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Serodiagnosis of Human Cytomegalovirus in Iraqi Breast cancer and fibroadenoma patients

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ABSTRACT

Human cytomegalovirus (HCMV) has a worldwide distribution and extremely common infections. The presence of HCMV genome and antigens has been detected in many kinds of human cancers especially breast cancer. In Iraq, the incidence of breast cancer generally exceeds any other type of malignancies among Iraqi population. The study was performed in the period between October 2016 and June 2017 in Central public health laboratory/Baghdad. It involve samples from 90 women including 60 breast cancer patients, 20 benign tumor patients, and 10 normal breast tissues. A blood sample was obtained from each woman included in this study. Anti-HCMV IgG antibody was presented in 9/10 (90%) of normal women, benign breast tumor patients 19/20 (95%) and malignant breast tumor patients 60/60 (100%) while anti-HCMV IgM antibody was only detected in breast cancer patients 5/60 (8.3%). The results of ELISA technique confirmed the seroprevalence of HCMV infection among Iraqi women and negates that the virus has a role in cancer or fibroadenoma progression.

Keywords: Human cytomegalovirus, Breast Cancer patients, Elisa technique, Anti-HCMV antibodies.

1. INTRODUCTION

Breast cancer is a frequent malignancy among women worldwide with an estimation of 1.67 million new cases in 2012 (25% of all cancers) [1]. The incidence of breast cancer generally exceeds any other type of malignancies among Iraqi population since 2011[2].

The etiology of breast cancer is poorly understood, environmental and genetic factors may contribute in the development of the disease [3]. It was demonstrated that several viruses contribute in the development of human malignancies [4]. Studies revealed a great association between Human cytomegalovirus (HCMV) and breast cancer development [5].

Human cytomegalovirus, also referred as human herpes virus 5 (HHV-5), belongs to the Herpesviridae family, subfamily Beta herpesviridae. Cytomegalovirus has the largest genetic content of the human herpesviruses. The genome designed as a double-stranded DNA with approximately 240kbp which encodes more than 200 proteins. The virus enclosed by an icosahedral type capsid. The capsid is100–110 nm in diameter and consist of 162 capsomers. Between the capsid and the virus envelope, a protein layer known as tegument was present. There are at least eight different viral glycoproteins which embedded in the lipid bilayer. The mature viral particle has a diameter of 150–200 nm [6, 7].

Human cytomegalovirus has a worldwide distribution and extremely common infections. Seroprevalences in the adult population vary from 45% to 100%, increasing with age and changed by geographic, social and economic background [8]. In Iraqi infants the anti-HCMV IgG and IgM percentages were 22.2% and 15.5% respectively [9]. Cox *et al*. (2010) hypothesized that incidence of breast cancer are related to seroprevalence of cytomegalovirus and could be raised by late exposure to HCMV. They researched for the

correlation between HCMV IgG levels and development of breast cancer [10]. Counter studies reported no significant association between the virus infection and breast cancer progression. That's why further studies should be applied on this field [11].

Since the significance of higher prevalence of HCMV in breast cancer tissue is poorly understood and since the presence of HCMV in breast tissue could boost malignant transformation of infected breast tissue, examination was applied to detect HCMV IgG and IgM in malignant, benign and normal breast tissues and to find whether the virus has a role in conversion of normal tissues to malignant or benign or not.

2. MATERIALS AND METHODS

2.1 Patients and sampling

This study was performed in a period between October 2016 and June 2017 in Central public health laboratory. Blood samples were collected from Al Alweiya teaching hospital according to ethical considerations and the hospital approval. Five milliliters (5ml) of blood was taken from sixty (60) breast carcinoma patients, twenty (20) benign breast lesion patients and ten (10) apparently healthy females. Samples obtained by venipuncture and collected in gel tubes. Blood samples were centrifuged at 2500 r.p.m. for five minutes to separate the serum from the other blood components. Serum in each tube was divided into two portions and placed in Eppendorf tubes for the evidence of Cytomegalovirus IgM and IgG. All Eppendorf tubes were marked and stored at -20C° prior to test time.

