

## Frequency of Human Cytomegalovirus and Human Herpesvirus-1 Antigen in Product of Conceptus Tissues of Pregnant Women with Spontaneous Abortion

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### Abstract

- Background** Viral infections in pregnancy are major causes of maternal and fetal morbidity and mortality. Infections develop in the neonate transplacentally, perinatally or postnatally.
- Objective** To determine the frequency of cytomegalovirus and human herpesvirus-1 antigens in product of conceptus tissues of pregnant women with spontaneous abortion and to study the association of the various socio-demographic and antenatal factors.
- Methods** Fifty (50) product of conceptus tissues samples were collected during the period from September 2013 till April 2014 from pregnant women with spontaneous abortion attended at Al-Batool Teaching Hospital for Maternity and Children in Baqubah city. All sociodemographic and antenatal characteristics such as age, education level, economic level, occupation, residence, gestational age, history of abortion and gravity were recorded. Human cytomegalovirus and human herpes virus-1 antigens were detected by immunohistochemistry technique in Department of Microbiology, College of Medicine, University of Diyala.
- Results** Among 50 formalin-fixed, paraffin embedded product of conceptus tissues blocks, the results showed that positivity frequency of human cytomegalovirus and human herpesvirus-1 antigens were 36% (18 out of 50) for each one. Patients age varied from (15-45) years with mean of 29.48 years. The highest rate of viral infection was diagnosed in the age 26-35 years. Multiple variables regarding frequencies of cytomegalovirus and human herpesvirus-1 antigens simultaneously, was evaluated, and none of the variables had significant difference.
- Conclusion** Human cytomegalovirus and human herpesvirus-1 seems to play a significant role in first trimester pregnancy loss and its infection rate is comparable with that in the other countries, also pregnant women in Baqubah city may have the risk for acquiring viral infection during pregnancy and consequently adverse pregnancy outcomes.
- Keywords** Abortion, cytomegalovirus, human herpes virus, immunohistochemistry, pregnant women
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**List of abbreviation:** B19V = Human parvovirus B19, HCMV = Human cytomegalovirus, HHSV-1/2 = Human herpes simplex virus, PBS = Phosphate buffers saline

### Introduction

Spontaneous abortion or pregnancy loss is the natural death of an embryo or fetus before it is able to survive independently<sup>(1)</sup>. The cutoff of 20 weeks of gestation after

which, fetal death is known as a stillbirth<sup>(2)</sup>. There are several factors related to spontaneous abortion, such as genetic abnormalities and infections<sup>(3)</sup>. Many studies showed virus such as human cytomegalovirus (HCMV), human herpes simplex virus (HHSV-1/2), human parvovirus B19 (B19V), enterovirus, adenovirus, and varicella-zoster virus are causative agents of spontaneous abortion<sup>(4-6)</sup>.

Herpesviruses comprise the largest family of viruses with oral manifestations. Eight types of herpesvirus are known to be pathogenic in human<sup>(7)</sup>.

Human cytomegalovirus is the most common congenital infection, occurring in 0.3-1 % of all live births worldwide. It may lead to permanent disabilities in the unborn child, such as deafness, blindness and mental impairments<sup>(8)</sup>. Primary HCMV infection results in life-long latent infection, and although congenital infection after reactivation and re-infection with a different HCMV strain may occur, the risk of congenital infection is highest for seronegative women<sup>(9,10)</sup>.

Another virus that could be implicated in recurrent abortion is herpes simplex. Genital herpes is the result of infection by human herpes simplex virus type 2 (HHSV-2) and to a lesser extent human herpesvirus type 1 (HHSV-1). There has been a rise in the prevalence of genital human herpesvirus infections in both industrialized and developing countries. The main factors attributed to the spread of human herpesvirus include asymptomatic virus shedding and under recognition and underdiagnoses of the disease. At the level of the individual patient, genital herpes is associated with significant psychological morbidity and complications such as neonatal herpes, the result of transmission of HHSV from mother to baby<sup>(11,12)</sup>. The incidence of asymptomatic cervical HHSV-2 infections was considerably higher in patients with a history of spontaneous abortion with a possible etiologic connection between human herpesvirus and spontaneous abortion<sup>(13)</sup>.

Serologic assays were not very useful for the elucidation of the role of human herpesvirus in inducing spontaneous abortions, although they indicate that the state of pregnancy predisposes to human herpesvirus reactivation<sup>(14)</sup>.

So, this study aims to determine the frequency of HCMV and HHSV-1 antigens in product of conceptus tissues of pregnant women with spontaneous abortion and study the possible association of the various socio-demographic and antenatal factors.

