

Bacterial vaginosis and other infectious agents in preterm labour in Kirkuk province.

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Abstract

Preterm labour was known as occurrence of delivery before completing 37 weeks of gestation, through which reduction in normal vaginal flora will occur specially lactobacilli in addition to change of pH, which may lead to increases of some aerobic bacteria such Gardnerella vaginalis , so this study was conducted to assess bacterial vaginosis rate among women with preterm labour. From the period of 1st of December2008 to 31th 2009 retrospective study was carried on in Suliamania teaching and Azadi teaching Hospitals. Seventy five women were enrolled in the study, their age ranged from 15 to 45 years. For each patient special questionnaire form was filled ,also for each high vaginal swab(HVS) was tested for color,pH,whiff test and Clue cells in addition to cultivation of HVS & urine for detecting other bacterial isolates. Women with polyhydraminos, multiple pregnancy, cervical incompetence, malformed uterine & drug addict were discharged from the study. The results were revealed the all rate of infection 76.67 % which included bacterial infection 42.67%(high rate 16.07 % with Escherichia coli and the low rate was with Proteus species),followed by 33.33 %,18.67 % & 8 % for, Gardnerella vaginalis, Candiada albicans & Trichomonas vaginalis respectively P<0.05.Gardnerella vaginalis was seen in 21 samples as pure cases with the rate 84 % mostly associated with clue cells & pH ranged from 6 to 8 with the rate of 26.42 %, Moblincus species rate was 9.12 %. Relationship between patient residency, age in regard bacterial vaginosis & other microorganisms' distribution were not statistically significant. History of vaginal discharge, disparunia, rupture membrane & vaginal itching in association to microbial distribution were significant P<0.05.

Introduction

Preterm labour is the greatest cause of prenatal morbidity and mortality in industrial countries (Nejad, Shahla,2008) . Preterm labour is delivery of a baby before 37 completed weeks of pregnancy. It's reported in about 12% of pregnant women, Bacterial vaginosis (BV) double risk of preterm labour [Keith 2007]. Several factors which predispose to preterm delivery. It seems that activation of hypothalamus – pituitary – adrenal axis of mother or the fetus , decidual hemorrhage, pathological uterine distension and infection are influencing factors (Lockwood, 2004) . Epidemiologic studies have shown that stress and infection of maternal genitourinary system independently and significantly increase the risk of preterm labor. (Gity, *et.al*; 2008) Theoretically, pathogenic organisms ascend via lower genital organs

to uterine cavity and may cause fetal membrane inflammation which lead to preterm rupture of membrane and labor. Maternal genitourinary infection can initiate the process of labor and cause preterm labor via different mechanisms including activation of cytokine cycle and acceleration of labor process (Menard,*et.al*;2010).

Bacterial vaginosis (BV) is a common condition characterized by a polymicrobial disorder, with an overgrowth of several anaerobic or facultative bacteria with a reduction or absence of lactobacillus colonization. (Guaschino,*et.al*;2008) The prevalence of BV ranges from 4 to 64%, depending on the racial, geographic and clinical characteristics of the study population. In asymptomatic women, the prevalence varies from 12 to 25%, and similar percentages were observed in pregnant women [Nejad, Shahla,2008]. .The microbiologic changes that characterize BV have been well studied. In women with BV, lactobacilli (especially hydrogen peroxide - producing strains) are greatly diminished, and large numbers of anaerobic and facultative anaerobic bacteria seem to "take over." These organisms include Gardnerella vaginalis, Mycoplasma hominis, Bacteroides species, Prevotella and Porphyromonas species Peptostreptococcus, and Mobiluncus species (Menard,*et.al*;2010).

The Amsel's criteria defines BV by the presence of three of four factors: (1) a homogenous vaginal discharge, (2) a vaginal pH greater than 4.5) (3) a positive "whiff" test result (a fishy odor when vaginal secretions are mixed with 10% potassium hydroxide), and (4) clue cells in the vaginal fluid wet preparation, (Spiegel, *et.al*, 1980 and Jack, *et.al*. 2008).

