

## Risk factors and level of interleukin-8 associated with bacterial vaginosis (B.V) in Iraqi pregnant women

By

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### Abstract :

**Background :** The most prevalent gynecological infection in women who are pregnant or of reproductive age is bacterial vaginosis (BV) , This study set out to ascertain the incidence of BV and related risk factors among pregnant Iraqi women because the role of vaginal innate immunity in response to microbial disturbance is still little known and may be critical for protecting against unfavorable outcomes. This investigation also looked at the level of interleukin (IL)-8 in the vaginal fluid collected from 51 pregnant BV-positive women and 60 healthy women. **Patients and methods:** In all, 422 expectant patients from the Tikrit Teaching Hospital took part in the research. Clinical and sociodemographic information was documented. Vaginal swabs were obtained and employed in wet mount and Gram stain techniques to assess the BV scores based on Nugent's and Donder's criteria, respectively. Enzyme-linked immunosorbent assays were utilized to quantify the amounts of interleukin (IL)-8. **Results:** the prevalence rates of BV were (30.7%) (43/140) in pregnant women compare to non-pregnant 10(20), Out of the 43 bacterial strains that were identified from BV patients, the most common ones were *Staphylococcus aureus* (23.30%) and *E. coli*, which accounted for 21.0% and 29.5% of the total, , followed by both of *Lactobacilli* and *B-hemolytic strep.* 7 (16.20%) ,the age group of 27–31 years old comprised 37.20% of study participants, followed by 22-26 years old 12(27.90%) , so more prevalent of B.V in in Third trimesters of pregnant women 20(46.50%) , About the majority 43(100%) of participants were married, whilst only held a primary education 11.(25.60%), followed by Secondary 9 (20.9%), Furthermore, 27(62.80%) of the survey participants lived in urban areas , as well as the cases had considerably higher serum levels of IL-8 ( $64.10 \pm 30.93$ ) compared to the controls ( $20.11 \pm 9.30$ ) pg/ml ( $P < 0.001$ ). **Conclusion:** the prevalence rates of BV in pregnant women more than non-pregnant , so most common ones were *Staphylococcus aureus* and *E. coli*, and more incidence in the age group of 22–31 years old , and in Third trimesters of pregnant women , whilst only held a primary education and lived in urban areas , as well as the cases had considerably higher levels of IL-8 in pregnant women compared to non-pregnant women.

**Keywords :** bacterial vaginosis (BV) ; Pregnancy, interleukin(IL) -8; risk factors.

## Introduction :

Bacterial vaginosis (BV) is the most common vaginal illness among both pregnant and non-pregnant women<sup>(1)</sup>. The most prevalent kind of aberrant vaginal flora, bacterial vaginosis, is common throughout both pregnancy and adolescence<sup>(2)</sup>. Bacterial vaginosis (BV) is defined by aberrant vaginal microbiota that includes aerobic and enteric bacteria such as group B streptococcus (GBS), *Klebsiella* spp., *Acinetobacter* spp., *Staphylococcus* spp., and *Escherichia coli*, as well as inflammation of the vaginal epithelium<sup>(3)</sup>, BV is a complex polymicrobial infection characterized by the proliferation of a mixed variable anaerobic and facultative flora, including gram-positive cocci, genital mycoplasma, *Prevotella* spp., *Bacteroides* spp., *Gardnerella vaginalis*, and *Mobiluncus* spp.<sup>(1)</sup>. For certain women, the vaginal microbiota is a dynamic community that shifts every day.<sup>31–34</sup> Further research is beginning to show that women with and without BV express distinct genes in species like *L. iners*<sup>(4)</sup>.

Immune mediator levels in the lower genital tract are known to alter during both adolescence and pregnancy<sup>(5&6)</sup>. Interleukin (IL)-8 have elevated levels when they have bacterial vaginosis<sup>(2)</sup>. As well as strong chemokine IL-8 (CXCL8) is linked to the number of vaginal neutrophils in vaginal fluid in both healthy and BV-positive women<sup>(7&8)</sup>, Although vaginal immunity in BV positive women has been the subject of numerous studies, few have examined the impact of *T vaginalis* on this immune system. A study found that vaginal secretions from trichomoniasis patients exhibiting symptoms had elevated levels of IL-8<sup>(9)</sup>.

