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Bacterial vaginosis and infertility: cause or association?

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ABSTRACT

Objectives: To estimate the prevalence of bacterial vaginosis (BV) in infertile women and evaluate the effect of treatment of BV on the pregnancy rate in patients with polycystic ovarian disease (PCOD) and unexplained infertility.
Study design: Cohort study conducted at the Department of Obstetrics and Gynecology in collaboration with the Microbiology Department of Sohag University Hospital, Egypt. All eligible women with female factor infertility ($n = 874$) were enrolled and all asymptomatic fertile women ($n = 382$) attending the family planning clinic of the study hospital were recruited as a control group. The study was in two phases: the first included screening all participants for BV after Gram-staining of the vaginal discharge. The second phase was concerned with evaluating the effect of treatment of BV on the cumulative pregnancy rate (CPP) in patients with PCOD (group I; $n = 278$) and unexplained infertility (group II; $n = 170$). Each group was divided into three sub-groups: groups Ia ($n = 129$) and IIa ($n = 73$) were BV positive and treated for BV; groups Ib ($n = 61$) and IIb ($n = 49$) were BV positive and did not receive treatment for BV, and groups Ic ($n = 88$) and IIc ($n = 48$) were BV negative. The prevalence of BV was compared using the Chi-square. The long rank test of Kaplan-Meier life table analysis was used to compare the CPR. A multivariate regression model was designed to define the most significant variable which affected the pregnancy rate in patients with PCOD.
Results: The prevalence of BV was significantly higher in infertile than fertile women (45.5% vs 15.4%). The highest prevalence was found in patients with PCOD (60.1%) and unexplained infertility (37.4%). The CPR in both patients with PCOD and unexplained infertility were significantly higher in the patients who were treated for BV. Regression model showed that BV was one of the significant factors interfering with pregnancy.
Conclusions: BV is strongly implicated in female infertility and is probably an underestimated cause of unexplained infertility. Screening and treatment of BV in patients with PCOD and unexplained infertility improved the pregnancy rate considerably.

1. Introduction

Bacterial vaginosis (BV) is considered the most common cause of vaginitis in sexually active women during the reproductive years [1]. The infection is characterized primarily by paucity or depletion of the vaginal lacto-bacilli and their replacement by an outgrowth of different micro-organisms including Gardnerella vaginalis (GV), anaerobic rods, pepto-streptococci, and mycoplasma species [2]. Although the exact cause of this disruption of the normal vaginal milieu is still not fully elucidated, previous studies pointed to a role of hormonal disturbances [3–5].

From the obstetric point of view, BV is associated with many complications including abortion [6], premature rupture of membranes and preterm delivery [7]. The implication of BV in infertility, on the contrary, is still controversial and precarious. While some studies linked bacterial vaginosis to pelvic inflammatory disease (PID) and hence tubal infertility [5,8–10], others disputed any relationship [11,12]. Moreover, previous studies reported a high prevalence of BV in both non-tubal and unexplained infertility [5,13,14].

All studies concerned with the issue of BV and infertility, however, addressed mainly the prevalence of BV amongst infertile women and none tested the effect of treatment of BV on the pregnancy rate in infertile women. Moreover, to our knowledge, the prevalence of BV in patients with polycystic ovarian disease

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(PCOD), with its associated hormonal disturbances, has not previously been addressed. Accordingly, the aim of the present study was to assess the prevalence of BV and evaluate the effect of treatment of BV on the pregnancy rate in infertile women.

2. Materials and methods

The present prospective cohort study was conducted from 1st March 2009 till 1st September 2011 at the Obstetrics and Gynecology Department in collaboration with the Department of Microbiology and Immunology of Sohag University Hospital, Egypt. During the study period, all women with female factor infertility were invited to participate in the study and were assigned as a study group ($n = 979$). All asymptomatic fertile women ($n = 382$) attending the family planning clinic of the study hospital who agreed to participate in the study were recruited as a control group. Local institutional ethical committee provided approval and written consent was obtained from all participants.

