

VCAM-1 Expression in Endometrium with Human Cytomegalovirus Infection

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Abstract

Background: To elucidate the possible role of human cytomegalovirus in pregnancy loss through induction of certain pro-inflammatory adhesion molecules.

Methods: Paraffin embedded sections of curate samples were obtained from 34 women had spontaneous abortion, and 5 women had elective termination of pregnancy (as control), and then subjected for immunohistochemistry analysis to detect human cytomegalovirus (HCMV) early protein and VCAM-1 molecule.

Results: Nine out of 34 women with spontaneous abortion were positive for HCMV early protein, with a

significantly higher expression of VCAM-1 in HCMV positive cases as compared with HCMV negative and the control groups ($p = 0.05, 0.001$ respectively).

Conclusion: HCMV infection may play an important role in the pathology of pregnancy loss on multidirectional bases include inducing the surface expression of pro-inflammatory adhesion molecules like VCAM-1.

Key words: human cytomegalovirus, VCAM-1, Abortion

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Introduction

Human cytomegalovirus (HCMV), a member of Herpesviridae family, is a common infection and found in 50-90% of adults⁽¹⁾. HCMV is rarely associated with clinical symptoms in immune competent individuals. However, Infection with HCMV can have serious consequences in individuals with acquired immune deficiency syndrome or in those who have been immunocompromised, and to the fetus during pregnancy^(1,2,3). Infection with HCMV has been also associated with vascular disease processes such as vascular allograft rejection, transplantation vasculopathy and native atherosclerosis⁽⁴⁾.

VCAM-1 is a 110 kDa protein binding to the integrin $\alpha 4\beta 1$ very late antigen (VLA)-4 cell surface heterodimer, but its also known to interact weakly with the closely related integrin $\alpha 4\beta 7$ ⁽⁵⁾. VCAM-1 was originally identified as a cytokine inducible surface protein (pro-inflammatory adhesion molecule), that mediate adhesion of a number of leukocytes including lymphocytes, monocytes, mast cells, eosinophils, and tumor cells to umbilical vein endothelial cells^(4,6,7).

HCMV viral proteins are potent transactivators that are necessary for productive infection and are involved in the regulation of cellular gene expression. HCMV also encodes a variety of other homologues with distinct subversive functions and which mimic the behavior of host proteins to divert the immune response.^(1,4,8) The major infiltrating leukocytes in villitis associated with HCMV are mononuclear phagocytes. Monocyte adhesion to syncytiotrophoblast (ST) is mediated by intercellular cell adhesion molecule which can be stimulated by the inflammatory cytokines interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , and interferon (IFN)- γ . Such adhesion leads to TNF- α -mediated apoptosis^(3,6,8).

In this study we tried to delineate the relation between the expression of VCAM-1 and HCMV protein in endometrium in women with spontaneous abortion, to find out whether HCMV plays a role in inducing this adhesion molecule that mediate the inflammatory

process which participate in the pathology of pregnancy loss.

Methods

Curate samples were obtained from 34 women had spontaneous abortion in the first trimester and undergone evacuation curate operation, while the control were 5 women had (legal) elective termination of apparently normal pregnancy in the first trimester for a maternal medical indication under approved consent of two senior gynecologists and a physician.

Samples were fixed in 10% buffered formalin then embedded in paraffin, then processed routinely and subjected for immunohistochemistry analysis to detect HCMV early protein using monoclonal antibodies for HCMV early non-structural protein of 68 KDa (BioGenex, USA) in a dilution of 1:100, and VCAM-1 as anti-CD106 (DAKO, Denmark) in a dilution of 1:50. The immunohistochemistry procedure and VCAM-1 signal evaluation were conducted as in (Vailhe et al, 1999)⁽⁹⁾.

Statistics: The *t* test of significance was used to compare expression of VCAM-1 among HCMV positive and negative cases and the control group. (The statistical analysis was done by using Statistical Package of Social Science "SPSS" program, version 15).

Results

HCMV early protein was detected in the trophoblasts of nine out of 34 endometrial biopsies in the study group (fig 1A), and none of the control group was positive for the virus. Positive cases showed dark brown blue dots in the cytoplasm and the nucleus of the trophoblast cells.

VCAM-1 expression displayed brown granular cytoplasmic staining of the endothelial cells (fig 1B). Table (1) shows the percentages of VCAM-1 expression in terms of mean \pm SE, minimum and maximum values in HCMV positive and negative cases and the control group.

