

Bacterial Vaginosis in Patients with Threatened Midtrimester Miscarriage

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Abstract:

Background: Miscarriage when fetus or embryo dies in the uterus during pregnancy .threatend miscarriage is one of the types of miscarriage that is associated with high potential for pregnancy loss. Bacterial vaginosis (BV) in pregnancy is a risk factor for various types of complications in pregnancy, including premature rupture of membranes, Second trimester miscarriage and Post C/S endometritis

Aim of the Study: Is to determine the relationship between bacterial vaginosis infection and second trimester threatened miscarriage.

Method: Case-control study involved 200 pregnant women in the second trimester of pregnancy, their gestational age ranged from(13-24 weeks) , 100 of them presented with lower abdominal pain and history of vaginal bleeding,by examination closed cervical os with ultrasound finding of normal viable fetus, diagnosed as threatened miscarriage (study group) . The other 100 women were healthy pregnant women (control group) . History taken, General & abdominal examination was done followed by vaginal examination. Diagnosis of bacterial vaginosis depen on Amsel criteria.

Results: The result indicated that 61% of patients with mid trimester threatened miscarriage have bacterial vaginosis according to Amsels' criteria. The most presenting symptoms in patients are vaginal discharge and abdominal pain. The most specific criterion is the whiff test, while the most sensitive one was the homogenous vaginal discharge, 94.8% & 96.7%, respectively. Lastly Threatened mid trimester miscarriage group, positive diagnosis by Amsel's criteria showed total 61 (61%) while control group showed 1%

Conclusions: Bacterial vaginosis consist about 61 % of patients with second trimester threatened miscarriage .. Multiparous women are more prone for bacterial vaginosis infection than primigravida .The most common symptoms that indicates bacterial vaginosis infection in pregnant females are abdominal pain and vaginal discharge respectively. Clue cells and homogenous vaginal discharge are more prevalent than vaginal PH and Whiff test.

Keywords: Bacterial Vaginosis, Threatened Miscarriage, Second Trimester Pregnancy, Midtrimester Miscarriage, Amsel Criteria, Pregnancy Loss, Vaginal Discharge, Clue Cells.

Introduction: Miscarriage is a medical term for a pregnancy loss before 20 weeks of gestation. Spontaneous miscarriage includes threatened, inevitable, incomplete, complete, septic, and missed miscarriage (1).the Royal college of obstetrics and gynecologist define miscarriage as spontaneous loss of

pregnancy before the fetus reaches viability. the term therefore includes all pregnancy loss from the time of conception until 24 weeks of gestation(2). . The definition of early miscarriage is defined by the Royal College of Obstetrics and Gynecologist (RCOG 2006) as loss within the first 12 completed weeks of pregnancy

(2).

A fetus or embryo that is expelled from the uterus before 20 weeks of gestation or with a birth weight of less than 500 g is said to have experienced a miscarriage. A reasonably frequent pregnancy problem that affects 20% of all pregnancies is threatened miscarriage (3).

The incidence of miscarriage varies depending on the gestational age and maternal age. Up to 50% of early pregnancies will fail within 4 weeks from the last menstrual period (LMP) so-called biochemical pregnancies. By 6 weeks gestation, the rate is one in five pregnancies and by the second trimester it falls to 1 in 40 (4).

A threatened miscarriage is defined as vaginal bleeding before 20 weeks of gestation with a closed cervical os, without passage of products of conception and without evidence of a fetal or embryonic demise (4).

It has been confirmed that experiencing threatened miscarriage may suggest potential placental dysfunction, which may manifest in subsequent pregnancy, leading to poor fetal and maternal outcomes such as second trimester miscarriage, preterm premature rupture of membranes (PPROM), placenta previa, pregnancy induced hypertension (PIH)/preeclampsia (PE), preterm delivery, fetal growth restriction (FGR) and neonatal intensive care unit (NICU) admission (5-8).

Alterations in placentation can manifest in the second and third trimesters in addition to their immediate impacts, such as spontaneous miscarriage. Preeclampsia, intrauterine growth restriction, and hypertension in pregnancy—all of which appear at various stages of pregnancy—have all been extensively researched to determine their shared cause (16).

