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Expression of Lymphtoxin - α and their Receptor among Aborted Women Infected with Human Cytomegalovirus

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Abstract: ***Background: Human cytomegalovirus infection is significant health problems in pregnant women around the world and considered major causes of morbidity in the fetus and neonate placenta. Objectives: To assess the frequency of human cytomegalovirus infections in product of conceptus tissues of pregnant women with spontaneous abortion in relation to expression of lymphotoxin alpha and their receptor. Patients and methods: Fifty products of conceptus tissues samples were collected from pregnant women who referred due to a spontaneous abortion attended at Al-Batool Teaching Hospital for Maternity and Children in Baqubah City, Iraq during the period from November 2013 till April 2014. Some characteristics such as age, gestational age, last history of abortion and gravity were recorded. The human cytomegalovirus antigen, lymphotoxin alpha and lymphotoxin beta receptor were detected by immunohistochemistry technique (IHC). Result: Eighteen out of 50 (36%) in product of conceptus tissues of aborted women cases were positive for human cytomegalovirus, but statistical analysis showed insignificant difference at P>0.05. The expressions of lymphotoxin α and receptor TLβRs were detected in most aborted women infected with human cytomegalovirus, 23 (46%) and 16 (32%) respectively. Multiple variables were evaluated, the positive cases of lymphotoxin alpha and lymphotoxin beta receptor showed that 19 about (82.60%) and 14 about (87.5%) respectively were aborted in the first trimester; most women had third and fourth time abortion respectively. Majority of women were multiparous 12(52.17%) and 10(62.50%) respectively. Non-significant difference was noticed with different parameters difference. Conclusion: Lymphotoxin α and their receptor TLβRs may play an important role in recurrent abortions with human cytomegalovirus, further delineation of the molecular mechanisms controlling by lymphotoxin to designing strategies for targeted antiviral therapies.***

Keywords: Spontaneous abortion, human cytomegalovirus, immunohistochemistry, lymphtoxin-α and their receptor.

1. Introduction

Human cytomegalovirus (HCMV) is a ubiquitous beta human herpesvirus type 5, compared to other human herpesviruses, HCMV is the largest, with a genome of approximately 235 kb containing approximately 250 open reading fream with the potential to encode proteins. Asymptomatically infects the host during childhood, and establishes life-long latency [1]. Approximately 10%-15% of congenitally infected newborns have detectable manifestations, most commonly sensorineural hearing loss, organomegaly, microcephaly, intracranial calcification and chorioretinitis, resulting from direct viral cytopathic effect on the fetus [2].

Immune control of HCMV is requires innate immune defenses mediated by interferon's (IFNs) and natural killer (NK cells), as well as adaptive immune responses, NK cells can function as effector cells by using both cytolytic and non-cytolytic mechanisms. Cytolytic pathways include the secretion of granules containing perforin and granzymes and non-cytolytic mechanism involving induction of IFN-β in the virus infected cell[3].

Evidence from some studies suggests infection with HCMV changes the Th1/Th2 balance during pregnancy through altered cytokine expression in placental cells [4]. And increased expression of Th1 cytokines in amniotic fluid [5]. In the absence of infection, altered placental cytokine expression is associated with intrauterine growth restriction, prematurity, and early membrane rupture [6]. It is plausible that HCMV-induced changes in cytokine levels result in proinflammatory changes in the placenta, with important consequences for placental development, placental function, virus transmission and fetal viability [7].

Lymphotoxins α and β are known to be responsible for organogenesis and lymphoid tissue maintenance [8]. They are generally produced, under normal physiological circumstances, by activated T, B and NK lymphocytes and other lymphoid tissue components [9].

Antiviral therapy is limited number not only by toxicity but also by the continual emergence of drug-resistant viruses. The limitations of this current therapeutics provide a strong impetus to develop novel approaches that will enhance the host's immune responsiveness while at the same time effectively controlling virus replication. Type I interferon (IFN) plays a critical role in initiating innate antiviral defenses and promoting adaptive responses and lymphotoxin (LT)-α β has recently been identified as an essential effector cytokine regulating the induction of type I IFN during human cytomegalovirus infection [10].

