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Efficacy of an acidic vaginal gel on vaginal pH and interleukin-6 levels in low-risk pregnant women: a double-blind, randomized placebo-controlled trial

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Background: Increased interleukin-6 (IL-6) levels and a vaginal pH of > 4.7 are associated with obstetric complications such as preterm delivery and low birth weight. Topical treatments, able to maintain a physiological vaginal pH, could help in the prevention of vaginal infections.

Study aim: In a randomized, double-blind, placebo-controlled trial, we evaluated the effects of an acidic buffering vaginal gel (Miphil®) on vaginal pH and IL-6 levels in pregnant women.

Patients and methods: Seventy low-risk women pregnant with a singleton (second trimester) were enrolled in the trial. Thirty-five were randomized to the acidic gel, 2.5 g every 3 days for 12 weeks, and 35 to the corresponding placebo. Vaginal pH and vaginal IL-6 level were measured at baseline and after 12 weeks. Women were then followed until delivery. The main outcome measures were vaginal pH, vaginal pH normalization (pH < 4.5) and vaginal IL-6 levels.

Results: Vaginal pH at baseline was 4.6 ± 0.4 and 4.4 ± 0.3 in the acidic gel and the placebo group, respectively. At baseline, a total of 40% (14/35) and 22% (8/35) of women in each group, respectively, had a vaginal pH of ≥ 4.7. At week 12, the vaginal pH was 4.3 ± 0.3 in the acidic gel group and 4.3 ± 0.3 in the placebo group (NS). The acidic gel normalized the vaginal pH in ten out of 14 women ($p = 0.04$) in comparison with only one out of eight women in the placebo group (NS). The acidic gel induced a significant ($p < 0.02$) reduction of vaginal IL-6 from 12.0 ± 7 to 8.9 ± 5 pg/l (–36%). In the placebo group, IL-6 increased from 9.0 ± 5 to 13.5 ± 6.8 pg/l (+50%) ($p = 0.05$). Birth weight was 2978 ± 700 g in the placebo group and 3241 ± 477 g in the acidic gel group ($p = 0.06$).

Conclusions: The use of the acidic gel in low-risk pregnant women is able to maintain a physiological vaginal ecosystem and prevents the increases of vaginal pH and vaginal IL-6. Prospective and controlled trials are warranted to evaluate whether this acidic gel can reduce obstetric complications linked to vaginal inflammation during pregnancy.

Key Words: ACIDIC VAGINAL GEL; pH; INTERLEUKIN 6; PREGNANCY

INTRODUCTION

Preterm delivery remains one of the most important issues in reproductive medicine, complicating about 9% of all pregnancies[1]. There is a strict link between maternal infections and obstetric complications such as preterm delivery[2]. In particular, bacterial vaginosis, a common vaginal infection, is a well-known risk factor for preterm birth and low birth weight[3]. Symptomatic or asymptomatic bacterial vaginosis could be detected in up to 20% of pregnant women[4]. Bacterial vaginosis is characterized by a vaginal pH of > 4.5, the presence of pathognomonic 'clue cells' and by a positive fishy odor[5]. It is microbiologically characterized by an overgrowth of several micro-organisms such as *Gardnerella vaginalis*, *Mycoplasma hominis* and *Bacteroides* spp[5]. The hallmark of bacterial vaginosis is a lack or a great reduction of the presence of vaginal lactobacilli[6]. Through the metabolism of glycogen, lactobacilli are responsible for the physiological mild acidity (i.e. a vaginal pH of < 4.5) of the vaginal secretions. The physiological vaginal pH during pregnancy is in the range of 4.0–4.5. In non-pregnant women the mild acidity of the

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healthy vagina has been shown to correlate with decreased risk for *Chlamydia*, *Trichomonas* and urinary infections[7]. Several studies have shown that an acidic vaginal pH significantly increases the binding capacity of lactobacilli to the vaginal epithelium and reduces the activity of several pathogenic bacterial enzymes such as sialidase[8]. Adhesion of *Gardnerella* to vaginal epithelial cells is pH-dependent, with a maximum attachment occurring between pH 5 and 6[9]. The vaginal pH is thus recognized as the most significant predictor of the status of the vaginal ecosystem. Interleukin 6 (IL-6), an inflammatory cytokine, is a major mediator of the host response to inflammation and infection[10]. High pH and vaginal levels of IL-6 are detected during vaginal infections[11]. Elevated vaginal IL-6 levels and a vaginal pH of > 4.7 are associated with preterm delivery and low birth weight[12]. Miphil™ (Mipharm, Milan, Italy) is an acidic bioadhesive polymer, polycarbophil-carbopol, with buffering capacity. Polycarbophil and carbopol, weak polyacids, are large molecules that are able to adhere to vaginal epithelial cells until they divide (up to 3–5 days), and buffer the vaginal secretions near their pK_a (i.e. 4.3). In women with suspected bacterial vaginosis[13] the polycarbophil-carbopol vaginal gel has been demonstrated to reduce the vaginal pH from 5.4 to 4.6. In patients with bacterial vaginosis, the combination of an antibiotic with this acidic gel achieved a more rapid normalization of the vaginal microflora, with an higher cure rate in comparison with antibiotic therapy alone[14].

