



STUDY OF VAGINAL MICROFLORA IN LOW RISK PREGNANT WOMEN DURING EARLY LABOR AT TERM PREGNANCY AND CHANGE IN IT 48 HOURS AFTER DELIVERY AND ITS ASSOCIATION TO CLINICAL FINDINGS

Gynecology

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ABSTRACT

Introduction

The association of lactobacilli with other vaginal microflora has been documented in the development of Aerobic Vaginitis and Bacterial Vaginosis. As the numbers of lactobacilli dwindle, proliferation of other aerobic microflora, predominantly enteric commensals occurs. In our practice we find E coli as the most common organism present in lochia of puerperal sepsis cases. In present study an attempt was made to study the relation between the Lactobacilli and other pathogenic organisms commonly present in the vagina during early labor and after delivery.

Aims and Objectives

To study normal and abnormal vaginal microflora in low risk pregnant women during early labor at term pregnancy, 48 hours after delivery and its association to clinical findings.

Material and Methods

50 low risk pregnant women in early labor at term pregnancy without any obstetric complications were studied for vaginal microflora. A vaginal swab was taken from all fornices and submitted for gram stain and culture. After 48 hrs of delivery, repeat swabs were taken and submitted for gram stain and culture.

Abnormal vaginal microflora was defined as the absence or diminished amount of lactobacilli (BV, AV and intermediate vaginal microflora) or a positive culture for Candida sp.

In Gram stain, vaginal microflora was studied according to grading.

Grade 1- Predominance of lactobacilli and diminished ancillary flora

Grade 2- Intermediary with a diminished number of lactobacilli coexisting with other bacteria

Grade 3- Markedly diminished or absence of lactobacilli and predominance of bacterial morphotypes

Results

Proportion of normal vaginal microflora (Grade 1) was 34% and abnormal vaginal microflora (Grade 2 & 3) was 66 % in the swabs taken during early labor at term pregnancy. The most frequent types among abnormal flora were Aerobic Vaginitis (34%), intermediate flora including BV (32%), Candidiasis (26%) and cytolytic vaginosis (6%). In spite of detection of abnormal vaginal microflora in 66% patients during early labor, none had developed complications like preterm labor, PROM, PPROM or chorioamnionitis. Grade 2 and Grade 3 microflora (abnormal flora) were more common in multigravida patients, 61.1%, 88.8%, and 100% in 2nd, 3rd and 4th gravida patients respectively. In spite of strict aseptic precautions, abnormal vaginal microflora was present in 96% patients 48 hours after delivery. Thus increased parity had relation with abnormal flora in our study.

Itching was present in 81.8% patients with abnormal vaginal microflora with P value <0.05.

Altered vaginal pH was present in 69.7% patients with abnormal microflora with P value <0.05.

In bacterial morphotypes, E coli was the predominant bacterial morphotype isolated in culture in majority of patients (72 %) after delivery and other organisms include staph epidermidis, pseudomonas, strepto pyogenes, enterobacter aeruginosa. After delivery, only 4% patients had normal or Grade 1 flora.

No patient had fever, subinvolution of uterus, foul smelling discharge P/V, episiotomy wound infection or gap in the puerperium.

Conclusion

Data from this study indicate a high proportion of alterations in vaginal microflora of low-risk pregnant women in early labor at term pregnancy without development of complications like preterm labor, PROM, PPROM or chorioamnionitis.

There is significant association between complaint of itching, altered vaginal PH and abnormal vaginal microflora.

E coli were the most common organisms isolated after delivery but none had developed puerperal sepsis. The ability of pathogenic organism to replicate is potentially governed by inhibitory or synergistic interrelationship with other microbes.

According to our study, the abnormal vaginal flora was more common with high parity. Does parity have any influence on vaginal microflora?