In particular, human cytomegalovirus infection of immune-compromised mice has revealed the immunotherapeutic potential of the lymphotoxin-beta receptor (LTβR) signaling pathway to restore immune function and provide protection from human cytomegalovirus mortality.

So this study aims to assess the frequency of human cytomegalovirus infections in product of conceptus tissues of pregnant women with spontaneous abortion and relation to expression of lymphotoxin alpha and their receptor.

2. Patients and Methods

Study design

A hospital based cross sectional study was conducted from November 2013 to April 2014 in Al-Batool Teaching Hospital for Maternity and Children in Baqubah, City, Diyala, Iraq.

Ethical approval

The proposal was reviewed and approved by the College of Medicine; University of Diyala, permission to conduct this study was obtained from Al-Batool Teaching Hospital for Maternity and Children administrator in Baqubah City, Diyala, Iraq. Human privacy was respected by taken patient's oral consensus.

Sample collection

Fifteen product of conceptus tissues were collected from previous spontaneous abortion women, ranging in age from (15-45) years at various trimester of pregnancy were included in this study. All criteria were collected through interviewed with patients by using pre-designed questionnaire, the questionnaire which including the following information such as age, gestational age, last history of abortion and gravity.

Sample processing

From each participant women, tissues sample were collected in a clean container with 10% formaldehyde and used to prepared formalin fixed paraffin embedded tissues block. Immunohistochemical staining (IHC) was carried out using late antigen (Cat. No. ab 49214, Cambridge Science Park - England), mouse anti-human lymphotoxin alpha (Cat. Number L2610-03B, US Biological- USA), mouse anti-human lymphotoxin beta receptor (Cat. Number L8015-03L, US Biological- USA) and immune-histochemistry detection kit (Cat. Number 17506, US Biological/USA). The slides were deparaffinized and rehydrated by xylene and serially graded alcohol for 5 minutes each , then distilled water. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 minutes. Slides were washed in phosphate-buffered saline, then treated with protein block, and incubated at 37°C for 5 minutes, slides washed with PBS thereafter. Primary antibody was applied to cover slides and incubated for 1 hours in humidity chamber at 37°C (primary antibody was prepared at dilution 1:100). Slides were rinsed gently in PBS. The secondary antibody was added for 10 minutes at room temperature, followed by the addition of Streptavidin-HRP antibodies for 10 minutes at 37˚C. After washing, samples were stained with diluted liquid DAB for 15-45 minutes at room temperature. Slides were counterstained with hematoxylin for 30 second and washed well in running tap water, then dehydrated and mounting with permanent-mounting medium (DPX), examined under light microscope first at 10 then at 400 magnifications was finally done.

All positive cases to human cytomegalovirus, lymphtoxin-α and their receptor were negative for Human anti-herpes simplex virus 1 and 2 IgM (ab 108742, Cambridge, UK), Human parvovirus B19 IgM (Ridascreen-K6031-Germany) and human anti-*Toxoplasma gondii* IgM (ab 108778, Cambridge, UK)

Statistical analysis

Date was analyzed using statistical package of social sciences (SPSS) version 16. Differences in proportions were assessed by Chi-square test, and P-values < 0.05 were considered statistically significant.

3. Results

The results of IHC have demonstrated that 18 out of 50 (36%) in product of conceptus tissues of aborted women cases were positive for HCMV, but statistical analysis showed insignificant difference at P>0.05 as shows in figure 1. While 23 (46%) and 16 (32%) were positive for lymphtoxin-α and their receptor as shown in figure (2)

