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Metronidazole Vaginal Gel 0.75% (Zidoval™) for Suppresion of Recurrent Bacterial Vaginosis (BV): A Pilot Clinical Trial

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Abstract

Objectives: A pilot efficacy and tolerability trial of suppressive metronidazole vaginal gel in reducing recurrent symptomatic relapses of bacterial vaginosis (BV).

Study design: A prospective open labeled study. Women with recurrent BV were assigned to metronidazole gel 3 times monthly for 24 weeks. The mean episodes of relapses of recurrence of BV after metronidazole suppression was compared to historical mean obtained from the same participants by a paired-sample T test. An exit interview was used to record patient satisfaction.

Results: The mean symptomatic relapses of BV was 0.44 (SD1.09). Historically, participants had a mean of 6.5 (SD3.09) by clinical or laboratory criteria, and a mean of 3.75 (1.06) by laboratory criteria only. Compared with each of the historical means by paired-sample T test, the difference is significant in both at P<0.0001. Adverse effects were uncommon.

Conclusions: Suppressive therapy of BV with metronidazole gel for 3 days after the menstrual cycle resulted in significant reduction in symptomatic relapses. Adverse effects were uncommon. A randomized controlled trial is needed to further validate these results.

Keywords: Recurrent bacterial vaginosis (BV); Metronidazole gel; Suppression; Prophylaxis

Introduction

Bacterial vaginosis (BV) is the commonest cause of abnormal vaginal discharge in women of reproductive age. The prevalence ranges from 5% in asymptomatic college students in the USA, 12% in a population of an antenatal clinic attendees in the UK, and 50% in rural Uganda [1,2]. BV is no longer considered just a nuisance as increasingly associated obstetric complications, and sexually transmitted diseases including HIV are reported. Several studies showed that women with BV are at an increased risk of developing endometritis or pelvic inflammatory disease following termination of pregnancy; furthermore pregnant women have a significantly increased incidence of intrauterine death, late miscarriage and pre-term birth [3-6]. These complications and associations were mostly reported in the absence of BV symptoms [7].

The etiology of BV is unknown but it is characterized by replacement of the usual lactobacillus-dominated flora with overgrowth of many non-lactobacillus and anaerobic organisms. The protective hydrogen peroxide (H_2O_2) producing lactobacillus species is replaced with *Gardnerella vaginalis*, obligatory anaerobic Gram negative rods, *Mobilincus* species, and *Mycoplasma hominis* [8]. More recently novel bacteria associated with BV such as *Atopobium vaginae* have been discovered by utilizing modern molecular biology techniques [9,10]. This disruption of the normal vaginal flora can be temporarily corrected by antibiotics but the rate of recurrence is high and, in symptomatic cases, recurrence can be up to monthly [11,12].

Epidemiological studies have reported several groups of women at risk of BV. Some of these included risk factors similar to sexually transmitted infections (STIs) such as young age, black ethnicity douching, smoking, use of intra uterine contraceptive devices (IUCD), multiple sexual partners and recent change of partners [13-15]. This suggests that a sexually transmissible agent is responsible for the changes of vaginal flora that underpin BV. However, other studies clearly showed that BV also occurs in virgin girls which indicate that BV is not exclusively sexually transmitted [16]. Also, treatment of male sexual contact of BV cases did not reduce the recurrence adding further evidence against the STIs theory [17]. Women who have sex with women (WSW) have high prevalence of BV and her there is an association with high life time number of female partners, shared use of sex toys, and oral-anal sex [18,19] . Bradshaw and co-workers reported that the rate of recurrent BV increased 2 folds in women with a history of BV, sexual workers, and those who had a regular male sexual partner during the study [15]. Also, the recurrence rate in WSW increased by 3 folds, and use of hormonal contraception was protective and recurrence commonly occurs around the time of menstruation [15,20].