1.6.4: Infections:

Infection has been implicated in 10 to 25 percent of second trimester pregnancy losses (9). Many infectious agents have been suggested, including bacteria, spirochetes, protozoa, viruses, and fungi (10). Infection is more closely linked to pregnancy loss in developing countries (9, 11). Bacterial vaginosis infection has been linked to second trimester loss (12). As with other etiologies that are difficult to confirm, there is limited proven benefit of intervention. Treating bacterial vaginosis may prevent premature labor in women with a history of preterm birth (13).

Bacterial vaginosis may be asymptomatic. It usually responds to treatment with antibiotics but can relapse rapidly, and reported rates of relapse are more than 50% within 3 to 6 months. There is a need alternatives to antibiotics and to find a way to prevent relapse. Some studies reported that sexually transmitted, associated with more pathogenic strains of *E. coli*, *Streptococcus* spp, *Gardnerella vaginalis* and *Atopobium vaginae*, *Leptotrichia amnionii*, *Mobiluncus* spp, *Prevotella* spp, *Mycoplasma hominis*, *Bacteroides* spp. Bacterial vaginosis is a risk factor for adverse pregnancy outcomes, including second-trimester miscarriage, spontaneous preterm birth, and post-Caesarean section endometritis (14).

Bacterial vaginosis is present in 50% of pregnant women and is a risk factor for mid trimester miscarriage and preterm labor (15).

The etiology and pathogenesis of BV are not entirely clear. This disorder is characterized by reduced *Lactobacillus* spp., high differences in bacterial species and excessive increases in anaerobic, facultative bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae* and other BV-related bacteria including *Megasphaera*, *Sneathia*, and *Clostridiales* spp. compared to healthy individuals (16, 17). So bacterial vaginosis is mostly caused by the synergistic interaction of *G. vaginalis* with obligate anaerobes (18). Seventy seven percent of patients had bacterial vaginosis during the second trimester compared to 9% who had it in the first trimester of pregnancy (19).

1.9: Aim of study:

Is to determine the relationship between bacterial vaginosis infection and second trimester threatened miscarriage.

Method

Study design and setting: A case control study was conducted in the department of obstetrics and gynecology in Babylon maternity and Children Teaching Hospital during the period from first of February 2023 to first of December 2023

Ethical approval: Approval from Iraqi council of medical specialization and scientific council of obstetrics and gynecology in Babylon Maternity and Children Teaching Hospital was obtained. Main objectives of current study were described and explained, and verbal consent was taken from all participants.

Table 2.1: Instruments, equipment, and their producing-company and original country.

NO.	Instruments	Company	Source
1-	Cover slides	AFCO	China
2-	Jar	Oxford	united kingdom
3-	Test tube	AFCO	Jordan
4-	Transport swab	Spine	China
5-	Vaginal PH strep	Hangzhou	China

Sample and sampling: the Study involved 200 pregnant women in the second trimester of pregnancy, their gestational age ranged from (13-24 weeks) , 100 of them presented with lower abdominal pain and history of vaginal bleeding, by examination closed cervical os with ultrasound finding of normal viable fetus, diagnosed as threatened miscarriage (study group) . The other 100 women were healthy pregnant women (control group). Patients and controls who were involved in this study, were collected from emergency department in Babylon Teaching Hospital for Maternity and children and outpatients clinic.

All cases were assessed by history taking and physical examination. History taken according to well-structured questionnaire which include maternal age, gestational age, LMP, gravidity and parity, previous miscarriage ,address, symptoms at presentation, past gynecological history, past obstetric history, past medical history, family history and drug & smoking history.

General examination including blood pressure, temperature measurement, respiratory and pulse rate. Abdominal examination including fundal height assessment.

Vaginal examination in lithotomy position for inspection and taking sample by high vaginal swab using a sterile non-lubricated Cusco's speculum. Then looked over vagina and cervix for any sign of inflammation (erythematous, abnormal discharges).then the procedure for diagnosis made

depend on Amsel criteria .