## Methods

### Study design

This cross-sectional study was done during the period from September 2013 to April 2014 in Al-Batool Teaching Hospital for Maternity and Children, pregnant women with spontaneous abortion were enrolled in the study. The age of women ranged from (15-45) years. Product of conceptus tissues samples were collected from all participants after the abortion and all sociodemographic and antenatal characteristics such as age, education level, economic level, occupation, residence, gestational age, history of abortion and gravidity were recorded.

### Ethical approval

The proposal was reviewed and approved by 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. Participants were also informed that they have full right to discontinue or refuse to participate in this study.

### Sampling and processing

From each participating woman, data were obtained and product of conceptus tissues sample were collected in a clean container with 10% formaldehyde and used to prepared formalin fixed paraffin embedded tissues block, then immunohistochemistry used for the detection of anti-cytomegalovirus ppm

antibody or late Ag (CMV- Cat. No. ab 49214. Cambridge Science Park - England) and specific human herpes virus type 1 envelop antigen (HHV-1 - Cat. No. ab9533. Cambridge Science Park - England).

According to manufacturer's protocol. The slides were deparaffinized and rehydrated by xylene and serially graded alcohol for 5 minutes each and 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, incubated at 37 °C for 5 minutes and washed with phosphate buffers saline (PBS). 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 Streptavidine-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 was finally done.

### **Statistical analysis**

Chi-square test were used to analyzed the data of present study and P-values <0.05 were considered statistically significant.

### **Results**

This study comprised of 50 pregnant women with spontaneous abortion among these women minimum age was 15 years and maximum 45 years. Mean age of the aborted women was 29.48 years; majority of pregnant

women with spontaneous abortion were among the age group 15-35 years. According to education level most participants had primary school education 28 (56%), regarding occupation and economic level, high percentages were recorded within housewives and intermediate level and they are accounted 86% and 78% respectively. There were highly significant differences ( $P<0.05$ ) noticed between pregnant women with spontaneous abortion and different parameters while non-significant correlation noticed only among age groups as shows in table (1).

The number of pregnant women with spontaneous abortion in the first trimester was 41 cases (82%) while in the second trimester were 9 cases (18%). There was highly significant differences ( $P<0.05$ ) noticed as shows in table (2). Second time abortion was very high 23 (46%) compared with other, multiparty in was higher among aborted women and they were accounted 31 (62%), but the difference was not significant.

Immunohistochemistry results have demonstrated that 18 out of 50 (36%) in product of conceptus tissues of aborted women cases were positive for HCMV and HHV-1, but statistical analysis showed insignificant difference at  $P>0.05$  as shown in table (3) and figure (1).

Table (4) and (5) demonstrate the correlation between expression of HCMV and HHV-1 with different variables. The results of present study showed that there were no significant differences between immunohistochemistry expression of both HCMV and HHV-1 with age, education level, economic level, occupation, residence, gestational age, last history of abortion and gravidity. Based on Chi-square test of analysis and Fischer exact test.

**Table 1. Socio-demographic characteristics of subjects**

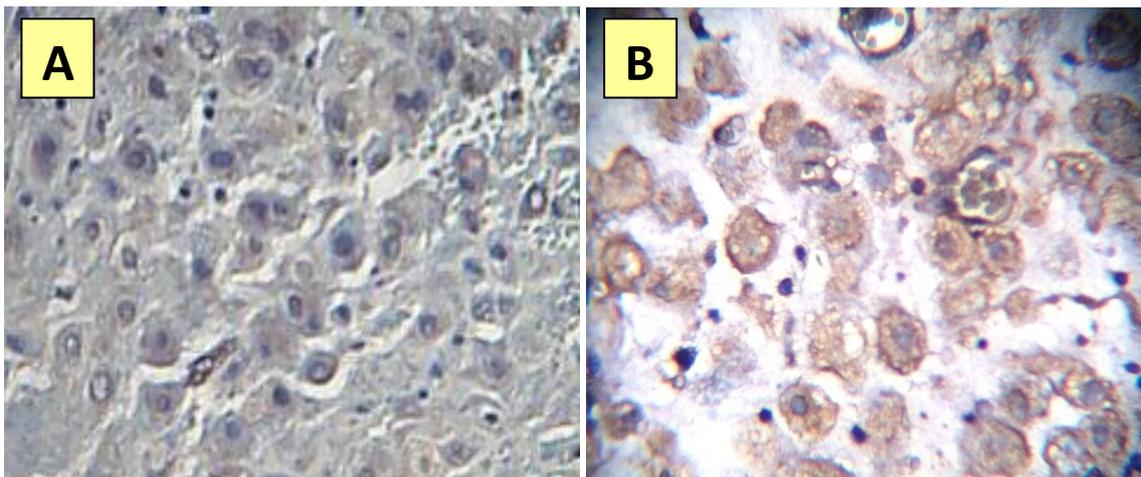
Variable		Number	Percentage	Comparison of Significance P-value
Age stratum	15-25 years	19	38%	0.375
	26-35 years	19	38%	
	36-45 years	12	24%	
Education level	Illiterate	8	16%	0.000
	Primary School	28	56%	
	Secondary School	6	12%	
	Tertiary level	8	16%	
Occupation	Working	7	14%	0.000
	Housewives	43	86%	
Economic level	Low	8	16%	0.000
	Intermediate	39	78%	
	High	3	6%	
Residence	Urban	36	72%	0.002
	Rural	14	28%	
Total		50	100%	