For pregnant women, the Centers for Disease Control and Prevention recommend using metronidazole, 250 mg three times daily for 7 days, on the basis of data from a preterm delivery prevention study (Hauth, *et.al*, 1995 ; Sobel and Leaman, 1998). There is concern that topical preparations used to eradicate BV in this setting may not be adequate for the treatment of possible subclinical upper genital tract infections, but limited data are available. (Hillier, *et.al*; 1998) Treatment of male partners is currently not recommended. There are no definite recommendations for the treatment or prevention of recurrent BV, but there is interest in using intermittent dosing of metronidazole gel, condom use & as prophylaxis for recurrent infection (Goering, *et.al*; 2008). Anecdotally, women report that consistent use of condoms is helpful in preventing recurrence. Another suggested treatment is recolonization of the vaginal flora with a vaginal suppository that contains an exogenous strain of hydrogen peroxide-producing *Lactobacillus crispatus*. This product is currently being studied in clinical trials. (Burhan, 2008) . This study was conducted to estimate the rate of bacterial vaginosis & other microorganisms that cause vaginal discharge during preterm labour and to detect the relationships between occurrence of Bacterial vaginosis with other factors like age, pH & color of discharges.

Materials & methods:

Site of study, patients' selection & samples collection:-

The study was carried on from 1st of December 2008 to 1st of July 2009 in Kirkuk and Sulaimani cities among women, who attended outpatient clinics of Azadi Teaching Hospital in Kirkuk city and Maternity Teaching Hospital in Sulaimani city. A total of 75 women, (48 women from Kirkuk city and 27 women from Sulaimani) ,in their reproductive age, suffering from preterm labour from (24 to 37) weeks

of pregnancy ,without other risk factors such as: uterine over distension, cervical incompetence, congenital abnormality of uterus and drugs including caffeine ,smoking and alcohol/.

A careful history was taken from them according to questionnaire prepared for this purpose including general information, obstetrical history, vital signs examination and abdominal examination. For each patient two samples were taken first urine sample (for culture and sensitivity using bacteriological artificial culture media under aerobic condition of cultivation (Cowen and Talaro, 2009), then after urine deposit was examined under microscope for detecting *Trichomonas vaginalis* in wet preparation(Markell and Voges,2006) .While the second sample high vaginal swab(HVS) which was taken under good source of light and soaked in transport media(Stuart's media) at once transported to the lab for direct examination as full and for culture and

Identification of microorganism

High vaginal swab was used for detection of the following:

-Vaginal pH: Vaginal pH measurement was made by flooding a piece of graduated pH paper into vaginal discharge on the vaginal swab. The color is then compared to the color and corresponding pH values on a standard chart with a scale pH ranging from 1-14.

Whiff test: was done by adding few drops of 10 % of KOH solution on clean microscopic slide containing small portion of vaginal discharge .A fishy odor due to liberation of amine group is an indicator for *Gardnerella vaginalis*(Burhan,2008).

-Detection of clue cell: Gram stain was used to detect the presence of clue cells using oil emersion objective lens X100 which can reveal attachments of coccobacilli to epithelial cells. Microscopical examination of wet mount preparation also used to detect a clue cell which is one of Amsel's criteria used to diagnose bacterial vaginosis[Lamont and Fisk,1993].

Results:

During the period of the study, a total of 75 Patients with preterm

labour, attending Gynaecology /Obstetrical department in Maternity Teaching Hospital in Sulaimani & Azadi Teaching Hospital in Kirkuk , primi or multi-gravdia,in their reproductive age, history of vaginal discharge was found in 65.33% Of patients. Only 8% was with history of vaginal bleeding .Considering to frequency; urgency and dysuria were seen 30.67% patients. In patients with history of rupture membrane, 34.67% were positive, only 18.67% had history of mal-odor after intercourse; also 2.67% had history of dyspareunia. Regarding Puritus and those with previous history of preterm labour were seen in 32%, 9.33% of patients respectively, (table 1).

Table (1) Clinical history in relation to vaginal infection

Clinical histories	No. +ve	% +ve
Vaginal discharge	49	65.33
History of bleeding	6	8
Disurea,frequency & urgency	23	30.67
Mal-odor after coitus	14	18.67
Disparunia	2	2.67
Peruritis(vaginal itching)	24	32
Previous history of preterm labour	7	9.33
History of rupture of membrane	26	34.67

The relationship between clinical history and type of microorganisms distribution was significant $P < 0.05$, via which the history of vaginal discharge and rupture of membrane were showed high rates of Gardnerella vaginalis (68 % & 60%) & the lower rate (4 %) was with the history of disparunia. While Trichomonas vaginalis infections were widely recorded among women with history of vaginal discharge, mal odor, pururitis (100% ,50 %and 50 %) respectively. Other bacterial infections with the different parameters presented in women enrolling the study shows fluctuate rates mostly high (59.38 %) in women with history of vaginal discharge followed by (34.38 %)

in women with dyspareunia while the lower rate(9.8 %)was recorded in women with malodor after sexual intercourse,(table-2).

