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SEROPREVALENCE OF HUMAN CYTOMEGALOVIRUS IN IRAQI BREAST CANCER PATIENTS

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Abstract

The current study was conducted in the period extending from November 2018 to October 2019 and designed as a case-control study and aimed to assess the seroprevalence of HCMV. However, a total number of 91serum specimens were collected to fulfill this purpose from females (71 breast cancer patients, and control group of 20 females) attending Al-Amal hospital for cancer management and Baghdad teaching hospital and the practical part was performed in College of Science, University of Baghdad. The study protocol was approved by the Ethics Committee at the Department of Biology (Reference: BEC/0220/0011). The immunological part for evaluation of seroprevalence of HCMV was accomplished by ELISA technique which revealed that anti-HCMV IgG was scored positive in 67/71 (94.36%) and 19/20 (95%) of breast cancer patients and control group respectively, whereas anti-HCMV IgM was only detected in 6/71 (8.45%) of the patients of breast cancer. Moreover, levels of IgG were elevated in 81.69% of breast cancer patients compared to 40% only of control group.
Keywords: HCMV, Seroprevalence, Breast Cancer.

Introduction

HCMV is among the most successful opportunistic pathogens of humans with seroprevalence that exceeds up to 100% in developing countries and influenced by different factors such as the age and socioeconomic status (Mocarski *et al*., 2007; Beam and Razonable 2012; Manicklal *et al.,* 2013). Regardless the high rate of infection, HCMV rarely results in clinical disease among immunocompetent individuals but may cause severe illness in immunocompromised patients (Ryu, 2017). Moreover, resulted from the numerous and unique immune-modulatory strategies that are shown by this virus, HCMV is also considered as a paradigm for viral immune evasion (Powers *et al*., 2008). The capability of HCMV to infect and to replicate in most types of cells such as epithelial cells, hepatocytes, and macrophages has facilitated systemic dissemination through human body and enabled the virus to participate in a range of diseases and syndromes including brain damage, hepatitis, and pneumonia (Sinzger *et al.,* 2008; Burrell *et al*., 2017). Furthermore, despite that the role of HCMV as an oncovirus is not fully established but HCMV has been found to be associated with different types of malignant diseases including breast cancer (Taher *et al*., 2013; Landi *et al*., 2014). The aims of the current study were to evaluate of seroprevalence of HCMV infection in breast cancer patients as well as to assess possible correlation between HCMV infection and the development of breast cancer.

Materials and Methods

Age Distribution of the Study groups

The total number of the study groups (breast cancer patients & controls) was 91 females with ages ranging from 30 to 65 years, the patients' group was consisted of 71 females and the other 20 females, without previous history of any malignancy, were enrolled as a control group. However, the mean ages of both groups were 47.33 and 46.35 years respectively.

Specimen Collection

Blood samples were collected from each patient and control via vein- puncture method (according to guidelines of Centers for Disease Control and Prevention, CDC, 2018). These samples were then divided into two portions to obtain serum and plasma which are used for detection of anti-CMV antibodies (serum) and as follows: 3 ml of blood were transferred into clot- activator gel tubes (Vacurate, Lebanon) and allowed to clot for ~30 minutes, these tubes are centrifuged for 3min. at 4000 rpm. The resulted serum was collected and divided into several aliquots of 0.3ml in eppendorf tubes and stored at -20˚C till used for detection of anti-HCMV antibodies.

Detection of Anti-Human Cytomegalovirus IgM/IgG By Enzyme Linked Immunosorbent Assay (ELISA)

Serum samples from the entire study group (71 breast cancer patients and 20 for control group) were tested for the presence of anti-HCMV antibodies (IgM/IgG) by using ELISA technique (Human, Germany). The assay was carried out according the instructions of the manufacturer as follows: All reagents of the ELISA kit were allowed to reach room temperature (18~25˚C) before preparation. Washing solution was prepared by adding 1 volume of the washing concentrate (50 ml) into 950 ml of deionized distilled water to make a final volume of 1000 ml, while samples were prepared by diluting 10 µl of patient's serum with 1000 µl of dilution buffer. A volume of 100 µl of both negative and positive controls was added into their respective wells (in duplicates) and the first well was left as a blank. While for patients, a volume of 100 µl of each pre-diluted sample was dispensed into its appropriate well. The microtiter plate was covered with the adhesive strip and incubated at 17- 25 ˚C for 30 minutes and then the wells of the microtiter plate were washed with the diluted washing solution for 4 times. Subsequently, a volume of 100 µl of the enzyme conjugate was added into all wells and incubated for 30 minutes at 17-25 ˚C after covering with the adhesive strips. The wells were washed with the washing solution for 5 times and then a volume of 100 µl of the substrate solution (TMB) was added to each well in the plate and then incubated for 15 minutes at