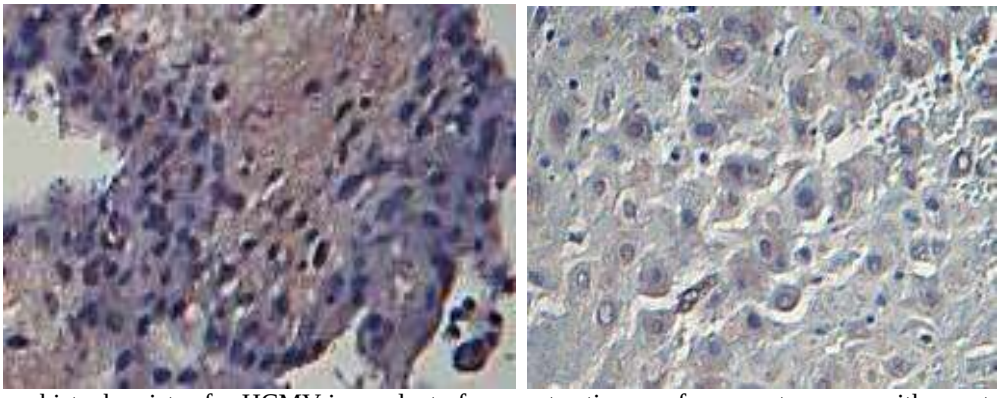

Figure 1: Immunohistochemistry for HCMV in product of conceptus tissues of pregnant women with spontaneous abortion section, stained by DAB chromogen and counter stained with heamatoxylin is shown as radish brown in positive cases (magnification power, 400)

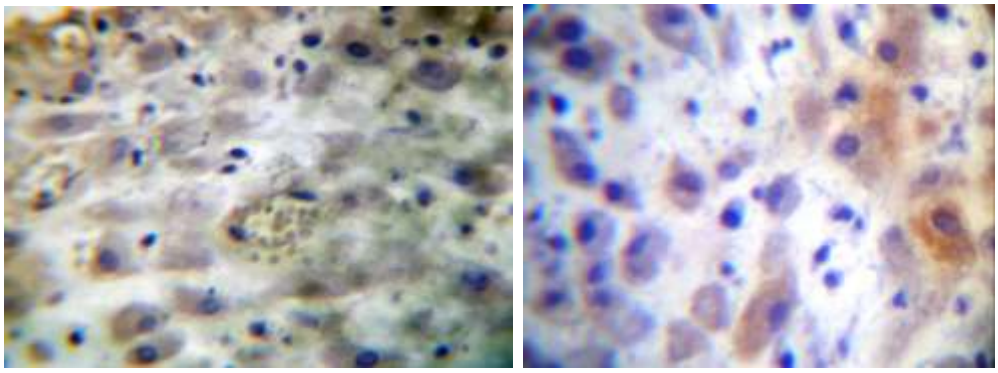

Figure 2: Immunohistochemistry of lymphtoxin-α and their receptor in product of conceptus tissues of pregnant women with spontaneous abortion section, stained by DAB chromogen and counter stained with heamatoxylin is shown as radish brown in positive cases (magnification power, 400). A- Positive expression of lymphtoxin-α , B- Positive expression of their receptor

The present study shows that mean age of the aborted women was 29.48 years; the high rates of pregnant women with spontaneous abortion were in the age group 26-35 years. According to positive expression of lymphtoxin-α and their receptor the data showed that 19 about (82.60%) and 14 about (87.5%) respectively were aborted in the first trimester. Adequate number of abortion, most women with positive result had third and fourth time abortion. Majority of women were multiparous 12(52.17%) and 10(62.50%) respectively. Non-significant difference was noticed with different parameters as shown in table1 and 2.

Table 1: Distribution of positive and negative lymphotoxin-α results according to different parameters

Variables	IHC results Positive	Negative	Comparison of Significance P-value
Age			
15-25	10(43.47%)	9(33.33%)	0.062*
26-35	11(47.82%)	8(29.62%)	
36-45	2(8.69%)	10(37.03%)	
Duration of pregnancy			
1St	19(82.60%)	22(81.48%)	0. 918*
2nd	4(17.39%)	5(18.51%)	
Number of abortion			
First time	3(11.11%)	3(11.11)	
Second time	8(34.78%)	15(55.55%)	0.513*
Third time	11(47.82%)	8(29.62%)	
Fourth time	1(4.34%)	1(3.70%)	
Parity			
Nulliparaous	3(13.07%)	2(7.40%)	
primiparaous	7(30.43%)	4(14.81%)	0.452*
Multiparaous	12(52.17%)	19(70.37%)	
Gravid	1(4.34%)	2(7.40%)	

***Non-significant**

Table 2: Distribution of positive and negative receptor results according to different parameters

