



MOLECULAR DETECTION OF CYTOMEGALOVIRUS (CMV) AND PRESENCE OF BACTERIAL INFECTION IN ABORTED WOMEN

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Abstract

Spontaneous abortion (SA), also known as miscarriage, is considered as one of the most frequent problems a woman may face during early pregnancy. An abortion is the removal or expulsion of an embryo or fetus from the uterus, resulting in/or caused by its death, the loss of pregnancy occurs within the first 20-24 weeks of gestation, after which, fetal death is known as a stillbirth. Prenatal cytomegalovirus in addition to bacterial infection may cause pregnancy problems could lead to abortion, so the aim of this study was to investigate the prevalence of bacterial vaginitis in women with abortions and the recent incidence of high-density cytomegalovirus to determining the percentage of bacterial and viral infections among women. In this study, 119 PAP specimens from aborted patients were amplified using PCR amplification of the UL97 gene, and 119 vaginal swap to detect related bacterial infection. The present study showed that HCMV DNA was detected in 35/94 (37.2%) of the patient group tested, and bacterial infection that recovered from vaginal swabs, of which 22 (23.4%) were Gram-negative and 77 (81.9 %) were infected with candida of the Gram-negative bacteria *Escherichia coli* 14 (14.9%) followed by *Klebsiella* 5 (5.3%) and *Pseudomonas* 3 (3.2%), there was a mixed infection of bacteria with *Candida* spp. was investigated in the current study and revealed that *Candida* was co-existing with *E.coli* and *Klebsiella* in 2 vaginal swabs, *E.coli* in 12 vaginal swabs and with three swabs of *Klebsiella* while no mixed infection was observed with *P. aeruginosa*.

Key words: Molecular detection, CMV, aborted women

Introduction

Abortion in medicine is defined as the withdrawal of the contents of the pregnancy before the completion of twenty weeks, including types of abortions, abortions, miscarriages and repeated miscarriages known as pregnancy loss 20 weeks ago and 15-20% of abortion rates in general (WHO., 2008). It is caused by chromosomal abnormalities occurring in women over the age of 35, uterine deformities, ovarian malformation, coagulation, as well as the use of vaginal detergents containing chemicals and bacterial vaginal infections. Prenatal cytomegalovirus infection may cause pregnancy complications such as intrauterine growth restriction and birth defects (Gao, 2013). Bacterial vaginitis BV is the most common non ulcerative lower genital tract disease and is characterized by alteration of the natural vaginal flora, most notably a marked decrease in numbers of lactobacilli (Hay, 2017). Bacterial vaginosis occurs after the bacteria enter the uterus during abortion and cause infection in the pelvic area. It is possible to treat infections with antibiotics, women feel weak and fever lasting more

than 24 hours or higher, abdominal pain, or bleeding a lot or for a long time. Any serious bacterial or viral infection can lead to miscarriage (Giakoumelou *et al.*, 2016). Human cytomegalovirus (HCMV) infection is a major cause of morbidity and mortality in immunocompromised patients; including solid organ transplant, HIV infected patients and congenitally or prenatally infected children (Keyvani *et al.*, 2016). This virus belongs to the Betaherpes virus family and leads, after primary infection, to a lifelong latency. Under special conditions such as immunosuppression, the virus can be reactivated and may lead to severe disease (Göhring *et al.*, 2015).

Ganciclovir (GCV), cidofovir and foscarnet are antiviral drugs used for the therapy of HCMV infection. The target of these drugs is the UL54 gene which encodes viral DNA polymerase. GCV, a nucleoside analog, is a pro drug which must be activated by phosphorylation, this phosphorylation is carried out by the viral kinase (UL97) and the activated drug inhibits the viral DNA polymerase (UL54) (Fisher *et al.*, 2017). The study focused on the prevalence of bacterial vaginitis in women

with abortions and the recent incidence of high-density cytomegalovirus to determining the percentage of bacterial and viral infections among women.