METHODS

We aimed to evaluate the effects of this acidic vaginal gel with buffering activity and high mucosal bioadhesion, 2.5 g applied every 3 days, on vaginal pH and vaginal IL-6 levels in low-risk pregnant women in the second trimester. The study primary outcomes were the values of vaginal pH and IL-6 levels in the two groups in comparison with baseline values. Secondary outcomes were the vaginal pH normalization (i.e. vaginal pH of ≤ 4.7) rates and the birth weight.

The study was a prospective, randomized, double-blind, parallel group, placebo-controlled trial. Two gynecology clinics took part in this trial. The local Institutional Review Board approved the study protocol. Seventy pregnant women were enrolled in the study, after they had provided their written informed consent. The main inclusion criteria were low-risk singleton pregnancy in women aged 18–40 years, at 12–14 weeks of gestation at randomization, and who provided written informed consent. The main exclusion criteria were a previous complicated delivery (preterm delivery and/or a low birth weight), diabetes mellitus or arterial hypertension, symptomatic vaginal infection at randomization or recent use of vaginal antibiotic or anticandidal drugs. Randomization was performed using a computer-generated randomization list (Arcus Quickstat) with a block of eight in a 1 : 1 ratio. Vaginal pH and was measured at baseline and after 12 weeks of treatment. Vaginal pH was measured in the lateral vaginal fornix using color strip indicator papers with a range of 4–7.0 (Merck Diagnostics, Darmstadt, Germany). Vaginal pH at week 12 was measured 72 h after the last application of the acidic gel or the corresponding placebo. The placebo gel, with an appearance similar to that of the study preparation, was made using a polymer (hydroxyethylcellulose) with no buffering activity. The placebo vaginal gel pH was 4.0. Vaginal IL-6 was measured, at baseline and after 12 week of treatment, with a chemiluminescent immunometric assay (DPC, Los Angeles, CA, USA) and expressed in picograms per liter.

Statistical methods

Il-6 vaginal levels were considered the primary endpoint of the study. In consideration of previous data, the sample size was based on the hypothesis of finding an absolute difference in IL-6 levels of at least 20 ± 40 pg/l after active treatment in comparison with baseline values. With a power of 80% and a type-I error of 0.05, a minimum of 64 (32 per arm) patients should be recruited in the trial. The Wilk–Shapiro test was used to check the normality of distribution of the main variables. The Fisher exact test was used to compare categorical variables and the Wilcoxon signed rank test and the paired *t* test were used to compare continuous variables. A p value of < 0.05 was considered significant. Statistical analysis was performed using the SPSS version 11.0 software package.

RESULTS

Between December 2000 and June 2001, 98 pregnant women were screened for the study. Seventy women met the inclusion criteria and were enrolled in the trial. Thirty-five were randomized to the acidic gel 2.5 g, applied every 3 days for 12 consecutive weeks, and 35 to the corresponding placebo. All patients were valuable for the efficacy and safety analysis on an intention-to-treat basis. For patients who prematurely concluded the trial, the last observation carried forward (LOCF) method was utilized. Figure 1 shows the study flow chart. Baseline demographic and clinical characteristics were similar in the two groups. Demographic and clinical data are shown in Table 1. Three patients (one in the acidic gel group and two in the placebo group) were prematurely withdrawn from the study as they did not attend the study visit (two patients) and because of the detection of pre-eclampsia (one patient in the placebo arm). Vaginal pH at baseline was 4.6 ± 0.4 and 4.4 ± 0.4 in acidic gel and placebo groups, respectively. At baseline, a total of 40% (14/35) and 22%

(8/35) women, respectively, had a vaginal pH of ≥ 4.7. At week 12, the vaginal pH wase 4.3 in the acidic gel group and 4.3 in the placebo group (NS). Treatment with the acidic gel normalized the vaginal pH (i.e. pH ≤ 4.7) in ten out of 14 women ($p = 0.04$; Fisher's exact test) in comparison with only one out of eight patients in the placebo group (NS). At week 12, a significantly ($p = 0.004$, Yates corrected χ^2 test) lower percentage of women in the acidic gel group had a vaginal pH of > 4.7 in comparison with the placebo group (11% vs. 21%). The acidic gel induced a significant ($p < 0.02$ Wilcoxon test) reduction of vaginal IL-6 levels from 12.0 ± 7 to 8.9 ± 5 pg/l (–36%). In the placebo group, the IL-6 level significantly increased from 9 ± 5 to 13.5 ± 6.8 pg/l (+50%) ($p = 0.05$). Gestational weeks at delivery were 39 ± 1 in the acidic gel group and 38 ± 2 in the placebo group. This difference was not statistically significant. Preterm delivery (< 37 weeks) was observed in two women in the placebo group (delivery at 29 and 36 weeks of gestation) and in one woman in the acidic gel group (delivery at 37 weeks). The birth weight was 2935 ± 806 g in the placebo group and 3241 ± 477 g in the acidic gel group ($p = 0.06$, unpaired t test) (Table 2). A negative correlation was found between IL-6 vaginal levels at baseline and birth weight in the placebo group ($r = 0.4$, $p = 0.05$, Pearson correlation test) but not in the acidic gel-treated group.