Table (2) Relation of clinical history to different types of infective micro-organisms

Type of infection	Gardnella vaginalis No.+ve & %	Trichomonas vaginalis NO.+ve & %	Other bacterial infections No+ve & %
Vaginal discharge	17 68	6 100	19 59.38
Vaginal bleeding	3 12		
Disuria,frequency&Urgency	11 44	2 33.33	11 34.38
Rupture membrane	15 60	2 33.33	16 50
Mal-odor after coitus	3 36	3 50	3 9.38
Dyspareunia	1 4	1 16.67	
Pruritus (Vaginal itching)	13 52	3 50	10 31.25

In spite of high rates obtaining in regard of vaginal infection with microorganisms and women ages but the relationship was statistically not significant, $p>0.05$ (table -3).

Table (3) Distribution of vaginal infection according to different age groups

Age /years	No.Exam.	%	No.+ve	% +ve	No.-ve	% -
15 to 25	37	49.33	28	75.68	9	24
26 to 35	32	42.67	24	75	8	25
36 to 45	6	8	6	100		
Total	75	100	58	77.33	17	22.67

From a total of 75 cases ,high vaginal bacterial infections were detected in 32 cases with rate of 42.67%(included both Gram positive and negative bacteria),while BV detected in 25 cases and the rate was 33.33% followed urinary tract infection (UTI),fungal and protozoa infection in 20,14&6 cases and the rates were 26.6%,18.67%&18.67% respectively (table 4).

Table (4) Different type of infection in preterm labour.

Type of infection	No.+ve	% +ve
Vaginal other bacterial infections	32	42.67

Gardnerella vaginalis	25	33.33
Urinary tract infection*	20	26.66
Candida albicans	14	18.67
Trichomonas vaginalis	6	8.0

*Only 2 cases pure UTI, others combined with vaginal infection. Due to co-infections, so the of +ve cases number were more than 56 in this table.

Considering mode of infection, the rate of single infection was 69.64 % while double infection rate was 23.22 % and triple infection rate was low 7.14 , $P < 0.05$ (table-5) .

Table (5) Mode of vaginal infections in preterm labour

Type of infections/	No.+ve	%+ve
Single infection	39	69.64
Other bacterial infection	20	35.71
Gardnerella vaginalis	10	17.85
Candida albicans	7	12.5
Trichomonas vaginalis	2	3.57
Double infections	13	23.22
Garnerella & Trichomonas	6	10.71
Gardnerella +Candida	3	5.35
Gardnerella+other bacterial infections	2	3.57
other bacterial infections + Candida	2	3.57
Triple infection	4	7.14

Direct chemical examination as Amsel's criteria, (table 6), reveal the followings: thin homogenous vaginal discharge 56%, pH

>4.5 100, whiff test 84%, clue cells on wet mount preparation were positive for Gardnerella in 21 samples 84% .

Table (6) Amsel's criteria predictor for diagnosis of Gardnerella vaginalis

Amsels criteria	NO. +ve	% +ve
Clue cells	21	84
Whiff tes	21	84
pH >4.5	25	100
Thin homogenous vaginal discharge	14	56

*Total No. of cases 25, **pH mostly between 6-8.

Bacterial vaginosis due to Gardnerella & mobiluncus were seen in 26 sample, through which Gardnerella vaginalis was higher than Mobiluncus, the rates were 37.5 % & 9.12 respectively P<0.05. The high rate of Mobiluncus was recorded as triple infections ,while Gardnella infections were recorded as double & single infections.

TABLE (7) vaginal infection with Gardnerella and mobiluncus as single, double and triple vaginal infections

Type of bacterial vaginosis	NO.+ve % +ve	Single		Double		Tripi No.+
		NO.+ve %	%	No.+ve	%	
Gardnerella vaginalis	21 37.5	9 3.57	16.07	10	17.85	2
Mobiluncus species	5 9.12	1 5.35	1.75	1	1.75	3
Total	26 46.42	10 8.92	17.85	11	19.64	5

Total number positive=56

From a total of 56 of high vaginal swab cultivation only

31 swabs (41.33%) were positive for bacterial infections, the high rate 16.07% was recorded with *Escherichia coli*, followed by 10.71%, 8.92%, 7.1% for *Enterobacter* species, *Staphylococcus aureus* and *Klebsiella* species while the lower rate 1.78% was with *Proteus* species (table-8).