Inclusion Criteria:

- Singleton pregnancy
- Gestational Age 13-24 wk

The exclusion criteria:

- Using antibiotic drugs therapy in last two weeks
- Vaginal bleeding at time of taking swab
- Rupture membranes
- Recent use of vaginal douches and cleanser.
- Recent intercourse
- Patients had chronic debilitating diseases e.g. diabetes mellitus, hypertension, renal disease etc.
- Systemic & urinary tract infection
- Ultrasound evidence of cervical incompetence.

Diagnosis of Bacterial vaginosis

Diagnosis of bacterial vaginosis depended on Amsel criteria ([bv125]), presence of three of the 4 criteria indicate its existence:

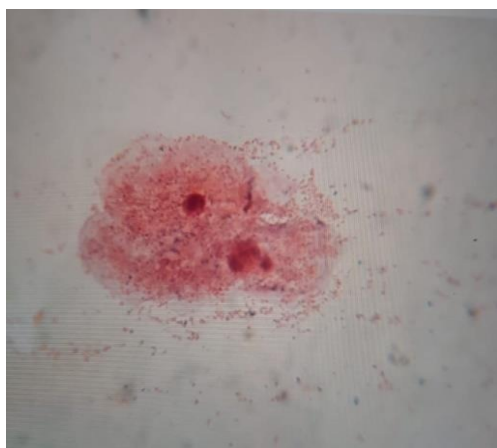
1. Vaginal discharge: An evaluation of the nature of vaginal discharge during pelvic examination. discharge was reported as positive for BV if it was thin , homogenous, white to greyish color
2. The vaginal acidity was checked by applying PH strip on lateral wall of vagina for 1 minute and avoid PH paper reach to cervix as to it has alkaline environment. PH > 4.5.



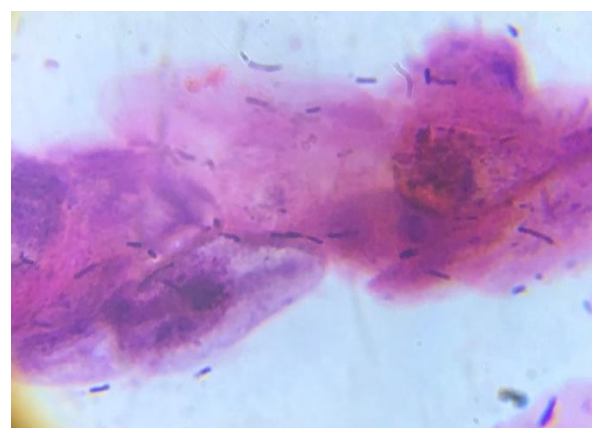
Figure 2.1: Strip of vaginal PH measure

3. A sample of vaginal discharge was taken by a sterile cotton swab which smeared from high vaginal swab, then fixed of slide ,staining of it by Gram stain

and examined under 40x (high power) of microscope, and checked for Clue cells (20% of epithelial cells with indistinct border due to adherent bacteria).



A



B

Figure 2.2: (A) and (B) pictures showed clue cell in sample of gram stained vaginal smear from patients with bacterial vaginosis (from our patients)

4. Whiff test was done, a drop of potassium hydroxide solution 10% was added to vaginal discharge and examined for fishy odor smell (amine compounds) which indicate the presence of a volatile amines (such as trimethylamine) .

Results

Case-control research which included 200 participants, were distributing as 100 pregnant with threatened mid trimester miscarriage as case group and second group were 100 pregnant mid trimester as control group.

In analysis of demographic data (as seen in table 3.1),

mean of maternal age exhibited 25.78 and 24.63 and mean gestational age displayed 18.4 and 19.38 among case and control group respectively, with no statistical significant difference (p value= 0.131 maternal age, 0.092 gestational age).

Similarities, address and previous miscarriage showed insignificant association (p value = 0.544 and 0.104 respectively), with regarding urban address 66 (66%) and 70 (70%) and no previous miscarriage 77 (77%) and 87 (87%) in case and control groups respectively.

Accepting parity groups had significant association (p value = 0.041) with more presenting parity 1-3 that showed 65 (65%) and 56 (56%) in case and control group respectively.

Table 3.1: Demographic data in threatened mid trimester miscarriage patients and control groups.

