

# The association between abnormal vaginal microbial flora and preterm labor. Does a simple Gram's stain of vaginal swab gives more useful data than culture ?

Haider Al Shamma'a\* FICMS, OBGYN

## Summary:

**Background:** Preterm labor is the major cause of perinatal mortality and morbidity and it is a challenging obstetrical complication as treatment is marginally effective if ever and no improvement in incidence over the last 5 decades , although many causes were identified the majority are idiopathic and in this category infection seems to be involved as a causative agent

**Objectives:** to evaluate the association of abnormal vaginal flora diagnosed by direct Gram's staining and vaginal culture with preterm labor.

**Patients, material and methods:** 25 women with preterm labor compared with 25 women at full term pregnancy, high vaginal swabs for direct Gram's staining (Spiegel criteria for abnormal vaginal flora) and culture. The results were compared in both groups

**Results:** this study showed significant association between preterm labor and abnormal vaginal flora 7 (28%) and 12 (48%) of grade II and III respectively . and a significant association between term pregnancy and normal vaginal flora 16 (64%) of grade I. Also in the presence of abnormal vaginal flora, no significant association with type of pathogenic microorganism and preterm labor as all types may be associated with PTL , and the presence of Lactobacillus spp. (normal grade I flora ) is associated with term pregnancy.

**Conclusions:** Gram's staining of high vaginal swab using Spiegel criteria can be useful simple test to predict preterm labor

**Key words:** preterm labor, vaginal flora grading, vaginal infection.

*Fac Med Baghdad*  
2014; Vol.56, No.2  
Received: Jan., 2014  
Accepted March. 2014

## Introduction:

Preterm labor is one of the most challenging obstetrical complications faced by the obstetricians ( 1, 2 ) , for the last 5 decades the incidence of PTL has changed little in spite of the advances of medical services as treatment is only marginally effective in the best situation and there is no agreeable method for prediction of PTL ( 3 ) .

This obstetrical complication is responsible for 70% of perinatal mortalities and 50% of long term morbidities in the developed world. In developing countries the PTL related mortality even higher ( 3 ) , and the improvement in perinatal mortality is largely attributed to improved neonatal care services rather than to reduction in PTL incidence

PTL is the direct cause of perinatal morbidity due to organ system immaturity which may be serious, with life long effects causing great deal of physical, mental, social and financial burden to the affected neonates and their families and the health system ( 3 , 4 )

while there are many well known readily explainable causes of PTL like multiple pregnancies, cervical incompetence, uterine anomalies, iatrogenic for fetal and maternal indications, the majority are idiopathic for which finding direct causative

factors are difficult, some factors are associated with PTL as social, racial, smoking, work and above all infection of the uterus and the amniotic sac appear to be an important factor( 1,2, 5 ,6 ). Infection reaches the deciduas and amniotic sac mainly by ascending through the lower genital tract rather than other routes as blood born from systemic infection (7, 8, 9). The treatment of PTL is only marginally effective ( 5 ) so there is a need for a method for prediction, early diagnosis , and finding a reliable effective treatment for PTL .

## Patients, materials and methods

Fifty women participated in this study age 19 – 34 years of average weight and middle class socioeconomically divided into two groups

First group complaining of idiopathic preterm labor, all were singleton uncomplicated pregnancy apart from preterm labor (PTL).

Patients with obstetrical complications known to be associated with PTL were excluded from the study as multiple gestation, polyhydramnios, antepartum hemorrhage, uterine anomalies, preterm rupture of membranes, diabetes, hypertension, fibroids, fever, trauma to the abdomen and other obstetrical complications. Second group 25 women with term pregnancy, also without obstetrical complications matching with the first group regarding age, weight and social status. After

\*corresponding author:

Dept. Obgyn. College of medicine/ university of Baghdad.  
hshammaa@yahoo.com

taken verbal consent from all women in the study, they were put in lithotomy position, a saline lubricated sterile Cusco's speculum inserted inside the vagina, three swabs taken from each women from the lateral vaginal wall (high vaginal swabs). First smeared on a glass slide and immediately fixed with methanol for direct Gram's staining

The other two swabs and the slide send to the bacteriology laboratory for direct wet preparation examination, Gram's staining and culture in blood, chocolate agar and Mc Cunkey media.

The results of the direct Gram's stain evaluated according to Spiegel et al criteria( 10 )

Grade I:- normal flora predominantly Lactobacillus morphotype

Grade II:- intermediate (reduced Lactobacillus mixed with other morphotypes )

Grade III :- abnormal ( few or no lactobacillus with greatly increased number of other morphotypes as Gardenerella vaginalis and clue cells.

