

## RESEARCH ARTICLE

# Immunological Detection of Cytomegalovirus and Bacterial Vaginosis Associated with *Trichomonas vaginalis* in Women with Miscarriage

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## ABSTRACT

Miscarriage is a complicated pregnancy and known as a spontaneous ending of pregnancy itself before the baby has attained a level of viability. Miscarriages may be sporadic or recurrent. A total of (113) a high vaginal swabs and serum was collected. According to sample size, samples from aborted women were obtained from hospitals in Maysan Governorate, Iraq (cervical swabs and serum) in the obstetric and gynecology outpatients department during period from November 2020 to May 2021. Some variables were been investigated in accurate study like age, education, residences and diseases and others. The SPSS version (23) is used for a statistical analysis, and a *p-value* of (0.05) was considered statistically significant. The aim of the study is immunological detection of IgM and IgG cytomegalovirus in blood of infected women suffering with *Trichomonas vaginalis* and bacterial vaginosis. Associated between cytomegalovirus, *T. vaginalis* and bacterial vaginosis in a abortion women.

**Keywords:** Bacterial vaginosis, Cytomegalovirus (CMV), Immunological detection, Recurrent miscarriage, *Trichomonas vaginalis*.

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## INTRODUCTION

Miscarriage is one of the most problems a woman faced with early pregnancy. It causes morbidity and mortality, significant social and psychological impacts on women.<sup>1</sup> There are two stages of miscarriages, early miscarriage is defined as the loss of a pregnancy in less than 12 weeks or in first trimester. Late miscarriage occur in the second trimester 12 to 24 weeks of pregnancy and rare, about (1–2)% of pregnancies.<sup>2</sup> Bacterial vaginosis is a most common problem was caused of vaginal secretion and characterized by a malodorous vaginal discharge, in a women of childbearing age.<sup>3</sup> All women has more affected experience an offensive, fishy-smelling secretion, often during menstruation.<sup>4</sup> Bacterial vaginosis can be transient and asymptomatic in nature.<sup>5</sup>

The more affected of experience a fishy odor secretion which sometimes occurs around menstruation. Others may have bacterial vaginosis transiently and asymptotically. It always responds to treatment with antibiotics but can relapse rapidly, and reported rates of relapse are more than 50% within (3–6) months. Some studies imply that it is sexually transmitted, with strains as *Escherichia coli*, *Streptococcus*, *Gardnerella* and *Atopobium vaginae*, *Leptotrichia aminionii*,

*Mobiluncus spp*, *Prevotella*, *Mycoplasma hominis*, *Bacteroides* being identified.<sup>6</sup>

*Trichomonas vaginalis*, a flagellate parasite, is a causative agents of most common non-viral sexually transmitted infection. The world's non-viral sexually transmitted infection (STI) is *T. vaginalis*.<sup>7</sup> Trichomoniasis can cause major health problems such as low-birth-weight babies and premature rupture of the placental membranes, as well as pelvic inflammatory illness, cervical cancer, and infertility. Viruses can infect and kill infants by transplacental or ascending routes. The most commonly virus as which was cause by cytomegalovirus, which can induce chronic or recurrent maternal infection of cervical mucosa. Cytomegalovirus can infect the placenta through the cervix or by viremia after primary and recurrent maternal infection, causing vascular insufficiency, tissue destruction, and transmission to the fetus.<sup>8</sup> Infections that occur during pregnancy effect on the fetus. For example, congenital abnormalities caused by (CMV), rubella virus, and herpes simplex virus (HSV). Congenital cytomegalovirus affects cognition, motor function, hearing, vestibular function, and vision and is the leading cause of central nervous system (CNS) and sensory abnormalities.<sup>9</sup>

**MATERIALS AND METHODS**

**Sample Collection**

A total of (113) from vaginal samples from females attending to obstetrics gynecology Out patients department in Al-Sadder Hospital, Misan City (Misan Province) and collage of Sciences in Misan university during the period from (November 2020 to may 2021). High vaginal swabs were taken from females in age range from (15–45) years based on side and symptoms of vaginosis like vaginosis like, vaginal discharge, itching, bad odor and other clinical manifestations by sterile metal speculum was inserted into the vagina without any lubricate and solution.

**Wet Amount Preparation and Staining**

After vaginal discharge have been taken smear was provided. Then put 1 to 2 drops of normal saline solution were placed on the prepared smear with cover slip and examined under microscopic<sup>10</sup> gram stain kits provided by Hi-media, India, to differentiate the morphology of bacterial isolates.

