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Possible Role of Bacterial and CMV Infections in Miscarriages

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Abstract--- Background: *Miscarriages are considered to be the most common complication in pregnancy. The incidence of miscarriages in clinically established pregnancies (over the sixth gestational week) is approximately 12%–15%. Several possible causes of miscarriage have been considered, the major ones being genetic abnormalities of the fetus. It has been established that more than 50% of miscarried fetuses are genetically abnormal.*

Aim of study: *To determine the role of CMV and bacterial infections in miscarriages in Hilla city.*

Methods: *One hundred thirty (130) samples of blood and high vaginal swabs were collected from married vaginosis women who visited hospital of Babylon city and private clinics with age ranging in from (18 to 47) years. All the Patients and control sera were screening for IgG antibodies to CMV is useful to detect previous exposure to CMV. Cervical swab\secretion was inoculated into Nutrient agar and was incubated at (37^oC) for (24) hours aerobically. The isolates were identified on the basis of colony morphology from colony Gram-staining, and colonies were identified to negative and positive isolates in compacted-vatik-2 system. Some bacteria were detection by PCR technique.*

Results: *The higher concentration of IgG at age group (18-23) and at age group (24-29) about 73.52600± 2.663314 and 55.69333 ± 2.904884 respectively, and absent of IgM level. The highest rate of isolation of bacteria from vaginitis in women with abortions is Lactobacillus in percentage 22(33.84%) followed by Escherichia coli 10(15.38%) Staphylococcus aureus 10(15.38%), Staphylococcus pyogene 7(10.76%), Proteus 5(7.69%), Pseudomonas aerogenosa 4(6.17%), Streptococcus agalactiae 4(6.15%) and Listeria momocytogen 3(4.62%).*

Conclusion: *CMV and bacterial infection were played important role in Miscarriages.*

Keywords--- *Miscarriages, Cytomegalovirus, Bacterial Vaginosis, Bacterial Infection.*

I. INTRODUCTION

Miscarriages are considered to be the most common complication in pregnancy. The incidence of miscarriages in clinically established pregnancies (over the sixth gestational week) is approximately 12%–15% [1]. Recurrent (three or more consecutive) miscarriages are a special problem and result in serious physical as well as psychological damage to women. Unfortunately, recurrent miscarriages occur quite often, in one out of 300 pregnancies [2]. Several possible causes of miscarriage have been considered, the major ones being genetic abnormalities of the fetus. It has been established that more than 50% of miscarried fetuses are genetically abnormal [3]. Genetic and anatomical abnormalities have been proven to be direct causes of miscarriages, whereas intrauterine infections have