KEYWORDS:

Abnormal vaginal microflora, Aerobic Vaginitis, Lactobacilli, E coli, Puerperal sepsis, PH

INTRODUCTION

The primary colonizing bacteria of a healthy individual are of the genus Lactobacillus. Since the first description of lactobacilli by Döderlein, lactobacilli have been generally considered as the gatekeepers of the vaginal ecosystem. The Lactobacillus SP is a gram-positive bacillus that produces components such as lactic acid, bacteriocin and hydrogen and which has properties that protect the

vaginal microflora. These components determine an acidic vaginal pH, lower than 4.5, inhibiting the growth of pathogenic bacteria.¹

The vaginal microflora is also composed of other commensal microorganisms, which in certain situations can become pathogenic.¹

The association of lactobacilli with other vaginal microflora has been

documented in development of Aerobic Vaginitis. As the numbers of lactobacilli dwindle, proliferation of other aerobic microflora, predominantly enteric commensals occurs.

In normal pregnancy, the resident vaginal microbial flora is thought to provide protection against infection. The microbiota during pregnancy is predominantly *Lactobacillus* spp. Microbiota composition can change during the course of the pregnancy. If the microbiota populations become more diverse, indicating that the normal *Lactobacillus*-dominated population has changed to a bacterial vaginosis population, risks of adverse pregnancy outcomes increase.²

Aerobic Vaginitis (AV) raises an important inflammatory response that can cause complications during pregnancy such as ascending chorioamnionitis, premature rupture of membranes and preterm labor. One of the most frequently cultured microorganisms in AV is *E. coli*, sometimes isolated as the sole organism.

Aerobic Vaginitis is a newly recognized vaginal microflora disorder, and should not be confused with Bacterial Vaginosis (BV). Although BV is a condition of abnormal vaginal microflora, abnormal vaginal microflora is not always BV.³

The abnormal flora in BV is anaerobic overgrowth (*Gardnerellavaginalis*, *Mobiluncus* sp., *Bacteroides* sp., *Prevotella* sp., *Peptostreptococcus* sp., etc.), in AV aerobic enteric commensals such as *Escherichia coli*, *Staphylococcus aureus*, group B streptococcus (GBS), and enterococci, are recovered.³

As both BV and AV are characterized by decreased concentrations of lactobacilli, vaginal lactate production is low in both the conditions.

We must take into account that under normal conditions, progesterone increases the number of intermediate epithelial cells resulting in a higher availability of glycogen and decreased vaginal pH. Even though these factors favor the presence of lactobacilli, they are also associated with the development of *Candida* sp.¹ Vulvovaginal Candidiasis is caused by several species of *Candida* sp.

The high levels of vaginal glycogen, local heat and humidity, are conducive to fungus activation, especially in the second trimester of pregnancy. Its common symptoms are itching, urinary discomfort, perineal irritation, and whitish discharge in plaques, though around 25% to 40% of women are asymptomatic.¹

Even though cytolytic vaginosis is not an infectious pathology, it is a condition frequently diagnosed as Vaginal Candidiasis because of its similar symptoms. Diagnosis should include microscopic examination of samples directly collected from vaginal content through Gram's method, to exclude the possibility of infection by *Candida* sp, observing a significant increase in the number of lactobacilli, generally adhered to epithelial cells, which are also in a larger number, while leucocytes, when present, are rare.⁴

Puerperal sepsis is infection of the genital tract commonly occurring within 14 to 21 days after childbirth or abortion. In our practice we find *E. coli* as most common organism present in lochia of puerperal sepsis cases. Other common organisms are Streptococci, Staphylococci, *B. fragilis*. Most common symptoms are fever, tachycardia, sub involution of uterus, foul smelling vaginal discharge and wound gap.

AIMS AND OBJECTIVES

To study the normal and abnormal vaginal microflora in low risk pregnant women during early labor and its association to clinical findings.

To study the change in vaginal microflora 48 hours after delivery and its association to clinical findings.

To study the predominant organism in culture after delivery and its association to puerperal sepsis.

MATERIAL & METHODS

This is a prospective study of vaginal microflora in low risk pregnant women during early labor at term pregnancy and change in it 48 hrs after delivery and its association to clinical findings conducted in 50 patients admitted in labor room at Civil Hospital, Ahmedabad between June to August 2016.

