ORIGINAL ARTICLE
Bacterial Vaginosis and *C. trachomatis* as Risk Factors for First Trimester Miscarriage

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ABSTRACT

Background: *Miscarriage is the most common adverse outcome of pregnancy. Bacterial vaginosis (BV) is known as alteration of vaginal milieu.* C. trachomatis *is intracellular, sexually transmitted bacteria. Objectives: we aimed to investigating the role of BV and* C. trachomatis *in 1st trimester miscarriage.* ***Methodology:*** *This study included 95 pregnant women with gestational age < 14 weeks. For each subject we investigate BV by Amsel's criteria and Nugent scoring. Anti chlamydial IgG antibodies were detected using qualitative ELISA.* C. trachomatis *DNA of cryptic plasmid were detected in urine sample by polymerase chain reaction.* ***Results:*** *BV was not risk factor for 1st trimester miscarriage, however it was significantly associated with smoking (*P= 0.001*), vaginal douching (*P= 0.001*), intrauterine device (*P= 0.001*) and history of preterm labor (*P= 0.002*).* C. trachomatis *infection was significantly associated with 1st trimester miscarriage (*P= 0.001*), hormonal contraception (*P= 0.03*) and BV (*P= 0.004*). Being older than 30 years was risk factor for miscarriage.* ***Recommendations:*** *We recommend screening of BV and* C. trachomatis *preconception and in pregnant women for early diagnosis and treatment and to prevent recurrent miscarriage or preter.*

INTRODUCTION

Miscarriage is the most common adverse outcome of pregnancy. Miscarriage is spontaneous loss of pregnancy during first 24 weeks. Miscarriage is linked with both psychological and physical complications.

Chlamydia trachomatis and bacterial vaginosis (BV) are sexually transmitted diseases.

Bacterial vaginosis is known as alternation of vaginal ecology. Normally Lactobacilli are dominant flora in vagina. In BV Lactobacilli are replaced by mixed bacterial species such as *G. vaginalis*, Mobiluncus spp, *Mycoplasma homonis*, bacteroides spp. and other anaerobes [1]. BV can be diagnosed by examination of vaginal discharge by direct microscopy of gram stained smear and by aerobic and anaerobic cultures [2].

Infections with *C. trachomatis* in women often produce no symptoms. Clinical conditions of *C. trachomatis* infections vary from cervicitis with mucopurulent discharge, urithritis and pelvic inflammatory diseases. *C. trachomatis* is also considered a risk factor of tubal infertility, ectopic pregnancy, preterm labor and premature rupture of membrane [3]. Its diagnosis could be carried out by detection of its different and specific antigens or nucleic acid in cervical samples, urine or output of conception. Anti chlamydial antibodies can be detect in serum by serological tests, However is not recommended in condition of infections of lower genital tract or in asymptomatic cases. The strict intracellular life cycle of *C. trachomatis* makes its culture difficult, requiring tissue culture techniques [4].

Previous studies on BV reported its association with late miscarriage (from 12 to 24 weeks) and preterm labor [5]. For *C. trachomatis* previous studies investigated its role in preterm labor and premature rupture of membrane [6]. However the role of BV and *C. trachomatis* in first trimester miscarriage (before 14 weeks of gestation) is not sufficiently investigated in healthy pregnant women. The aim of our study was to compare the incidence of 1st trimester miscarriage in women with normal vaginal flora against women with BV and to investigate the role of *C. trachomatis* infections in 1st trimester miscarriage.

METHODOLOGY

This study is a cohort study including all pregnant women in 1st trimester who attending Antenatal Care Clinic of Zagazig University Hospitals during the year 2016.

Patients:

This study included 95 pregnant women (18- 38 years old). Gestational age was calculated (< 14 weeks). Written informed consents were given by all participants. The protocol approved by the institutional review board (IRB).

Exclusion criteria:

- Multiple gestations.
- Predisposing conditions of abortion such as the following medical conditions: diabetes mellitus,

chronic hypertension, endocrinal diseases, autoimmune diseases, renal diseases, blood diseases, heart diseases, cervical incompetence or cerculage.