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been established as a cause of premature rupture of fetal membranes and preterm labor, but their role in miscarriage is still not completely clear. Several studies have confirmed the role of infections as a cause of miscarriage, especially during the second trimester of pregnancy, but the role of infection in first trimester miscarriages is still questionable [4]. Since the main route of infection to the placenta and fetus is from the vagina and cervix, most studies have attempted to find an association between miscarriage and abnormal bacterial flora in the lower genital tract, vagina, and cervix, respectively. Cytomegalovirus (CMV) is an important opportunistic pathogen in immunocompromised individuals and a frequent cause of congenital infection. Sexual transmission plays an important role in the acquisition of CMV [5]. CMV is a highly complex virus with considerable genomic variation among strains. The amino-terminal region of the envelope glycoprotein gpUL73 (gN) is highly polymorphic, and the genomic variants have been shown to cluster into 4 distinct genotypes [6]. Bacterial vaginosis (BV) is the most prevalent lower genital tract infection in women of reproductive age worldwide [7]. Women with BV typically report symptoms that include a thin vaginal discharge and a fihy malodor [8]. However, a substantial portion of affected women is asymptomatic [9]. The etiology of BV is not completely understood. No single etiological agent is the known cause of BV, and the syndrome is considered an ecological disorder of the vaginal microbiota. BV is characterized by a reduction of lactic acid-producing bacteria (mainly Lactobacillus spp.) and an increase in the number and diversity of facultative and strictly anaerobic bacteria [9, 10]. BV has been associated with the acquisition and transmission of other sexually transmitted viruses, including HIV and herpes simplex virus [11]. Although the acquisition and transmission of CMV is associated with STDs, including BV, the pathogenesis and the exact mechanisms of this relationship have not been defined [7, 12]. To characterize the association between genital tract CMV infection and BV, we analyzed vaginal wash specimens from women attending an STD clinic. In addition, CMV strain diversity in the vaginal wash specimens from the study women was examined by determining the gpUL73 (gN) genotypes. A microorganism needs access to the intrauterine surroundings to cause an inflammatory reaction of endometrium or fetal tissues that is responsible for a miscarriage. Infectious agents present inside the uterus can cause infections between decidual tissue and fetal membranes (amnion and chorion), inside placenta, in the amniotic fluid (amnionitis), or in the umbilical cord (funisitis), infections of the placenta are rare [13]. The main route of infection to the placenta and fetus is from the vagina and cervix, most studies have attempted to find an association between miscarriage and abnormal bacterial flora in the lower genital tract, vagina, and cervix, respectively. *Gardnerella vaginalis* and *Mycoplasma hominis* both cause vaginitis and are often found in mixed infections as a cause of amnionitis, whereas *Ureaplasma urealyticum* alone is found as a cause of amnionitis [14]. These pathogens are also considered as a cause of preterm labor, along with *Escherichia coli*, enterococci, staphylococci, and group B streptococci [15]. A microorganism needs access to the intrauterine surroundings to cause an inflammatory reaction of endometrium or fetal tissues that is responsible for a miscarriage. Infectious agents present inside the uterus can cause infections between decidual tissue and fetal membranes (amnion and chorion), inside placenta, in the amniotic fluid (amnionitis), or in the umbilical cord (funisitis). Infections of the placenta are rare, and the most common causative agents found are *Treponema pallidum*, *Mycobacterium tuberculosis*, *chlamydias*, *mycoplasma*, Rubella virus, human cytomegalovirus (HCMV), herpes simplex virus (HSV), Toxoplasma gondii, and Candida albicans [16].

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Aim of study

To determine the role of CMV and bacterial infections in miscarriages in Hilla city.

II. MATERIALS AND METHODS

Study design

This study was designed as Retrospective (cross-section).

Study setting

The study was carried out in hospital of Babylon city and private clinics.

Period of study

The study conducted between June 2019 to March 2020.

Study samples

One hundred thirty (130) samples of blood and high vaginal swabs were collected from married vaginosis women with age ranging in from (18 to 47) years. The samples were divided in to two groups (65) samples (recurrent miscarriages), and (65) samples as control patients. Serum was separated from all blood samples to Screening for IgG antibodies to CMV by using CMV-specific IgG and IgM ELISA technique The Anti CMV IgG and IgM antibodies concentrations were measured in international unit / ml using standard curve. Vaginal swab was taken after obtaining the permission from the subjects for examination and sampling, patient was rested in lithotomy-dorsal position. Inspection was done for any lesion and vaginal/cervical discharge. Sterile vaginal bivalve speculum was introduced and fixed by the physician, using proper lightening and environment, then three cotton swabs of vaginal discharge were obtained from each woman by brushing a swab across the vaginal wall; as specimens were collected by inserting swab into the high vagina and were rubbed by rotating it. Then swabs were removed carefully to avoid any contact with vaginal secretions. The first swab was used for clinical diagnosis (Amsel's criteria and Nugent criteria), while the second swab was immersed in plain tube-containing 5ml of Brain Heart Infusion broth supplemented with 15% glycerol and frozen immediately at -20°C to be used for molecular diagnosis. The third swab was immediately placed into Amie's Transport Media to be used for the bacteriological diagnosis by culturing and identification of vaginal flora and other pathogenic bacteria. The rest of the specimen was transferred to the Department of Microbiology for further investigations. Wet Film and Smear were prepared from each of the cervical sample. Prepared Smear was stained by Gram staining [17]. Wet Film and the stained smears were searched for observing morphology of relevant organisms and number of pus cell. Cervical swab\secretion was inoculated into Nutrient agar and was incubated at (37^{o}C) for (24) hours aerobically. The isolates were identified on the basis of colony morphology from colony Gram-staining, and colonies were identified to negative and positive isolates in compacted-vatik-2 system. Some bacteria were detection by PCR technique.