Materials and Methods

Patients and sampling

Samples of PAP smear and high vaginal swap were obtained from patients of a pathologist from Al Alweiya teaching hospital and Al Furat hospital for the period from beginning of November 2018 to October 2019 according to ethical considerations and the hospital approval. High vaginal swabs and PAP specimen were received from aborted patients seen within the hospital either on admission or from outpatient, specimens were collected by trained nurses and patients were instructed to go quickly to the Public Health laboratory. On arrival at the public health laboratory, patients demographics were collected.

Two Vaginal swabs from 119 women were taken, Twenty five of control samples were collected from healthy women using a special vaginal opening machine and then Sim's Speculum then insert sterile cotton swabs within 2 cm inside the vagina and gently spin towards the vaginal wall before pulling the scanner.

The swabs were transported to the laboratory by inoculating the swab into a sterile plastic tubes containing gel media, one of the swabs was directly inoculated onto Blood agar, MacConkey agar and chocolate agar for microbiological investigation, the other swab was used for direct examination by Gram stained for detection of bacteria, inoculated culture plates were incubated at 37°C for 24-72 hr. The Vitek 2 test was used in this study in order to diagnosis the bacterial isolates.

PAP specimen add to a plane tube containing phosphate buffer saline preparing for molecular detection.

Statistical Analysis

The statistical analysis was performed using chi-square test. P values less than 0.05 were considered statistically significant.

Results

Characteristics of patients

A total of ninety four aborted female with significant difference ($P \leq 0.05$) in addition to 25 apparently healthy female used as control table 1.

All 119 subjects were at age ranged from 20 – 44 year (< 20), (20 - 29) (30 - 39) and (>40).

Patients were located into four categories depending on their age. The results exposed that the high percentage

Table 1: Distribution of patients and control according to age.

Age group		Group		Total N (%)
		Patient N (%)	Control N (%)	
Age group	<20	20 (21.3%)	6 (24.0%)	26 (21.8%)
	20-29	33 (35.1%)	16 (64.0%)	49 (41.2%)
	30-39	33 (35.1%)	2 (8.0%)	35 (29.4%)
	>40	8 (8.5%)	1 (4.0%)	9 (7.6%)
Total		94 (100%)	25 (25%)	119 (100%)

of patients (35.1%) found in the second and third group, followed by the patients of first group (21.3%) and the lowest percentage (8.5%) was for the latest group. Statically there was high significant difference ($P \leq 0.05$) between age groups.

In current study the higher rate of aborted women in age group between 20- 29 years old (35.1%). These results agree with many previous Iraqi studies which showed that the higher rate in abortion in ages group between 20- 29 years (Mohammed and Hadeel, 2011, Hussein *et al.*, 2014). The abortion rates show an inverted u-shaped pattern by women's age. It was observed that the highest rate of abortion was in age group between 20–24 year olds, in twenty three out of forty one countries (Gilda *et al.*, 2012), also many studies reported that the increased risk of abortion and infertility with women age, also showed that the older women have fertility problems because declining egg quality, and other problem which that more current in older women that disagree with our study (Elisem and Patrick, 2002).

The molecular assays for HCMV diagnosis are considered to be beneficial and fast tools especially to susceptible groups such as transplant recipients and AIDS patients as well as aborted women (Lashini *et al.*, 2011). The PCR results were interpreted by the presence or absence of specific bands of amplified gene on 2% of agarose. The current study was conducted for amplification of HCMV phospho transferase gene (Thymidine kinase, UL97) from PAP smear of aborted women. The amplified products of this gene (UL97) was 650 bp as in Fig. 1.

The present study included also a molecular diagnosis of HCMV in aborted female, and related bacterial infection in Baghdad city. HCMV DNA was detected in 35/94 (37.2%) of the patient group tested as shown in table below.