The results analyzed using the chi-square test (X<sup>2</sup>) and Fisher's exact test, the statistical significance P<0.05.

#### Results:

Nineteen out of 25 women ( 76% ) with preterm labor showed abnormal vaginal flora , while in normal term pregnancy abnormal flora found in 9 women only ( 36% ) , and the majority show normal flora 16 out of 25 ( 64%) as shown in ( table 1 ) . this was statistically significant association p= 0.01.

**Table1. the relation between the grade of vaginal flora and the preterm labor**

	PTL n = 25	TERM n = 25	Total N = 50
Grade I vaginal flora	6 ( 24% )	16 ( 64% )	22 ( 44% )
Grade II vaginal flora	7 ( 28% )	5 ( 20% )	12 ( 24% )
Grade III Vaginal flora	12 ( 48% )	4 ( 16% )	16 ( 32% )
<b>Total</b>	<b>25 ( 100% )</b>	<b>25 ( 100% )</b>	<b>50 (100% )</b>
X <sup>2</sup> = 8.88	P< 0.01 significant		

Table 2 showed the association of the grade of vaginal flora with the time till delivery in preterm labor and it shows that in the presence of abnormal flora, the patient is more likely to deliver earlier than those who have a normal flora, in spite of tocholytic treatment. Although it did not reach a statistically significant level.

**Table 2: the association between the grade of vaginal flora and the duration till delivery**

	Delivery within 48 hrs	Delivery 2-7 days	Delivery after > 7 days	Total n=25
Grade I	1 (16.7%)	2 (33.3%)	3 (50%)	6 (100%)
Grade II	4 (57.1%)	2 (28.6%)	1 (14.3%)	7 (100%)
Grade III	9 (75 %)	3 (25 %)	0 (0%)	12 (100%)
<b>Total</b>	<b>14</b>	<b>7</b>	<b>4</b>	<b>25</b>

Table 3: showed the type of bacteria isolated from the vagina. There are many types of microorganisms that may be associated with preterm labor non of them was significantly more than others, On the other hand Lactobacillus species were found significantly associated with term pregnancy. This makes the interpretation of the results of culture difficult as many types of pathological microorganisms singly or in combination may be associated with preterm labor although not reach a statistically significant level .

This means that the presence of lactobacilli can be regarded as a protective against preterm labor. and the absence of it is associated with preterm labor.

**Table 3: the association between the type of microorganisms and preterm labor**

Type of organism	Preterm	Term	Total	P
Staph. Aureus	4	3	7	P > 0.05
Staph. Epidermidis	2	3	5	P > 0.05
E. coli	4	3	7	P > 0.05
Gardenerella	5	1	6	P = 0.25
Trichomonas vaginalis	4	1	5	P > 0.05
Klebsiella	2	3	5	P > 0.05
Neiseria gonorrhoea	1	0	1	P > 0.05
Candida spp.	3	4	7	P > 0.05
Non haemolytic streptococci	0	1	1	P > 0.05
<b>Lactobacilli</b>	<b>13</b>	<b>22</b>	<b>35</b>	<b>P &lt; 0.015*</b>

(\*) statistically significant level P < 0.05

Table 4: showed the presence of symptoms as discharge ,pruritis and soreness are not reliable evidences for the diagnosis of abnormal bacterial colonization of the vagina as tow thirds of women with abnormal flora are asymptomatic.

**Table4: the association between vaginal colonization and the presence of symptoms.**

	Symptomatic	Asymptomatic	Total
<b>Normal flora ( Grade I )</b>	<b>8 36.4%</b>	<b>14 63.6%</b>	<b>22</b>
<b>Abnormal flora ( Grade II &amp; III )</b>	<b>10 35.7%</b>	<b>18 64.3%</b>	<b>28</b>
<b>Total</b>	<b>18</b>	<b>32</b>	<b>50</b>

P= 0.8 NS

**Discussion:**

preterm labor is still a challenge to the obstetricians as most of the current treatments with tocolytics are marginally effective ( 1, 2, 3, 4, 5 ), and many hypotheses were discussed and the hypothesis of infection is among the most promising theory as there are a lot of evidences to support it ( 4,5,6,7,8 ) as it has been found that there is an association between microorganisms isolated from the amniotic cavity , the decidua , the cervix and the vaginal flora ( 6, 7, 8 ) but unfortunately treatment with antibiotics during the onset of preterm labor was inconclusive may be because the treatment with antibiotic was initiated late after the triggering an irreversible sequences of labor ( 7, 12, 13 ) according to that there is a need to identify infection early enough to prevent PTL