**Biochemical Examination**

Smear gram staining and culture on blood agar, chocolate agar, and MacConkey agar were done on one swab. Biochemical examination, urease, oxidase, coagulase, and catalase tests with suspicious culture were incubated at 37°C.<sup>11-13</sup>

**VITEK-2 Diagnostic System of Bacterial Vaginosis**

The Vitek-2 device has been used to diagnose of bacteria isolates from culture media.<sup>14</sup>

Detection of CMV IgM by Minividias: Use a VIDAS CMV IgM and IgG avidity. IgG avidity was determined using a commercial kit VIDAS CMV IgG avidity II (Biomérieux, France). The assay uses the VIDAS CMV IgG kit.

**Diagnosis of *T. vaginalis***

A clinical diagnosis used to diagnose *T. vaginalis*. The “strawberry” cervix, a yellowish green frothy vaginal secretions, vaginal smear, dysuria, dyspareunia, and yellowish-green frothy vaginal secretions are all symptoms associated with *T. vaginalis*.<sup>15</sup>

**Microscopically Examination**

Wet amount technique and Giemsa staining: To detect *T. vaginalis* and preparation, motile *T. vaginalis* can be diagnosed by moving with a Jerky movement.<sup>16,17</sup>

**RESULTS**

**Age Groups with Recurrent Miscarriage Women**

Age is a very important factor associated with recurrent miscarriage in women. Table 1 describes the number of miscarriages with age. One miscarriage was a high percentage, recorded in age group (25–34) year (20%) and low percentage in age (≤45 ) year (3%). Twice miscarriage the age (25–34) year was recorded low rate (1%), more than twice miscarriage, a high rate in age (14–25) year (5%), and a low percentage in age groups (35–44) year (2%) and Figure 1 show miscarriage fetus. The high percentage occurs due to high levels of estrogen

hormone, elevated of pH level and change in the secretion of glycogen in vaginal miscarriage women, which lead to the change of growth of pathogen miscarriage.<sup>17</sup> Researchers studied the role of material age of pregnancy time in risk of miscarriage and recorded the age groups range between (30–34) year was recorded a high rate 11921 (10.8%) and this study disagree with the accurate study.

**Recurrent Miscarriage with Different Pathogenic**

Bacterial vaginosis was isolated from 113/7 (100%) as in total count in all cases of miscarriage (Table 2, Figure 2). Twice miscarriage was documented 7/5 (100%). In cytomegalovirus, rate was 113/29 (100%) as in total count and in one and twice miscarriages were recorded 2/1 (50%), respectively. *T. vaginalis* was recorded 113/2 (100%) in one, twice miscarriage but mix infection was 113/102 (100%) as total count was 113/102 (100%) and in one miscarriage 102/46 (45.17%) as a high rate and low rate in more than one miscarriage 102/113 (12.7%) with no statistical significance.<sup>18</sup> Researchers recorded abnormal birth as a high ratio (49.2%) and death fetal with birth was lower ratio (2.9%) (65), this associated to effect cytomegalovirus on growth of fetus in uterus and causing congenital cytomegalovirus or lower immunity, having contraceptives, change of normal flora count in uterus and vagina lead to courage CMV to increased. This results agreement with<sup>19</sup> was reported in her study a high ratio in premature delivery or abortion in (21 weeks) from pregnancy. These results were similar with<sup>20</sup> who recorded that *Staphylococcus aureus* are the common causative agent in asymptomatic lower genital tract infection, followed by *E. coli* in miscarriage women. In Iraq, Al-Mousawi *et al.* (2006)<sup>21</sup> recorded that *S. aureus* was the dominant one that colonized vaginal mucosa and caused toxic shock syndrome. Burtin *et al.*, 1995<sup>22</sup> reported an analysis of seven studies, which

**Table 1:** Age-associated with miscarriage

Age (group)	One miscarriage	Twice miscarriage	More than one	p-value
14–24	17 (34.0)	12 (24)	(38.5)5	0.045*
25–34	20 (40)	25 (50)	3 (23.1)	
35–44	10 (20)	12 (24)	2 (15.4)	
≤45	3 (6)	1 (2)	3 (23.1)	
Total	50 (100)	50 (100)	13 (100)	

