higher mortality rates in older people and immune-disorder patients.

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