

# Frequency of Bacterial Vaginosis in Babylon Governorate and its association with intrauterine contraceptive device

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## Abstract

This study aimed to assess the frequency of bacterial vaginosis (BV) and its association with some risk factors like intrauterine contraceptive device (IUCD). A total number of 111 patients with vaginal discharge were enrolled in this study in addition to control group (30 women). Case and control groups were taken from Babylon Hospital and some out patients clinics between January and December 2009. Only 46 out of 111 (41.44%) patients had BV while it was only (10%) in the control group with statistically significance (P value < 0.05). The most commonly affected age groups were (20-29 years) and (30-39 years) with the following percentages respectively (28.26 % ; 36.95%). The leukocyte cells in gram staining of vaginal swabs showed that ( 84.79% ) of BV have leukocyte cells less than 10 cells /HPF while only (15.21%) have leukocyte cells > 10/HPF (P value < 0.05). Thirty three out of 46 (71.73% ) patients with BV had urinary tract infections (UTI) in comparison to (28.27%) without UTI ( P value <0.05). The study showed that BV had a high frequency rate (67.39%) in pregnant women when compared to non-pregnant women (32.61%). Twenty out of 31 (64.51%) pregnant women have premature uterine contractions (PUC ) while only 11 out of 31 (35.49%) presented without PUC ( P value <0.05). Ten out of 15 (66.66%) non pregnant women patients were used IUCD in comparison to only 5 out of 15 (33.34%) patients with BV. PCR detection of *l6S rRNA* gene for *Gardnerella vaginalis* (*G. vaginalis*) reveal insignificant change in frequency of *G. vaginalis* in patients with BV (45.65 % ) in comparison to control group (36.6%) ( P value > 0.05). As the BV is treatable medical disorder and it's frequently ignored as the symptoms are frequently irrelevant but the clinical consequences could be vital, it can be concluded that medical screening for BV is mandatory specially in antenatal care of pregnancy in Primary Health Care Centers and women using IUCD to avoid future complications.

**Keywords:** Bacterial Vaginosis , intrauterine contraceptive device, urine examination

## الخلاصة

الهدف من هذه الدراسة هو لتقييم نسبة حدوث الالتهابات المهبيلة ومدى علاقتها مع بعض عوامل الخطورة مثل مانع الحمل الرحمي. تمت دراسة (١١١) حالة مرضية لنساء تعاني من افرازات مهبيلة بالإضافة الى ٣٠ حالة كمجموعة سيطرة. تم اخذ العينات المرضية وعينات السيطرة من مستشفى بابل وبعض العيادات الخاصة في الفترة (كانون الثاني- تشرين الثاني ٢٠٠٩). تبين من هذه الدراسة ان ٤٦ من اصل ١١١ (٤١.٤٤%) حالة كانت تعاني من الالتهابات المهبيلة بالمقارنة مع (١٠%) من حالات السيطرة مع فارق معنوي. كانت اكثر الحالات المرضية في الاعمار (٢٠-٢٩ سنة) و(٣٠-٣٩ سنة) بالنسبة التالية (٢٨.٢٦% : ٣٦.٩٥% بالتتابع). أظهر تعداد الخلايا البيضاء في صبغة الغرام للمسحات المهبيلة ان ٨٤.٧٩% من الحالات المرضية هي اقل من ١٠ خلية و (١٥.٢١%) من الحالات اكثر من ١٠ خلية مع فارق معنوي. كذلك تبين من هذه الدراسة ان ٣٣ من اصل ٤٦ (٧١.٧٣%) حالة التهاب مهبل كانت تعاني من التهاب المجاري البولية بالمقارنة مع (٢٨.٢٧%) حالة بدون التهاب المجاري البولية مع فارق معنوي. كذلك وجد ان نسبة حدوث الالتهابات المهبيلة تكون اكثر في النساء الحوامل (٦٧.٣٩%) بالمقارنة مع غير الحوامل (٣٢.٦١%). وكان ٢٠ من اصل ٣١ (٦٤.٥١%) حالة التهاب مهبل مع الحمل تعاني من تقلصات رحمية مبكرة بالمقارنة مع ١١ من اصل ٣١ (٣٢.٦١%) بدون تقلصات رحمية. وتبين كذلك ان ١٠ من اصل ١٥ (٦٦.٦٦%) حالة التهاب مهبل بدون الحمل كان لديها مانع حمل رحمي بالمقارنة مع ٥ من اصل ١٥ (٣٣.٣٤%) حالة وبينت نتائج فحص تفاعل البلمرة المتسلسل عدم وجود فرق احصائي في الكشف الجيني عن بكتريا الغاردنريلا المهبيلة بين المرضى الذين يعانون من التهاب مهبل (٤٥.٦٥%) ومجموعة السيطرة (٣٦.٦%). ولكون مرض الالتهابات المهبيلة من الامراض التي من الممكن علاجها والتي يتم تجاهلها في اغلب الاحيان بسبب قلة اعراضها ولكون مضاعفاتها مهمة للغاية، يستنتج من هذه الدراسة انه من الواجب ان يتم التحري الطبي خصوصاً اثناء مراجعة المرضى الحوامل لمراكز الرعاية الصحية وكذلك النساء اللاتي يستخدمن مانع الحمل الرحمي لتجنب المضاعفات المستقبلية.