According to Carrazzo *et al.*, 2010, women with BV may be more susceptible to HIV and other STDs<sup>(10)</sup>. Like the cervix, the vagina is prone to infection; however, due to ecological variations between the two sites, the etiological agents are different. A healthy vagina has a pH of 3.5–4.5, whereas the cervix of women who are of reproductive age has a pH of 7.0<sup>(11)</sup>. Therefore, To better comprehend the condition and manage its consequences, one must be aware of the risk factors linked to the acquisition and removal of BV<sup>(12)</sup>.

## Patients and methods

**Study design:** This cross-sectional study included pregnant women who visited the Tikrit Hospital's obstetrical and gynecological clinic were included in the

study, between January and October of 2023 , gave vaginal swab specimens, and adequately completed the questionnaire. The lead investigator used an interview-based structural questionnaire to collect sociodemographic information (age, marital status, educational attainment, place of residence), clinical observations (abnormal vaginal fluid or discharge), as well as behavioral and sexual traits (cleanliness of the vagina).

**Exclusion criteria:** The study eliminated women who were under legal age, had genital cancer, were pregnant, had taken antibiotics within the two weeks before collected data , had vaginal bleeding from an unknown source, and had doused their vagina with chemicals.

### Methods :

Vaginal swabs were taken from each patient right away following a physical and gynecological examination by inserting a sterile, unlubricated speculum into the vagina. The samples were appropriately labeled and two sterile vaginal swabs were utilized. The swabs were carefully removed to prevent contamination with vulva and introitus microorganisms. One of them was placed on a glass slide, covered with regular saline, and KOH (10%) was added for the wet mount procedure. The wet mount was then investigated to look at *Trichomonas vaginalis* (TV). To isolate and identify *Candida albicans*, additional tests were performed on the materials, including the germ tube test and Sabouraud Dextrose agar (SDA). This yeast had the capacity to generate, also used Enzyme-linked immunosorbent assays were utilized to quantify the amounts of interleukin (IL)-8.

**Data analysis:** The Statistical Package for Social Sciences (SPSS) version 22 was used to code and analyze the quantitative data that had been collected, a P-value of less than 0.05 was deemed statistically significant.

### Results :

Of these, 49 (35%) had a diagnosis of candidiasis isolated from pregnant women compare to non-pregnant 23(46), as well as 43 (30.7%) had bacterial vaginosis isolated from them, compare to non-pregnant 10(20), and 14 (10%) had *Trichomonas virginals* isolated , Then, out of 50 non- pregnant women (the control group) (Table 1).

**Table(1): Type of infections in pregnant women and Control (healthy women)**

Type of infections	pregnant women		Control (non- pregnant women)	
	No.	%	No.	%
Bacterial vaginosis	43	30.7	10	20
Candidiasis	49	35	23	46
Trichomonas vaginalis	14	10	1	2
Non-infected	34	24.3	16	32
Total	140	100	50	100

Ten (23.30%) of the pregnant women had bacterial vaginosis. *Staph. aureus* and *E. coli* were the most frequently isolated BV-associated organisms, accounting for roughly 9 (21.0%) of the total, followed by both of *Lactobacilli* and *B-hemolytic strep.* 7 (16.20%) and *Proteus* 6 (14%) , *Chlamydia* 4 (9.30%) was one of the other infections found (Table 2).

**Table(2): Distribution type of Bacterial vaginosis (B.V) in pregnant women**

Bacterial vaginosis (B.V)	NO.	%
<i>Lactobacilli</i>	7	16.20
<i>E.coli</i>	9	21.00
Chlamydia	4	9.30
<i>Gonorrhea</i>	0	0
<i>Staph.aureus</i>	10	23.30
<i>B-hemolytic strep.</i>	7	16.20
<i>Proteus</i>	6	14.0
Total	43	100

The age group of 27–31 years old comprised 37.20% of study participants, followed by 22-26 years old 12(27.90%) and 16-21 years old 8(18.60%), compare to microbial infections.

**Table(3):prevalence of B.N and Microbial infections in pregnant women according to age groups.**

Age groups (years)	Microbial infection		B.N Infection	
	No.	%	No.	%
16-21	15	23.80	8	18.60
22-26	19	30.1	12	27.90
27-31	13	20.70	16	37.20
32-36	8	12.70	4	9.30
≤ 37	8	12.70	3	7
Total	63	100	43	100

Results in table (4) showed the B.V incidence in Third trimesters of pregnant women 20(46.50%) more than second 17 (39.50%) and first trimesters of pregnant women 6( 14.0%).