Di Giulio et al (2010 ) and Han et al (2009) found that the major rout for bacterial amniotic colonization is from bacteria colonizing the vagina by ascending through the cervix with and without rupture of amniotic membranes (8, 9), this makes estimation of vaginal microorganisms an important tool trying to predict PTL

In this study , bacterial vaginal flora was determined by high vaginal swab and a direct Gram's staining using the Spiegel criteria ( 10 ) as it is the method used routinely by the microbiology lab in Baghdad teaching hospital.

It has been found that there is a significant associations between abnormal vaginal flora and preterm labor. This result was compatible with a lot of body of informations(1, 2, 4, 5, 6 7, ).Found this association this means that when there is reduction in the number of Lactobacillus spp. other bacteria predominates and the explanation is the Lactobacillus spp. Convert glycogen to lactic acid reducing the vaginal pH become unsuitable for the pathogenic bacteria to thrive , also Sabah Kalyosef et Al et al found that in addition to the acidity produced by the Lactobacilli , they also produce hydrogen peroxide ( H<sub>2</sub>O<sub>2</sub> ) and a specific antibacterial proteins (bacteriocins ) which directly inhibits the growth of other bacteria(11) ,this can explain why grade I vaginal flora rich in Lactobacilli associated with term pregnancy. Finding an infectious cause of PTL is difficult as this requires the

identification of organism in diseased person then treatment of that organism should cure the disease and then finally introduction of that microorganism should create PTL Kock's postulate, and this is very difficult so most studies looking for proven associations rather than causative relation.

Many types of microorganisms can be associated with PTL especially Gardnerella vaginalis this study agrees with Di Giulio (2010) et al and Akila et al who found that PTL was associated in addition to G. vaginalis ,with Ureaplasma, Mycoplasma, Klebsiella , streptococci , staphelococci ,E.colli , yeast Candida and the parasite trichomonas vaginalis ( 3,7, 8 , 15 ) . Although Gardnerella was blamed for PTL , prophylactic metronidazole alone did not reduce the incidence of PTL( 3, 13, 16 ) , Gelberet al(2008) describes the bacterial vaginosis as a complex and the exact role of Gardnerella although important, is not fully understood (7). Swidinski et al described the role of this bacteria in creation of a biofilm in which many types of microorganisms live and interact together enjoining an environment that resist the body defenses mechanisms (16) and systemic antibiotics as these organisms live in vaginal cavity adherent to the vaginal epithelium but do not invade the tissues.

Many trials of using antibiotics for the treatment of abnormal vaginal flora fail to prevent PTL ( 3, 7 , 8 , 15, 17 ) and Kimberly (2012) gave the following explanations 1-the antibiotics kill the bacteria but their lipid polysaccharide wall remain and trigger the inflammatory reaction that stimulate labor( 13 ). 2- may be the antibiotics were given late after the triggering of the inflammatory response . 3- metronidazole may not able to kill the bacteria that present in a biofilm.(16) 4- metronidazole may not be effective against other types of bacteria which are associated bacterial vaginosis(18) . so if these explanations are true then earlier detection and treatment of abnormal vaginal flora may be crucial for the prevention of PTL

A trial of a combination antibiotics that cover aerobic, anaerobic and mycoplasma showed a significant reduction in PTL but the results needs further evaluation( 3, 17 )

High vaginal swab culture and sensitivity needs 72 hours to be ready and this with the difficult interpretation of the results reduces the practical usefulness in dealing with patients at risk of PTL.

On the other hand direct Gram's staining can detect derangement from normal flora whatever the type of bacteria and it is simple and the results can be available on the same day , this makes it a practical test to be used in antenatal clinic. The severity of disruption of normal vaginal flora may play a role in reducing tocolytic treatment effectiveness as show in this study in table 2. The higher the grade of vaginal flora the less the duration till delivery, this may reflect the intensity of inflammatory response which trigger the process of labor and the failure of tocolytic treatment although this did not give

a statistical significance due to small number of cases. This study agreed with most literatures regarding the poor reliability of symptoms related to abnormal vaginal colonization as about two thirds of women with abnormal vaginal flora were asymptomatic (3, 6). In summery, vaginal infection is associated with PTL, the vagina is the site from which the bacteria reach the uterus, many types of microorganisms can be associated with PTL, Lactobacilli are protective against abnormal vaginal colonization this mean protect against PTL. Testing of abnormal vaginal colonization by culture and sensitivity is time consuming

#### **Conclusions:**

Normal vaginal flora of Lactobacillus spp. are protective against PTL while abnormal vaginal flora is associated with PTL. Gram's staining of high vaginal swab can detect abnormal flora rapidly and can predict PTL, while culture is time consuming and may be difficult to interpret.