Low risk pregnant women with term pregnancy in early labor without any obstetrical complications were included in the study after informed and written consent.

Proper antenatal history was taken and patients were inquired about symptoms like white discharge per vaginum (P/V), itching, burning micturition, leaking P/V, bleeding P/V etc. apart from pain in lower abdomen. Per speculum examination was done by sterilized speculum and anterior vaginal wall retractor without using any lubricant. Vaginal discharge, cervical erosion, show, leaking, bleeding etc. including other pathologies of cervix and vagina were observed. Patients in advanced labor and with obstetrical complications were excluded from the study. Patients who required instrumental or cesarean delivery were also excluded from the study.

pH measurement was done by pressing pH paper against the middle third of lateral vaginal wall for one minute and color of the pH paper was compared with the chart provided with test kit. Normal vaginal pH: 3.8-4.5.

In early labor, vaginal swab was taken from all fornices and slide was prepared by heating method for gram stain and swab sent for culture in Microaid culture medium and studied for microorganisms. After taking swab, injectable antibiotic (ceftriaxone) was given to all patients as a part of routine protocol in our department. Since ours is a government hospital and a tertiary care centre, most of the patients are illiterate, underprivileged, and not reliable to take care of personal hygiene. Due to this, we usually give a single dose of prophylactic antibiotics to all the patients upon admission to the Labor Room.

All patients were delivered vaginally spontaneously with strict aseptic precautions and general infection control measures.

After 48 hrs of delivery, all patients were examined for fundal height, foul smelling discharge and the condition of episiotomy wound apart from routine examination. Post delivery swab was taken from all fornices. Slide was prepared for gram stain by heating method and swab was sent for culture in Microaid culture medium and studied for microorganisms. Proportion of Abnormal vaginal microflora was studied and its association to clinical findings was recorded. Patients were discharged two to three days after delivery following clinical examination and blood test for CBC. They were advised follow up 15 days after delivery.

Changes in vaginal microbial flora after delivery were recorded. The proportion between *Lactobacillus* SP and other microorganisms determines the type of vaginal microbiota: Spiegel et al criteria
Grade 1- Predominance of lactobacilli and diminished ancillary flora
Grade 2 –Intermediary with a diminished number of lactobacilli coexisting with other bacteria
Grade 3- Markedly diminished or absence of lactobacilli and predominance of bacterial morphotypes
The diagnosis of Candidiasis was based on cultivation of *Candida* in culture medium.

Diagnosis of abnormal vaginal microflora

Grade 1 vaginal microflora was considered as normal vaginal microflora. Grade 2 and 3 vaginal microflora and a positive culture for *Candida* sp. were considered as abnormal vaginal microflora.

RESULTS

Table 1: Type of vaginal microflora during early labor

Grade	Primigravida	2ndgravida	3rdgravida	4thgravida
Grade 1	9 (42.8%)	7 (38.9%)	1 (11.1%)	0
Grade 2	7 (33.3%)	5 (27.8%)	3 (33.3%)	1 (50%)
Grade 3	5 (23.8%)	6 (33.3%)	5 (55.5%)	1 (50%)
Total	21(42%)	18(36%)	9(18%)	2(4%)

Table 1 shows the type of vaginal microflora in primi and multigravida patients. Out of 50 patients, majority of patients (42%) were primigravida and 36% patients were 2ndgravida.

During early labor, in primigravida patients, Grade 1 microflora was most common (42.8 %) as compared to multigravida patients. Grade 2 and Grade 3 microflora (abnormal flora) were more common in multigravida patients, 61.1%, 88.8%, and 100% in 2nd, 3rd and 4th

gravida patients respectively.

Table 2: Proportion of vaginal microflora during early labor

Vaginal microflora	No. of patients	Percentage
Normal vaginal microflora (Grade 1)	17	34%
Abnormal vaginal microflora (Grade 2 & 3)	33	66%

Table 2 shows the proportion of vaginal microflora during early labor. Normal vaginal microfloras were 34% and abnormal vaginal microfloras were 66%.

