Original Article

Human Cytomegalovirus Up-regulates Nuclear Factor-κB in Women with Spontaneous Abortion

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Summary:

Background: Human cytomegalovirus (HCMV) infection during pregnancy causing serious problems through induction of viral genes that enhance the expression of different cellular factors ending in failure of pregnancy.

Patients and 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 in situ hybridization technique to detect nuclear factor-κB (NF- κB) mRNA.

Results: Only nine out of 34 women with spontaneous abortion were positive for HCMV early protein, with a significantly higher expression of NF-κB in HCMV positive cases as compared with HCMV negative and the control group ($p = 0.001$).

Conclusion: This study strengthen the possibility that HCMV infection may play an important role in the pathology of pregnancy loss on multidirectional bases include inducing the expression of the transcriptional factor; NF-κB.

Key wards: HCMV, NF-κB, abortion

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Introduction

Human cytomegalovirus (HCMV) is a double-stranded DNA virus, a member of Herpesviridae family (1); it's a ubiquitous virus that infects more than 60% of the general population and as much as 100% of some populations and/or geographical areas. HCMV is rarely associated with sever clinical symptoms in immunocompetent individuals. However, in immunocompromised individuals and transplant patients and during pregnancy, HCMV infection can manifest itself in sever and often fetal conditions (1, 2, 3, 4). A Primary infection is asymptomatic in immunologically healthy women however; fetal infection by maternal HCMV is a serious problem. A congenital HCMV infection can result in abortion or stillbirth, with symptomatic survivors displaying sequelae such as thrombocytopenia, hepatosplenomegaly, vision loss, sensorineural deficits and mental retardation (1, 2, 4). Primary infection occurs in 2% of women during pregnancy. Serologic or cultural evidence of in utero HCMV infection is present in 0.2-2.2% of all live borns (3, 5).One ubiquitous transcription factor of particular importance in immune and inflammatory responses is nuclear factor-κB (NF- κB) (6). Many stimuli activate this transcriptional factor, including cytokines, viruses like cytomegalovirus, EBV and influenza viruses, activators of protein kinase C and oxidants (6, 7). NF- κB acts on genes of pro-inflammatory cytokines, chmokines (chemotactic cytokines that attract inflammatory cells to sites of inflammation), enzymes that generate mediators of inflammation, immune receptors and adhesion molecules (8, 9,10).NF-κB and its inhibitors were shown to be down regulated during normal pregnancy, which subsequently inhibit Th1 cytokine production help in maintaining pregnancy (11). In this study, we attempted to delineate the relation between the in situ expression of NF-κB and HCMV infection in women with spontaneous abortion, to find out whether or not HCMV induces this transcriptional factor leading to pregnancy loss

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Patients and 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 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, and then subjected for immunohistochemistry analysis to detect HCMV early protein using monoclonal antibody for HCMV early non-structural protein of 68 KDa (BioGenex, USA) in a dilution of 1:100, refer to the immunohistochemistry procedure in reference (12). And in situ hybridization technique for detection of NF- κB; using biotin-labeled DNA probe for human NF-κB (371 bp), (Maxim

Biotech, Inc., USA), refer to the in situ hybridization procedure in reference (13).

Statistics: The t test of significance was used to compare the in situ expression of NF-κB among HCMV positive and negative cases and the control group.

Results

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

The expression of NF-κB was detected using in situ hybridization technique which showed typical granulated blue-black nuclear staining (fig 2). Table (1) shows the percentages of NF-κB in situ expression in terms of mean ± SE, minimum and maximum values in HCMV positive and negative cases and the control group.

Table 1. The expression of NF-κB in HCMV positive and negative cases and The control grou

Groups	n	Mean ± S.E.ψ	Min. Value	Max. Value
NF-κB in HCMV positive cases	9	62.97 ± 3.5	54	83
NF-κB in HCMV positive cases	25	43.5 ± 2.6	10.3	60
NF-κB in the control group	5	34.9 ± 2.8	25.5	42

Ψ Standard error

The t test of significance revealed significantly higher expression of NF-κB in HCMV positive cases as compared with its expression in HCMV negative and the control group ($p = 0.001$).