Table (8) Frequency of bacterial isolates from HVS & urine samples

Type of bacterial isolates	No.+ve	% +ve
<i>Escherichia coli</i>	9	16.07*
<i>Enterobacter</i> species	6	10.71
<i>Staphylococcus aureus</i>	5	8.92
<i>Klebsiella</i> species	4	7.1
Alpha-haemolytic <i>Streptococcus</i>	3	5.35
<i>Streptococcus faecalis</i>	3	5.35
<i>Proteus</i> species	1	1.78
Total	31	41.33

Total number Exam = 75. Total number positive = 56. *P<0.05

Discharges of vagina were checked for pH, the results showed distribution of micro-organism, in two scale of pH: 4-6 which revealed 33.33% of microorganisms, while the second scale of pH: 6-8 showed 41.33%, statistical analysis was not significant P>0.05 (Table-9).

Table 9-Incidence of micro- organisms in relation to vaginal pH

Vaginal PH	No.exam	%	No.+ve	% -
4 to 6	32	42.67	25 *	33
6 to 8 **	43	57.33	3**	4
Total	75	100	56	74

*2 cases of pure UTI within this scale, **.most of Bacterial vaginosis within this range.

Distribution of microorganisms according to color of discharge, (table 10) showing that white-translucent discharge was with high rates 70.67% through which 14 samples (56%) were positive for *Gardnerella Vaginalis* (GV), while other color of discharge revealed low rates, P<0.05 (Table-10).

Table (10) Distribution of infectious agents in relation to color of

vaginal Discharge

Color of vaginal discharge	No.exam &%	Gardnella NO.+ve & % +ve	Mobiluncus +ve & %	Trichomonas No,+ve & % +ve *	Other Bacteria Infectio
White	53 70.67	14 56 *	4 * 80		4 12
Bloody	16 21.34	2 8.0	1 20	1 16.66	2 6.
Yellow	4 5.33	3 12.0		1 16.66	20 64
Green	1 1.33	3 12.0		4 66.66	2 6.
Brown	1 1.33	3 12.0			3 9.
Total	75 100	25 100	5 100	6 100	31 100

*P<0.05.

Discussion

The prevalence of bacterial vaginosis (BV) in the present study among women in their reproductive age, either prime or multigravida suffered from preterm labour, was 33.33% ,this agrees with a study which showed the prevalence of BV in preterm delivery ranging from 10-30% in England& New York by (Lamont et.al.1993,and Chery et.al.1996) respectively, this rate is higher than that recorded in Kerman in Iran by (Nejad, Shahla,2008) , whom they recorded 25%, also it is higher than 27.7 % that recorded in Isfahan in the same country by (Simoes et.al.2008) Also prevalence of BV is higher than those recorded in Thailand,USA& in Denemark the rates were25.8%.&16%,16% respectively(Awassanan et.al.2007, Sharon

Significant relation between preterm labour and rupture membrane with BV in the present study in a rate of 60%, this rate when it was compared with a study in Tehran/Iran(Gity et,al;2008) which reveal 64.29% .Considering the rate of BV 40% in present study with preterm labour associated with intact membrane , was agree with what found in same study as 35.71% but higher than that recorded in Paris as 6.8% (Goffinet et.al.,2003,). Also the relationship between preterm labour with previous history of preterm labour in present study 9.33% is agree with a study done in England by (Hay et.al.,1994) ,but ,this rate disagreed with that recorded in the study in Kerman/Iran whom they show 25% of preterm labour had previous history of preterm labour (Vida et.al.,2008).

Comparing the rate in present study with the scattered studies that recorded in Iraq, BV infection was underestimated in Iraq, because its real cause is still controversial (Al-Zubaidy, 2001). The comparative study which was carried on in both Tikrit & Kirkuk cities by (Burhan, 2008) revealed the prevalence of BV among women in third trimester than in second and first trimesters the rates were 33.33 % and 20% respectively. In Najaf city , increased risk of preterm labour in women with history of preterm labour was 1.9 time ,and this also agrees with a study of[Sharon et.al.,1995] whom they shows 1.4 time ,the mechanism by which BV predispose to preterm labour is not known ,but there is evidence that it can cause infection of upper genital tract ,moreover it had been found that this disease was associated with 2-3 folds increase infection of amniotic fluid (Watts et.al.,1990and Sharon et.al.1995) and infection of chorion and amnion, (Sharon *et.al.*,1988,US). Variances in all rates might be due several factors including type of laboratory methods, size of samples, level of sanitation & others. The color of discharge that most commonly associated with BV White-translucent vaginal discharge in a rate of 56% which agreed with a study in Tikrit in a rate of 60.86% while it was less than that recorded in Kirkuk in a rate 30.35%, (Burhan,2008) .In the present study the pH of vagina in those who had BV mostly between (6-8) 57.33% , when it was compared with a study about the impact of regular vaginal pH screening on the diagnosis of bacterial vaginosis in pregnancy show that regular vaginal pH tested had higher rate detection of bacterial vaginosis than control group [10.10% versus 20.10% and this increase the incidence of diagnosis of preterm delivery 1.5 fold than control group[Dwenda,2000].