Table 3: Proportion of various abnormal vaginal microfloras during early labor

Abnormal vaginal microflora	Number of patients	Percentage
Aerobic Vaginitis (Grade 3)	17	34%
Intermediate flora (Grade 2)	16	32%
Candidiasis	13	26%
Cytolytic vaginosis	3	6%

Proportion of abnormal vaginal microflora was 66% in the study group among which the most frequent ones were Aerobic Vaginitis in 34%, intermediate flora including BV in 32%, Candidiasis in 26% and cytolytic vaginosis in 6% patients (Table 3). BV was diagnosed by presence of clue cells and PH more than 4.5.

Table 4: Association between type of vaginal microflora and clinical findings during early labor

Clinical findings	Normal vaginal microflora	Abnormal vaginal microflora	P value	Total
Whitish discharge	4 (23.5%)	4 (12.1%)	0.403	8 (16%)
Itching	5 (29.4%)	27 (81.8%)	0.0008	32 (64%)
Alkaline pH	3 (17.6%)	23 (69.7%)	0.0014	26 (52%)
Acidic pH	14 (82.4%)	10 (30.3%)		24 (48%)

White discharge was reported in 12.1% patients with abnormal vaginal microflora. Itching was present in 81.8% patients with abnormal vaginal microflora. P value for itching was <0.05 so there was significant association between itching and abnormal vaginal microflora. (Table 4)

Altered vaginal pH was present in 69.7% patients with abnormal vaginal microflora. P value for vaginal pH was <0.05 so there was significant association between altered vaginal pH and abnormal vaginal microflora.

Table 5: Proportion of vaginal microflora during early labor at term pregnancy and change in it 48 hours after delivery

Grade	Early labor	48 hrs after delivery
Grade 1	17 (34%)	2 (4%)
Grade 2	16 (32%)	7 (14%)
Grade 3	17 (34%)	41 (82%)
Total	50	50

During early labor, lactobacilli were predominant organisms (Grade 1) in 34% patients, reduced lactobacilli with other morphotypes (Grade 2) in 32% patients and other bacterial morphotypes with no lactobacilli (Grade 3) in 34% patients.

48 hours after delivery, other bacterial morphotypes were predominant with no lactobacilli (Grade 3) in 82% patients, reduced lactobacilli with other morphotypes (Grade 2) in 14% patients and predominant lactobacilli (Grade 1) only in 4% patients.

So during early labor, Grade 1 vaginal microflora was common and after delivery, Grade 3 vaginal microflora was common.

Table 6: Organisms isolated in culture during early labor and 48 hours after delivery

Organism	Early labor	48 hrs after delivery
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E coli	22 (44%)	36 (72%)
Lacto	13 (26%)	3 (6%)
Candida	13 (26%)	5 (10%)
Staph aureus	9 (18%)	5 (10%)
Streptococci	8 (16%)	5 (10%)
Staph sapro	5 (10%)	3 (6%)
Pseudomonas	5 (10%)	5 (10%)
Yeast	6 (12%)	-
Strepto pyogenes	4 (8%)	4 (8%)
GPC	3 (6%)	-
Staph epidermidis	2 (4%)	5 (10%)
Bacillus subtilis	1 (2%)	1 (2%)
Neiseria ulcerans	1 (2%)	-
Enterobacter aeruginosa	-	1 (2%)

E coli were the predominant bacterial morphotypes isolated after delivery in majority of patients (72%). Other organisms newly isolated or increased after delivery were staph epidermidis and enterobacter aeruginosa.

In puerperium no patient had fever, subinvolution of uterus, foul smelling discharge P/V and episiotomy wound infection or gap. Post delivery total WBC counts were within normal limit.

DISCUSSION

This study enabled the identification of the proportion of abnormal vaginal microflora in low-risk pregnant women during early labor at term pregnancy regardless the reported complaint.