For UL97 gene, the high percentage was reported in different age group varying from 20-39 years. The current results were disagreed with Yasir, (2012) who showed low positivity of viral DNA in his selected groups, this

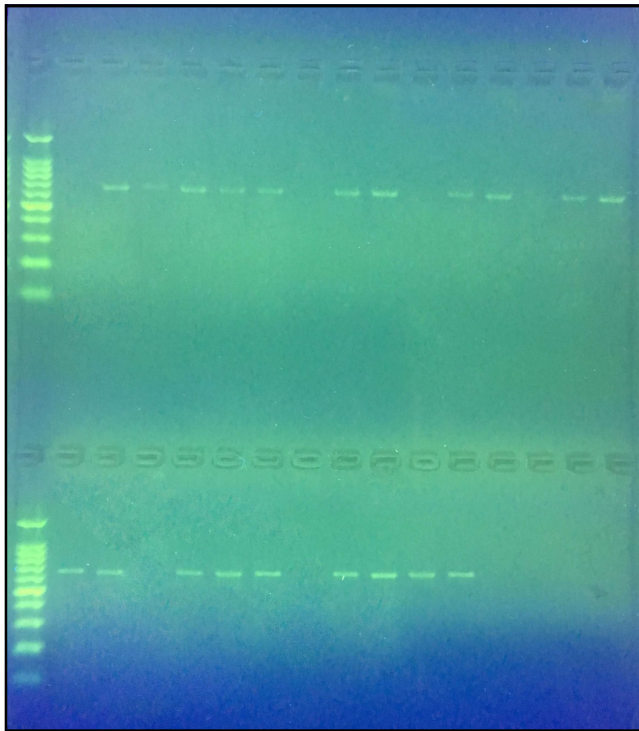


Fig. 1: PCR product band size 650 bp for UL97 gene. The product was electrophoresis on 2% agarose at 5 volt/cm². 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

Table 2: The presence of UL97 gene between patients and control.

650 bp		Group		P Value
		Patient N (%)	Control N (%)	
650 bp	-ve	59 (62.8%)	25 (100.0%)	0.001**
	+ve	35 (37.2%)	0 (0.0%)	
Total		94 (100%)	25 (100%)	

may be due to the use of the other gene which was major immediate early gene (MIE) and it was detected at active replicating state. However, these results were in line of Zhang *et al.*, (2000) and (2010) who showed the high sensitivity of PCR for detection of HCMV which detect even latent non replicating virus in asymptomatic patients in young or old ages.

The current results are supported by Hussein *et al.*, 2014 when recorded UL97 gene in 75/98 (77%) of women suffering from infertility and 20/30(67%) of breast cancer infected female. Mengelle *et al.*, (2003) who showed nearly same results when he was used whole blood.

It is apparent that there is a very substantial difference in detection of CMV DNA in the reviewed articles. Some studies used cervical scrapes and the others biopsied tissue specimens. We used cervical swabs, the most frequent source of cells used today for routine viral studies. The primers used for CMV DNA detection were different

between all studies. Thus, the different results can be attributed to differences in the laboratory assays, the primers and probes used, different types of specimens studied, or to differences in populations where the studies were performed.

Bacterial and fungi ratios of aborted women related with the presence of UL97 gene

Many bacterial genera and fungi were isolated from vaginal swabs of aborted women, A total of 119 women were included in the study, the overall prevalence of bacterial vaginosis was 23.40% in ninety four aborted women with no infection in 25 apparently healthy women. Spectrum of bacteria causing aerobic vaginosis that recovered from vaginal swabs, of which 22 (23.4%) were Gram-negative and 77 (81.9 %) were infected with *Candida* of the Gram-negative bacteria *Escherichia coli* 14 (14.9%) followed by *Klebsiella* 5 (5.3%) and

Table 3: The distribution of *Candida* infection between patient and control.