Symptomatic BV is characterized by an offensive and homogenous vaginal discharge. The odour is often described as fishy and associated with significant embarrassment and it is frustrating to both patient and clinician due to the lack of curative therapy. However, in about half of microbiologically diagnosed BV patients are asymptomatic [8].

Conventionally, the diagnosis of BV is made by the presence of at least three of four composite (Amsel's) criteria: the presence of a typical thin homogeneous vaginal discharge; a pH of vaginal fluid

greater than 4.5; the release of a strong fishy smell on adding 10% KOH to a sample of vaginal fluid and the presence of clue cells on microscopic examination. In research settings and increasingly in clinical settings the Nugent scoring system is adopted as an alternative to the Amsel criteria. The Nugent scoring is based on the structured scoring of characteristic changes on the Gram-stained vaginal smear [21,22].

BV can be treated successfully with antibiotics effective against anaerobic bacteria [23]. Metronidazole is the mainstay of treatment of BV and intravaginal treatments are equally effective [24], alternatives include oral and topical clindamycin. Although some women have spontaneous resolution of symptoms, others get frequent relapses post treatment. Clinical trials reported a cure rate of 80%-90% but about 30% had recurrence within 3 month and with long term follow up (mean 6.5 year) 52% of these women had at least one more episode of BV [12]. In another study of women with symptomatic BV treated with oral metronidazole, 58% and 84% had recurrence of symptoms and abnormal flora respectively by 12 month. Thus currently available therapy for BV is not effective in preventing recurrence of symptoms and or normalizing the vaginal flora [15]. The current practice is not to treat asymptomatic BV except in surgical prophylaxis in women undergoing s gynecological procedures [22].

There is no agreed and acceptable definition of "recurrent bacterial vaginosis". In the few previous publications a threshold of 2-3 or more proven episodes per year, diagnosed by either Amsel's or microscopic criteria, was used [25,26]. However, in clinical practice the patients often decline confirmation of every single episode of BV only treatment on the basis of "offensive-fishy smelling discharge" is often sought.

Recurrent BV can be very upsetting and women often experience embarrassing symptoms that interfere with their daily living, particularly their sexual lives. In a recent study such impact was described by 80% of participants as severe [15]. There is little evidence base on BV suppression and the evidence available supports long term oral metronidazole but the side effects may be unacceptable [7]. Other options such as repopulation of the vagina with lactobacilli, and acidification of the vagina with acid gel were not as successful [27,28]. A large well-designed study reported significant reduction of BV recurrence with using twice weekly metronidazole vaginal gel compared to placebo. However, half the women in this trial developed vaginal candidiasis, and the authors recommended concomitant antifungal therapy which is a drawback [7]. In another study by Pulkkinen et al BV relapse was prevented with a combination of metronidazole and nystatin vaginal pessaries (used for 3 nights after menses for 6 menstrual cycles). This combination prevented relapse for six months in 32/32 (100%) compared to 26/34 (76%) given placebo pessaries [29-31].

We hypothesized that lower doses of topical metronidazole used less frequently than in the licensed dose may suppress recurrent BV, and reduce side effects and hence the need to take candidiasis prophylaxis. We propose a pilot clinical trial to provide an indication of efficacy and acceptability/tolerability of the use of metronidazole vaginal gel to suppress recurrent BV as a proof of principle for a future randomized clinical trial [32].

Study Objectives

- To assess the efficacy and acceptability/tolerability of cyclical metronidazole vaginal gel 0.75% (Zidoval™) in suppressing symptomatic recurrent BV.
- To describe the participants' experience of cyclical metronidazole as a method of prevention of recurrent BV.

Methods

Study subjects

A sample of women from a population of genitourinary clinic attendees who were diagnosed as recurrent BV. The inclusion criteria were females aged>18<55 years who had at least four symptomatic episodes of BV in the last year, of which at least three were proven by the Nugent or Amsel's criteria, all were not on any BV medication at base line, willing to comply with study protocol and sign an informed consent after reading the study patient information leaflet. Patients were excluded if they did not consent, had other sexually transmitted disease, pregnant, lactating, or intolerant to metronidazole.