The results in our study reveal a high rate of abnormal vaginal microflora (66%) during early labor at term pregnancy, which is in agreement with other recent studies involving pregnant women.¹ Aerobic Vaginitis was the most frequent alteration in the flora, with an isolated proportion of 34%.

In spite of having 66% of abnormal flora during early labor, the patients had not developed preterm labor, PROM or chorioamnionitis and majority were asymptomatic except having localized itching because some of the organisms are commensals and become pathogenic only in the presence of predisposing factors. Inhibitory or synergistic interrelationship with other microbes is responsible for disease caused by exogenous or endogenous bacteria that possess virulence.⁶ Although lactobacilli are key regulators when they are dominant in number, their ability to maintain governance is influenced by other bacterial species within the micro flora of genital tract.⁶

Candida was present in 26% patients during early labor. Under normal conditions, progesterone increases the number of intermediate epithelial cells, resulting in a higher availability of glycogen and decreased vaginal pH. Even though these factors favor the presence of lactobacilli, they are also associated with the development of Candida sp.¹

The complaint of whitish discharge P/V was present in 12.1% patients with abnormal flora and in 23.5% patients with normal flora. Due to presence of show, proper examination of type of vaginal discharge was not possible.

Itching was the most common complaint in 81.8% patients and altered vaginal pH was present in 69.7% patients having abnormal vaginal microflora. Itching and altered vaginal pH have significant association with abnormal vaginal microflora with P value of <0.05. With altered pH chances of Aerobic Vaginitis (AV) and Bacterial Vaginosis (BV) are increased.

In one study, using pH as a screening tool in pregnancy, only 40% of women with increased vaginal pH had BV.⁷ In a random screening test of women in Uganda, BV was discovered in 27% of women having vaginal pH of 4.4-4.7 and in 39% of women having pH above 4.7. Coccid Aerobic Vaginitis was discovered in 11% of women having vaginal pH of 4.4-4.7 and in 25% women having vaginal pH above 4.7.⁸

Abnormal vaginal microflora (Grade 2 & 3) were more common in

multigravida patients, 88.8% in 3rd gravida and 100% in 4th gravida patients in comparison to primigravida (57.1%) and 2nd gravida (61.1%). It indicates that in spite of full aseptic precautions and prophylactic antibiotics, high parity is likely to alter the vaginal microflora. It is also proved by the fact that during early labor the proportion of abnormal vaginal microflora was 66% while it was 96% after delivery in our study. So there was decrease in the proportion of Grade 1 flora from 34% to 4%. It might be due to the process of delivery itself in spite of full aseptic precautions.

The risk of puerperal sepsis after vaginal delivery significantly increases in presence of Bacterial Vaginosis, Aerobic Vaginitis and gram negative bacilli.

In spite of isolation of E coli as the most common organism in culture media after delivery in 72 % patients, no patient developed abnormal symptoms in puerperium. The ability of pathogenic organism to replicate is potentially governed by inhibitory or synergistic interrelationship with other microbes. All aseptic precautions were taken during vaginal examination, labor and after delivery and general infection control measures were applied. All patients were treated with prophylactic antibiotics and perineal care was given.

CONCLUSION

Data from this study indicates a high proportion of alterations in vaginal microflora of low-risk pregnant women in early labor at term pregnancy without development of complications like preterm labor, PROM, PPROM or chorioamnionitis.

There is significant association between itching, Alkaline PH and abnormal vaginal microflora.

E coli were the most common organism isolated after delivery. Majority of patients having high proportion of abnormal vaginal microflora following delivery were asymptomatic because these microorganisms become pathogenic only in the presence of predisposing factors. The ability of pathogenic organism to replicate is potentially governed by inhibitory or synergistic interrelationship with other microbes.

According to our study, the abnormal vaginal flora was more common with high parity. Does parity have any influence on vaginal microflora?

Proper aseptic precaution during vaginal examination, labor and after delivery and application of general infection control measures are preventive steps for preventing puerperal sepsis in low risk pregnant women without obstetrical complication.

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Disclosure of Interests

There are no interests of any sort involved with the study.

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