2.2 Enzyme Linked Immunosorbent assay (ELISA) protocol

Cytomegalovirus IgM kit and Cytomegalovirus IgG kit (Bioactiva diagnostica / Germany) were used in this experiment. The procedure was applied for both Cytomegalovirus IgM and cytomegalovirus IgG. All reagents and serum samples were mixed gently and allowed to reach room temperature before use. The microtitration strips were marked according to the marking of the serum samples. Serum samples were diluted by sample diluent and mixed well for homogenization. Prepared standards (positive, negative and cut off) and diluted serum samples were added to wells. The micotitration strips were covered and incubated for 30 minutes at 37C° using heating block microelisa system. After incubation; the wells were aspirated and washed four times for 30 seconds with washing solution using an automatic microplate washer. The microtiter then dried by inverting the plate on absorbent material. By using multichannel micropipette, 100µl of HCMV-HRP conjugate was added to each well. The plate incubated at 37C° for 30 minutes using heating block microelisa system. After incubation, the wells were aspirated and washed four times for 30 seconds with washing solution using an automatic microplate washer. The microtiter then dried by inverting the plate on absorbent material. Using multichannel micropipette, 100µl of TMB chromogen solution was added to each well in dark. The plate was incubated in dark for 15 minutes at room temperature. A volume of 100µl of stopping solution was added to each well using multichannel micropipette, the colour of wells changed from blue to yellow color. The absorbance of the solution in wells was read within 30 minutes, using a microplate reader (HumaReader Hs). The instrument was set to dual wavelength measurement at 450nm with background wavelength correction set at 620nm.

3. RESULTS AND DISCUSSION

Enzyme-linked immunosorbent assay of anti-HCMV antibody of two classes IgG and IgM performed on a total of 60 patient's serum with breast carcinoma, in addition to 20 benign tumor women's serum and 10 apparently healthy women. The results showed the presence of anti-HCMV antibody IgG in 60/60(100%) of patients' serum with breast carcinoma while IgM presented in 5/60(8.3%) of them. Benign tumors serum and normal tissues also demonstrated the prevalence of anti-HCMV IgG antibody 19/20(95%) and 9/10(90%) respectively. Anti-HCMV antibody IgM has not been detected in any case of benign and normal tissue cases (table 1). Statistical analysis of IgM and IgG levels shows no significant differences ($P<0.01$) between the studied groups (figure1 and figure2).

Table 1: Seroprevalence of HCMV antibodies in the studied groups.

Studied groups	Total (N.)	Anti-HCMV IgM				Anti-HCMV IgG			
		Positive		Negative		Positive		Negative	
		N	%	N	%	N	%	N	%
Control	10	0	0	10	100	9	90	1	10
Benign	20	0	0	20	100	19	95	1	5
Malignant	60	5	8.3	55	91.7	60	100	0	0

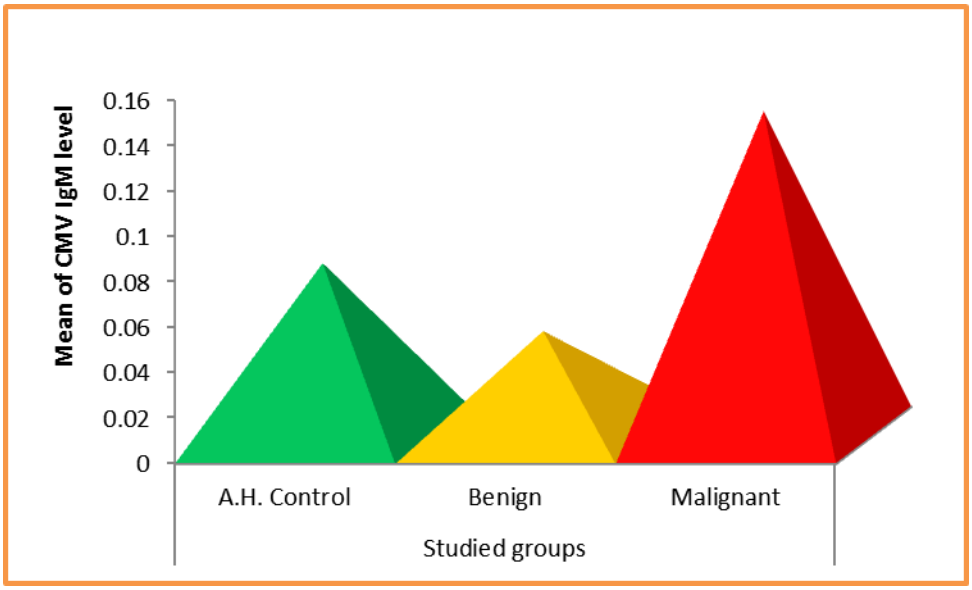

Figure 1: Mean comparison of serum HCMV IgM level among studied groups.