Study design

The study is a proof of principle, pilot, open label clinical trial. Participants were enrolled consecutively as they attended the clinic. Recruitment completed over a period of 6 month. We enrolled 16 patients who consented out of 20 invited to participate. With this sample size, we expected to obtain sufficient indication of the effect of metronidazole-the drug of first choice for treating BV- on recurrent BV to guide a future randomized controlled trial.

Data collection

We collected data using a questionnaire and case notes at enrolment, structured interview on socio demographic characteristics such as age, ethnicity, sexual and behavioural characteristics: number of sexual partners in the previous year and last 4 weeks, age at first sexual intercourse (coitarche), douching and bubble path practice, smoking, clinical characteristics: number of BV diagnosis in the previous year, number of years since first BV diagnosis, history of pregnancy and contraception. In addition, at enrolment participants were trained to take self-vaginal swabs and smear it on a slide and mark it on a symptoms diary (both provided). At 6 month data was collected from the symptoms diary, and exit survey: number of relapses, need for therapeutic dose of trial medication, laboratory confirmation of relapses, and patient satisfaction.

Measures

Self-taken vaginal swab is a validated method of obtaining vaginal smears and was proven to be more convenient to participants compared to practitioner taken swabs. Participants filled in a symptoms diary in a standard form that included time of symptoms, time of periods, time of taking the metronidazole gel, and time of bringing a vaginal smear to the in-house microscopy lab, and time therapeutic doses of metronidazole if any.

Symptomatic BV was diagnosed if vaginal odour, and or discharge are noted on the symptom diary, and a vaginal swab with Nugent score ≥ 7. The slides will be reported as BV (score 7-10) or no BV (score<7). Additional treatment (the full-licensed-dose of

metronidazole gel) will be offered when symptomatic attacks occur. A designated qualified Medical Laboratory officer performed all the microscopy. As we did not plan to monitor asymptomatic BV or changes in vaginal flora, swabs were only taken if participants are symptomatic [15].

A visual analogue scale of (1-10) with 1=definitely no, and 10=definitely yes was used to measure patient satisfaction: improvement of symptoms, adverse effects, and willingness to continue on metronidazole prophylaxis if offered.

Pharmacology

Metronidazole vaginal gel 0.75% (Zidoval™): Metronidazole is a synthetic antibacterial agent and has anti-protozoal activity. Antimicrobial effects result from inhibition of nucleic acid synthesis and disruption of DNA. Metronidazole vaginal gel 0.75% (Zidoval™) is only 2% bio-available compared with a 500 mg tablet taken orally. It is metabolized in the liver and 35%-65% is excreted in urine as metabolites and unchanged drug. Side effects include disulfiram like reaction with alcohol, this is however unlikely with the vaginal gel formulation. In addition, nausea, metallic taste in the mouth, diarrhoea and constipation and worsening of vaginal candidiasis symptoms and it may interact with oral anticoagulants. The standard licensed dose for treating BV is a 5-gram applicator-full for 5 nights but clinical experience has shown 3 nights to be adequate in prevention. One 5-gram applicator full of metronidazole vaginal gel 0.75% (Zidoval™) was inserted for three consecutive nights following the menses for six consecutive menstrual cycles.

Ethics

The study was conducted according to the good clinical practice (GCP/ICH) guidance. The study was approved by the local REC. In addition, an exemption to use metronidazole as in the study-dosing schedule was obtained from the drugs licensing authority.

Statistics

The primary outcomes measure is the mean incident of relapses of symptomatic BV during the study time, and the mean difference compared with the historical mean by a paired-sample T test. The null hypothesis (H_o) is that there is no difference between the mean relapses of recurrent BV in participant taking Metronidazole prophylaxis from their mean relapses before metronidazole, at 5% alpha. In addition patient satisfaction with Metronidazole prophylaxis is calculated as frequency (%) of responses to the 3 categories of patient satisfaction questions and reported in a bar chart.

