Cytomegalovirus Infection as Risk of Atherogenic Lipid Profile in Iraqi Aborted Women

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ABSTRACT

Atherosclerosis is a form of the cardiovascular diseases which may occur due to a chronic inflammatory reaction to endothelial damage caused by multiple causes which include infection by microorganisms like Cytomegalovirus (CMV). The present study aims to evaluate the effect of cytomegalovirus infection in patients and its relation to atherosclerosis and to measure the monocyte chemoattractant protein-1 (MCP-1) as a predictive marker for inflammatory process. A total of 30 women who were attending the Gynecology outpatient clinics and were suffering from abortion for first time or with recurrent abortion due to CMV infection were enrolled in the current study. For comparison, twenty blood samples were collected from healthy apparent women as a control group in this study. Serum MCP-1, IL-6 and CMV-IgM were measured by using ELISA technique. Lipid profile which includes total cholesterol, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL) was measured by spectrophotometer. The results of this study showed a significant difference in mean of IL-6 in patients was (570.67 ± 199.27) when compared to control group (p>0.0001). While there is no significant difference of MCP-1 level between patients and control group (P=0.134). And the results showed a significant elevation of cholesterol (P<0.05) and a highly significant elevation of triglyceride and LDL in CMV infected patients (P<0.01), while no elevation occurred in HDL values (P=0.977), according to the present data there is a correlation between CMV infection and atherosclerosis.

Keywords: Atherosclerosis, Cytomegalovirus, CMP-1, Cholesterol.

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INTRODUCTION

Atherosclerosis is considered as inflammatory changes that occurred in the artery wall triggered by an injury of the artery endothelium. There are many reasons of endothelial damage include elevation of blood pressure, smoking, elevated LDL, and infection with viruses such as cytomegalovirus infection [1]. In addition to that other causative factors have been implicated such as the association between infections and the occurrence of atherosclerosis, especially the diseases of coronary artery [2]. The relationship between atherogenesis and infectious agents such as CMV has been discussed in many researches which approved that atherosclerosis is responsible for most of the pathology of the cardiovascular disease which is considered as an inflammatory disease with multiple cytokines, adhesion molecules, and MCP-1 associated with the occurring of atherogenesis. MCP-1 is one of the chemokine secreted by endothelial cells, monocyte, and smooth muscle cells; all these cells are involved in atherogenesis [3-4]. This chemokine induces chemo taxis of monocyte and T lymphocytes, which are the major inflammatory cells involved in athermanous lesions. Several epidemiological researches have attempted to associate between the CMV IgG seropositivity and atherogenesis improved that the early infection with CMV is specified by the presence of CMV antibodies in patients with atherosclerosis. Odds ratios in relation to CMV antibodies and cardiovascular disease have been showed in some epidemiologic studies [5]. Other epidemiologic studies showed a relation between various forms of vascular disease and the detection of viral antibodies. While several studies showed that there was a presence of virus, viral antigens or nucleic acid in atherosclerotic lesions, regarding the studies in animal models and cell-culture, many studies showed attractive mechanisms when the virus might play a role as a causative agent of the atherosclerosis [6-7]. A possible role of CMV as a cofactor in atherogenesis was studied previously, in accordance with the great public health problem associated with CMV infection, with the occurrence in 0.7% of overall live newborns worldwide [8-9]. There is informative data available for the association between CMV and cells relevant to the pathogenesis of atherosclerosis, the most important included cells are smooth muscle cells and endothelial cells. The results from such studies concentrate on the possible mechanisms that can explain this association. Human CMV may infect the deep layers of blood vessels rather than endothelial cells and smooth muscle cells. The ability of CMV to infect endothelial cells of different types of vascular tissues is varied. Depending on the cell type, CMV can produce infection or enter in the latent period and endothelial cells may consider as one of the main sites of latency of CMV.

AIM OF THE STUDY

The main objective of the present study was to show a possible association between CMV and atherosclerosis in aborted Iraqi women who showed elevation in their lipid profile.

SUBJECTS AND METHODS

Thirty blood samples were collected from women suffering from abortion for first time or with recurrent abortion who were attending the Gynecology outpatient clinics, and twenty blood samples were collected from healthy apparent women as a control group in this study during the period

from1/6/2017 to 1/3/2018. This research project was approved by the Ethics Committee of College of Pharmacy / Mustansiriyah University (No. 126109-A1).