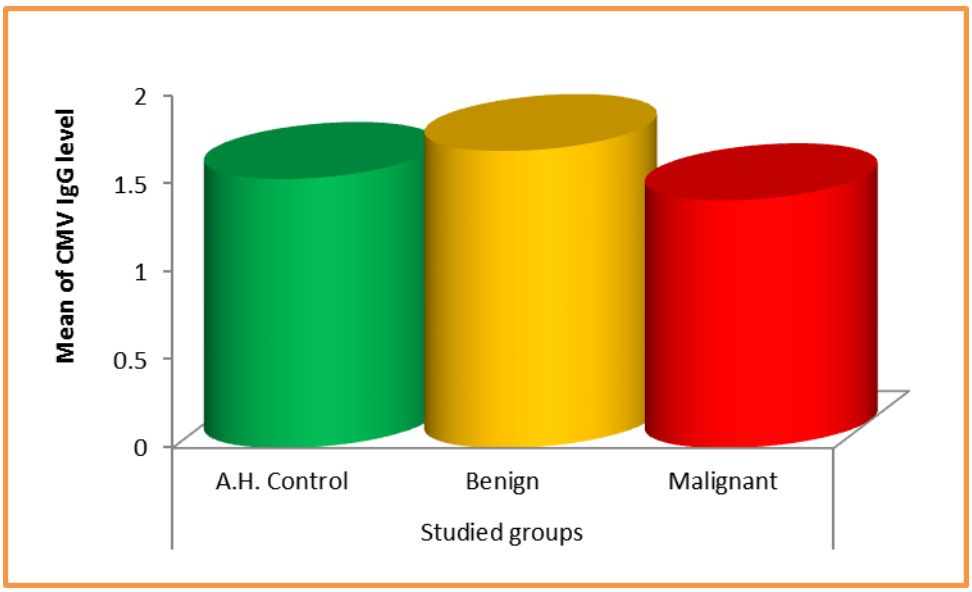

Figure 2: Mean comparison of serum HCMV IgG level among studied groups.

The present study showed non-significant differences between IgG levels of normal breast women and IgG levels of benign breast women. Also, non-significant differences were shown in this experiment between IgG levels of breast cancer and IgG levels of normal breast. These results negates that the virus has a role in breast cancer progression and/or fibroadenoma development.

Regarding to IgM levels, non-significant differences was demonstrated between IgM levels of breast cancer patients and IgM levels of normal women. Also, the same result was shown between benign breast patients and normal women. The seropositive IgM women are indicating an individual under acute infection or may be reactivation of latent infection [12].

The present results were supported by Taher *et al*., (2013) who revealed the high prevalence of HCMV (100%) in the cases of breast cancer women and (91%) in case of metastatic sentinel lymph node [13]. Furthermore, these results were in line with Al-Baiati *et al*. (2014) who used Enzyme Linked Immunosorbent Assay test (ELISA) to detect anti-human cytomegalovirus IgM and IgG. The percentages were 93% and 12% respectively among infertile women, and 100% and 60% respectively among breast cancer women [14]. In Iran, a study by Eghbali *et al*. (2012) regarded on the frequency of human cytomegalovirus in benign and malignant breast tumors. The study shows no relationship between CMV infection and breast benign and malignant tumors [15]. In another study of pregnant Iraqi women the blood samples were positive for CMV-IgM and both IgG/IgM in 44 out of 145 (28.27 %) [9]. Other study on pre-marital women in Baghdad province, without any clinical evidence of cytomegalovirus (CMV) infection, were screened for the presence of IgG and IgM antibodies against CMV by ELISA test and the result showed the IgG antibodies were percentage of 36%, while the percentage of IgM antibodies were 9.9% [16]. The study of Abdul hussein and Al-azzawi (2015) on two hundred and seventeen (47 and 170 subjects suffering from renal transplant

and hematological malignancies patients (adults and child), respectively) showed that percentages of anti-HCMV IgG was (50.23%), while for anti -HCMV IgM was (5.5%) [17].