Thorough clinical and sonographic assessments of the participants were undertaken. Blood samples were drawn from the study group for assay of the basal hormonal profile (FSH, LH, free androgen index [FAI], T3, T4, and prolactin). The study group was categorized according to the cause of infertility into those with PCOD ($n = 371$), unexplained infertility ($n = 289$), tubal infertility ($n = 126$), and endometriosis ($n = 88$). PCOD was diagnosed according to the Rotterdam criteria [15] while tubal infertility and endometriosis were diagnosed after conducting laparoscopy (Olympus, Germany). According to our protocol, unexplained infertility entailed infertility for more than one year despite regular marital life, regular cycles, uneventful clinical examination, normal husband semen analysis according to the WHO criteria [16], normal basal hormonal level (FSH, LH, T3, T4, and prolactin), regular ovulation for at least three consecutive cycles (documented by serial folliculometry and midluteal serum progesterone >10 ng/dl), normal hysterosalpingography, and normal laparoscopic findings.

The study was in two phases. The first phase aimed at evaluating the prevalence of BV. This was done through screening all eligible participants (those with PCOD, unexplained infertility, tubal infertility, and endometriosis) for BV. Exclusion criteria of participants during this stage were refusal to participate in the study and abnormal husband semen analysis. The second phase was concerned with evaluating the effect of treatment of BV on the pregnancy rate in infertile women. In order to accomplish this, patients with tubal infertility and endometriosis were excluded during this stage and only those with PCOD and unexplained infertility were followed up for six months to calculate the cumulative pregnancy rate (CPR). Exclusion criteria during this stage were refusal to participate in the study, presence of uterine or adnexal pathology, previous PID, previous pelvic surgery, referral to assisted reproduction, previous laparoscopic ovarian drilling (LOD) in patients with PCOD, and obesity (BMI > 30 kg/m^2) in patients with unexplained infertility.

During the second phase, patients with PCOD and unexplained infertility were assigned as groups I and II respectively. Without randomization, some of the BV-positive patients in the two aforementioned groups were treated for BV while the rest of the patients were not treated. Each group was divided into three subgroups: groups Ia ($n = 129$) and IIa ($n = 73$) were BV positive and received treatment for BV; groups Ib ($n = 61$) and IIb ($n = 49$) were BV positive and did not receive treatment for BV, and groups Ic ($n = 88$) and IIc ($n = 48$) were BV negative and considered as a control subgroup. Both partners were treated for BV using a single dose of 2 g secnidazole (Secnidazole, IEPICO, Egypt) which was repeated every month in the patients who did not conceive.

Patients with PCOD were treated with clomiphene citrate (Clomid, Sanafi Aventis, France) 50 mg twice daily for five consecutive days starting from the second day of a spontaneous or induced cycle. Trans-vaginal folliculometry (Acuson XP, USA) was conducted and when al least one follicle measured $\geq$18 mm, 10,000 IU of human chorionic gonadotropin was given. Midluteal serum progesterone level was assayed and a value of >10 ng/dl was indicative of ovulation. If the patient failed to ovulate, the dose of clomiphene citrate was increased during the subsequent cycles to a maximum of 200 mg/day. The patients who failed to ovulate were excluded from the statistical analysis only during the same cycle. Patients with unexplained infertility were treated with gonadotropins using the standardized step-up protocol described elsewhere [17] or referred to assisted reproduction.