Amsel's criteria: according to [18].

Nugent's Criteria: according to [19].

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Enzyme Linked Immuno Sorbent Assay (ELISA)

Was used according to the instruction for the detection of CMV IgG/IgM (BioCheck, Inc. Foster City, CA).

Identification Bacterial isolates with compacted Vitek-2 System

All the isolates were screened and identified via the VITEK-2 System (BioMerieux). This is a phenotypic type of identification, which depends on biochemical reactions to identify the isolates.

Genomic Bacterial DNA Extraction from High Vaginal Swab Samples:

In this study, the collected samples that include high vaginal swabs from patients were subjected to DNA extraction by using G-SpinTM. Total DNA extraction kit (iNtRON/ Korea) and done according to company instruction

Detection of non-cultural bacterial isolated from vaginal swabs by 16sPCR technique

The RNA genes specific primers which are used in PCR for detection of some bacterial isolated by PCR technique [20] and provided from Macrogen Company as shown in the Table (1).

Table 1: DNA genes specific primers which are used in PCR for detection of some bacterial isolated [20]

Bacterium	**Primer Sequence (5' -------- 3')**	**PCR product size**
Gardenrella vaginalis	F-GACGACGGCGAATGGCACGA R-AGTCGCACTCCGCGCAAGTC	331
Mycoplasma hominis	F: CAA TGG CTA ATG CCG GAT ACG C R: GGT ACC TGC AGT CTG CAA T	335
Chlamydia trachomatis	F: GGGATTCCTGTAACAACAAGTCAGG R: CCTCTTCCCCAGAACAATAAGAACAC	207

Table 2: The level of IgG in CMV infections patients miscarriages

Age range	Variables	No.	mean± SD	p-value
18-23	Patient	15	73.52600± 2.663314	**0.0001**
	control	15	2.46800 ± 0.536233	
24-29	Patient	15	55.69333 ± 2.904884	**0.0001**
	control	15	1.6769± 0.51790	
30-35	Patient	15	43.1633± 3.01980	**0.0001**
	control	15	2.2000±0.45670	
36-41	Patient	10	33.7660±2.01036	**0.0001**
	control	10	1.8000±0.56372	
42-47	Patient	10	24.1510±0.44809	0.067
	control	10	0.25940±0.317355	

p-value of ≤ 0.05 was considered as significant

Inclusion criteria

These females were diagnosed by the gynecologist as BV according to the characteristic criteria (Amsel's criteria and Nugent criteria).The sampling was carried out by specialized clinicians and all subjects underwent speculum examination. Each female were underwent-detailed history regarding age, symptoms of infection, gestation age, number of miscarriage, education level, and treatment received.

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Exclusion criteria

In this study, unmarried females, known causes of miscarriage, females with recent usage (within 7 days) of antibiotic treatment ,patients who had history of diabetes mellitus, immune deficiency disease and patients who show negative BV by Amsel's criteria and Nugent criteria are excluded from sampling in the study. Women using intrauterine contraceptive devices and those who used antibiotics or vaginal creams and douching (during the last two weeks) were excluded.

Ethical consideration

The necessary ethical approval from ethical committee of the Hospitals and patients and their followers must obtained. Moreover, all subjects involved in this work are informed and the agreement required for doing the experiments and publication of this work is obtained from each one prior the collection of samples. For every female or her followers, the procedure had been informed before the samples were collected, making absolutely sure that they understood the procedure that is to be carried out. The subjects are sentient that they had the right to reject to be included in the study without any detrimental effects.