**الكلمات المفتاحية:** التهاب المهبل، موانع الحمل، التهاب الادراس.

## Introduction

BV is the most frequent vaginal infection in child bearing age women (Reid ,2003). It is characterized by a polymicrobial illness and the first signs of BV are fundamental variations in vaginal ecosystem (Guaschino, *et al.*,2008). H<sub>2</sub>O<sub>2</sub>-producing lactobacilli, which represent 96 % of normal vaginal bacterial flora in female, are obviously diminished or missed, while microorganisms like *G. vaginalis* and facultative and obligate anaerobes prevail (*Prevotella spp.*, *Atopobium vaginae*, *Mycoplasma hominis* and *Mobiluncus spp.*),( Nam ,2007 ; Menard ,2010 ; Fredricks *et al.*, 2005 ; Lamont *et al.*, 2011). The prevalence of BV was (4-64%) depending on the geographic ,racial, and clinical characteristics of the study population. In asymptomatic women, the incidence fluctuated from 12 -25%, and similar percentages were observed in pregnant women [Nejad & Shahla ,2008]. Women with BV typically complained of vaginal discomfort and homogeneous malodorous vaginal discharge, that was more obvious subsequently with unprotected intercourse, while a considerable number of female were asymptomatic (Klebanoff, *et al.*, 2004). In fact, the changes in the vaginal microbiota had associated with infections of upper genital tract and obstetric complications (Romero, *et al.*, 2004 ; Koumans *et al.*, 2002) as well as with UTI (Harmanli, *et al.*, 2000) that result in adverse pregnancy outcomes such as amniotic fluid infection, preterm delivery, and premature rupture of the membranes (McGregor, and French, 2000; Goldenberg, and Culhane, 2003). Many bacteria were associated with urogenital tract infection specially in women with IUCD such as *Klebsellia*, *E. coli*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Streptococcus* , *Enterobacter* (Abdul Razzak *et al.*, 2008). IUCD was frequently used birth-spacing manner which is formfitting into maternal system. Microbial , clinical and cytopathological observation of women using these procedures are essential for establishing their side effects, threat of genital tract infection and carcinogenic effect (Agarwal, *et al.*, 2004) .The presence of IUCD might alter the bacterial flora of genital tract in female. Furthermore, the insertion of an IUCD breaches the protective barrier of the cervical mucus, and its tail creates transmission link into uterus (Elhag, *et al.* ,1988). For this purposes, the aim of this study is to detect the BV frequency and its association with some risk factor specially IUCD and some of its possible complications.

## Patient and materials

### Patients:

A total of (111) female patients were enrolled in this study and 30 women as a control group and both tested for BV. Case and control groups were taken from Babylon Hospital between January and December 2009. The inclusion criterion for case group was vaginal infection according to Amsel's criteria (Amsel,1983) and the suggestive discharge defined as a thin, dark or dull, homogenous malodorous discharge when careful history was taken. Patients with vaginal discharge due to other causes like candidiasis and Trichomoniasis were excluded. Control group (30 women) include patients attending hospitals for other reasons (Hypertension, leg swelling, DVT and other for routine prenatal care). The healthy control group consisted of women without vaginal symptoms in the last 2 months and no evidence of pathological vaginal condition.