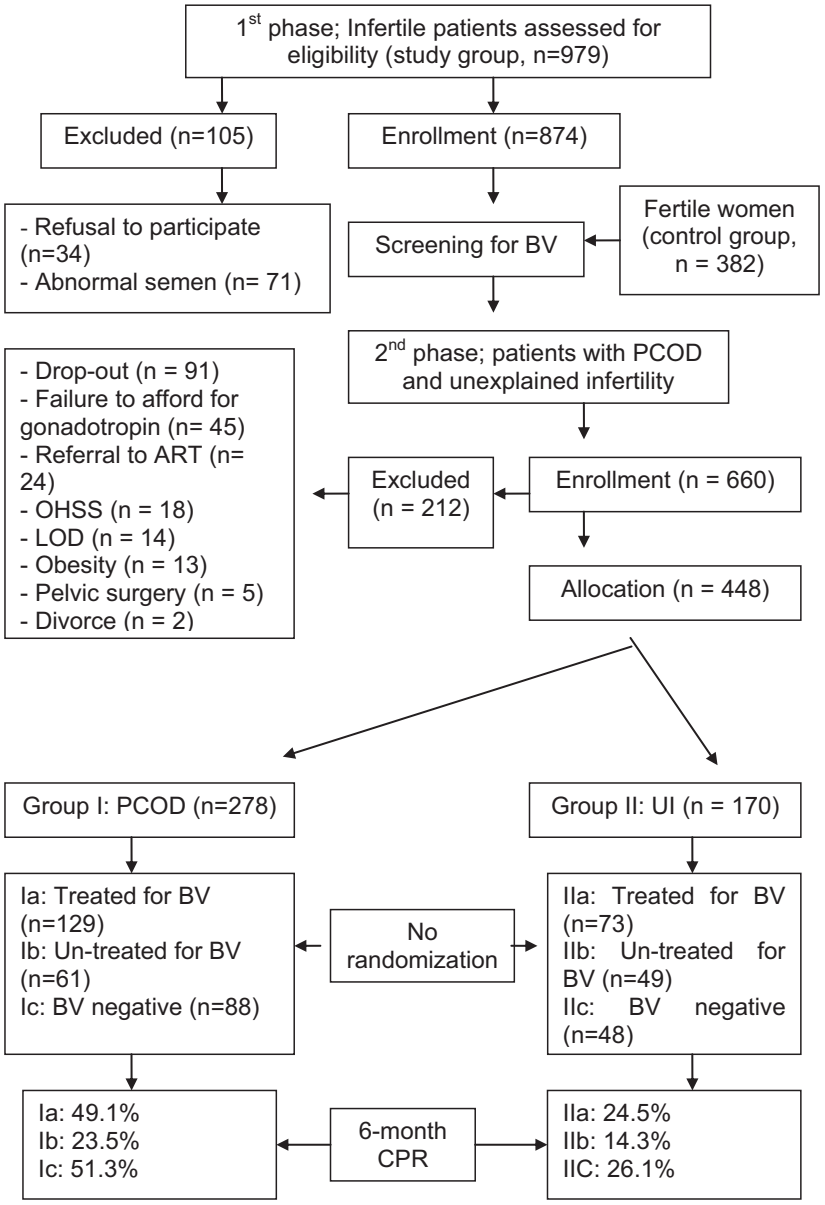
Screening for BV was undertaken during the initial clinical evaluation of the participants. A sterile non-lubricated Cusco's speculum was introduced into the vagina and a swab from the discharge of the posterior fornix was taken using a sterile cotton swab. The swab was immediately smeared onto a glass slide, left to dry then Gram-stained and examined under the microscope ($1000\times$ magnification). BV was diagnosed according to the modified Spiegel's criteria [18] which categorize the vaginal flora into three grades: normal (mainly lacto-bacilli), intermediate (reduced lacto-bacilli with increased number of other morphotypes), and BV (depleted or absent lacto-bacilli with predominance of other morphotypes; mainly GV).

The sample size of the study was calculated so as to achieve 80% power and 5% confidence of significance. Depending upon the results of published studies, we assumed 12% and 24% prevalence of BV in the fertile and infertile women respectively. According to these proportions, the calculated sample size needed for the study was 246 patients. Owing to the expected high drop-out rate and the design of the study which required multiple sub-groupings of the participants, more than three times the calculated sample size was enrolled into the study. The prevalence of BV in the different causes of infertility was compared using the Chi-square and the real variables were compared using Student's t-test (p value <0.05 was considered significant). Kaplan-Meier life table analysis was used to calculate the CPR during the 6-month follow-up period and the log rank test was used to compare the statistical difference. Cox regression was used to perform a univariate analysis of the possible variables which may affect the pregnancy rate in patients with PCOD. A forwards multivariate step-wise regression model was then designed to define the most significant variable which affected the pregnancy rate in patients with PCOD. The statistical analysis was done according to the per protocol rule.