Statistical Data analysis

Statistical analysis was carried out using SPSS version 24. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). p-value of ≤ 0.05 was considered as significant.

III. Results And Dissections

The level of IgG and IgM in CMV infections patients miscarriages

Cytomegalovirus infections were being reported to be the causative of abortion in women, the infection was higher in groups of age range from (18-23) and age range (24-29) years old about 73.52600± 2.663314 and 55.69333 ± 2.904884 respectively. The humoral immune profile for these patients were showed seroconvertion ($P \leq 0.05$) in IgG isotype. The absence of IgM antibodies with Positive IgG results, in, most often are indicative of past Cytomegalovirus (CMV) infection and do not necessarily assure protection from future infection with CMV. Data from a Malaysian study [21] showed that anti-CMV IgG antibody was detected in 84% of healthy pregnant women as well as women with adverse pregnancy outcome, including (17) cases of miscarriage. Other study found that, a group of seropositive (59) women with peri-conceptional CMV infection, which occurred between 4 weeks prior to the last reported menstrual period and up to 3 weeks after the expected date of the period. Out of these women, four had miscarriages before undergoing amniocentesis to confirm intrauterine infection. The remaining patients either elected to terminate the pregnancy or gave birth to live infants [22]. CMV infection can result in placental dysfunctions. However, further studies were required to elucidate the true role of CMV in adverse pregnancy out comes. CMV can cause illness in pregnant women and borns. These entire infectious agents induce a shift of immune response during pregnancy from Th2 to Th1 and apoptosis which can be observed clinically as an abortion process [23]. The results of the present study suggest that CMV was facilitated by the presence of genital tract infection due to BV. Significant inflammatory changes in the genital tract have been documented in women

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with BV, and treatment of BV has been shown to result in a decrease in inflammatory cytokine levels in the cervix [24]. CMV is a complex virus with many immunomodulatory activities, and some of the CMV gene products promote local inflammation [25]. The cytomegalovirus causes serious fetal malformations such as intrauterine growth disorder, smallness of the head, intracranial calcification, hearing and vision impairment, etc. The virus travels through body fluids and through sexual contact. The virus can also spread from mother to fetus, the mother was infected at the beginning of pregnancy [26].

Detection of bacterial isolated from vaginal swabs

Detection of bacterial isolated from cultural vaginal swabs by Vitek2 System

This technique is characterized by fast detection of bacteria without need for many of culture media as well as reduces cultural contamination. A total of (56) women diagnosis as suffering from vaginosis according to Amsel's and Nugent criteria were investigated by using Vitek2 System. The result showed that all of 65(100%) samples of miscarriage women were positive for bacterial culture isolates. *Lactobacillus* have been isolated in high percentage 22(33.84%) followed by *Escherichia coli* 10(15.38%) *Staphylococcus aureus* 10(15.38%), *Staphylococcus pyogene* 7(10.76%), *Proteus* 5(7.69%), *Pseudomonas aerogenosa* 4(6.17%), *Streptococcus agalactiae* 4(6.15%) and *Listeria momocytogen* 3(4.62%) as shown in Table (3). This result was agreement with [27] who found that the percentage of *Lactobacillus* spp. was (40%), and agreement with [28] found that the percentage was (32.5%). The percentage of *Lactobacillus* spp. as the predominant in vaginal flora, is an indicator of healthy vagina but its presence in low amounts with a high proportion of other pathogens is pathogenic. They play an important role in protecting against pathogens invasion or overgrowth by production of hydrogen peroxide, bacteriocins, and lactic acid [29]. This result in agreement with result proposed by [30] who found a significant reduction of the proportion of the *Lactobacillus* flora in the first trimester of pregnancy in patients having a history of miscarriage, there was a significant increase in the abundance of opportunistic microorganisms, mostly aerobic flora, such as Enterobacteriaceae, *Streptococcus* spp., and *Staphylococcus* spp. Ascending uterine infection from the lower genital tract due to bacteria has been implicated as an important causative factor for many pregnancy complication one of them spontaneous miscarriage [31]. A number of Enterobacteriaceae members has been isolated in this study. Colonization of the introitus with Enterobacteriaceae species like *E coli K. pneumonia, P. mirabilis* and *Enterobacter* spp. were predisposing factor for urinary tract infection [32].