- Patients who receive antimicrobial therapy 2 weeks before sampling.

Full history was taken from all participating women, general and abdominal examinations, vaginal examination and ultrasound examination.

Sampling:

From each subject we obtained: High vaginal swabs for diagnosis of BV, blood sample for serum separation and detection of *anti- C. trachomatis* IgG and first voided urine (FVU) sample for extraction of *C. trachomatis* nucleic acid and amplification by PCR.

All samples were delivered and processed in Medical Microbiology and Immunology department, Faculty of Medicine, Zagazig University.

Diagnosis of bacterial vaginosis:

- Using sterile, disposable and not lubricated speculum, vaginal pH was measured using (color pHast, MCB Reagents, Gibbstown, N.J.). Sterile cotton swabs were used to obtain material from posterior vaginal fornix, and then spread on slides. Smears were used for Wiff test (amine test), wet mount and Gram stain.
- Bacterial vaginosis was diagnosed using Amsel's composite criteria [7]. Cases which gave positive results with Amsel's criteria were then scored by Nugent's scoring [8].
- Amsel criteria: BV is diagnosed when fulfilling three of four of the following:
  - Vaginal fluid pH $\leq$ 4.6.
  - Thin gray and homogenous vaginal discharge.
  - Positive Whiff test (amine test).
  - Presence of clue cells in wet mounts.
- Nugent's scoring 7 or higher are considered diagnostic for BV[8].

Detection of anti- C. trachomatis IgG in serum by ELISA:

For Diagnosis of past or chronic *C. trachomatis* infection we detected the presence of anti chlamydial IgG antibodies in pregnant women's serum. This is carried out using qualitative ELISA [RIDASCREEN *Chlamydia trachomatis* IgG] kits (R- Biopharm, Germany). This kit detected a specific antibody against highly conserved complexes of outer membrane protein (COMP) of *C. trachomatis.*

Interpretation of the results:

- We calculated the average absorbance of cut- off control.
- We calculated sample index from the equation:

$$\text{Sample index} = \frac{\text{Absorbance of sample}}{\text{The calculated average value}}.$$

- The sample index was evaluated as: Negative if sample index < 0.9, equivocal if sample index 0.9-1.1 and positive if sample index > 1.1.

Detection of C. trachomatis DNA in urine specimens:
For the diagnosis of current *C. trachomatis* infection we examined first voided urine samples for the presence of chlamydial DNA using PCR technique.

- Urine samples were obtained a septicale centrifuged and separate the pellet and stored at 20°C until DNA extraction and amplification were performed[9&10].
- DNA extraction: *C. trachomatis* nucleic acid was extracted using the commercially available kits [QIA amp viral RNA kit] from QIAgen- Germany). DNA extraction was done following manufacturer instruction using spin column protocol. After extraction, DNA was stored at - 70°C till amplification steps were carried out.
- PCR amplification was done using the primers pair PC_{24} and $P\ C_{27}$ [11]. This primer is designed to amplify 207 bp within the cryptic plasmid of *C. trachomatis*:
  - PC_{24} :(5`GGG ATT CTT GTA ACA ACA AGT CAG G3 `)
  - PC_{27} :(5`CCT CTT CCC CAG A AC AAT AAG AAC AC 3`)

Reaction mixture: The volume was100 ul: 1 xPCR buffer, 2.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate, 2.5U Taq DNA polymerase (Prom ega, Mad ison WI, USA), 50 Pmole of each primer. Ten micro liters of extracted DNA were added to the reaction tube. Amplification conditions: An initial denaturation step for 5 min at 94° C followed by 1min of denaturation at 94° C for 35 cycles, annealing at 60° C for 1 min, then primer extension at72° C for 1 min. Finally, cooling at 4° C to stop the reaction.