### Collection of Specimens:

By the aid of sterile vaginal speculum, two vaginal swabs from each patient were taken from the mid- lateral vaginal sidewall, by means of disposable cotton swabs. These swabs

were rotated before withdrawing. An antiseptic creams and lubricants were avoided to prevent their inhibitory effect on bacteria.

### **Diagnosis of BV**

#### **1-Clinical diagnosis**

The clinical diagnosis of BV is established when 3 out of the 4 criteria are present, which called "Amsel's criteria" (Amsel,1983):This would confirm the diagnosis (National guideline ,2006).These were:

- 1-Clue cells which was visible on microscopical examination (>20%).
- 2- Vaginal fluid pH> 4.5.
- 3- Homogenous white gray discharge.
- 4- Positive whiff test.

Out of (111) female patients presented with abnormal vaginal discharge, only (46) cases had three or more of Amsel's criteria, hence the diagnosis of BV was made.

#### **2-Laboratory diagnosis**

##### **A-Macroscopical examinations**

**1-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 standard color and the corresponding pH values on a standard chart (with a scale pH ranging from 1-14) would be taken. (Goldenberg, *et al.*, 2000).

**2-Whiff test:** The addition of a small amount of potassium hydroxide (10% KOH) to a microscopic slide containing the vaginal discharge causes the release of amine that produces sharp fishy odor. A characteristic "fishy" odor was considered as a positive whiff test and was suggestive of BV (Burhan & Ali ,2008).

##### **B-Microscopical examinations:**

##### **1.Wet-mount preparation: done according to (McComack, and Evans, 1989):**

A wet-mount preparation was obtained by putting a vaginal swab into 2 mL test tube containing saline solution and then placed on a slide with a cover slip. The slide was examined microscopically with scanning of several fields. Microscopic examination of wet-mount preparation was done for:

- 1- Clue cells detection which were vaginal epithelial cells that coated with coccobacilli. Microscopic examination of *G. vaginalis* showed that they were polymorphic cells to double bacilli or single, gram negative to variable gram stain (Holt,1994). The long rod-shaped bacteria were characteristic of normal lactobacillus morphotype. Looking for background bacterial flora whether they appeared greatly increased in number, in addition to short rods and coccobacillary forms predominance. In addition, *Mobiluncus* bacterial species may be recognized as spiral or serpent-like motility. Observing the amount of bacteria in the wet preparation and decrease in the normal lactobacillus type bacteria aided in identification of BV on microscopic examination
- 2.Exclusion of *Trichomonas vaginalis* according to (Kriger,1988) and
3. Exclusion of vaginal candidiasis according to (Ellis,1994).

##### **2-Gram stain**

Gram stained method was done as a confirmatory test to wet mount for detection of clue cells to avoid false positive results of wet mount since it is more reliable and it is one of the predictor for diagnosis of BV. As *lactobacilli* and rarely *Streptococci* stick to desquamated vaginal epithelial cells, *Lactobacilli* will be clarified by gram stain as gram

positive rods. In addition to that it was used for leukocyte counts (Coonrod ,2008 ; Nurgent,1991).

**3.A general urine examination:** was done to see the presence of pus cells for the diagnosis of UTI.

#### C-Culture

For diagnosis of *G. vaginalis* , Columbia blood agar base (Oxoid) in addition to nalidix and gentamicin acid was used and cultivated in microaerophilic atmosphere (5–10% CO<sub>2</sub>) at 35–37 C° for 48–72 hours. The gram-negative or short rods gram-variable with  $\beta$ -hemolytic, transparent colonies, catalase-negative were presumptively diagnosed as *G. vaginalis*.

#### D-Molecular diagnosis

##### DNA extraction:

Vaginal samples total DNA was extracted through the use of DNA kit (Amplif Sens).

**Primer design:** The primes were designed in this study by using NCBI-GenBank recorded sequence and by using primer3 plus design online as following.

Primer		Sequence	Product size
16S rRNA gene	F	TTACTGGTGTATCACTGTAAGG	331 bp
	R	CCGTACAGGCTGAACAGT	

##### Polymerase chain reaction:

PCR was done using Taq DNA polymerase (Gene BIO).