3. Results

During the study period, a total of 979 infertile women were recruited for the study. A total of 874 infertile women fulfilled the inclusion criteria and were assigned as a study group while 382 asymptomatic fertile women agreed to participate as controls. The study group included 371 (42.4%) patients with PCOD, 289 (33.1%) with unexplained infertility, 126 (14.4%) with tubal infertility, and 88 (10.1%) with endometriosis. During the second phase of the study, a total of 212 (32.1%) patients (93 with PCOD and 119 with unexplained infertility) were excluded while the remaining 448 patients (278 with PCOD and 170 with unexplained infertility) were enrolled into the study (Fig. 1).

The average ages (25.8 ± 3.1 years vs 27.1 ± 2.2 years) and BMI (26.3 ± 2.1 vs 26.9 ± 1.8) were comparable in the fertile and infertile women. The prevalence of BV in the control women was 15.4% (59/382) compared to 45.5% (398/874) in infertile women ($p < 0.001$). The highest prevalence of BV (60.1%) was found in patients with PCOD



Abbreviations, BV; Bacterial vaginosis, PCOD; polycystic ovarian disease, ART; Assisted reproductive techniques, OHSS; Ovarian hyperstimulation syndrome, LOD; Laparoscopic ovarian drilling, UI; Unexplained infertility, CPR; Cumulative pregnancy rate.

Fig. 1. Flowchart of the study.

(OR = 7.11; $p < 0.001$) followed by those with unexplained infertility (OR = 3.24; $p = 0.001$) (Table 1).

During the second phase of the study, the average age, BMI, duration of infertility, ovarian volume, LH, FAI, and the ovulation rates in patients with PCOD were comparable in the three subgroups (data not shown). The pregnancy rate during the first cycle was significantly higher in group Ic (14.2%) than groups Ia (6.3%; $p = 0.03$) and Ib (5.9%; $p = 0.03$). The CPR was significantly higher in group Ia than Ib (49.1% vs 23.5%; $p = 0.001$) and comparable to group Ic (51.3%) (Fig. 2). The same trend was found in patients with unexplained infertility, where the CPR was higher in group IIa than IIb (24.5% vs 14.3%; $p = 0.04$) and comparable to group IIc (26.1%) (Fig. 3).

Table 1
The prevalence of bacterial vaginosis in infertile women with different causes of infertility.

	Prevalence (%)	OR (CI)	*p* Value[a]
Total infertility	45.5	5.23 (3.06 ± 8.12)	0.0001
PCOD	60.1	7.11 (4.56 ± 12.3)	0.0001
Unexplained infertility	37.4	3.24 (2.15 ± 5.16)	0.001
Tubal infertility	24.6	1.83 (0.90 ± 3.71)	0.09
Endometriosis	19.3	0.70 (0.83 ± 3.47)	0.32
Control	15.4		

All data were expressed as mean ± SD, unless otherwise indicated.
Abbreviations: OR: odd ratio; CI: confidence interval; PCOD: polycystic ovarian disease.
[a] Compared to the control group.

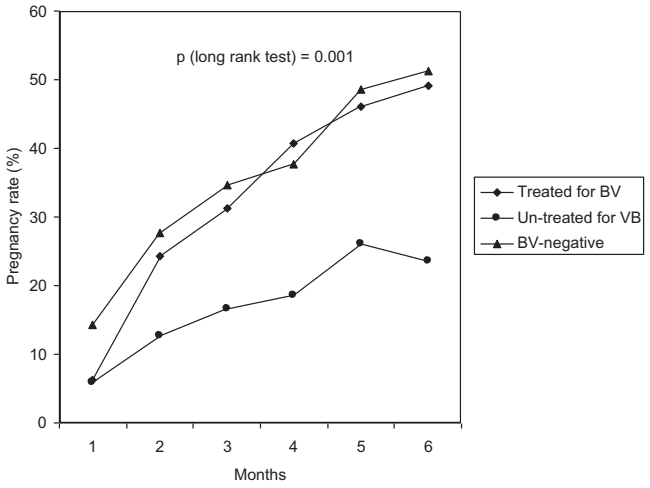


Fig. 2. Cumulative pregnancy rates in patients with PCOD.