Each amplification run contained negative and positive controls. Positive control: *C. trachomatis* cryptic plasmid DNA, was kindly provided from Egyptian company for production of Vaccines, Sera & Drugs (VCSERA). It was added to reaction tube instead of extracted DNA to test for the presence of inhibitors that might interfere with amplification. Negative control: PCR reaction was performed using Dnase/Rnase free water instead of the template DNA. After electrophoresis samples positive for chlamydial DNA showed bands of size 207 bp (fig. 1).
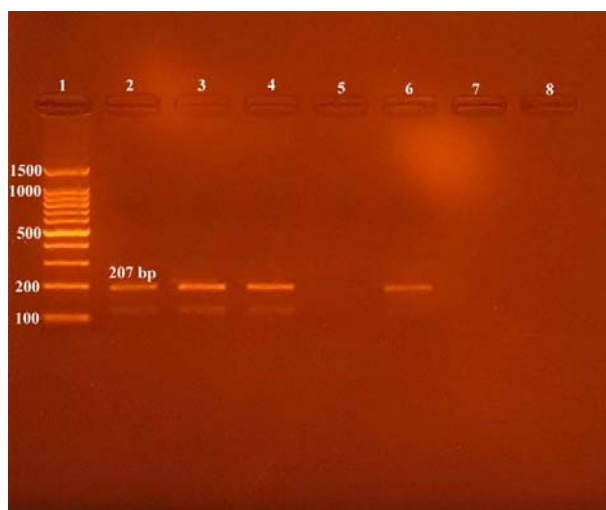
Fig. 1: Agarose gel electrophoresis: lane 1: DNA ladder, lanes 1,2 and 3: positive cases showing bands at 207 bp, lane 5: negative case, lane 6: positive control and lanes 7&8: negative control.

Statistical analysis:

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 18.0.Qualitative data were represented as frequencies and relative percentages. To calculate difference between qualitative variables, Chi square test and fisher exact test were used. Odds ratio was calculated to estimate risk among different variables. Quantitative data was expressed as mean and SD.

RESULTS

This study included all the pregnant women in 1st trimester who attending Antenatal Care Clinic of Zagazig University Hospitals during the year 2016. They was 95 women, with age range 18-38 (Mean ± SD=27.8±5.78). Eighteen women (18.9%) had 1st trimester miscarriage during the follow up period. As regard duration of pregnancy in miscarriage women its range was 3- 12 week and the mean ±SD was 6.94 ± 2.71 weeks (Table 1). Missed abortion was the most common type of abortion representing 50% of cases of miscarriage (9/18) (fig. 2).

Table 1: Prevalence of 1st trimester miscarriage in the studied group:

	Variable	*(n=95)*
Miscarriage	*No N (%)*	77 (81.1%)
	Yes N (%)	18 (18.9%)
Type:	*Missed N(%)*	9 (9.5%)
	Incomplete N(%)	4 (4.2%)
	Inevitable N(%)	3 (3.2%)
	Complete N(%)	2 (2.1%)
Weeks of	*Mean ± SD*	6.94 ± 2.71
abortion	*Range*	3 – 12

Fig. 2: Type of 1st trimester miscarriage in the studied group

Table 2 shows the demographic, history and clinical data of pregnant women with and without Bacterial vaginosis (BV). By using Amsel's criteria 29 pregnant women were positive for BV. Five of them were Nugent's scoring less than 7, and thus were considered negative for BV. We categorized educational level as poor if six years or less of education, fare if 6-12 years and good if high educated women. From table 2 we noticed that poor education was highly statistically significant associated with BV with OR = 3.48(1.30-9.25) and *P*=0.004. Also smoking and vaginal douching were risk factors of BV with highly statistically significant difference (*P*<0.001 for both). As regard the methods used for contraception, IUD was significantly associated with BV with OR = 5.1(1.89-13.78) and *P*= 0.001. On comparing the presence of past history of preterm labor between women with BV and those without BV, it was significantly associated with BV *P* = 0.002. *C. trachomatis* DNA or recent infection was significantly present in women with BV *P* < 0.001. We found no significant association between BV in pregnant women and age groups, number of pregnancies, 1st trimester miscarriage or previous *C. trachomatis* infections.