Collectively, the present study and many other studies negates that the virus causes conversion of normal breast tissues to malignant or benign tissues. Further studies by different methods should be applied on this topic.

4. CONCLUSION

The Prevalence of Human cytomegalovirus infection among Iraqi women was confirmed in the present study by the results of Enzyme-linked immunosorbent assay (ELISA) of anti-HCMV antibody IgG. It was showed that IgG was common in malignant, benign and normal patients' serum with poor possibility that the virus may a have a role in cancer progression or fibroadenoma development.

5. REFERENCES

1. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, s.; Mathers, C.; Rebelo, M.; Parkin, D.; Forman, D.; and Bray, F. (2015). Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer.136:359-386.
2. Iraqi cancer board. (2014). Iraqi cancer registry 2011. Ministry of health and environment. Republic of Iraq.
3. Esteva, F. And Hortobagyi,G. (2008). Gaining ground on breast cancer. Scientific American, INC.
4. Mclaughlin-Drubin, M.; and Munger, K. (2008) .Viruses associated with Human Cancer. Biochimica. Et. Biophysica. Acta. 1782: 127–150.
5. Al-Alwany, S. And Ali, S. (2013). Molecular detection of Human Cytomegalovirus in Iraqi patients with breast cancer. I.J.A.B.R. 3: 454-459.
6. Blut, A. (2010). Human Cytomegalovirus (HCMV) – Revised. Transfus Med. Hemother. 7:365–375.
7. Miller, S. (2016). Virology, p. 457-479. In: K. C. Carroll, S. A. Morse, and s. Miller (ed.). Jawetz, Melnick,& Adelberg's Medeical Microbiology, 27th ed. Mcgraw-Hill Education, United States.
8. Cannon, M.; Schmid, D.; Hyde, T. (2010). Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev. Med. Virol. 20:202–213.
9. Abdulhussein, T. A.; and Al-azzawi, R.H.(2015). Genotyping of Human Cytomegalovirus Envelop Glycoprotein B in Iraqi Pregnant Women and Infants by Multiplex nested PCR. European Journal of Scientific Research. 136 (3): 252-259.
10. Cox, B.; Richardson, A.;Graham, P.; Gislefoss, R.; Jellum, E.; and Rollag, H.(2010). Breast cancer, Cytomegalovirus and Epstein-Barr virus: a nested case-control study. Br. J. Cancer 102:1665–1669.
11. Utrera-Barillas, D.; Valdez-Salazar, H.; Gómez-Range, D.; Alvarado-Cabrero, I.; Aguilera, P.; Delgado, G.; et al. (2013). Is human cytomegalovirus associated with breast cancer progression? Infect. Agent Cancer 8:12-17.
12. Rachel, L. ; Fatiha, N. ; Bertrand, K. ; Daniele, T. ; Sylvie, B. ; Jacqueline, L. ; Bruno, L. ; Jean-Francois, G. ; Michele, A. 1997. Detection of cytomegalovirus in semen from a population of men seeking infertility evaluation.Fertility and Sterility.Med. Publ. Else.Scie. Inc. 66 5:123-129.
13. Taher, C.; deboniface, J.; Mohammad, A.; Religa, P.; Hartman, J.; and Yaiw, K.; et al. (2013). High prevalence of human cytomegalovirus proteins and nucleic acids in primary Breast cancer and metastatic sentinel lymph nodes. Plos. One 8:56795-56803.
14. Al.Baiati, H.; Muhsin, M.; and Jabbar, R. (2014). Seroprevalence of Human Cytomegalovirus (HCMV) in aborted women in Baghdad province. Int. J. Curr. Microbiol. App. Sci 3: 97-102.
15. Eghbali, M.; Ghane, M.; and Mirinargesi, M. (2012). Frequency of Cytomegalovirus (CMV) in benign and malignant tumors. IJMCM .2:175-179.
16. Al-azzawi, R.H.(2012). Seroprevalence of cytomegalovirus infection in pre-marital women in some baghdad hospitals. Iraqi journal of Science. 53 (1):40-45.
17. Abdulhussein, T. A.; and Al-azzawi, R.H.(2015). Genotyping of Human Cytomegalovirus envelop glycoprotein B in Iraqi renal transplant and malignancy patients by multiplex nested PCR. World journal of experimental biosciences. 3 (2): 113-117.

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