(p-value ≤0.05)\*



**Figure 1:** Abortion fetus in age (3) months

**Table 2:** Pathogen associated with miscarriage

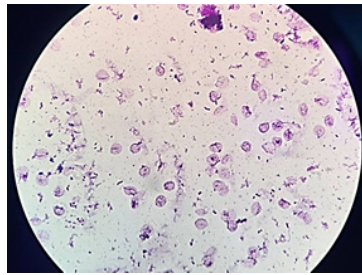
Pathogen	Once	Twice	More than	Total	p-value
Bacterial vaginosis	2 (28.6%)	5 (71.4%)	0	7 (100%)	
CMV	1 (50%)	1 (50%)	0	2 (100%)	
Trichomonas vaginalis	1 (50%)	1 (50%)	0	2 (100%)	0.35*
Mix infection	46 (45.17)	43 (42.2)	13 (12.7)	102 (100%)	
Total	50 (44.2)	50 (44.2)	13 (11.5)	113 (100%)	

(p-value ≤ 0.05)\*

**Table 3:** Immunoglobulin types associated with of miscarriage

Immunoglobulin	Once miscarriage	Twice miscarriage	More than one	Total	p-value
IgM	19 (52.8%)	15 (41.7%)	2 (5.6%)	36 (100%)	0.21*
IgG	21 (36.8%)	28 (49.1%)	8 (14%)	54 (100%)	
IgG and IGM	10 (50%)	7 (35%)	3 (15)	20 (100%)	
Total	50 (44.2%)	50 (44.2%)	13 (11.5%)	113 (100%)	

(p-value ≤ 0.05)\*



**Figure 2:** *T. vaginalis* with Giemsa stain under (1000 X)

**Table 4:** Bacterial vaginosis associated with miscarriage women

No	Bacterial vaginosis	Frequency	%
1	<i>Neisseria spp</i>	4	3.6
2	<i>Acinetobacter spp</i>	1	0.9
3	<i>Bacillus spp</i>	4	3.6
4	<i>Klebsiella spp</i>	26	23.6
5	<i>E. Coli</i>	25	22.7
6	<i>Staphylococcus Sciuri</i>	1	0.9
7	<i>Micrococcus Luteus</i>	5	4.5
8	<i>Enterobacter Cloacae</i>	1	0.9
9	<i>Kocuria Kristinae</i>	4	23.6
10	<i>Staphylococcus Haemolyticus</i>	7	6.3
11	<i>Enterococcus Faecalis</i>	9	8.1
12	<i>S. aureus</i>	10	9.09
13	<i>Burkholderia Cepacia</i>	3	2.7
14	<i>Pseudomonas spp</i>	2	1.8
15	<i>Staphylococcus Warneri</i>	2	1.8
16	<i>Enterococcus Facium</i>	2	2.7
17	<i>Streptococcus spp</i>	3	2.7
18	<i>Proteus spp</i>	1	0.9
Total		110	100%

favorite that metronidazole doesn't increase a risk for birth defects in fetus with the first trimester, metronidazole use is currently recommended only with second and third trimesters.

**Immunoglobulin (IgM and IgG) of Cytomegalovirus in X Women with Miscarriage**

According to immunoglobulins of cytomegalovirus in miscarriage women. It was seen that for women with IgM a positive was indicate to new infection with cytomegalovirus, but with IgG, a positive refers to old infection or reactivation and mix with IgM positive and IgG positive indicates to reactivation or recent infection. The high rate was recorded 21 (36.8%), 15 (41.7%) and 2 (5.6%) but IgM was reported<sup>18</sup> (52.8%) ,15 (41.7%), 2 (5.6%) and lower rate with mix IgM and IgG 10 (50%),7 (35%), 3 (15%) as in Table 3. Accurate study agreement with<sup>23</sup> was recorded a higher rate with CMV- IgG, 27 (21.6%) this study disagree with this present study. He reported a higher rate with CMV- IgG 27 (21.6%) (66). Salih *et al.* 2016<sup>24</sup> studied human CMV IgM and IgG seropositive in pregnant women and their relation during an abortion in (185) patients of women in Salaimani city, they noticed 17 (9.18%) were positive anti-CMV- IgG.

**Bacterial vaginosis associated with miscarriage women**

The percentage of bacterial vaginosis in the present study was 103 (93.7%) as in Table 4 some study show a percentage of other non-bacterial vaginosis was (76.3%) There exists a relation between bacterial vaginosis and recurrent spontaneous abortion. According to the findings of this study, the percentage of gram-positive bacteria isolates was 47 (43%) and a percentage of gram-negative bacteria isolates was (63) (57%), which contrasts with the findings of a study to identify bacterial vaginosis in women with recurrent spontaneous abortion, in which, a significantly higher frequency with bacterial vaginosis in women with a recurrent spontaneous abortion (21%) this data is agree with this study. Ralph *et al.*, 2009<sup>24</sup> recorded that women with one late miscarriage

had a significantly greater prevalence ( $p = 0.001$ ) of bacterial vaginosis (21%).

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