Table 2: Relation between Demographic, history and clinical data of the studied group and Bacterial vagiosis (BV):

Variable		No BV (n=71)		BV (n=24)		P	OR (95%CI)
		N	%	N	%		
Age group	< 30 years	41	57.7	16	66.7	0.44	0.68(0.26-1.80)
	≥ 30 years	30	42.3	8	33.3		
Education	Poor	18	25.4	13	54.2	**0.004****	**3.48(1.30-9.25)**
	Fare	25	35.2	6	25	0.36 NS	1.63(0.58-4.63)
	Good	28	39.4	5	20.7	0.09 NS	2.47(0.83-7.39)
Smoking	No	70	98.6	18	75	**<0.001****	**23.3(2.64-60.3)**
	Yes	1	1.4	6	25		
Vaginal douche	No	64	90.1	8	33.3	**<0.001****	**18.3(5.77-7.92)**
	Yes	7	9.9	16	66.7		
Contraception	No	25	35.2	4	16.7	0.08 NS	0.37(0.11-1.20)
	Hormonal	22	31	3	12.5	0.07 NS	0.32(0.09-1.18)
	IUD	20	28.2	16	66.7	**0.001****	**5.1(1.89-13.78)**
	Barrier	4	5.6	1	4.2	0.78 NS	0.73(0.08-6.85)
No. of pregnancies	Primi	21	29.6	6	25	0.68 NS	1.26(0.44-3.62)
	1 – 2	32	45.1	14	58.3	0.26 NS	0.59(0.23-1.5)
	3 – 4	18	25.4	4	16.7	0.38 NS	1.70(0.52-5.63)
History of preterm	No	64	90.1	15	62.5	**0.002****	**5.5(1.76-17.09)**
	Yes	7	9.9	9	37.5		
Miscarriage	No	57	80.3	20	83.3	0.74	0.81(0.24-2.77)
	Yes	14	19.7	4	16.7	NS	
Type	Missed	7	9.9	2	8.3	0.83 NS	0.83(0.16-4.3)
	Incomplete	3	4.2	1	4.2	0.99 NS	1(0.10-9.94)
	Inevitable	2	2.8	1	4.2	0.74 NS	1.5(0.13-17.32)
	Complete	2	2.8	0	0	0.41 NS	------
***C. trachomatis* IgG**	-ve	49	69	18	75	0.58	0.74(0.26-2.13)
	+ve	22	31	6	25	NS	
***C. trachomatis* DNA**	-ve	70	98.6	17	70.8	**<0.001****	**28.82(3.32- 0.25)**
	+ve	1	1.4	7	29.2		

* Significant.   ** Highly significant.

Table 3 presents relation between demographic, history and clinical data of pregnant women and *C. trachomatis* infection. In this study we diagnosed previous or chronic *C. trachomatis* infection by qualitative assay of anti chlamydial IgG antibody in serum samples. However current infection is diagnosed by detecting chlamydial DNA in first voided urine samples. From table 3 we noticed that *C. trachomatis* infection was significantly associated with hormonal contraceptive methods with OR= 2.78 (1.09- 7.09) and *P*=0.03. Thirty two pregnant women out of 36 (88.9%)

having *C. trachomatis* infection were asymptomatic *P*= 0.04. From table 3 we found that BV is significantly associated with *C. trachomatis* infection *P*= 0.004. Also *C. trachomatis* infection is risk for 1st trimester miscarriage with OR= 6.10(1.95- 19.11) and *P*= 0.001. As regard type of miscarriage, incomplete abortion was significantly found with *C. trachomatis*. We found no significant association between *C. trachomatis* infection and age groups, smoking, vaginal douching or history of preterm.

Table 3: Relation between Demographic, history and clinical data of the studied group and *C. trachomatis*:

| Variable		No *C. trach.* (n=59)		*C. trachomatis* (n=36)		P	OR (95%CI)
		N	%	N	%		
Age group	*< 30 years*	33	55.9	24	66.7	0.30	0.63(0.27-1.50)
	≥ 30 years	26	44.1	12	33.3	NS	
Education	*Poor*	15	25.4	16	44.4	0.06 NS	0.43(0.18-1.03)
	Fare	18	30.5	13	36.1	0.57 NS	0.78(0.32-1.87)
	Good	26	44.1	7	19.4	0.07 NS	0.64(0.23-1.63)
Smoking	*No*	56	94.9	32	88.9	0.28	2.33(0.49-11.09)
	Yes	3	5.1	4	11.1	NS	
Vaginal douch	*No*	44	74.6	28	77.8	0.72	0.84(0.31-2.23)
	Yes	15	25.4	8	22.1	NS	
Contraception	*No*	20	33.9	9	25	0.36 NS	1.54(0.61-4.03)
	Hormonal	11	18.6	14	38.9	**0.03***	**2.78(1.09-7.09)**
	IUD	25	42.4	11	30.6	0.25 NS	1.67(0.70-4.02)
	Barrier	3	5.1	2	5.5	0.92 NS	(0.15-5.73)ʼ0.9
No. of pregnancy	*Primi*	18	30.5	9	25	0.56 NS	1.32(0.52-3.36)
	1 – 2	25	42.4	21	58.3	0.13 NS	0.52(0.23-1.22)
	3 – 4	16	27.1	6	16.7	0.24 NS	1.86(0.65-5.30)
History of preterm	*No*	50	84.7	29	80.6	0.60	1.34(0.45-3.98)
	Yes	9	15.3	7	19.4	NS	
Symptoms of chalmydia	*No*	58	98.3	32	88.9	**0.04***	**7.25(1.78-67.65)**
	Yes	1	1.7	4	11.1		
BV	*No*	50	84.7	21	58.3	**0.004****	**3.97(1.50-10.48)**
	Yes	9	15.3	15	41.7		
Miscarriage	*No*	54	91.5	23	63.9	**0.001****	**6.10(195-19.11)**
	Yes	5	8.5	13	36.1		
Type of abortion	*Missed*	2	3.4	7	19.4	**0.01***	**6.88(1.34-35.25)**
	Incomplete	0	0	4	11.1	**0.009****	---
	Inevitable	1	1.7	2	5.6	0.30 NS	3.41(0.30-39.05)
	Complete	2	3.4	0	0	0.26 NS	-----

* Significant.   ** Highly significant.

Table 4 shows the relation between demographic, clinical and history data and 1st trimester miscarriage in the studied group. Being older than 30 years is a risk factor of 1st trimester miscarriage with OR = 3.92 (1.32-11.64) and P = 0.01. Past history of preterm labor is significantly associated with 1st trimester miscarriage with $P < 0.001$. We also found that both *C. trachomatis* serological tests (previous infection) and molecular diagnosis (current infection) were risk factors for 1st trimester miscarriage, with P= 0.001 and 0.01 respectively.

Table 4: Demographic, clinical and history data of the studied group and relation between these factors and 1ˢᵗ trimester miscarriage:

Variable		Total (n=95)	No miscarriage (n=77)	Miscarriage (n=18)	P	OR (95%CI)
Age group	*< 30 years*	57 (60%)	51 (66.2%)	6 (33.3%)	**0.01***	**3.92(1.32-11.64)**
	≥ 30 years	38 (40%)	26 (33.8%)	12(66.7%)		
Education	*Poor N (%)*	31 (32.6%)	24 (31.8%)	7 (38.9%)	0.53 NS	0.71 (0.25-2.06)
	Fare N (%)	31 (32.6%)	26 (33.8%)	5 (27.8%)	0.63 NS	1.33(0.43-4.12)
	Good N (%)	33 (34.8%)	27 (35.1%)	6 (33.3%)	0.89 NS	1.08(0.36-3.20)
Smoking	*No N (%)*	88 (92.6%)	73 (94.8%)	15 (83.3%)	0.09	3.65(0.74-18.02)
	Yes N (%)	7 (7.4%)	4 (5.2%)	3 (16.7%)	NS	
Vaginal douche	*No N (%)*	72 (75.8%)	59 (76.6%)	13 (72.2%)	0.70	1.26(0.40-4.02)
	Yes N(%)	23 (24.2%)	18 (23.4%)	5 (27.8%)	NS	
Contraception	*No N (%)*	29 (30.5%)	25 (32.5%)	4 (22.2%)	0.40NS	0.59(0.18-1.99)
	Hormonal N (%)	25 (26.3%)	19 (24.7%)	6(33.3%)	0.45 NS	1.53(0.50-4.62)
	IUD N (%)	36 (37.9%)	29 (37.7%)	7 (38.9%)	0.92 NS	1.05(0.37-3.02)
	Barrier N (%)	5 (5.3%)	4(5.2%)	1(5.6%)	0.95 NS	1.07(0.11-10.23)
No. of pregnancy	*Primi N (%)*	27 (28.4%)	22 (28.6%)	5 (27.8%)	0.95 NS	1.04(0.33-3.26)
	1 – 2 N (%)	46 (48.4%)	37 (48.1%)	9 (50%)	0.88 NS	0.92(0.33-2.58)
	3 – 4 N (%)	22 (23.2%)	18 (23.4%)	4 (22.2%)	0.92 NS	1.07(0.31-3.66)
History of preterm	*No N (%)*	79 (83.2%)	69 (89.6%)	10 (55.6%)	**<0.001****	**6.9(2.11-22.53)**
	Yes N (%)	16 (16.8%)	8 (10.4%)	8 (44.4%)		
BV	*-ve N (%)*	71 (74.7%)	57 (74%)	14 (77.8%)	0.74	0.81(0.24-2.77)
	+ve N (%)	24 (25.3%)	20 (26%)	4 (22.2%)	NS	
***C. trachomatis* IgG**	*-ve N (%)*	67 (70.5%)	60 (77.9%)	7 (38.9%)	**0.001****	**5.55(1.87-16.5)**
	+ve N (%)	28 (29.5%)	17 (22.1%)	11 (61.1%)		
***C. trachomatis* DNA**	*-ve N (%)*	87 (91.6%)	74 (96.1%)	13 (72.2%)	**0.01***	**9.49(2.02-44.6)**
	+ve N (%)	8 (8.4%)	3 (3.9%)	5 (27.8%)		

* Significant.   ** Highly significant.

Figure (3) demonstrated that the prevalence of BV was 25.3% and *C. trachomatis* was 37.9% in the studied group.
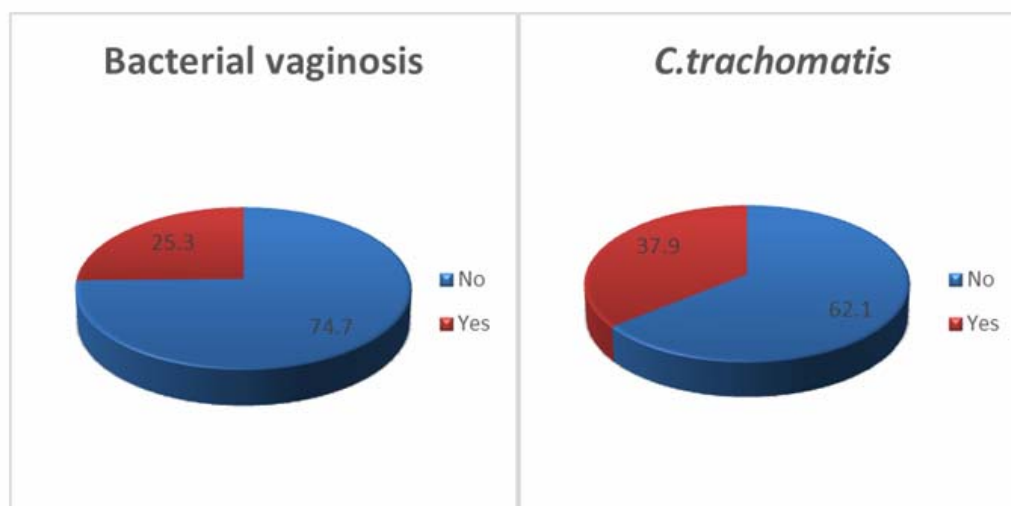
Fig. 3: Prevalence of bacterial vaginosis and *C. trachomatis* in the studied group

DISCUSSION

Miscarriage is inevitable spontaneous loss of pregnancy during first 24 weeks. Early miscarriage occurs within 12 weeks of pregnancy. Fetal loss causes considerable morbidity for women. Early diagnosis and treatment of the causes of miscarriage are important to avoid such maternal harmful[12].