17-25 ˚C. Moreover, A volume of 100 µl of sulphuric acid (as stop solution) was dispensed to each well and the absorbance was of the microtiter plate measured at 450 nm (within 30 minutes) after the terminating of the reaction.

Results and Discussion

Detection of Immune Response against HCMV by ELISA

Enzyme Linked Immunosorbent Assay was employed to assess the presence of anti-HCMV antibodies (IgM/IgG) in the sera that were obtained from the study groups. However, 8.45% and 94.36% out of 71 females as the breast cancer group were positive for anti-HCMV IgM and IgG respectively, whereas the percentages were 0.0% and 95.0% out of a total 20 females representing the control group. The statistical analysis of these results revealed a high significant difference between each of the immunoglobulin classes for both groups and no significant variation between IgG prevalence in both groups as shown in Table 1.

Table 1: Immune Response against HCMV by ELISA.

Group	Total	Anti-HCMV IgM				Anti-HCMV IgG				Chi-Square -χ^2(P-value)
		Positive		Negative		Positive		Negative		
		No.	%	No.	%	No.	%	No.	%	
Patient	71	6	8.45	65	91.54	67	94.36	4	5.63	50.972 ** (0.0001)
Control	20	0	0.0	20	100	19	95.0	1	5.0	15.210 ** (0.0001)
Chi-Square -χ^2 (P-value)		4.378 * (0.0417)				0.572 NS (0.721)				** P≤0.01).
* (P≤0.05), NS: Non-Significant.										

The obtained results of this study reflect the ubiquitous nature of HCMV through different human populations across the world and are supported by global, regional and local studies. As stated by some researchers such as Beam & Razonable (2012), HCMV may reach 60-70% of seroprevalence in developed countries and this percentage may reach even higher up to 100% in developing countries, while the local studies have clarified that seropositivity for HCMV IgG were as high as 100% and 8.3% for IgM (Al-Nuaimi *et al*., 2018). However, detection of IgM was frequently low and not relied on due to the lacking of specificity for primary infection, the possibility of false positive results and its uselessness in diagnosis of immunocompromised patients (Dollard *et al*., 2011; Pass, 2018). On the other hand, detection of the IgG class is considered as a good indicator for presence of HCMV infection (Baroco and Oldfield, 2008). Although in the recent study the prevalence of HCMV between the breast cancer patients and the control group was statistically insignificant, nevertheless the titer of anti-HCMV IgG in patients' group (81.69%) was as double as compared with the group of controls (40.0 %) at (P≤0.01), as shown in table 2.

Table 2: Immune Response against HCMV According to the Elevated IgG Level.

Patients' group (Breast Cancer).			Chi-Square -χ^2 (P-value)
Total	Elevated IgG	Percentage	
71	58	81.69%	37.878 ** (0.0001)
Control group.			
20	8	40.0 %	
**** (P≤0.01).**			

The findings of this study are concurred with observations of Cox *et al.,* in 2010 who reported the occurrence of elevation in anti–HCMV IgG titers in breast cancer patients as compared with normal controls, nevertheless they also suggested that the elevation of anti-HCMV IgG levels precedes the development of breast cancer. Moreover, Yousif in 2016 indicated that the highest levels of IgG were ~50% more in malignant breast cancer patients than their levels in other studied groups.

Conclusion

The infection of HCMV among the Iraqi population is highly prevalent without a statistical significance between breast cancer patients and control group as revealed by anti-HCMV IgG immune response via ELISA technique. However, breast cancer patients showed an observable elevation in the titers of anti-HCMV IgG as compared with group of normal controls with a high statistical significance at (P≤0.01). and thus, the results obtained from this study support the suggestion that HCMV may be involved at some point in the process of development of breast cancer.

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