Variables	IHC results Positive	Negative	Comparison of Significance P-value
Age			
15-25	7(43.75%)	12(35.29%)	
26-35	8(50%)	11(32.35%)	0 .123*
36-45	1(6.25%)	11(32.35%)	
Duration of pregnancy			
1St	14(87.5%)	27(79.41%)	0.487*
2nd	2(12.50%)	7(20.58%)	
Number of abortion			
First time	1(6.25%)	5(14.70)	
Second time	10(62.5%)	13(38.23%)	0.336*
Third time	4(25%)	15(44.11%)	
Fourth time	1(6.25%)	1(2.94%)	
Parity			
Nulliparaous	2(12.5%)	3(8.82%)	
primiparaous	3(18.75%)	8(23.52%)	0.967*
Multiparaous	10(62.5%)	21(61.76%)	
Gravid	1(6.25%)	2(5.88%)	

***Non-significant**

4. Discussion

Vertical transmission of viruses is an important cause of morbidity in the fetus and neonate placental viral infection indicates risk of vertical transmission, but not always transmission to or disease of the fetus [11]. The result of present study focused on the role of lymphtoxin-α and their receptor as essential effectors for host defense against HCMV infection.

The result showed that 36% of the pregnant women with spontaneous abortion tested had positive HCMV, indicating that the virus infection rate is high in Baqubah city. This result is comparable with result of a study done in Erbil city which found that the seropositivity was 30.05% [12]. However, other studies showed higher rate than present study, which reported a infection rates ranged between 62.3%, 81.1% and 96,3% [13][14][15]. While the rate was lower in study done by Zaki and Hossam in Egypt that achieved 12% [16]. Even within countries the rates of human cytomegalovirus seropositivity in women vary by socio-economic status and ethnicity [17][18].

In addition, the limited studies in developing countries, including Latin America (Chile, Brazil, Mexico and Panama), Africa (Ivory Coast and Gambia) and Asia (Korea, Taiwan, China and India) have reported a birth prevalence of congenital human cytomegalovirus infection ranging from 0.6 to 6.1% of pregnancies [19]. Based on the number of live births per year [20]. And reported congenital HCMV prevalence [21].

The age in this study showed statistically non-significant difference for aborted women with spontaneous abortion, that is mean the age may did not influence in human cytomegalovirus (P >0.05). This may be related with fact human cytomegalovirus is a ubiquitous virus and it infects human of all ages. Acquisition of the virus as a general in the population usually occurs early in life, mainly during the first two decades, and often during the first year [22][23][24].

Most lymphotoxin alpha and lymphotoxin beta receptor positive cases were recorded in aborted women with first trimester, this result agree with [25][26]. Anytime during pregnancy, primary or non-primary maternal infection (i.e. reactivation of a woman's latent virus or re-infection with a different strain) can lead to HCMV crossing the placental barrier and infecting the fetus, resulting in congenital human cytomegalovirus infection.

The present study showed expression in lymphotoxin alph and their receptor in tissues from aborted women, this finding agreement with [3]. Who indicate activated NK cells expressed lymphotoxin alph and beta on their cell surface during human cytomegalovirus infection, these protein bind with glycoprotein D on human cytomegalovirus and potent inhibitors of viral replication.

Tumor necrosis factor (TNF) and lymphotoxin-α induce antiviral activity to a broad range of viruses in tissue culture by causing premature death of the virus-infected cell, thus limiting the production of new virions [27]. Other in vitro study showed that both mouse and human cytomegalovirus are controlled at least, in part, by the regulation of the type1 IFN system through lymphotoxin signaling [28].

In study done by Banks *et al*.,(2006) who tried to develop novel approaches of therapy that will enhance the host's immune responsiveness by controlling virus replication through type I interferon (IFN) plays a critical role in initiating innate antiviral defenses and promoting adaptive responses and lymphotoxin (LT)-αβ has been identified as an essential effector cytokine regulating the induction of type I IFN during human cytomegalovirus infection [29].

In the present study, different variables like age, duration of pregnancy, number of abortion and parity were not significantly associated with the expression of lymphotoxin alph and their receptor during HCMV infection (P>0.05). However, it is difficult to rule out the associations of these variables totally with HCMV infection also may be related with limited sample size.

We conclude that lymphotoxin α and their receptor TLβRs may play an important role in recurrent abortions with HCMV carful investigation should be depended during HCMV infection also further delineation of the molecular mechanisms controlling by lymphotoxin to designing strategies for targeted antiviral therapies.

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