**Table(4): Relationship between BV and trimesters of pregnant women**

Trimester	B.V	
	No.	%
First	6	14.0
Second	17	39.50
Third	20	46.50
Total	43	100

About the majority 43(100%) of participants were married, whilst only held a primary education 11.(25.60%), followed by Secondary 9 (20.9%), Furthermore, 27(62.80%) of the survey participants lived in urban areas ( table-5).

**Table(5): Relationship between BV infection and socio-demographic characteristics**

Demographic characteristics	Patients		Control	
	No.	%	No.	%
<b>Marital status</b>				
Single	0	0	0	0
married	43	100	50	100
<b>Educational level</b>				
Primary	11	25.60	17	34
Secondary	9	20.90	10	20
Tertiary	8	18.60	13	26
High	7	16.30	8	16
No-education	8	18.60	2	4

Residence				
Urbane	27	62.80	30	60
Rural	16	37.20	20	40
Total	43	100	50	100

The cases had considerably higher levels of IL-8 ( $64.10 \pm 30.93$ ) compared to the controls ( $20.11 \pm 9.30$ ) pg/ml ( $P < 0.001$ ) (table-6).

**Table(6):level of Interleukin -8 in patients with B.V and control group(healthy)**

study groups	Levels of IL-8(Pg/ml)	p-value
	M $\pm$ S.D	
Patients	$64.10 \pm 30.93$	$P < 0.001$
Control	$20.11 \pm 9.30$	

## Discussion :

The most common type of vaginitis in women is called bacterial vaginosis (BV), formerly known as "nonspecific vaginitis." It is linked to the overgrowth of several vaginal anaerobic flora members, genital mycoplasmas, and a small Gram-negative rod called Gardnerella vaginalis. Previously, BV was thought to be the only cause of the illness<sup>(13)</sup>, Whilst the A healthy pregnancy may be associated with the abundance of a complex vaginal microbial community of CST-IV, which includes Gardnerella, Prevotella, Chlamydia, and bacterial vaginosis (BV)-associated bacterium-I (BVAB-I). This community has been linked to an increased risk for unfavorable pregnancy outcomes and fetal infection<sup>(14&15)</sup>.

Our findings are in line with a recent large community-based study of low-risk pregnant women, which discovered that BV was linked to a four-fold greater risk of pregnancy loss in the early second trimester, between 13 and 15 weeks gestation (RR=3.5, 95% CI: 1.2–10.3)<sup>(16)</sup>.

According to Larsson *et al.*, the younger age group had a much higher prevalence of BV, but not those who had previously experienced a preterm delivery<sup>(17)</sup>, On the other hand, Beng *et al.*, study from 2023 revealed no evidence

of a significant correlation between mother age, marital status, and educational parity and the risk of BV<sup>(18)</sup>.

Women between the ages of 21 and 29 had the highest frequency, according to Mengistie *et al.*<sup>(19)</sup>, These age groups have the highest rates of sexual activity and, thus, the largest risk of BV and STDs<sup>(20&21)</sup>, The highest prevalence of BV was seen in women aged 30 to 40<sup>(22 &23)</sup>, however these were not pregnant women. Though the difference was not statistically significant, individuals who were primigravidae had a greater risk of BV than those who were multigravidae, which is consistent with the results of a study conducted in Nigeria<sup>(24)</sup>.

However, during pregnancy, IL-8 levels are noticeably greater (Beigi *et al.*, 2007)<sup>(25)</sup>. found that elevated IL-8 levels were independently linked to bacterial vaginosis and pregnancy<sup>(6)</sup>. However, Madan *et al.*, 2012)<sup>(7)</sup> found that there hasn't been any correlation between adolescence and cervicovaginal IL-8 levels. Therefore, rather than the population's younger age, we propose that the elevated cervicovaginal IL-8 levels found here may have been caused by pregnant status based on findings from the literature.

**Conclusion :** current study concluded the following :

- 1- the prevalence rates of BV in pregnant women more than non-pregnant
- 2- The most common bacterial isolates were *Staphylococcus aureus* and *E. coli*.
- 3- B.V was more incidence in the age group of 22–31 years old , and in Third trimesters of pregnant women , whilst only held a primary education and lived in urban areas.
- 4- Higher levels of interleukin (IL-8) in pregnant women compared to non-pregnant women.

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