Most of previous studies have been concerned with the role of BV and *C. trachomatis* in preterm labor and fetal birth weight. However the role of BV and *C. trachomatis* in 1st trimester miscarriage still needs more investigations.

In this study we examined the association between BV and *C. trachomatis* infections and first trimester miscarriage.

BV exists when Lactobacilli, the normal vaginal colonizing flora, are substituted with anaerobic bacteria, *G. vaginalis*, group B streptococci, *Mycoplasma homonis* or *Ureaplasma urealyticum* [13].

In our study, we used two approved methods for diagnosis of BV. First method was the composite Amsel's clinical criteria. We found some difficulties on carrying out these methods such as determination of the color and consistency of the discharge as well as to judge the fishy odor. Thus we proceed to the second method which is Nugent scoring by Gram stain. Both methods are simple, acceptable and reliable for diagnosis of BV [14]. More over Kurki and his colleagues[2] concluded in their study that all cases of culture diagnosed BV could be detected by Gram stain.

The BV was found in 24/ 95 (25%) of pregnant women included in this study , this prevalence come in accordance with Ralph et al. study [15].

We found relation between BV and practicing vaginal douching *(P<0.001)* similar to a previous study[14]. Douching may alter vaginal pH by destruction of Lactobacilli.

As regard methods of contraception, we found statistically significant association ($P = 0.001$) between using IUD and BV, as in another study [16].

Among the 95 women included in this study 7 women gave history of smoking and 6 of them had BV. This was statistically significant ($P < 0.001$) similer to a previous study [14].

Smoking leads to accumulation of large amount of (amines) like nicotine and cotinine in the vagina and cervical secretions that cause disturbance of vaginal flora [17].

On comparing the rate of 1st trimester miscarriage between women with and without BV, we found no statistically significant diffecrence ($P= 0.74$). This is similar to Oostrum et al. study on naturally conceived women [18]. In contrast to our finding Donder and his colleagues[19] reported that BV before 14 gestational weeks predicted early miscarriage in 36% of pregnant.

In our study BV was significantly present in women with past history of preterm labor ($P = 0.002$). This agreed with previous studies [20&21]. Several published theories can explain the relationship between BV and preterm delivery. First theory, has suggested that bacteria causing BV destroy connective tissue of fetal membranes by producing proteases that leads to premature rupture of membrane. Another theory proposes that bacteria may also produce mucinase and sialidase that destroy the protective cervical mucosa permitting infection to ascend to uterus and amniotic fluid [5]. Also, prostaglandins, endotoxines and interlukin-1a, were found in high concentration in vaginal and cervical secretion in pregnant women with BV[22].

C. trachomatis is sexually transmitted bacteria. It is intracellular bacteria depending on host cell ATP. *C. trachomatis* infection in women is usually asymptomatic or produces minimal symptoms [23].

In our study we used qualitative ELISA assay to detect serum antibodies to highly conserved complexes of outer membrane protein of *C. trachomatis*. Serological testing is useful in diagnosis of previous or chronic infections and in seroepidemiological studies [4]. To diagnose asymptomatic or current *C. trachomatis* infection we used PCR as rapid, accurate and reliable test to detect the DNA. PCR was done on; first voided urine sample (FVU).Urine samples are noninvasive, simply collected specimens and cause no discomfort to women. Moreover Rours and his colleagues[24] declared that female urine is almost as good as an endocervical swab for detection of female genital tract infection with *C. trachomatis*. We chose *C. trachomatis* cryptic plasmid as target DNA for amplification because there are 10 copies of it per elementary body; thus the sensitivity of our procedure is strongly increased [25].

C. trahomatis was detected in 36/ 95 (37.9 %) of pregnant women. Authors of previous Egyptian studies reported the prevalence of *C. trachomatis* infection range from 15 to 45% depending on the method of diagnosis [26&27]. Another study in Arab world was carried by Al- Hindi and his colleagues on Palestinian women[28]. In their study they determined the seroprevalence of *C. trachomatis*. It was 12.8% a lower than that in this study. This difference is attributed to including both infertile and aborted women as well as estimating anti chlamydia IgM antibodies in their study.