Exclusion criteria: individuals who are taking lipid lowering drugs or dietary treatment.

Inclusion criteria: All women were enrolled in this study were in the reproductive age.

Estimation of CMV antibody: Blood samples were collected from all patients enrolled in the current study and tested for anti-CMV IgM antibodies by enzyme-linked immunosorbent assay (Ray Biotech Co., USA). Antibody results were calculated from standard curves provided with the manufactured kit. The threshold value for a "positive" result was determined prospectively, concerning a negative result by ELISA value less than (0.25 units), and a positive result by a value equal to and more than (0.25 units) which indicates prior exposure to CMV. All samples were triplicate tested for anti-CMV IgG antibodies and in two separate experiments. Both Serum MCP-1and IL-6 also measured by using ELISA (Ray Biotech Co. /USA, Anogen/Canada, respectively) and according to manufacturer's instructions. Lipid profile including total cholesterol, triglycerides, HDL, LDL, and were measured by spectrophotometer technique (Bio Merieux AS, France) [10].

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used in this study to find to the differences in the parameters of studied groups. T-test was used to significant compare between means, estimate of correlation coefficient between variables in this study [11].

RESULTS

The mean CMV-IgM of patients and control group enrolled in the current study was (1.298 ± 0.24), (0.641 ± 0.17) respectively. There is a highly significant difference in the mean CMV-IgM of patients group in comparison with control group (P<0.0001). And the mean of IL-6 in patients was (570.67 ± 199.27), and (57.50 ± 23.38) in control group with highly significant difference (0.0001). While there is no significant difference of MCP-1 level between patients and control group (P=0.134), table 1.

Table 1: Mean CMV-IgM, MCP-1 and IL-6 in patients and control group

Group	Mean ± SD		
	CMV-IgM (U/ml)	MCP-1(ng/ml)	IL-6 (pg/ml)
Patients (No.=30)	1.298 ± 0.24	39.93 ± 13.23	570.67 ± 199.27
Control (No. =20)	0.641 ± 0.17	34.85 ± 8.42	57.50 ± 23.38
T-test	0.126 **	6.715 NS	90.379 **
P-value	0.0001	0.134	0.0001

** (P<0.01), NS: Non-Significant.

The results of this study showed that there is a relation between the elevation of lipid profile and the infection with CMV, this is revealed by a significant elevation of cholesterol (P<0.05) and a highly significant elevation of triglyceride and LDL in CMV infected patients (P<0.01), while no elevation occurred in HDL values (P=0.977), table 2.

Table 2: Lipid panel in patients and control group

Group	Mean ± SD			
	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Patients (No.=30)	190.90 ± 22.26	177.67 ± 26.18	44.80 ± 6.17	111.83 ± 16.29
Control (No. =20)	179.05 ± 16.02	159.45 ± 9.28	44.75 ± 6.06	88.50 ± 8.12
T-test	11.625 *	12.290 **	3.558 NS	7.927 **
P-value	0.0459	0.0045	0.977	0.0001

* (P<0.05) , ** (P<0.01) , NS: Non-Significant.

In the current study, the results showed there is no relationship between the age and CMV infection in patients and control group, but there is a relationship between the number of abortion and the infection with CMV in patients and control group, table 3.

Table 3: Mean age and abortion values in patients and control group

Group	Mean ± SD (Range)	
	Age (year)	No. of Abortion
Patients (No.=30)	26.40 ± 4.43 (18.00-35.00)	2.17 ± 0.94 (1-4)

Control	25.40 ± 4.82	0.00 ± 0.00
(No. =20)	(18.00-35.00)	(0-0)
T-test	2.667 NS	0.428 **
P-value	0.454	0.0001

** (P<0.01), NS: Non-Significant.

Regarding the age of patients in this study, the correlation showed a significant elevation of triglyceride and highly significant elevation of cholesterol level. And according to number of abortions the results of the current study revealed a significant difference in the titer of CMV-IgM and highly significant elevation in IL-6, and LDL levels, table 4.

Table 4: Correlation coefficient for age and abortion in different studied parameters

Parameters	Correlation coefficient-r	
	Age	No. of Abortion
CMV-IgM	0.04 NS	0.72 **
MCP-1 (ng/ml)	0.10 NS	0.07 NS
IL-6	0.03 NS	0.73 **
Cholesterol	0.35 **	0.08 NS
Triglyceride	0.26 *	0.15 NS
HDL	-0.09 NS	0.15 NS
LDL	0.17 NS	0.42 **

* Correlation is significant at (P<0.05)
** Correlation is highly significant at (P<0.01), NS: Non-Significant.