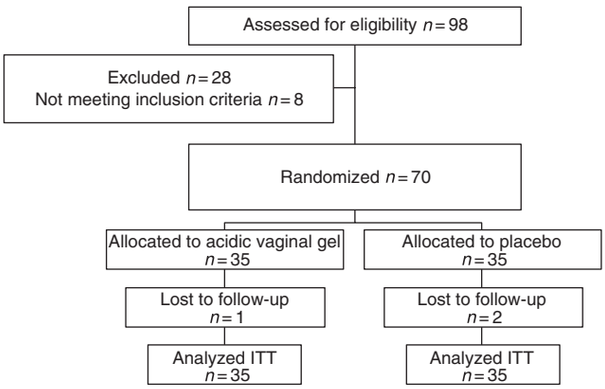

Figure 1 Study flow chart. ITT, intention to treat

Table 1 Baseline characteristics of the study population

	Acidic gel (n = 35)	Placebo (n = 35)	p Value
Age (years)	32 ± 4	31 ± 4	NS
Smokers (%)	26	15	NS
Gestational weeks at randomization	13 ± 1	13 ± 1	NS
Parity	1	1	NS
Gestational weeks at delivery	39 ± 1	38 ± 2	NS

CONCLUSIONS

The results of our study have demonstrated that the use of an acidic vaginal gel with buffering activity was safe in pregnant women. Furthermore, the use of the acidic gel in low-risk pregnant women was shown to normalize the vaginal pH and prevent an increase of vaginal IL-6, contributing to the maintenance of a 'physiological' vaginal ecosystem during pregnancy. The mild acidity of the healthy vagina has been shown to correlate with a decreased risk for *Chlamydia*, *Trichomonas* and urinary infections[7]. Several studies have shown that an acidic vaginal pH significantly increases the binding capacity of lactobacilli to the vaginal epithelium and reduces the activity of several pathogenic bacterial enzymes such as sialidase[15]. Adhesion of *Gardnerella* to vaginal epithelial cells is pH-dependent with a maximum attachment occurring between pH 5 and 6[9]. The vaginal pH is thus recognized as the most significant predictor of the status of the vaginal ecosystem. IL-6 is an important mediator of inflammation. Therefore, vaginal pH and IL-6 level are considered practical predictors of the status of the vaginal ecosystem. High pH values and increased vaginal levels of IL-6 are commonly detected during vaginal infections such as bacterial vaginosis[11]. Bacterial vaginosis is

Table 2 Study variables endpoints

	Baseline		After treatment		
	Acidic gel (n = 35)	Placebo (n = 35)	Acidic gel (n = 35)	Placebo (n = 35)	p Value
Vaginal pH	4.6 ± 0.4	4.3 ± 0.3	4.3 ± 0.3	4.3 ± 0.3	NS
Patients with pH ≥ 4.7 (%)	39	22	11*†	20	*$p = 0.04$ vs. baseline †$p = 0.04$ vs. placebo group
Interleukin 6 (pg/l)	12.0 ± 7	9.0 ± 5	8.3 ± 5*	13.5 ± 6.8†	*$p = 0.02$ vs. baseline †$p = 0.05$ vs. baseline

associated with gynecological and obstetric complications. Our group have demonstrated that increased vaginal IL-6 levels and a vaginal pH of > 4.7 are associated with preterm delivery and low birth weight[12]. The acidic vaginal gel is a bioadhesive compound with buffering activity. After vaginal application, the gel is able to adhere to the vaginal epithelial cells until they divide (in 3–5 days), and buffers the vaginal secretions near its pK_a (i.e. 4.3). Previous randomized controlled studies have shown that the clinical use of this acidic gel normalized the vaginal pH in women with suspected bacterial vagionsis[13]. In women with confirmed bacterial vaginosis, a 4-week application of the acidic gel, after antibiotic treatment, contributed to the maintenance of a normal vaginal pH and reduced the recurrence of vaginal infections[14]. Our study has shown that the use of the acidic gel in low-risk pregnant women was associated with a positive effect on vaginal pH and vaginal IL-6 levels. However, some study limitations have to be considered in evaluating our results. First, the primary study endpoints were so-called surrogate variables (IL-6 and vaginal pH). Our trial was not powered to find any effects on 'hard' outcomes such as preterm and other obstetric complications. However, there are consistent data showing that vaginal pH and IL-6 are predictive of preterm delivery and low birth weight. We found a trend in favor of the acidic gel regarding a greater weight at birth in comparison to the placebo group. Furthermore, baseline IL-6 vaginal levels inversely correlated with birth weight in the placebo group but not in the group receiving the acidic gel. Finally, low-risk pregnant women were enrolled in this trial. Therefore, our results cannot be applied to pregnant women at higher risk. Large prospective and controlled trials are warranted to evaluate whether this treatment can reduce obstetric complications linked to vaginal inflammation and infection.

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