Parameters		Threatened mid trimester miscarriage group (n = 100)	control group (n = 100)	P value
Maternal age/years	mean±SD	25.78±3.976	24.63±6.454	*0.131
Gestational age/weeks	mean±SD	18.4±3.565	19.38±3.797	** 0.092
Parity	para 0	28(28%)	25(25%)	^0.041
	para 1-3	65(65%)	56(56%)	
	≥ para 4	7(7%)	19(19%)	
Address	Urban	66(66%)	70(70%)	^ 0.544
	Rural	34(34%)	30(30%)	
Previous miscarriage	0	77(77%)	87(87%)	^0.104
	1	15(15%)	6(6%)	
	≥ 2	8(8%)	7(7%)	

^Chi-Square Test, *Independent Samples T Test, **Mann-Whitney test

Based on analysis of Table 3.2, clinical symptoms had abdominal pain 95.1% , itching 63.9% , burning sensation 85.2% , dysuria 73.8% , and vaginal discharge 96.7% in patients with threatened mid trimester miscarriage with bacterial vaginosis .

Table 3.2: Clinical symptoms in patients with threatened mid trimester miscarriage with bacterial vaginosis.

Symptoms		Threatened mid trimester miscarriage with bacterial vaginosis group (n = 61)
		N (%)
Abdominal pain	symptomatic	58(95.1%)
	asymptomatic	3(4.9%)
Itching	symptomatic	39(63.9%)
	asymptomatic	22(36.1%)
Burning sensation	symptomatic	52 (85.2%)
	asymptomatic	9(14.8%)
Dysuria	symptomatic	45(73.8%)
	asymptomatic	16(26.2%)
Vaginal discharge	symptomatic	59(96.7%)
	asymptomatic	2(3.3%)

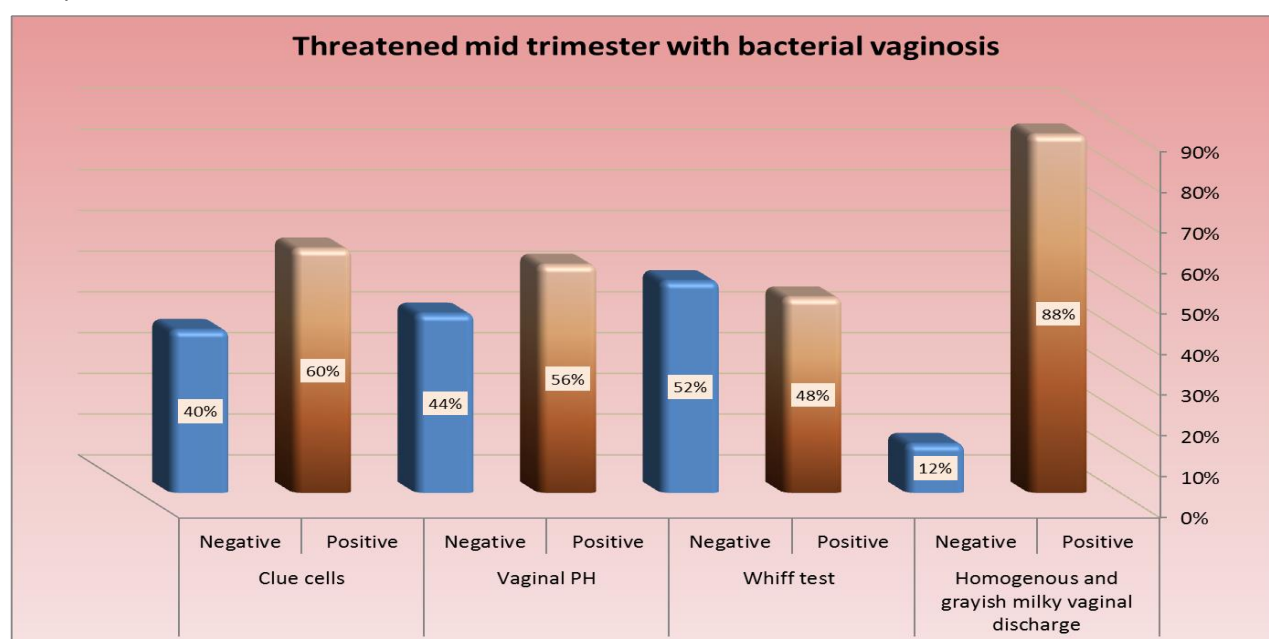
A commonly abnormal parameters in Amsel's criteria for Diagnosis bacterial vaginosis showed 88% homogenous grayish milky vaginal discharge , 48% whiff test , 56% vaginal PH, and 60% clue cells in threatened mid trimester with bacterial vaginosis group . While in control group were exhibited 3%

homogenous grayish milky vaginal discharge , 2% whiff test , 9% vaginal PH, and 24% clue cells as illustrated in table 3.3. there were a highly significant association between case and control groups regarding homogenous grayish milky vaginal discharge ,whiff test , vaginal PH, and clue cells (p value = < 0.001)