DISCUSSION

Previous studies which concentrate on the epidemiology, molecular, and animal studies of CMV infection have supposed that the CMV is implicated in atherogenesis. Because CMV is a ubiquitous herpes virus; the exposure to CMV may occur during any time in childhood, when atherogenic lesions may develop CMV will enter in latent infection that may be reactivated when immunosuppressant occurred. Since CMV infects endothelial cells especially monocyte, CMV is influence atherogenesis [12]. In the current study we investigated CMV-IgM rather than IgG because the infected individual with the virus will develop CMV IgM seropositivity for lifetime after primary infection [13], and non-primary [14]. The results of the current study showed elevation in CMV-IgM in patient group and bring to light recent active CMV infection. Previous studies indicated that CMV infection will increase the expression of some cytokines specifically interleukin-6 which will elicit hepatic synthesis of acute-phase reactants, and some of these reactants might promote atherogenesis, and some chemokine such as MCP-1 that produced by endothelial cells, epithelial, fibroblasts, smooth muscle, astrocytic, monocyte, mesangial, and microglial cells, all these cells are play a vital role in the peripheral circulation and tissues immune response against viral infection [15]. Infection with CMV may cause activation of monocyte/macrophages pulled to the areas of endothelial injury by secretion of MCP-1 and stimulation of atherogenesis, and other study found that MCP-1 was increased in patients who were suffered from angina which may indicate the relation between increased MCP-1 level and the damage in myocardial cells and the formation of thrombus [16]. The results of the current study didn't showed elevation in MCP-1 and disagreed with the previous studies which concentrated on the role of the proinflammatory cytokines in CMV infection that may be related to elevation of cholesterol and triglyceride leading to atherogenesis. In the present study, the results showed significant elevation in IL-6 levels in patients with CMV infection rather than control group and this result agreed with a result of Blankenberg et al., who conclude the elevation of IL-6 level increase the risk of future cardiac disease in patients infected with CMV [17]. Previous studies tried to associate between lipid panel of patients and their infection with CMV, while others have examined CMV IgG seropositivity and serum total cholesterol as a risk factor for cardiovascular disease, but with no statistical relation between CMV seropositivity and serum lipid levels [18-19]. In addition to that a research by Adam *et al.,* studied the CMV seropositivity in relation to serum triglyceride and total cholesterol concentrations in patients undergoing atherosclerosis-vascular surgery and they found no relation between CMV infection and lipid panel of male patients but an association was found between CMV seropositivity and elevation of cholesterol level in females less than 50 year of age [20], the results of Adam *et al.,* was compatible to the results of the current study. Depending on our knowledge, the previous studies didn't searched if there is an association between elevation of serum total cholesterol levels and CMV seropositivity in younger patients in Iraq. In the current study, the association between CMV seropositivity and serum total cholesterol levels were determined in patients who were in the productive age, with mean age about 26 year of age. The alterations in lipid and cholesterol levels noticed parallel with decreases in HDL in different types of inflammatory disorders and infectious diseases. And other consistent increase in lipid profile and small dense LDL [21], these results came in compatible with the results of the current study which revealed an elevation in total

cholesterol, triglycerides, and LDL while the level of HDL was within normal range.

CONCLUSIONS

The results of the current study proposed that infection with CMV accelerate the subclinical inflammatory response, and the individuals will be susceptible to the Atherogenic effects of CMV. In summary the current study gives an evidence that CMV seropositivity is correlated with elevation of serum total cholesterol level in female patients who are in reproductive age or under 35 year of age.