The correlation of demographic and behavioral patterns with the risk symptomatic recurrent BV was described using a predictive multivariate regression model. Descriptive statistics are analyzed and presented as means, minimum, maximum and standard deviation. Categorical variable were presented in cross tabulation.

Data was analyzed by SPSS (version 20.01, SPSS Inc.) statistical package.

Results

All sixteen women completed the study and are included in this analysis.

The great majority of participants 11/16 (69%) described their ethnicity as black, 3/16 (19%) white and 2/16 (12%) as other. The mean age of participants was 28.5 years (SD 4.5), duration of recurrent BV was 5 years (3.4) and mean episodes of BV episodes in the previous year was 6.56 (SD2.83) details of other behavioural characteristics are in table1. Table 2 summarizes the contraceptives used by participants split by smoking and practice of vaginal douche. It reveals that most participants 6/16 (37%) used condoms for contraception, 10/16 (62%) practiced douching and 12/16 (75%) patients are smokers.

Selected Demographic, Behavioral and Clinical Characteristics				
	Minimum	**Maximum**	**Mean**	**Std. Deviation**
BV episodes in last 12 month	4	12	6.56	2.83
Duration of recurrent BV in years	2	15	5.00	3.425
No of sexual partners in last 12 month	0	2	1.75	2.769
Age at first intercourse	13	19	16.19	1.471
No of sexual partners in last 4 weeks	0	1	0.75	0.447
Age (years)	21	36.5	28.5	4.5

Table1: Selected demographic, behavioral and clinical characteristics.

Smoking status, Use of contraception and Practice of Vaginal Douche Cross tabulation								
Practice of vaginal douche			**Use of contraception**					Total
			non	condoms	IUCD*	depo provera	Ocp**	
yes	Smoking status	yes	3	5	0		0	8
		no	0	0	1		1	2
	Total	3	5	1		1	10	
no	Smoking status	yes	2	0	1	1		4
		no	1	1	0	0		2
	Total	3	1	1	1		6	
Total	Smoking status	yes	5	5	1	1	0	12
		no	1	1	1	0	1	4
	Total	6	6	2	1	1	16	

*Intrauterine contraceptive device; **Oral contraceptive pill

Table 2: Smoking status, use of contraception and practice of vaginal douche cross tabulation.

A multivariate model was built using "backward fitting". Whereby, all variables were initially put into a random effects Poisson model and eliminated in a stepwise manner based on the significance of a likelihood ratio test (LRT). A variable is kept in the model if the P-value is below a threshold of 0.05. The final predictive model is described in Table 3. Future BV episode occurring was predicted by previous history of BV (OR1.43, P-0.003), and use of condoms (OR

3.35, P-0.013). There is a trend that douching (OR1.70, P-0.06) is also predictive. A higher number of pregnancies (OR 0.59, P-0.02) and being an x-smoker (OR, P-0.12, P-0.07) had a protective effect in our model.

Variable	Odds ratio	95% Confidence Interval	P-value
BV history	1.430	1.129-2.813	0.003
BV confirmed	0.571	0.106-1.080	0.515
Pregnancy	0.596	0.378-1.937	0.025
Coitarche	2.200	0.876-5.593	0.093
Contraception	0.7511*	0.039-1.464	0.012
	1.230**	0.928-1.840	0.004
	3.351***	2.090-5.470	0.013
Douching	1.700	0.765-3.773	0.062
Smoking status (ex-smoker)	0.1245	0.0129-1.208	0.073
*Hormonal contraception; **IUCD; ***Condom			

Table 3: Multivariate regression model.