Genital tracts of pregnant and non-pregnant women represent different environments for propagation of *E. coli*. *P. mirabilis* were more frequently isolated from urine [33]. The percentage of *P. mirabilis* that isolated from urinary tract infection reached to 50 % of *P. mirabilis*, this confirms the fact that *P. mirabilis* play important role in causing UTI and it may reach genital tract [34]. These organisms were also reported as vaginal pathogens by [32]. These bacteria had been isolated especially from women suffering from offensive odour and from vaginal discharge, besides, from non-pregnant women using intrauterine device. It is potentially opportunistic bacteria within the vagina. Such microorganism may become an increasing prevalent upon minor alterations of the vaginal environment. Other investigators have also isolated these bacteria from cases of vaginitis [35].

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Table 3: The level of Igm in CMV infections patients miscarriages

Age range	Variables	No.	mean± SD	p-value
18-23	Patient	15	0.23633 ± 0.002637	**0.0001**
	control	15	1.13467 ± 0.515404	
24-29	Patient	15	0.08513 ± 0.002696	**0.0001**
	control	15	0.8013 ± 0.36829	
30-35	Patient	15	0.3533 ± 0.20307	**0.0001**
	control	15	0.2553 ± 0.03701	
36-41	Patient	10	0.2542 ± 0.00290	**0.0001**
	control	10	0.20000 ± 0.081650	
42-47	Patient	10	0.1560 ± 0.02591	**0.088**
	control	10	0.17780 ± 0.211448	

p-value of ≤ 0.05 was considered as significant

Table 4: Bacterial isolated from cultural vaginal swabs

M.O isolates	**No. of isolates**	**Percentage**
Gram-positive		
Staphylococcus aureus	10	15.38%
Staphylococcus pyogene	7	10.76%
Streptococcus agalactiae	4	6.15%
Listeria momocytogen	3	4.62%
Gram-negative		
Lactobacillus spp.	22	33.84%
Escherichia coli	10	15.38%
Proteus spp.	5	7.69%
Pseudomonas aerogenosa	4	6.17%
Total	**65**	**100%**

Molecular detection of unculturable bacteria by PCR technique

DNA was extracted from all (65) collected high vaginal swab specimens from vaginosis women with miscarriage conventional PCR was carried out using these DNA samples for the amplification of specific 16SrRNA primer of positive indicators for bacterial vaginosis.

Molecular Detection of Gardenrella vaginalis

DNA was extracted from all (65) collected high vaginal swab specimens; conventional PCR was carried out using these DNA samples for the amplification of specific 16SrRNA primer; according to the sequences in Table (1). After that gel electrophoresis showed that, out of the (65) samples, only 22(33.84%) produced the specific (331)

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bp DNA fragment when compared with allelic ladder; as shown in Figure (1). The results of molecular detection for16s RNA gene for [36] showed that (48) out of (60) samples gave positive results to *G. vaginalis* with a specific primer the incidence of *G. vaginalis* was (31.1%). Whereas [37] has (21) positive results only out of (60) samples of *G. vaginalis*. The utility of 16SrRNA gene for clinical microbiology, they compared the efficacy of phenotypic and 16SrRNA identification and its suitability for medical clinical samples. As one PCR primer pair could target the 16S rRNA or even the other gene from a wide range of bacterial species as mentioned by [38]. The current study was similar with study that conducted by [39] who found *G. vaginalis* comprised high percentage (66.67%). Lactobacilli depletion, combined with the presence of either *G. vaginalis* or *A. vaginae*, is also a highly accurate predictor of BV [40]. Also our result similar to study done in Iraq by [41] who revealed that *G. vaginalis* was isolated from preterm labor in percentage (20%).