**Table 2. Antenatal characteristics of subjects**

Variable		Number	Percentage	Comparison of Significance P-value
Duration of pregnancy	1 <sup>st</sup> trimester	41	82%	0.000
	2 <sup>nd</sup> trimester	9	18%	
	3 <sup>rd</sup> trimester	0	0	
Number of abortion	First time	6	12%	0.000
	Second time	23	46%	
	Third time	19	38%	
	Fourth time	2	4%	
Parity	Nulliparaous	5	10%	0.000
	Primiparaous	11	22%	
	Multiparaous	31	62%	
	Grandmultipar	3	6%	

**Table 3. Positive and negative results for CMV and HHV-1 among studied group**

IHC results	Positive No. (%)	Negative No. (%)	Comparison of Significance P-value
CMV	18 (36%)	32 (64%)	1.000
HHV-1	18 (36%)	32 (64%)	



**Figure 1. Immunohistochemistry for HCMV and HHV-1 in product of conceptus tissues of pregnant women with spontaneous abortion section, stained by DAB chromogen and counter stained with hematoxylin is shown as radish brown in positive cases (magnification power, 400). A-HCMV positive expression, B- HHV-1 positive expression.**

## Discussion

Viral infections are more likely to occur at certain times in life such as childhood, adolescence and pregnancy. Pregnant women are at greatest risk of viral infection, due to the fact that they have defect in immune response. We chose in this study this segment of females for focus with a view two reasons the extent of the problem in our society. The idea was to investigate the viral infection rate among pregnant women with spontaneous abortion. Human cytomegalovirus is linked to late abortions and to stillbirth and reactivation of chronic HCMV infection in the course of pregnancy might result in fetal infection with spontaneous abortion<sup>(15-17)</sup>. The current study had demonstrated that HCMV infection in pregnant women with spontaneous abortion was 36%, this result is comparable with result of a study done in Erbil city, which found that the seropositivity was 30.05%<sup>(18)</sup>. However, other studies showed higher rate than result of present study, which reported a detection rates ranged between 62.3% and 81.1%<sup>(19, 20)</sup>. When compare to studies conducted in Arabic countries such as Tunisia, where a 96.3% seroprevalence of HCMV antibody was

reported among 404 Tunisian pregnant women<sup>(21)</sup>. High frequency rate could therefore be related to a higher risk of infection, but it may also be related to use less sensitive diagnostic methods such as virus culture in certain areas compare with other technique. While the rate was lower in study done by el-Sayed and Goda that achieved 12%<sup>(22)</sup>.

There is a 2% incidence of HHSV-1 or HHSV-2 infections among women during pregnancy<sup>(23)</sup>. Herpes virus 1 accounts to about one-third to a half of cases of neonatal herpes<sup>(24,25)</sup>.

The result demonstrated that the frequency of HHV-1 was 36% in product of conceptus tissues of pregnant women with portentous abortion. Similar study carried out by Frenkel, et al., 1993<sup>(26)</sup>, who reported that HSV was detected by serology in 36% in pregnant women with no history of genital herpes. Also, this result was in agreement with the findings el-Sayed and Goda (2007) who reported that 40% women with recurrent abortion have HSV IgM<sup>(22)</sup>. Increased risk of spontaneous abortion, stillbirth, and congenital anomalies have always been associated with herpes virus<sup>(27)</sup>.

**Table 4. Distribution of positive and negative HCMV-IHC results according to socio-demographic and antenatal characteristics of study**