The *t* test of significance revealed significantly higher expression of VCAM-1 in HCMV positive cases as compared with its expression in HCMV negative cases and the control group (p = 0.05, 0.001 respectively).
 Ψ Standard error

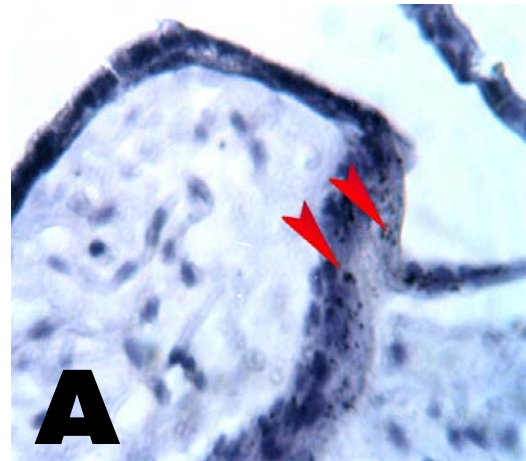
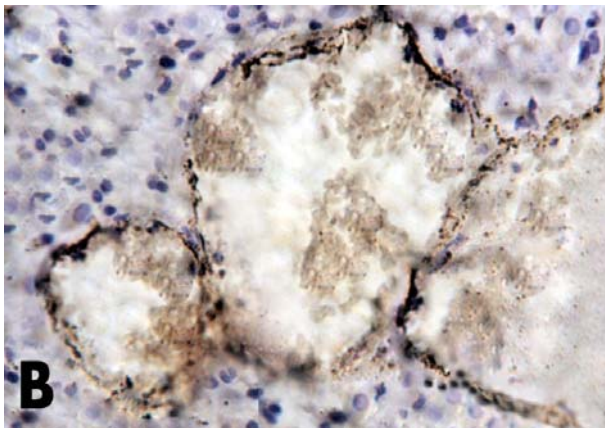
negative and the control group (p = 0.05, 0.001 respectively).

Table 1. The expression of VCAM-1 in HCMV positive and negative cases and The control group

Groups	n	Mean ± S.E.Ψ	Min. Value	Max. Value	P Value	
VCAM-1 in HCMV positive cases	9	67.9 ± 4.5	44	85	0.0321	0.0008
VCAM-1 in HCMV negative cases	25	53.1 ± 3.6	25	82		
VCAM-1 in the control group	5	37.2 ± 4.5	20	46		0.0008

Figure (1):

Detection of HCMV and VCAM-1 by immuno-histochemistry in women with pregnancy loss. (A) HCMV expression shows dark brown to black dots in the cytoplasm and the nuclei of the cytotrophoblasts (arrow heads). (B) VCAM-1 expression displayed brown granular cytoplasmic staining of the endothelial cells. Magnification power (X400).



Discussion

Human Cytomegalovirus was chosen in this study because it is the most common viral agent encountered in intrauterine infection^(10,11), also HCMV acts as an immune-modulator through elaborating an array of immune evasion strategies and its viral proteins are involved in regulation of cell gene expression⁽⁸⁾. Placental infections by HCMV are accompanied by villous inflammations (villitis), *in utero* transmission of infection from the mother to the fetus, and low birth weight babies⁽¹²⁾.

This study showed that HCMV early protein can be expressed and detected in the trophoblasts of women with spontaneous abortion which is in line with other studies documenting permissiveness of trophoblast for HCMV infection^(11,13,14), they also stated that the infection progress slowly and progeny viruses remain predominantly cell associated reflecting that, direct effect of HCMV as a cause of abortion, is unlikely unless the virus titer is high as in primary infection^(10,15), and because HCMV is a well known immuno- modulator, its proteins can stimulate, and through paracrine release of cytokines like IL1 β and TNF- α , up-regulation of adhesion molecules and enhance TNF- α mediated apoptosis^(3,4,8). This supports the finding in this study that positive HCMV expression in aborted endometrium is significantly associated with high expression of VCAM-1 molecule. The result agrees with another study showing increase in the expression of pro-inflammatory adhesion molecules like VCAM-1 AND ICAM-1 in HCMV infected vascular endothelial cells in patients with renal transplantation⁽¹⁶⁾, and pregnancy loss^(2,3,4). In conclusion, HCMV infection in the first trimester of pregnancy might induce surface expression of VCAM-1 on vascular endothelial cells that could participate or aggravate the villitis process that occurs during HCMV infection of the placenta⁽¹³⁾, playing a role in the pathology of pregnancy loss.

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