Table 3.3 : Amsel's criteria for diagnosis of bacterial vaginosis in cases and control group

Parameters		Threatened mid trimester miscarriage group (n = 100)	control group (n = 100)	*P value
		N (%)	N (%)	
Homogenous and grayish milky vaginal discharge	Positive	88(88%)	3(3%)	<0.001
	Negative	12(12%)	97(97%)	
Whiff test	Positive	48(48%)	2(2%)	<0.001
	Negative	52(52%)	98(98%)	
Vaginal PH	Positive	56(56%)	9(9%)	<0.001
	Negative	44(44%)	91(91%)	
Clue cells	Positive	60(60%)	24(24%)	<0.001
	Negative	40(40%)	76(76%)	

*Chi-SquareTest

**Figure 3.3: Comparing percentage of Amsel's criteria for diagnosis for bacterial vaginosis**

In accuracy of effective role of Amsel's criteria (table 3.4) for diagnosis bacterial vaginosis displayed a high level of sensitivity, specificity, PPV, and NPV, in which homogenous grayish milky vaginal discharge showed

96.7%, 25.6%, 67%, and 83.3%, whiff test exhibit 75%, 94.8%, 95.8%, and 71.1%, vaginal PH revealed 80.3%, 82%, 87.5%, 72.7%, and clue cells showed 90.1%, 87.1%, 91.6%, 85% that represented sensitivity, specificity, PPV, and NPV respectively.

Table 3.4 : Diagnostic parameters in Amsel's criteria in bacterial vaginosis.

Amsel's criteria	no. of positive	no. of negative	sensitivity %	Specificity %	PPV %	NPV %
Homogenous and grayish milky vaginal discharge	88	12	96.7%	25.6%	67.0%	83.3%

Whiff test	48	52	75.0%	94.8%	95.8%	71.1%
Vaginal PH	56	44	80.3%	82.0%	87.5%	72.7%
Clue cells	60	40	90.1%	87.1%	91.6%	85.0%

Lastly Threatened mid trimester miscarriage group , positive diagnosis by Amsel's criteria (table 3.5) showed total 61 (61%) from case group, in which the most parameter for diagnosis was patients have all

Abnormal Amsel's criteria 26 (26%) and less one was Patients have all clue cell with PH with Whiff test 2 (2%). While control group showed 1% in patients have Abnormal discharge+PH+clue cell

Table 3.5 : Frequency of Amsel's criteria in bacterial vaginosis

Abnormal Amsel's criteria	Threatened mid trimester miscarriage group (n = 100)	control group (n = 100)
Patients have Abnormal Amsel's criteria	26 (42.6%)	0
Patients have Abnormal discharge+PH+Whiff test	6 (9.8%)	0
Patients have clue cell +PH+Whiff test	2 (3.3%)	0
Patients have Abnormal discharge+PH+clue cell	15 (24.6%)	1(100%)
Patients have Abnormal discharge+clue cell+ Whiff test	12 (19.7%)	0
Total No. of Bacterial vaginosis	61 (100%)	1 (100%)

Discussion

Miscarriage has adverse effect on physical and emotional aspects of a pregnant female. The researches demonstrate 15% of early pregnancy loss and up to 66% of late pregnancy loss are related to infectious etiology (20).

GARRIDO-GIMENEZ, et al and Foessleitner, et al (21, 22) stated that untreated infections in pregnancy may remain for long period without any clinical signs or symptoms, and can result in miscarriage.

In current study, higher percentage of bacterial vaginosis was found in those are para 1-3 and lowest in those with parity ≥ 4 .