Candida		Group		P Value
		Patient N (%)	Control N (%)	
Candida	-ve	17 (18.1%)	25 (100.0%)	0.001**
	+ve	77 (81.9%)	0 (0.0%)	
Total		94 (100%)	25 (100%)	

Table 4: The distribution of *E.coli* infection between patient and control.

E. coli		Group		P Value
		Patient N (%)	Control N (%)	
<i>E.coli</i>	-ve	80 (85.1%)	25 (100.0%)	0.040 *
	+ve	14 (14.9%)	0 (0.0%)	
Total		94 (100%)	25 (100%)	

Table 5: The distribution of *Klebsiella* infection between patient and control.

Klebsiella		Group		P Value
		Patient N (%)	Control N (%)	
<i>Klebsiella</i>	-ve	89 (94.7%)	25 (100.0%)	0.239 ^{NS}
	+ve	5 (5.3%)	0 (0.0%)	
Total		94 (100%)	25 (100%)	

Table 6: The distribution of *Pseudomonas* infection between patient and control.

Pseudomonas		Group		P Value
		Patient N (%)	Control N (%)	
<i>Pseudo-</i> <i>monas</i>	-ve	91 (96.8%)	25 (100.0%)	0.366 ^{NS}
	+ve	3 (3.2%)	0 (0.0%)	
Total		94 (100%)	25 (100%)	

Pseudomonas 3 (3.2%) presented in table below :-

The results were less than the rate of Al-Mashhadani *et al.*, 2006, where it was isolated by (51.6%) but more than the isolation rate of Ali, 2010, isolating it by 1.2% and approaching to 7% in the study of Florica, 2013, due to the difference in the percentage of isolation of different methods of collection of samples and the difference in place and time of collection in addition to different methods of isolation of bacteria.

The prevalence rate of bacterial vaginosis in the present study was well within the reported range in the local study of Tsahel, 2019 which was *Escherichia coli* isolates (19.2%) and *Klebsiella* isolates by (10.3%), while Bushra, and Amina, 2010 confirmed higher results in gram negative infection, which was *E.coli* 21 isolates, then *P.aeruginosa* 18 isolates and *Klebsiella* 11 isolates. Sociodemographic characteristics, sexual activity, reproductive health information and behavioral and genital hygiene have been identified as causes of variation in the prevalence rates of bacterial vaginosis (Bautista *et al.*, 2016).

The variation in the proportions and numbers of isolated bacteria may be due to several factors including that some of the bacteria are due to the natural flora of the vagina, the digestive tract or flora of the skin or to external origin due to differences in cultural and educational level factors and the health status and living conditions of pregnant women under study (Alcendor, 2016).

The incidence of mixed infection of bacteria with *Candida* spp. was investigated in the current study and revealed that *Candida* was co-existing with *E.coli* and *Klebsiella* in 2 vaginal swabs, *E.coli* in 12 vaginal swabs and with three swabs of *Klebsiella* while no mixed infection was observed with *P. aeruginosa*.

Table 7: The distribution of mixed infection of bacteria.

Microorganisms	NO.	% of total
<i>E.coli</i> and <i>Klebsiella</i>	2	2.13
<i>E.coli</i>	12	12.77
<i>Klebsiella</i>	3	3.19
Total	17	18.09

The co-existence of *Candida* with bacteria may arise from competition for nutrients, contamination from other sites, or co-existing as secondary pathogens (Achkar and Fries., 2010). *Candida* consider as most common form of infectious vaginitis and is responsible for substantial morbidity among reproductive age women; a significant burden to the healthcare system due to rising vaginitis related health care costs. Its development is usually

attributed to the disturbance of the balance between *Candida* vaginal colonization and host environment (Mahmoudi *et al.*, 2011, Alexander *et al.*, 2016).