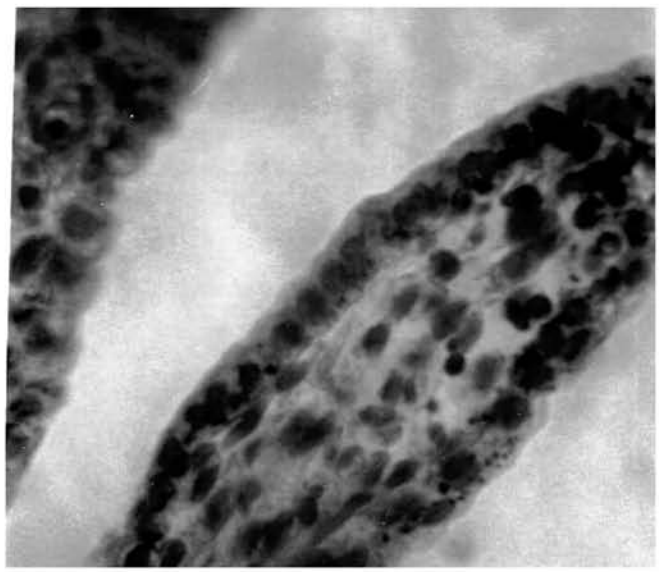

Figure (1): Detection of HCMV by immunohistochemistry in women with pregnancy loss. Dark brown to black dots is shown in the cytoplasm and the nuclei of the cytotrophoblasts in women with spontaneous abortion. Magnification power (X400).

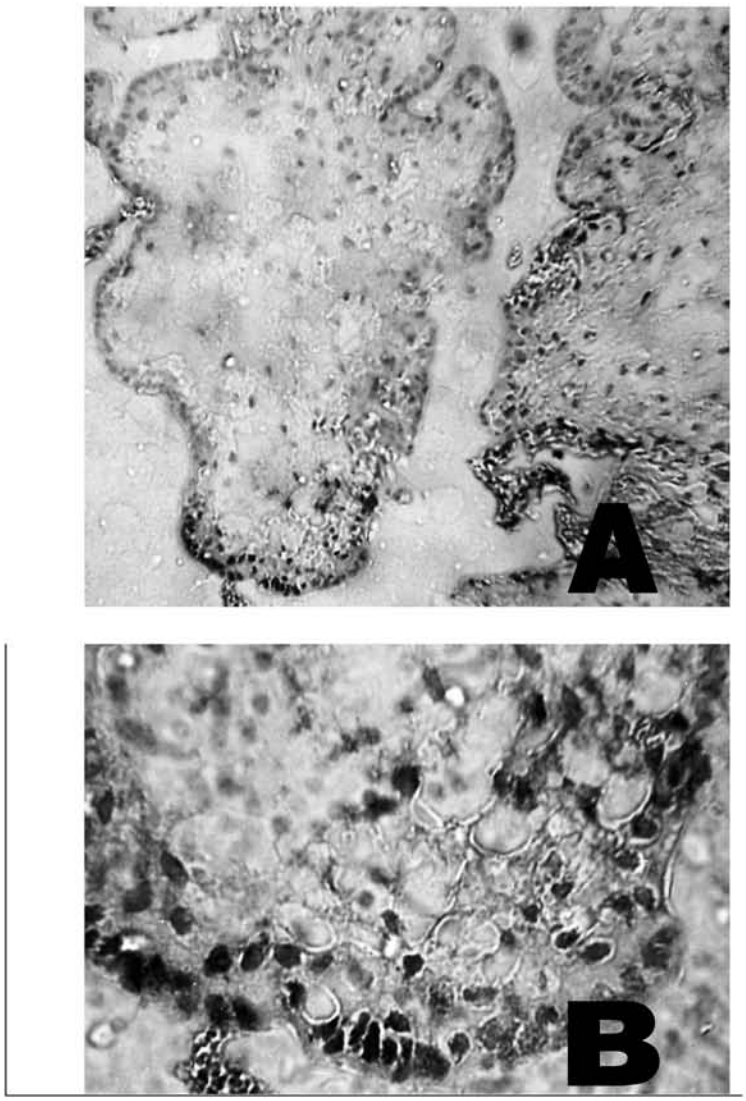

Figure (2): Detection of NF-κB in women with pregnancy loss by in situ hybridization. Staining of NF-κB mRNA by BCIP/NBT (blue-black) counterstained with nuclear fast red. (A) Tissue from woman with spontaneous abortion shows positive NF-κB hybridization signals. (B) Higher magnification of (A) demonstrates the typical heterogeneous granular nuclear staining pattern. Magnification power of A (X100), and B (X400).

Discussion

One of the common events that takes place during viral infection is the interaction of the virus with the host, this interplay between cellular and viral factors is critical for the regulation of viral gene expression, replication, and maturation and virion release (4). During HCMV infection a coordinated cascade of events takes place, including induction of viral immediate early, early and then late genes (3, 4).