According to our study younger age is not a risk factor for *C. trachomatis* infections. We found no statistical significant association between *C. trachomatis* infection and age groups ($P = 0.30$). In contrast many previous studies declared that age less than 25 years is risk factor of *C. trachomatis* infection. Their findings could be attributed to some factors; getting first coitus at early age, changing and having new sexual partners[29], practicing sexual intercourse with more than one sexual partner and having coitus without

using of condoms [30&31]. These factors are not common in our community due to religious and cultural believes and thus could explain the difference between our finding and theirs.

We found significant association between *C. trachomatis* infection and BV with OR = 3.97(1.50-10.48) and *P*= 0.004. This agreed with Rours and his colleagues, who suggested that previously diagnosed or present STD is risk factor for *C.trachomatis* infection[31]. Also, Menon and his colleagues concluded that *C. trachomatis* infection risk increases with BV [32]. This can be explained by two hypotheses. The first considered B.V. as predictor for *C. trachomatis* infection. As Lactobacilli produce H_2O_2 and confer acidic vaginal pH which plays a protective roles and antichlamydial activity[33]. The second hypothesis was given by Nelson and his colleagues, they declared that organisms in BV produce indole which enables *C. trachomatis* to produce amino acid tryptophan and subsequently evade the immunological activity of INF-γ and thus promote the infection [34].

We found statistically significant correlation between *C. trachomatis* infection and oral or hormonal contraception (*P* = 0.03). Barnes and his colleagues declared that, the quantitative culture of *C. trachomatis* from endocervical samples was higher in women using oral or hormonal contraceptive methods [35], such finding supports our result.

According to our results, *C. trachomatis* previous infection was a risk factor for miscarriage in first trimester with OR = 6.10 (1.95- 19.11) and this was highly statistically significant as *P* = 0.001, also current infection is significantly associated with early miscarriage (*P*= 0.01). This finding agrees with previous studies [36-37]. This finding indicated that 1st trimester miscarriage might be due to previous or current *C. trachomatis* infection. Two mechanisms were published to explain our findings. The first mechanism is concerned with chronic or previous *C. trachomatis* infection and related to a protein named heat shock protein 60 (Hsp60). This protein is highly conserved, 60 kD protein and presents both in Chlamydia (cHsp60) and human (hHsp60). About 50% of amino acids sequence is shared between hHsp60 and cHsp60. Human hsp60 is expressed during pregnancy on the surface of embryo and maternal decidiua [38]. Chronic *C. trachomatis* infection would promote prolonged exposure to cHsp60 of bacteria. That would lead to an immunological reaction and the production of antibodies against the conserved epitope of Hsp60. That in turn causes autoimmunity against human Hsp60 due to molecular mimicry. Autoimmunity reaction will lead to early destruction of emberyo[32]. The second mechanism was declared by Vagil and his colleagues. This mechanism concerned with current *C. trachomatis* infection. They referred that to either infected zygot or infected early emberyo. They proposed that infection of zygot results from infected spermatozoon which would transmit the organism to oocyte. They suggested also that early embryo might become infected from fallobian tube or from inside uterus. The infected zygot or infected early emberyo will result in lysis of the recent conception and early loss of pregnancy [39].

Among 95 women included in this study 18 (18.9 %) women had 1st trimester miscarriage, similer to Everttе [40], who reported 1st trimester abortion rate of 15-20%. According to our study risk factors of 1st trimester miscarriage were being ≥ 30 years, previous or current *C. trachomatis* infections.

We concluded that the risk of BV to cause loss of pregnancy is related to the gestational age and it is not predictor of 1st trimester miscarriage. However *C. trachomatis* infection is a risk factor of 1st trimester miscarriage.

Recommendations:
- Screening of B.V and *C.trachomatis* antenatal or preconception to reduce adverse pregnancy outcome.
- Screening of *C.trachomatis* in all women experienced miscarriage to provide suitable treatment to avoid recurrent miscarriage.
- Quantitative detection of antibodies against Hsp60.

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