Variable		Positive No. (%)	Negative No. (%)	Comparison of Significance P-value
Age (year)	15-25	6 (33.33%)	13 (40.62%)	0.111
	26-35	10 (55.55%)	9 (28.12%)	
	36-45	2 (11.11%)	10 (31.25%)	
Education level	Illiterate	1 (5.55%)	7 (21.87%)	0.294
	Primary School	13 (72.22%)	15 (46.87%)	
	Secondary School	2 (11.11%)	4 (12.50%)	
	Tertiary level	2 (11.11%)	6 (18.75%)	
Occupation	Working	2 (11.11%)	5 (15.62%)	0.659
	Housewives	16 (88.88%)	27 (84.37%)	
Economic level	Low	3 (16.66%)	5 (15.62%)	0.508
	Intermediate	13 (72.22%)	26 (81.25%)	
	High	2 (11.11%)	1 (3.12%)	
Residence	Urban	15 (83.33%)	21 (65.62%)	0.181
	Rural	3 (16.66%)	11 (34.37%)	
Duration of pregnancy	1 <sup>st</sup>	15 (83.33%)	26 (81.25%)	0.854
	2 <sup>nd</sup>	3 (16.66%)	6 (18.75%)	
	3 <sup>rd</sup>	0	0	
Number of abortion	First time	2 (11.11%)	4 (12.5%)	0.217
	Second time	9 (50%)	14 (43.74%)	
	Third time	5 (27.77%)	14 (43.75%)	
	Fourth time	2 (11.11%)	0	
Parity	Nulliparaous	1 (5.55%)	4 (12.5%)	0.705
	primiparaous	3 (16.66%)	8 (25.0%)	
	Multiparaous	13 (72.22%)	18 (56.25%)	
	Gravid	1 (5.55%)	2 (6.25%)	

The results of current study showed the highest HCMV and HHV-1 infection rate among those enrolled cases at age interval between 15-35 years, cases of HSV-1 infections are seen worldwide and do not discriminate by age<sup>(28)</sup>. Comparison of HCMV and HHV-1 positive results according to occupation and economical level, most positive cases occur within non-worker women and with intermediate economic level but no significant correlation occur among them. Human

cytomegalovirus is found universally throughout all geographic locations and in all socioeconomic groups<sup>(19)</sup>. Also, this result agrees with Pechaham et al. 2001<sup>(29)</sup>. Although the present study revealed that the HCMV and HHV-1 were highest in the first trimester, this result was consistent with those reported by other who found that the highest infection rate of HCMV and HHV-1 among aborted women<sup>(6,30)</sup>. Also, HHV found in the second trimester<sup>(27)</sup>. While other study done

by Skoczyński et al, (2015) who did not confirm the prenatal transmission of HPV and HSV during the investigation in 138 samples of amniotic fluid from pregnant women during the second trimester of gestation <sup>(31)</sup>. However,

the damage is still more severe in infections occurring during the first half of pregnancy, while infections in the second half would result in reduced mortality <sup>(32)</sup>.

**Table (5): Distribution of positive and negative human herpesvirus 1-IHC results according to socio-demographic and antenatal characteristics of study group**

Variable	Positive No. (%)	Negative No. (%)	Comparison of Significance P-value
Age (year)	15-25	7 (38.88%)	0.976
	26-35	7 (38.88%)	
	36-45	4 (22.22%)	
Education level	Illiterate	4 (22.22%)	0.203
	Primary School	7 (38.88%)	
	Secondary School	4 (22.22%)	
	Tertiary level	3 (16.66%)	
Occupation	Working	2 (11.11%)	0.956
	Housewives	16 (88.88%)	
Economic level	Low	4 (22.22%)	0.667
	Intermediate	13 (72.22%)	
	High	1 (5.55%)	
Residence	Urban	14 (77.77%)	0.495
	Rural	4 (22.22%)	
Duration of pregnancy	1 <sup>st</sup>	16 (88.88%)	0.459
	2 <sup>nd</sup>	2 (11.11%)	
	3 <sup>rd</sup>	0	
Number of abortion	First time	2 (11.11%)	0.848
	Second time	7 (38.88%)	
	Third time	8 (44.44%)	
	Fourth time	1 (5.55%)	
Parity	Nulliparaous	2 (11.11%)	0.694
	primiparaous	4 (22.22%)	
	Multiparaous	10 (55.55%)	
	Gravid	2 (11.11%)	

In the current study, there is no significant correlation between the HCMV and HHV-1 infection in aborted pregnant women with age, education level, occupation, economic level, residence, gestational age, history of abortions and parity; so, these cannot be considered as risk factors for infection.

The differences between the results of the previously mentioned studies and even with the results of present study could be related to many factors, like the methodology in the current study used molecular technique while other study may use serological methods, sample size, studied population different from one area to another, the duration of incubation

if samples collect in acute infection the result will different from chronic infection, individual's immune status, demographic and geographical variations season and etc.

The current study concludes that viral infections with HCMV and HHV-1 might play a role in recurrent abortions. A cytomegalovirus and human herpesvirus-1 antigen is comparable with that in the other countries, and pregnant women in Baqubah city may have the risk for acquiring viral infection during pregnancy and consequently adverse pregnancy outcomes. Careful investigation for such conditions must involve detecting the presence of these viruses. Further investigation is needed with large sample size to clarify this issue and studying the role of other viruses in the pregnant women such as parvovirus.

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#### Author contributions:

Authors participate in drafting the article and revising it critically for important intellectual content.

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