This study showed that HCMV early protein can be expressed and detected in the trophoblasts of women with spontaneous abortion which is supported by other studies (14, 15, 16), and this expression is significantly associated with high level of in situ expression of NF-κB which agrees with another study showing that NF-κB message is induced during HCMV infection and that the induction is biphasic, suggesting an initial induction at immediate early times and a second round of induction at early times. In addition, virus binding alone is sufficient to stimulate NF-κB DNA binding activity, supporting its role in the initial induction of NF-κB (3).

Upregulation of NF-κB may cause increase in the expression of genes on which NF-κB act like genes of pro-inflammatory cytokines (8, 10), which is supported by our previous studies on NF-κB induction of IFN-γ expression leading to pregnancy loss in patients having spontaneous abortion (13). In addition, NF-κB induction of proinflammatory adhesion molecules (8, 17), like VCAM-1 in women with spontaneous abortion (unpublished data). All these support the role of NF-κB which is induced by HCMV in the pathology of pregnancy loss.

References:

1- Mocarski ES. Cytomegaloviruses and their replication. Fields Virology. 3rd ed. Fields BN, Knipe DM, Howley PM, Chanock RM, Melnick JL, Monath TP, Roizman B and Straus SE eds. Lippincott-Raven, Philadelphia. 1996; pp. (2447-2492).

2- Fowler KB and Pass RF. Sexually transmitted diseases in mothers of neonates with congenital cytomegalovirus infection. J Infect Dis. 1991; 164: 259-264.

3- Yurochko AD, Kowalik TF, Huong SM and Huang ES. HCMVupregulates NF-κB activity by transactivating the NF-κB p105/p50 and p65 promoters. J of virolo. 1995; 69: 5391-5400.

4- Chan G, Hemmings DG, Yurochko AD and Guilbert LJ. HCMV caused damage to placental trophoblasts mediated by IE gene induced TNF-α. Am J Pathol. 2002; 161: 1371-1381.

5- Raynor BD. HCMV infection in pregnancy. Seminars in Perinatology. 1993; 17: 394-402.

6- Kopp EB and Ghosh S. NF-κB and rel proteins in innate immunity. Adv Immunol. 1995; 58: 1-27.

7- Bacucrle PA and Baltimore D. NF-κB ten years after. Cell. 1996; 87: 13-20.

8- Barnes PJ and Karin M. NF-κB – a pivotal transcriptional factor in chronic inflammatory diseases. The New Eng j of Med. 1997; 10: 1066-1071.

9- Chen F, Castranova V, Shi X and Demers M. New insights into the role of NF-κB, an ubiquitous transcription factor in the initiation of diseases. Clin Chem. 1999; 45: 7-12.

10- Aronica M A, Mora AL, Mitchell DB, Finn PW, Johnson JE et al. Preferential role for NF-κB/Rel signaling in the type 1 but not type 2 T cell-dependant immune response in vivo. J Immunol. 1999; 163: 5116-20.

11- McCraken SA, Gallery E and Morris JM. Pregnancy-specific down-regulation of NF-κB expression in T cells in humans is essential for Maintenance of the cytokine profile required for pregnancy success. J Immunol. 2004; 172: 4583-91.

12- Al-Obaidi AB, Hussain AG and Shamran HA. Spontaneous abortion and failure of human cytotrophoblasts to adopt a vascular adhesion phenotype. J Fac Med Baghdad. 2006; 48: 402-406.

13- Al-Obaidi AB, Habib MA, Ridha WK. Up-regulation of the in situ expression of NF-κB and IFN-γ in women with recurrent spontaneous abortion. JABMS. 2006; 8: 331-338.

14- Hemmings DG, Kilani R, Nykiforuk C, Preiksaitis JK and Guilbert LJ. Permissive cytomegalovirus infection of primary villous term and first trimester trophoblasts. J Virol. 1998; 72: 4790-4979.

15- Hemmings DG and Guilbert LJ. Polarized release of human cytomegalovirus from placental trophoblasts. J Virol. 2002; 76: 6710-6717.

16- Terauchi M, Koi H, Hayano C, Toyama-Sorimachi N, Karasuyama H et al. Placental extravillous cytotrophoblasts persistently express class I major histocompatibility complex molecules after human cytomegalovirus infection. J Virol. 2003; 77: 8187–8195.

17- Chan G, Stinski MF and Guilbert LJ. Human cytomegalovirus-induced up-regulation of intercellular cell adhesion molecule-1 on villous syncytiotrophoblasts. Biol Reprod. 2004; 104: 1-10.