##### DNA amplification protocol:

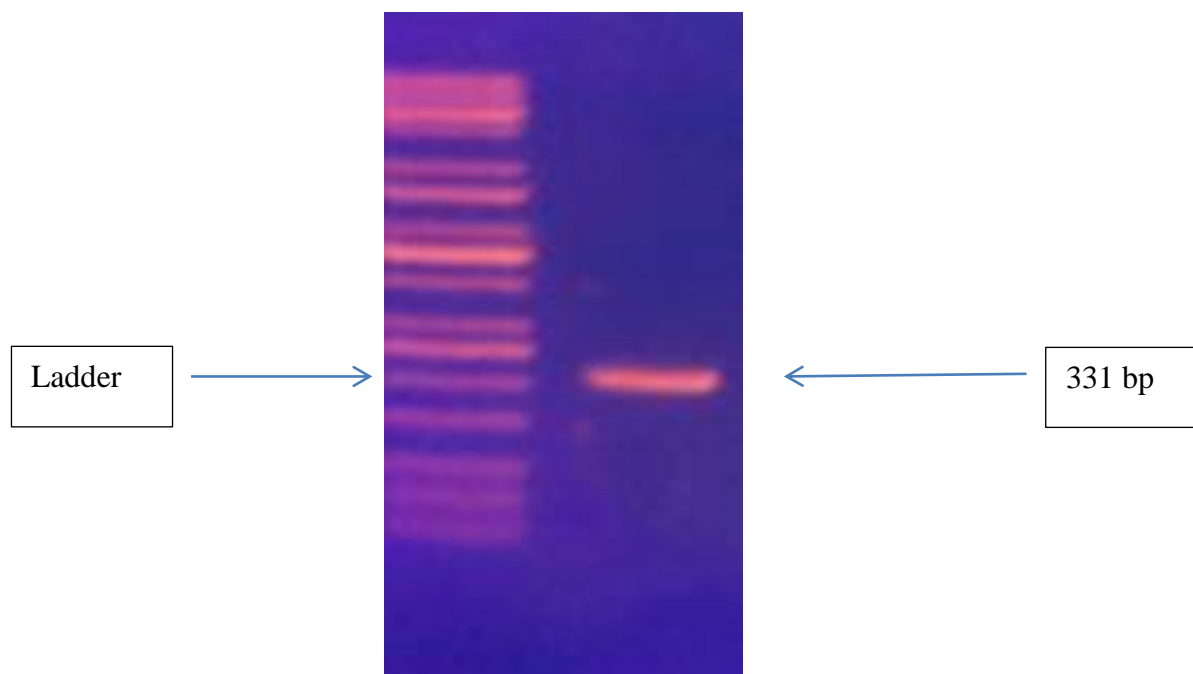
DNA protocol	
The initial denaturation	94 °C for 5 min
(The denaturation ) (30 cycles)	94 °C for 45 sec
The annealing	60 °C for 45 sec
The extension	72° C for 45 sec
The final single extension	7 min at 72 °C

##### Gel electrophoresis:

Separation of the products of PCR were done through 1% agarose gel for 45 minutes and ethidium bromide staining. Augmented genes were recognized by the foundation of their anticipated fragment size (331 bp). (Figure 1)

##### Statistical analysis:

Data are presented and tested for the significance of different data and independent-sample t-test , Chi-square and Fisher's exact tests were applied. Correlation analysis was done in SPSS version 11.0. P values below 0.05 were accepted as statistically significant.