The mean symptomatic relapse episodes of BV during the 6month follow up period was 0.44(SD1.09). Historically, the study group had a mean of 6.5(SD3.09) when BV was diagnosed by either clinical or laboratory criteria, and a mean of 3.75(1.06) by laboratory criteria alone. Compared with each of the historical means by paired-sample T test, the difference is significant in both at P<0.0001 as detailed in Table 4.

	Mean (SD)	N	P-value
Historical	6.56 (3.09)	16	1
Historical Confirmed	3.75(1.06)	16	1
Metronidazole* prophylaxis	0.44(1.09)	16	

Table 4: Paired-samples t test statistics.

At trial exit survey 11/16 (69%) of participant indicated they would definitely continue on Metronidazole Prophylaxis, that their BV symptoms improved, and they did not experience adverse effects. Likewise 4/16 (25%) said they would likely to continue, and 1/16 (6%) was unsatisfied with the regime because of candidiasis (Figure 1).

Discussion

We report significant suppression of BV over the course of 6 month follow up in women who had 4 or more episodes of BV within the preceding 12 month. In addition, the greater majority found suppression with metronidazole gel acceptable and 87.5% indicated that they are willing to continue. This is the first time, to our knowledge, that cyclical metronidazole, in a reduced dose, is used to suppress BV and the results appear promising.

Our efficacy results were in line with those of two previously published trials in which metronidazole gel was used to suppress recurrent BV [7,29]. However, we used fewer doses (twice weekly vs. 3 times monthly) than in Sobel's et al, and unsurprisingly the adverse effects particularly vaginal candidiasis was uncommon. Therefore, our regime is likely to be more convenient if these results are reproduced in a larger randomized controlled trial.

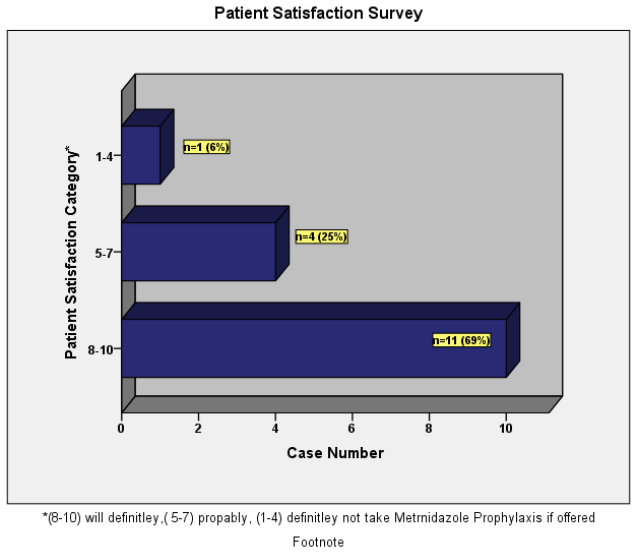

Figure 1: Patient satisfaction survey.

Fifteen self-taken swabs were obtained from 4 participants, 10 of them were from the same woman. All 4 had symptoms consistent with BV but microscopy reported Nugent score of>7 in only 3 samples from the same participant who provided 10 samples. One sample was reported as candida, and the rest were reported as normal flora (results not shown). If we only the consider confirmed episodes in our study recurrence would have been under estimated However, diagnosing BV on symptoms grounds only is likely to result in over diagnosis, and is not objective hence under estimating the effect of the intervention. On the other hand microscopic diagnosis on self-taken swabs tends to under estimate symptomatic BV as and many women have symptoms of BV with normal or intermediate vaginal flora on microscopy [15,32,33].

Most of the adverse effect were in a single participants who is known to suffer from both recurrent thrush (candidiasis) and recurrent BV, she indicated in the study exit questionnaire that since enrolment her thrush symptoms worsened, and therefore she would not use it again in the future. This due to metronidazole induced worsening of thrush a recognized adverse effect of this drug. Also, previous studies suggested that in some women antibiotic therapy is not sufficient to suppress the recurrence of BV and other additional therapy such as acidification, and lactobacillus repopulation of the vagina is needed too [8,34]. Another participant who at base line reported 12 relapses in the preceding year, continued to be symptomatic throughout the study period. Although we did not objectively monitor compliance with metronidazole, it seems it had no effect on her recurrent BV most likely due to resistance.