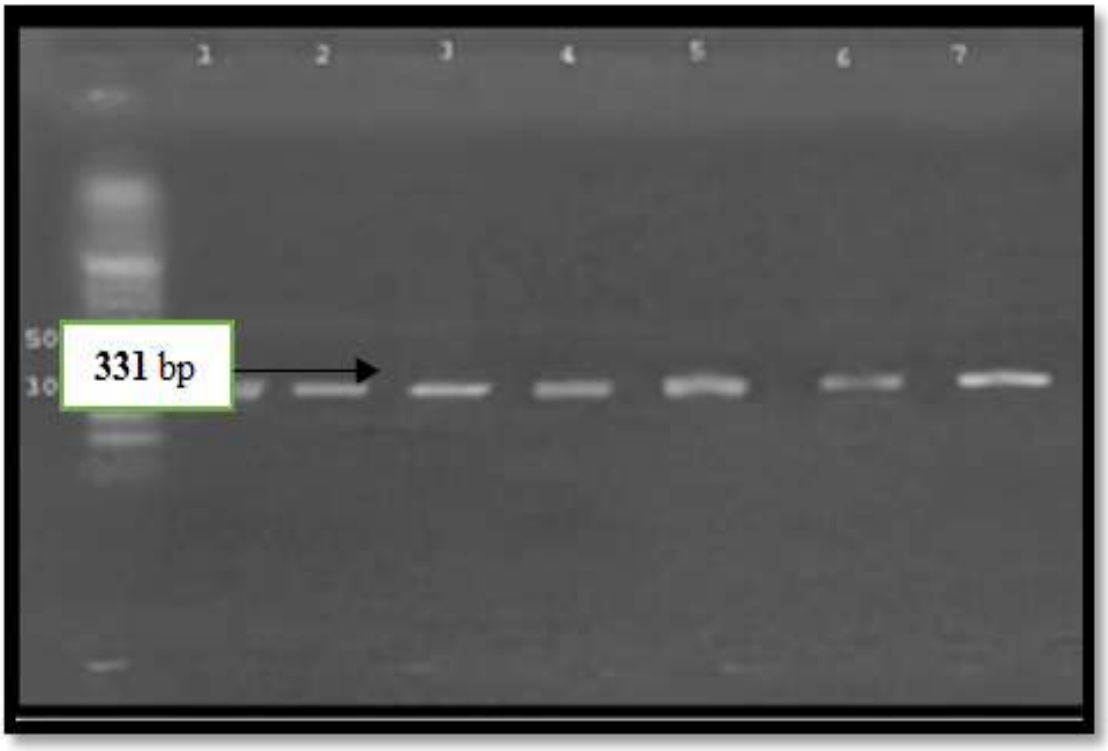

Figure 1: 1% Agarose gel electrophoresis at 70 volt for 50 min for *Gardenrella vaginalis* PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bp ladder; lane (1-10) were positive for this gene, the size of product is 331 bp.

Molecular detection of Mycoplasma hominis

Polymerase chain reaction technique was based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand and, the end of PCR reaction; the specific sequence will be accumulated in billions of copies (Amplicon). DNA was extracted from all suspected isolates. After that gel electrophoresis showed that, out of the (65) samples, only 5(7.69%) produced the specific (335bp) DNA fragment when compared with allelic ladder, as shown in Figure (2). [42] who found that, bacterial vaginosis and the presence

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of *M. hominis* in the vagina were associated with an increased risk of early miscarriage. *M. hominis* has also been found in the placenta and is associated with perinatal morbidity and mortality and preterm labor [43]. However, colonization of the uterine cervix with *M. hominis* is quite common in women who have not experienced any problems during pregnancy, so its possible role in the induction of miscarriage is questionable [44].

Figure 2: 1% Agarose gel electrophoresis at 70 volt for 50 min for *Mycoplasma hominis* PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bp ladder; lane (1-10) were positive for this gene, the size of product is 335 bp.