Clue cell was one of the predictor for diagnosis of BV in our study found in a rate of 84 % , which agrees with other study that shows sensitivity of clue cell for diagnosis of BV in a rate of 90%.[Jack et.al.2008] . In the present study the rate of candidiasis 18.67% which is close to 14%that recorded in Australia by (Herbert *et.al.*2004), while it was lower than that recorded by(Gravett *et.al.*,1986) whom show 45%. Also the rate of urinary tract infection(UTI) in present study was 26.67% , which is in agreement with that recorded by(Harmanli ,2000), who recorded 22.4% , also

with other study which shows 12.5% to 30%, (Little PJ, 1966).

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داء المهبل الجرثومي ومسببات عوامل الأمراض الأخرى في النساء اللواتي يعانين من الحمل السابق لأوانها في محافظة كركوك

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الخلاصة

تعرف الولادة السابقة لأوانها بولادة مولود قبل إتمام الأسبوع السابع و الثلاثون من الحمل. اختزال أعداد البكتريا الطبيعية للمهبل وبخاصة العصيات اللبنية وكذلك التغيير في باهء المهبل إلى المعتدل يؤدي إلى زيادة في تكاثر البكتريا المسببة لداء المهبل الجرثومي (Bacterial vaginosis) وتشمل: *Gardenella vaginalis* وغيرها من الجراثيم. تم اقتراح اجراء الدراسة لمعرفة نسبة انتشار الداء المهبلي بين النساء الواتي يعانين من الولادة السابقة لاوانها في مستشفى الولادة التعليمي في السليمانية و مستشفى ازادي التعليمي في كركوك، من كانون الاول 2008 ولغاية حزيران 2009. شملت الدراسة 75 امرأة تتراوح أعمارهم بين (15-45) سنة، حامل بين أسبوع (24-37) يعانون من الولاده السابقة لاوانها سواء ولادة بكر او ولادة متكررة، تم اختيارهم بطريقة عشوائية. تم جمع المعلومات عن طريق استبيان معد لهذا الغرض، و تضمنت الطرق المختبرية: فحص القطيلات المهبليية من حيث اللون، الباهء واستخدام طريقة الويف (Whiff test) لتحديد رائحة الأمين المتحررة من تواجد المستديمة المهبليية، وتم التأكد من الفحص بتحديد خلايا كلو (Clue cell) في المسحات المحضرة من الإفرازات المهبليية المصبوغة بطريقة غرام. المسحة الماخوذة من أعلى المهبل (high vaginal swab) لغرض الزرع الجرثومي وتضمن البحث ايضا فحص البول العام والأستنبات الجرثومي للبول ومسحات اعلى المهبل وتم استعمال فحص السونار واغراض تشخيصية أخرى. النساء الحوامل اللواتي تم استبعادهم من البحث: زيادة السائل الامنيوسي، الحمل متعدد الاجنه، رخاوة في عنق الرحم، تشوهات الرحم والمدمنات على الكحول، كافائين والتدخين.

النتائج: كانت نسبة الخمج الكلية 76.67% توزعت الى 42.67%، 33.33%، 18.67%، 8%.

للاخماج الجرثومية، المستديمة المهبليية، الفطريات، والابتدائيات على التوالي. $p < 0.05$.

أظهرت طرق الفحص الكيمائي المباشر باستخدام معايير امسيل (Amsel's criteria) (فحص الويف، الباهء، خلايا كلو وإفرازات مهبليية بيضاء شفافة متجانسة) بان المستديمة المهبليية كانت متواجدة في (84%) 21 نموذج وبنسبة 42.86% كحالات نقيه او منفردة. وكانت نسبة تواجد

جرثومة موبلنكس (Mobiluncus) في فحص الغرام كعصيات معقوفة بنسبة 9.12 %
توزعت المستديمة المهبلية في الإفرازات البيضاء ذات الباهاء المتراوحة بين 6-8 بنسبة
26.42%. العلاقة بين توزيع الإصابات المهبلية للمستديمة المهبلية والأحياء المجهرية الأخرى
بالنسبة للعمر وإقامة المرضى كانت غير معنوية.