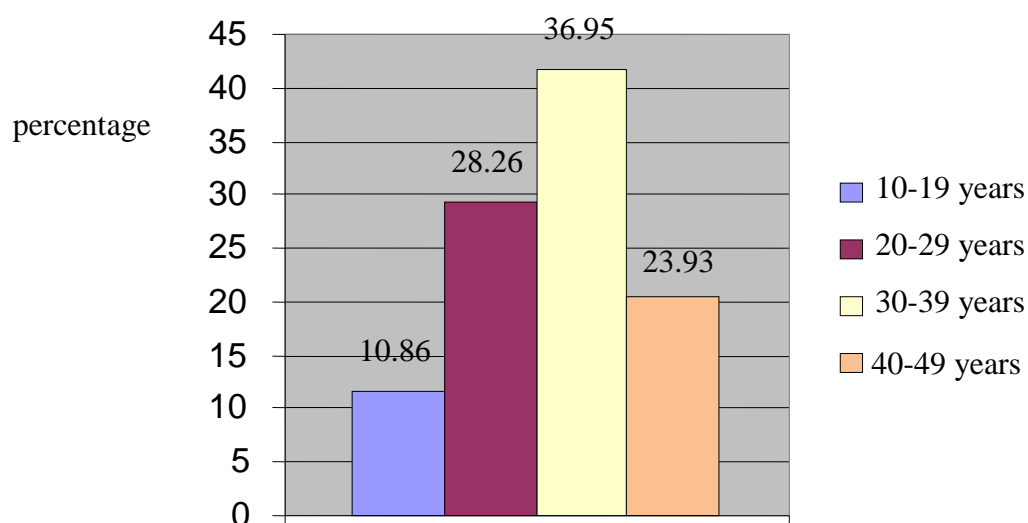


**Figure ( 1 ): Gel electrophoresis of PCR product of 16S rRNA gene**

## Results

**Table(1) : Frequency of BV according to Amsel's criteria**

Amsel's criteria	Patients with abnormal vaginal discharge (111)	%	Control (30 case)	%	P value
Suggestive discharge of BV	72	64.86	6	20	< 0.05
Positive (KOH)whiff test	57	51.3	5	16.5	< 0.05
Clue cells	46	41.44	5	16.5	< 0.05
PH>4.5	61	54.9	7	23	< 0.05
≥ 3 Amsel's Criteria	46	41.44	3	10	< 0.05



**Figure (2): Frequency of bacterial vaginosis according to age groups**

**Table ( 2 ): leukocyte cells in gram staining of vaginal swabs in relation to BV**

Parameters	BV	%	P value
leukocyte cells more than 10 /HPF	7	15.21	< 0.05
leukocyte cells less than 10 /HPF	39	84.79	
Total	46	100	

**Table (3): showed the frequency of BV in relation to UTI.**

Patients with vaginal discharge	BV	%	P value
With UTI	33	71.73	< 0.05
Without UTI	13	28.27	
Total	46	100	

**Table (4):Frequency of BV in relation to the pregnancy**

Parameters	BV	%	P value
Pregnant women	31	67.39	< 0.05
Non pregnant women	15	32.61	
Total	46	100	

**Table (5) : showed the frequency of BV in pregnant women in relation to PUC.**

Pregnant patients with vaginal discharge	BV	%	P value
With PUC	20	64.51	< 0.05
Without PUC	11	35.49	
Total	31	100	

**Table (6) : Frequency of BV in relation to IUCD**

Patients with vaginal discharge	BV	%	P value
With IUCD	10	66.66	< 0.05
Without IUCD	5	33.34	
Total	15	100	

**Table (7) : Frequency of *G. vaginalis*(positive of 16S rRNA gene) in patients with BV and control**

	PCR detection of 16S rRNA gene ( <i>G. vaginalis</i> )	Percentage	P value
Patients with BV ( $\geq 3$ Amsel's Criteria) (46)	21	45.65	> 0.05
Control group(30)	11	36.6	

## Discussion

Table -1- showed that 64.86 % of cases had suggestive discharge in comparison to 20 % of control group. This mean there was significant correlation of vaginal discharge with BV. Also there was significant correlation between case study and control group in regard to positive KOH (51.3% versus 16.5% respectively)( P value < 0.05). The overgrowth of vaginal anaerobes leads to an increased creation of amines (putrescine, trimethylamine and cadaverine) which turn out to be volatile in alkaline pH, i.e. afterward sexual intercourse and through the menstrual cycle which give the typical abnormal odor of vaginal discharge (Chen *et al.*, 1979). This finding might regard the whiff test as major dependent criteria in outpatient clinics to suspect BV. Also the number of patients who had clue cells was ( 46 out of 111) (41.44 %) which was statistically significant in relation to control group ( 5 out of 30 individuals) (16.5%)( P value < 0.05). This might make wet preparation suitable for use in general practice. In regard to vaginal PH, it had been shown that there was significant value ( P value < 0.05) in relation to higher PH more than 4.5 between case study and control group and this might be due to effect of volatile amines released by anaerobic organisms associated with BV (Eriksson *et al.*, 2005). Only 46 out of 111 (41.44%) had 3 or more of Amsel's criteria through which the diagnosis of BV was made. The frequency of BV might be underestimated because the enrolled study cases were only symptomatic patients with abnormal vaginal discharge and because many cases of BV might be asymptomatic (Klebanoff *et al.*, 2004) , so this reflects that the BV was not highly inflammatory , that was why it is referred to as a vaginosis and not a vaginitis (Donders,2002).