#### **References:**

- 1- Rannio F, Lamout M, Ruth, Pauls E.A, Adinkra . *Advance in the use of antibiotics in the prevention of preterm labor. Recent advance in obstetrics and gynecology* .21: P 35-44.
- 2- G.G.Dondres, K. Van Calsteren, G. Bellen, et al . *Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. B.J.O.G. 2009; 116 10: 1315-1324.*
- 3- *William's Obstetrics textbook 23edit. 804-831.*
- 4- Lawn, J. E., Gravett, M. G., Nunes, T. M. *Global report on preterm birth and stillbirth (1 of 7): Definitions, description of the burden and opportunities to improve data. BMC Pregnancy and Childbirth, 2010, 10(Suppl.), S1.*
- 5- Robert, L. Goldenberg, John, C. Hauth, Williams, W. Andrews. *Intrauterine infection and preterm delivery. The New England Journal of Medicine 2000;vol. 342, number 20.*
- 6- Rebecca, M. Brotman. *Vaginal microbiome and sexually transmitted infections: an epidemiology perspective. The Journal of Clinical investigation. 2011 Vol. 121 number 12 December.*
- 7- Kimberly K. Jefferson. *Advances in Applied Microbiology 2012, vol. 80 . ISSN 0065-2164.*
- 8- Di Giulio, D. B., Romero, R., Kusanovic, J. P. et al . *Prevalence and diversity of microbes in the amniotic fluid , the fetal inflammatory response, and pregnancy outcome in women with preterm labor pre-labor rupture of membranes . American journal of reproductive immunology, 2010, 64: 38-57.*
- 9- Han, Y. W., Shen, T., Chung, P., et al. *Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth. Journal of Clinical Microbiology, 2009, 47: 38-47.*
- 10- Carol A. Spiegel., Richard Holmes. *Diagnosis of bacterial*

*vaginosis by direct Gram stain of vaginal fluid. Journal of clinical microbiology 1983. 18: p170-177*

11- Sabah Kalyoussef, Edward Nieves, Ellen Dinerman, et al. *Lactobacillus proteins are associated with the bactericidal activity against E. coli of female genital tract secretions. Plos one November 2012. Vol. 7 issue 11. e49506.*

12- Paulo Cesar Geraldo, Edilson D. Araujo, Jose Eleuterio Junior, et al. *The prevalence of urogenital infections in pregnant women experiencing preterm and full-term labor. Infectious diseases in obstetrics and gynecology, vol. 2012, article id 878241, 4 pages.*

13- Dulay, A. T., Buhimschi, C. S., Zhao, G., et al . *Soluble TLR2 is present in human amniotic fluid and modulate the intra-amniotic inflammatory response to infection. Journal of immunology 2009. 182, 7244 – 7253.*

14- Gelber, S. E., Aguilar, J. L., Lewis, K. L., et al. *Functional and phylogenetic characterization of vaginolysin, the human specific cytolyisin from Gardnerella vaginalis. Journal of bacteriology, 2008. 190, 3896 – 3903.*

15- Akila Subramaniam, Adi Abramovici, William W. Andrews, et al. *Antimicrobials for preterm birth prevention. Infectious diseases in obstetrics and gynecology. Volume 2012, article ID 157159, 12 pages. Doi:10.1155/2012/157159*

16- Swidsinski, A., Mendling, W., Loening-Bauke, V., et al. *Adherent biofilm in bacterial vaginosis . Obstetrics and gynecology 2005. 106: 1013-1023.*

17- Morency, A. M., Bujold, E. *The effect of second trimester antibiotics therapy on the rate of preterm birth. journal of obstetrics and gynecology Canada, 2007, 29: 35 – 44.*

18- Mitchell, C. M., Hitti, J. E., Agnew, K. J., et al. *Comparison of oral and vaginal metronidazole for treatment of bacterial vaginosis in pregnancy; impact on fastidious bacteria BMC infectious diseases. 2009 ,9: 89.*