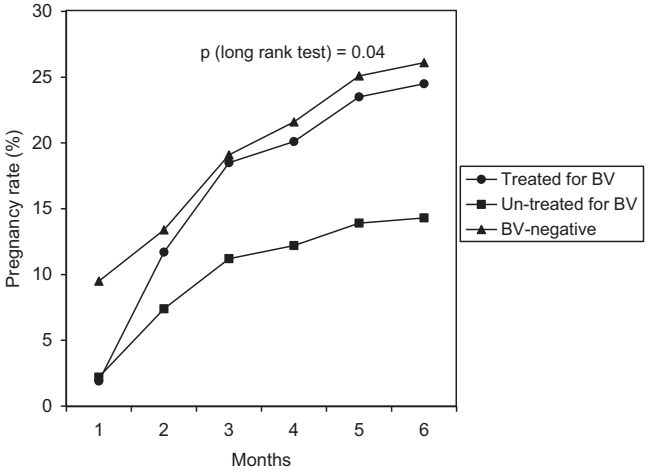


Fig. 3. Cumulative pregnancy rates in patients with unexplained infertility.

Univariate analysis showed that the age, duration of infertility, BMI, FAI, LH, and BV were the most significant variables which affected the pregnancy rate in patients with PCOD (Table 2). After adjustment for age, BMI, and LH using a multivariate analysis, BV still showed significant difference between the pregnant and non-pregnant patients. This difference became abolished only after adjustment for FAI (Table 3).

Table 2
Univariate analysis of the variables which affected the pregnancy rate in patients with PCOD.

	Pregnant (n = 143)	Not pregnant (n = (135)	p Value
Age (years)	22.5 ± 1.2	28.7 ± 0.9	0.02
Infertility (years)	1.7 ± 0.8	4.1 ± 1.1	0.02
BMI (kg/m^2)	28.3 ± 1.4	33.6 ± 1.4	0.01
LH (mIU/dl)	9.4 ± 0.6	16.1 ± 2.4	0.01
FIA	3.6 ± 0.99	7.5 ± 2.8	0.001
Ovarian volume (cm^3)	9.9 ± 2.2	11.6 ± 0.9	0.37
LH/FSH ratio	4.4 ± 0.9	6.2 ± 1.2	0.03
BV (%)	23.2 ± 4.1	44.7 ± 2.5	0.001

All data were expressed as mean ± SD, unless otherwise indicated.
Abbreviations: BMI: body mass index; LH: luteinizing hormone; FAI: free androgen index (serum testosterone × 100/sex hormone binding globulin); FSH: follicle stimulating hormone; BV: bacterial vaginosis.

Table 3
Step-wise multivariate logistic regression analysis of the variables which affected the pregnancy rate in patients with PCOD.

	Univariate analysis[a]	Multivariate analysis			
		Step 1	Step 2	Step 3	Step 4
Age (years)	0.02	In model	In model	In model	In model
Infertility (years)	0.02	0.03	In model	In model	In model
BMI (kg/m^2)	0.01	0.08	0.13	0.64	0.98
LH (mIu/dl)	0.01	0.01	0.04	In model	In model
FAI	0.001	0.001	0.01	0.02	In model
FSH/LH	0.03	0.04	0.08	0.15	0.23
BV	0.001	0.01	0.03	0.03	0.14

All data were expressed as mean ± SD, unless otherwise indicated.
Abbreviations: BMI: body mass index; LH: luteinizing hormone; FAI: free androgen index (serum testosterone × 100/sex hormone binding globulin); FSH: follicle stimulating hormone; BV: bacterial vaginosis.
[a] Only variables with statistical significance were included.