The reduced dose of suppressive metronidazole not has prevented all the episodes of BV but neither did it when used at higher doses, where it was associated with significant rate of vaginal thrush [7].

Sobel et al reported a 70% and 39% probability of remaining cured in the metronidazole gel and the placebo arms respectively, which declined to 34% and 18% by 28 weeks, follow up. Therefore, for a selected group of women, additional methods may be needed for BV suppression.

No participant reported a treatment limiting adverse effects associated with metronidazole, and they all completed the course of suppressive therapy including the one who noted worsening of her recurrent thrush. This is consistent with published results of metronidazole tolerability even when much higher/systemic regimens were used. Metronidazole gel may be an additional option for long-term suppression of BV for women who have frequent recurrence of BV. However, there is a cost implication as the gel formulation is 20 times more expensive than tablets in the UK [35].Therefore, the reduced dose provides financial as well as clinical benefit.

Consistent with previous cross sectional studies, women tended to have more recurrence if they are older, of black ethnicity, cigarette smokers and have a current sexual partner, and douching. However, bivariate analysis did not reveal significant associations of the rate of BV recurrence with the demographic, clinical, and behavioural independent variables. The multivariate model revealed significant relative risk of developing future BV episodes in women with higher number of BV episodes in the past (OR 1.43, P-0.003). Condom use was associated with the highest risk; women using this contraceptive method were at three times more risk to develop BV than those not using any contraception at all which is surprising given the likely sexually transmitted nature of BV. However, it is also biologically plausible that spermicides in condoms precipitated BV in some of the women. Similarly, there is a trend towards douching predicting the occurrence of future episodes (OR 1.70 P-0.06). Other factors that showed a protective effect were being an ex-smoker and having higher number of pregnancies; compared to women who smoked, an ex-smoker had a reduced risk of BV. As pregnancy was modelled as a continuous variable, a history of one more pregnancy resulted in 41% reduction in risk of experiencing future BV episodes (the P-value 0.072 was of borderline significance). Although this model is informative it should be interpreted with caution. The small sample size may have biased the findings. Moreover, using the stepwise approach have its inherent limitations e.g. the individuals for whom the outcome should be predicted may differ from those in this dataset in a way not captured by the variables measured.

There will be no perfect therapy for recurrent BV until the underlying etiology is identified; increasingly new evidence is emerging through advances in molecular biology suggesting that newer organisms may have a role in the pathogenesis of BV. Until then, women with symptomatic recurrent BV have to get by using less than satisfactory remedies.

The strengths in our study is that it evaluated in a systematic way a practice often used in the clinic based on clinical experience only, it is a prospective, hypothesis based study, utilized self-taken sampling which was shown to be more convenient to women. The weaknesses are: use of historical controls, subjective nature of outcomes, and the problematic case definition of recurrent BV. Also, the study is open to recall and selection biases as women may have tried to give favourable answers to the questionnaires; and those likely to agree to participate in our long term follow up study may have less risk or different health seeking behaviour.

In a future large randomized controlled trial, these shortcomings need to be addressed. For instance, a multi-centred approach will be more suitable to recruit enough women to show a difference between the metronidazole and placebo (dummy gel) arm due to the relatively low prevalence of recurrent BV if defined as>3-4 confirmed episodes. In addition, offering incentives and or postal delivery of samples may allow objective end points to be used.

Conclusions

Metronidazole vaginal gel may be effective in suppressing recurrent BV when used for three days following the menstrual cycle.

The majority of women found it acceptable and associated with little adverse effects. A larger double-blinded randomized controlled trial is needed to evaluate this condition.

Conflict of Interest

The authors declare no competing financial interests.

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