As shown in figure (2), the age groups (20-29) & (30-39) had a higher frequency rate of BV (28.26% and 36.95 % respectively). This might be due to high rate of pregnancy

(67.39%) and high rate of IUCD (66.66 %) used in these age group that lead to imbalance in the bacteria which was normally found in a woman's vagina (Reid, 2003). Also in this table the frequency of BV was more among patients aged (40-49) than those patients whose age less than 19 years and this might be caused by an alteration in the normal vaginal flora in which the normally predominant Lactobacilli are replaced by pathogenic bacteria (Bushra, 2010). The low pH generated by production of lactic acid reduces the adherence of the bacteria to the vaginal epithelium. In addition to that, some compounds produced by the lactobacilli such as lactacin B (Barefoot & Klaenhammer, 1983), acidolin (Hamden & Mikolajcik, 1974), and hydrogen peroxide (Eschenbach *et al*, 1989) inhibit the growth of other bacteria. Certain lactobacilli are capable of producing hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and had been shown to reduce BV (Hillier *et al*, 1992) and have bactericidal effect on *G.vaginalis* and *Prevotella bivia* in vitro (Klebanoff & Coombs, 1987).

Many cases give history of long infection period and as it had been noted in table (2) that there were no inflammatory cells in BV, so this might be due to absence of the lactic acid and the production of succinate, which also raises vaginal pH and blunt the chemotactic response of polymorphonuclear leukocytes and reduce their killing ability (Jeffrey *et al*, 2005), so BV produce no cellular inflammatory response despite the presence of high number of potentially pathogenic micro-organisms. The above finding support the previous mentioned paragraph concerning the BV of being not highly inflammatory condition. Hence, we might conclude that screening tests for leukocytes cells in vaginal swabs cannot reflect the presence of BV in routine diagnostic tests.

Table (3) showed that there was statistically significant relationship of occurrence of UTI in patients with BV and indicate a strong relationship (71.73% versus 28.27%). It had been reported previously that there was an association between BV and acute cystitis in females who used diaphragms (Preston, 1992). It is possible that women with BV developed UTI because of sexual activity (Reid, 1990). Three out of 46 (6.52%) women with UTI had history of spermicide usage as a mean of contraception which might increase risk of attaining UTI since many spermicides have nonoxynol-9, a nonionic detergent which in addition to its spermicidal action had negatively affect the composition of the urogenital micro flora by inhibits the growth of *Lactobacilli* (Majeed, 2004). Five out of 46 (10.86 %) had history of using douching for treatment of infections which might lead to increase in vaginal pH that finally leads to decrease the number of *Lactobacilli* and an increase in facultative and obligate anaerobic gram positive cocci (Boonnaert, 2000 ; Carroll, 1993). In addition to the effect of PUC, maternal genitourinary infection also might lead to preterm labor via different mechanisms including stimulation of cytokine progression and acceleration of labor process (Menard, *et al*, 2010). It is believed that altering the vaginal pH by repetitive alkalization can contribute to the development of bacterial vaginitis (Carpa, and Ackermann, 2006) and might facilitate colonization with uropathogens because of overgrowth of pathogenic bacteria and the absence of lactate and hydrogen peroxide producing *Lactobacilli* in the vagina, putting those women at risk for UTI (Larsen, and Monif, 2001). It had been hypothesized that Lactobacilli show a critical function in keeping the ordinary vaginal ecosystem by preventing overgrowth of pathogens and other opportunistic organisms by producing lactic acid, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), bacteriocins and other antimicrobial substances (Echenbach, 1989).

Table (4) showed that the frequency of BV was significantly more in pregnant than non-pregnant women (67.39 % versus 32.61%). This increase in bacterial infection rate in pregnancy might occur due to hormonal influence and change in vaginal flora associated



with pregnancy that favor increase in infection rate (Priestley, *et al.*, 1997; Schwebke, *et al.*, 1997 ; Keane, *et al.*, 1997). In addition to that during pregnancy, the glycogen content increased in the vaginal epithelium that would result in very favorable conditions for acid-tolerant bacteria (Schwebke, *et al.* , 1996; Biswas, 1993 ; Andrews, 2001; Julian, *et al.*, 2003) in addition to the change in cell-mediated immunity (decreased T cell activity) and impairing of neutrophils and monocytes ability to phagocytize, and provision of glycogen-rich vaginal epithelium ( Fleury, 1982).