Molecular detection of Chlamydia trachomatis

Molecular detection of *Chlamydia trachomatis* was carried out by using a specific PCR primer were done by comparison with allelic ladder which gave a (207bp) It was found that *Chlamydia trachomatis* present in 4(6.15%) positive samples out of (65) samples as shown in Figure (3). *C. trachomatis* is the most frequent cause of cervicitis. They can ascend to the uterus and ovaries and possibly by vertical transmission to the placenta and fetus. There are indications of the involvement of *C. trachomatis* in the development of miscarriage, however, there is not enough evidence to confirm them [45]. *C. trachomatis* infects placental trophoblast membrane cells *in vitro*. *C. trachomatis* plays a significant role in the induction of most miscarriages [46]. *Chlamydia trachomatis* could provoke miscarriage because of a strong immunological reaction to the specific bacterial protein. Moreover, *C. trachomatis* infections of the cervix have been associated with second trimester abortions and premature membrane rupture [47].

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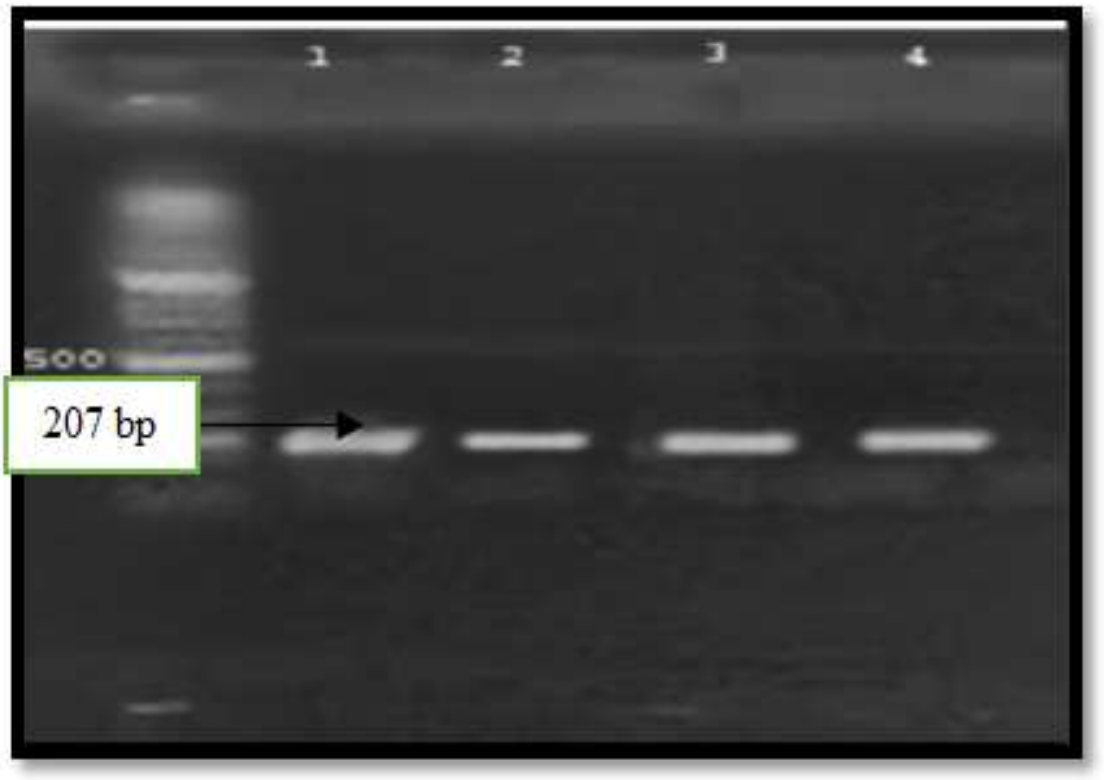

Figure 3: 1% Agarose gel electrophoresis at 70 volt for 50 min for *Chlamydia trachomatis* PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bp ladder; lane (1-10) were positive for this gene, the size of product is 207 bp.

IV. CONCLUSION

CMV and bacterial infection were played important role in Miscarriages

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