4. Comment

The present study reported a high prevalence of BV in infertile women, particularly those with PCOD and unexplained infertility, and to a lesser extent in those with tubal infertility. These results disagreed with those reported by Wilson et al. [5] who reported a higher prevalence of BV in patients with tubal infertility than those with unexplained and ovulatory infertility. Among our participants, however, the largest group had PCOD (42.5%) with its consequences of anovulation and hormonal disturbances. Many studies have suggested a possible role of hormonal imbalance in the acquisition of BV [3–5]. By contrast, in the study of Wilson et al., a large proportion of the patients had tubal infertility while a minority had anovulation. This difference in the proportions of the causes of infertility could explain the contradiction between the two studies.

The high prevalence of BV in infertile women may suggest either a possible role of BV on fertility or just an association between BV and infertility. Although some studies concluded that infertile patients were inherently predisposed to BV and disputed any role of BV in fertility [11,12,19,20], the results of the current study not only contradict this conclusion but also provide evidences for a possible role of BV in infertility. The high prevalence of BV in the different causes of infertility, even including those with endometriosis, is one piece of evidence. The results of the univariate analysis of the variables which affected the pregnancy rate in patients with PCOD provide another. These findings are further reinforced by the results of the regression model. Even after controlling for the different variables which influenced the pregnancy rate in patients with PCOD, BV still remained a significant factor impairing the pregnancy rate. Moreover the deleterious effect of BV on fertility was abolished only after adjustment for the FAI; a finding which may suggest a possible link between BV and high androgen levels.

Similar to our results, some studies have suggested a possible role of BV on female fertility [5,8–10,13]. None of these studies, however, evaluated the influence of treatment of BV on the pregnancy rate. To the best of our knowledge, the present study was the first to test the effect of treatment of BV on the pregnancy rate in infertile women. The present study showed that a single-dose treatment of BV was associated with a high CPR in patients with PCOD and unexplained infertility. These high pregnancy rates not only provide a clear evidence implicating BV in infertility, but also suggest BV as a new cause of unexplained infertility which has probably been underestimated.

One of the most interesting findings of the current study was the very high proportion of BV in patients with PCOD. This may seem illogical as it is well known that a high estrogen milieu, which

is the case in PCOD, increases the number of the lactobacilli and decreases the risk of BV [21]. PCOD is also associated with other hormonal disturbances, however, particularly elevated androgen levels and insulin resistance. Whether these hormonal disturbances were responsible for the high prevalence of BV in patients with PCOD is a question that remains unanswered. Nevertheless, the dramatic increase in the pregnancy rate after treatment of BV in patients with PCOD might suggest a hypothetical vicious circuit of hormonal disruption leading to increased risk of BV which may participate in perpetuating the problem of infertility.

Another interesting finding was the surprisingly low pregnancy rates during the first cycle after treatment of BV which was even lower than that of the control subgroups. This finding was consistent in patients with PCOD and unexplained infertility. Although the exact means by which BV may cause infertility is still unsettled, many mechanisms including plasma cell endometritis [10,22], tubal motility disorders [1], and auto-immune infertility [13,23] have been proposed. It is possible that one or more of the effects of these factors may still persist for some time after treatment of BV and may be responsible for the low pregnancy rate during the first cycle of treatment. Another possible explanation is resistance of BV to treatment, but the high pregnancy rate during the subsequent cycles precluded this explanation.

The most evident limitation of the present study was the lack of follow-up of the patients to detect resistance or recurrence of BV following treatment. Previous studies, however, reported high cure and low recurrence rates after treatment of BV with secnidazole [24]. Moreover, administering the drug every cycle might further decrease the recurrence rate. Another point of concern was the non-randomization of the participants. Although at the beginning of the study the authors designed it as a randomized one, but this randomization was not possible because a large number of participants refused to be included in the control sub-groups. A third shortcoming was lack of information about the methods of contraception (which may influence the prevalence of BV) used by the control group.

In conclusion, the prevalence of BV was very high in infertile women, particularly those with PCOD and unexplained infertility. BV is strongly implicated in female infertility and it is probably an underestimated cause of unexplained infertility. Screening and treatment of BV during the course of infertility treatment increased the pregnancy rate considerably. Randomized studies including larger number of participants are needed, however, to reach more validated conclusions. Moreover, research is strongly recommended on the mechanisms by which BV impairs fertility and on the link between BV and elevated androgen levels.

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