Table (5) : showed the frequency of BV in pregnant women in relation to PUC. This table showed statistically significant relationship of occurrence of PUC in patients with BV and indicate a strong relationship in form of cause and effect. So bacterial vaginosis might be the precipitating factor of preterm labor (Bradshaw, C. S,2005) through the significant frequency of PUC. As the BV is treatable medical condition , so the complications and morbidity of preterm labor (prenatal morbidity and mortality )( Nejad & Shahla, 2008) would be preventable.

Table (6) : showed increase in BV among patients with IUCD (66.66% versus 33.34 %) and this might be due to the irritation caused by and contamination in the area of IUCD (Pabich, ,2003; Klebano,1992), It has generally been claimed that the existence of a foreign body can facilitate the establishment of endogenous anaerobic infections. Recent reports indicated that a women using an IUCD had a threefold increase in risk for BV. It is believed that only sexual active women acquire it and perhaps semen alkalizes the vagina and influence for it ( Holmes, 1985). The use of IUCD itself clearly altered the normal vaginal flora. In addition to that , IUCD use resulted in cervical erosion and finally would decrease the immune response to the possible pathogen (Ocak, 2007). This data support the hypothesis that IUCD might change cervicovaginal environment, and suggests that women with IUCD may be at a greater risk for vulvovaginal infection, so the application or using of IUCD could be one of the contributing factors of BV ,hence female using IUCD needs an ordered follow up, clinical examination and additional investigation.

Table (7): showed that the frequency of *G. vaginalis* detected by PCR technique which was (45.65 % ) in BV while it was (36.6 %) in the control group with statistic insignificance ( P value >0.05). This finding might reflect the cause of BV which could be due to bacteria other than *G. vaginalis* like *Mobiluncus species*, *Mycoplasma hominis*, anaerobic gram-negative rods belonging to the genera *Prevotella* , *Porphyromonas* , *Bacteroides* and *Peptostreptococcus* species(Hillier, 1993). In addition to that, this table also showed that there was high frequency rate of *G. vaginalis* in the control group(36.6 %). This high frequency of *G. vaginalis* might be due to high carrier rate in asymptomatic female (Amsel's, *et al.*, 1983). Only three out of 11 case in the control group had  $\geq 3$  Amsel's Criteria. As there was much controversy adjoining BV can partly be linked to the emphasis placed on the mere existence of *G. vaginalis* in the vagina rather than on the entire disease entity that involve change in female vaginal flora (Holst ,1987), so the four diagnostic criteria for BV with gram stain and culture for facultative anaerobic should be the routine laboratory method to differentiate symptomatic carrier caused largely by *G. vaginalis* from that of asymptomatic colonization carrier state and the PCR technique was not a mandatory test in daily routine investigations.

## Conclusion

- BV was more common in young and middle aged patients.
- There was a significant correlation of suggestive discharge together with BV.
- Despite the BV was not highly inflammatory condition but significant UTI might be associated.
- In pregnant women, pregnancy increases BV while BV increased PUC in pregnancy which might lead to pregnancy complications.
- IUCD might increase the risk of BV.
- The high frequency of BV in Babylon young and middle aged patients is alarming, since BV upsurges body susceptibility to HPV, HIV, and other important diseases by sexual transmission. Consequently BV had to be time diagnosed and adequately treated correctly.
- As the BV is treatable medical condition and it's frequently ignored since the symptoms are frequently irrelevant but the clinical consequences could be important, so medical screening is mandatory specially in antenatal care of pregnant women in Primary Health Centers and non-pregnant women using IUCD.
- Despite PCR is the greatest sensitive technique for the recognition of *G. vaginalis*, however it is not recommended in routine examination for BV.

### Recommendations

- Independent techniques from culture based methods that involve the examination of rRNA gene sequences have to be recognized that will deliver powerful tools to disclose the phylogenetic diversity of all microorganisms found within the vaginal ecosystem to ascertain vaginal bacterial communities dynamics dramatic differences between women with and without BV.
- Further study regarding further pathogens complicated in BV like *Mobiluncus* spp. are necessary.

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