In Wiraguna, et al study (23) showed parity groups had insignificant association with BV with more presenting percentage in parity ≤ 1 . While Ahmed Elsayed Mansor et al study (24) showed that parity groups had no significant association.

Our study does not show any association between address and bacterial vaginosis infection, this may be attributed to that most of patients' lives in urban area.

The highest prevalence of BV was le-class commuobserved in the urban (38.6%), followed by the rural community (28.8%) and urban middnity (25.4%)(76).

Regarding history of previous miscarriage, there is no correlation with bacterial vaginosis infection.

In Ahmed Elsayed Mansor et al study (24), a history of previous miscarriage showed no significant difference between case & control groups, however, higher percentage of BV was found in those with no history of previous miscarriage.

Goffinet, et al. (25) found that history of spontaneous miscarriage was higher in pregnancy with BV.

IŞIK, Gözde, et al. study (26) showed that BV was more likely to be associated with mid trimester pregnancy

loss.

Regarding presenting symptoms in patients with threatened mid trimester miscarriage with bacterial vaginosis showed higher frequency of abdominal pain, vaginal discharge, itching, burning sensation, and dysuria. while S. Eroglu and N. Asgin study(27) showed frequency of clinical symptoms to be vaginal discharge 62.73%, vaginal itching 23.9%, pelvic pain 10.15%, and less presentation dysuria 1.1%.

The current study showed that commonly abnormal parameters in Amsel's criteria for diagnosis of bacterial vaginosis were 88% homogenous grayish milky vaginal discharge, 48% Whiff test, 56% Vaginal PH, and 60% Clue cells.

Atef m. Darwish et al (28), showed 49% homogenous grayish milky vaginal discharge, 75.6% whiff test, 100% vaginal PH, and 83.3% clue cells in threatened mid trimester with bacterial vaginosis group. While in control group were exhibited 6.4% homogenous grayish milky vaginal discharge, 4.8% whiff test, 31.1% vaginal PH, and 2.4% clue cells as compared with our results. there were a highly significant association between case and control groups (p value = < 0.001).

In current study, evaluation of Amsel's criteria have high predictive value for diagnosis of bacterial vaginosis because displayed a high level of sensitivity 96.7%, 75%, 80.3%, and 90.1%, specificity 25.6%, 94.8%, 82%, and 87.1% in homogenous grayish milky vaginal discharge, whiff test, vaginal PH, and clue cells respectively.

Moreover, the whiff test and pH were detected to have the lowest specificity and sensitivity, respectively. The researchers thus concluded that in the absence of Gram staining, Amsel's criteria could be used as a practical method for diagnosis of BV (29).

Mengistie et al. (29) compared various diagnostic methods for BV and suggested Amsel clinical criteria to have a sensitivity of 85.7% and a specificity of 98.0% compared with Nugent scoring system. They indicated the presence of clue cells as the individual Amsel's criterion with the highest specificity and sensitivity.

Taj et al. (30) observed that Amsel's criteria in comparing to other microbiological methods to diagnose BV, the Amsel's criteria are acceptable for diagnosing BV.

They concluded that Amsel's criteria without utilizing staining methods could be misleading (31).

The Nugent's criteria versus Amsel's criteria have comparable diagnostic value for BV as discussed by many research groups, with specificity and sensitivity of Amsel's criteria reaching as high as 95.2% and 91%, respectively, when compared with Nugent's

criteria(32-35).

They suggested diagnosis and treatment of bacterial vaginosis during pregnancy as soon as possible (36).

Conclusion

- Bacterial vaginosis consist about 61 % of patients with second trimester threatened miscarriage.
- Multiparous women are more prone for bacterial vaginosis infection than primigravida.
- The most common symptoms that indicates bacterial vaginosis infection in pregnant females are abdominal pain and vaginal discharge respectively.
- Clue cells and homogenous vaginal discharge are more prevalent than vaginal PH and Whiff test.

Recommendations

- Larger sample size are important to study the mechanism and effect of bacterial vaginosis in second trimester miscarriage.
- Early detection and treatment of pregnant female with bacterial vaginosis is crucial.
- Abdominal pain and vaginal discharge should raise the suspicion of bacterial vaginosis infection in pregnant females.

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