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REFERENCES

1. M. Kent Froberg, Alice Adams, Nicole Seacotte, Jan Parker-Thornburg, Pappachan Kolattukudy, Cytomegalovirus Infection Accelerates Inflammation in Vascular Tissue Over expressing Monocyte Chemoattractant Protein-1, Circulation Research, 2001,1-7.
2. M. Degré, Has cytomegalovirus infection any role in the development of atherosclerosis? Clinical Microbiology and Infection, Vol, April 2002, Pages 191-195.
3. Terkeltaub R, Boisvert W, Curtiss L. Chemokines and atherosclerosis. CurrOpinLipidol. 1998;9: 397–405.
4. Kolattukudy PE, Quach T, Bergese S, et al. Myocarditis induced by targeted expression of the MCP-1 gene in murine cardiac muscle, Am J Pathol. 1998; 152: 101–111.
5. Melnick JL, Adam E, DeBakeyMECytomegalovirus and atherosclerosis. Eur Heart J, 14 (suppl K) (1993), pp 30-38.
6. Nieto FJ, Adam E, Sorlie P, *et al.*Cohort study of cytomegalovirus infection as a risk factor for coronary intimal-medial thickening, a measure of subclinical atherosclerosis, Circulation, 94 (1996), pp. 922-927
7. Zhou YF, Leon MB, Waclawiw MA, *et al.*Association between prior cytomegalovirus infection and the risk of restenosis after coronary atherectomy. N Engl J Med, 335 (1996), pp. 624-630
8. Dollard S. C., Grosse S. D., Ross D. S. 2007. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev. Med. Virol. 17:355–363.
9. Kenneson A., Cannon M. J. 2007. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection, Rev. Med. Virol. 17:253–276.
10. Friedewald WT, Levy RI, Fredrickson DS.(1972). Estimation of the concentration of lowdensity lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.ClinChem; 18:499- 502.
11. SAS. 2012. Statistical Analysis System, User's Guide. Statistical.Version 9.1th ed. SAS.Inc. Cary.N.C. USA.
12. Guetta E, Guetta V, Shibutani T, Epstein SE. Monocytes harboring cytomegalovirus: Interactions with endothelial cells, smooth muscle cells, and oxidized low-density lipoprotein. Circulat Res 1997; 81:8-16.
13. Picone, O., et al. 2009. A 2-year study on cytomegalovirus infection during pregnancy in a French hospital, BJOG 116:818–823.
14. Sheila C. Dollard, Stephanie A. S. Staras, Minal M. Amin, D. Scott Schmid, and Michael J. Cannon.National Prevalence Estimates for Cytomegalovirus IgM and IgG Avidity and Association between High IgM Antibody Titer and Low IgG Avidity. CLINICAL AND VACCINE IMMUNOLOGY, Nov. 2011, 18(11): 1895–1899
15. Waldman WJ, Knight DA, Huang EH. An in vitro model of T-cell activation by antilogous cytomegalovirus (CMV)-infected human adult endothelial cells: Contribution of CMV-enhanced ICAM-1. J Immunol 1998; 160:3143-3151.
16. Mazzone, A., S. De Servi, I. Mazzucchelli, I. Bossi, E. Ottini, M. Vezzoli, F. Meloni, M. Lotzinker, and G. Mariani. 2001. Increased concentrations of inflammatory mediators in unstable angina: correlation with serum troponin T. Heart 85:571–575.
17. Blankenberg, S., H. J. Rupprecht, C. Bickel, C. Espinola-Klein, G. Rippin, G. Hafner, M. Ossendorf, K. Steinhagen, and J. Meyer for The AtheroGene Group. 2001. Cytomegalovirus infection with interleukin-6 response predicts cardiac mortality in patients with coronary artery disease. Circulation 103:2915–2921.
18. Danesh J, Wong Y, Ward M, Muir J. Chronic infection with Helicobacter pylori, Chlamydia pneumoniae, or cytomegalovirus: population based study of coronary heart disease. Heart 1999; 81:245-247.
19. Adler SP, Hur JK, Wang JB, Vetrovec GW. Prior infection with cytomegalovirus is not a major risk factor for angiographically demonstrated coronary artery atherosclerosis. J Infect Dis 1998;177: 209-212.
20. Adam E, Probtsfield JL, Burek J, McCollum CH, Melnick JL, Petrie BL, Bailey KR, DeBakey ME. High levels of cytomegalovirus antibodies in patients requiring vascular surgery for atherosclerosis.Lancet 1987; 2:291-293.
21. Rufail, M.L., et al., Atherogenic lipoprotein parameters in patients with aggressive periodontitis. J Periodontal Res, 2007, 42(6): p. 495-502.
22. Tchantchaleishvili, V. Preface to the Journal of Cardiovascular Disease Research, third issue 2011 (2011) Journal of Cardiovascular Disease Research, 2 (3), pp. 139-140. DOI: 10.4103/0975-3583.85259