Cytomegalovirus infections are being reported to be the causative of abortion in women, the infection was higher relation with presence of candida, UL97 gene recorded in 35 (37.2%) of aborted women, 30 (31.91%) of cmv infections were positive for candida, while only 14 (14.9%), 3 (3.2%), 2 (2.1%) showed positive detection for *E.coli*, *Klebsiella* and *Pseudomonas* respectively as shown in table (8, 9, 10, 11).

Table 8: The correlation between UL97 gene and *Candida*.

		Group		Total
		-ve	+ve	
UL97 gene	-ve	17 (18.16%)	42 (44.7%)	59 (62.8%)
	+ve	5 (5.31%)	30 (31.91%)	35 (37.2)
Total		22 (23.40%)	77 (81.9%)	94 (100%)
Chi sq. (P-value)		0.001 ^{NS}		

Table 9: The correlation between UL97 gene and *E. coli*.

		E.coli		Total
		-ve	+ve	
UL97 gene	-ve	59 (62.8%)	0 (0.0%)	59 (62.8%)
	+ve	21 (22.3%)	14 (14.9%)	35 (37.2)
Total		80 (85.1%)	14 (14.9%)	94 (100%)
Chi sq. (P-value)		0.001**		

Table 10: The correlation between UL97 gene and *Pseudomonas*.

		Pseudomonas		Total
		-ve	+ve	
UL97 gene	-ve	58 (61.7%)	1 (1.1%)	59 (62.8%)
	+ve	33 (35.1%)	2 (2.1%)	35 (37.2)
Total		91 (96.8%)	3 (3.2%)	94 (100%)
Chi sq. (P-value)		0.284 ^{NS}		

Table 11: The correlation between UL97 gene and *Klebsiella*.

		Klebsiella		Total
		-ve	+ve	
UL97 gene	-ve	57 (60.6%)	2 (2.1%)	59 (62.8%)
	+ve	32 (34.0%)	3 (3.2%)	35 (37.2)
Total		89 (94.7%)	5 (5.3%)	94 (100%)
Chi sq. (P-value)		0.279 ^{NS}		

Association between bacterial and viral infection can be attributed to change in the complex balance of microflora in the vagina. There is a decrease in Lactobacilli and an over growth of facultative and anaerobic bacteria either singly or in combination. The associated risk factors of BV are also poorly understood as reports on risk factors have been conflicting (Machado

et al., 2017). There are reports on the association of BV with demographic, behavioral and clinical characteristics of pregnant women (Bitew *et al.*, 2017) while other studies report no association with some of these factors. Also, there has been contradicting reports on sexual transmission as a necessary prerequisite to the acquisition of BV. Some studies have reported sexual involvement while others detected BV in sexually inexperienced women. As a result, Verstraelen suggested BV to be considered a sexually enhanced disease (SED) rather than a sexually transmitted disease (STD) (Verstraelen *et al.*, 2010). Other studies have reported its association with multiple sex partners. Because prevention strategies of any disease target risk factors, knowledge of risk factors of BV is important in the design of effective interventions to prevent poor pregnancy outcomes, particularly as many cases are asymptomatic (Li XD *et al.*, 2015).

Bacterial vaginitis is a common problem occurring among pregnant women and studies have consistently shown BV to be a risk factor for adverse obstetric and gynecological outcomes such as pre-term labor and delivery, premature rupture of membranes, low birth weight, spontaneous abortion, postpartum infections such as endometritis and caesarean section wound infections. Since most cases remain asymptomatic early detection, especially among pregnant women is essential for timely treatment and prevention of related complications and poor pregnancy outcomes (Mengistie *et al.*, 2014, Isik *et al.*, 2016, Das *et al.*, 2017).

Conclusion

Bacterial vaginitis prevalence was affected by some hygiene behaviors, socio-demographic and clinical factors. Screening and treatment of positive cases during antenatal visits to prevent adverse outcomes, as well as education of women on vaginal hygiene is highly recommended. Cytomegalovirus infections are being reported to be the causative of abortion in women, the infection was higher relation with presence of candida and with rare relation with other bacterial infection.

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