ORIGINAL ARTICLE

Reactional changes in short-term levonorgestrel-releasing intrauterine system (lng-ius) use

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SUMMARY

OBJECTIVE: *To evaluate endocervical and vaginal environment changes in women using a levonorgestrel-releasing intrauterine system (LNG-IUS).*

METHODS: *A quasi-experimental study included sixty women who had an LNG-IUS inserted in the Family Planning Clinic of UNICAMP between April and November of 2016. Women in reproductive age, non-pregnant, without the use of antibiotics and contraceptives seeking for LNG-IUS insertion were selected for this study. All women were evaluated with regard to vaginal and endocervical pH, vaginal and endocervical Gram-stained bacterioscopy, and Pap-smear before and two months after LNG-IUS insertion. Clinical aspects such as cervical mucus, vaginal discharge, and cervical ectopy were also observed.*

RESULTS: *After LNG-IUS insertion, there was an increase in the following parameters: endocervical pH>4.5 (p=0.02), endocervical neutrophil amount (p<0.0001), vaginal cytolysis (p=0.04). There was a decrease in vaginal discharge (p=0.01). No statistically significant changes were found in vaginal pH, neutrophils amount in the vaginal mucosa, vaginal discharge appearance, vaginal candidiasis, bacterial vaginosis, vaginal coccobacillary microbiota, cervical mucus appearance, or cervical ectopy size.*

CONCLUSIONS: *Short-term LNG-IUS use did not increase vulvovaginal candidiasis or bacterial vaginosis, and led to diminished vaginal discharge. Notwithstanding, this device promoted reactional changes in the vaginal and endocervical environment, without modification on cervical ectopy size.*

KEYWORDS: *Levonorgestrel/adverse effects. Contraceptive Agents/adverse effects. Vaginosis, bacterial. Vaginal discharge.*

INTRODUCTION

The levonorgestrel-releasing intrauterine system (LNG-IUS) has been used since the early 1990s and is considered one of the most effective methods of contraception[1]. LNG-IUS is also widely used for other clinical purposes, such as reducing heavy menstrual blood loss, symptoms of endometriosis, endometrial hyperplasia, and endometrial protection during post-menopausal estrogen replacement therapy[2-5].

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Several studies suggest that the progestogenic effects of LNG-IUS and the route of hormone delivery influence the vaginal microenvironment since the cervicovaginal epithelium is directly modulated by the action of hormones[6-9]. Estrogen induces the maturation of the cervicovaginal epithelium and promotes the accumulation of glycogen in the epithelial cell. Thus, women under a progestin-based contraception method have increased parabasal cells and decreased intermediate and superficial cells. This has a direct impact on the bacterial population, since glycogen is an important factor in *Lactobacillus* growth, protecting against genital infections via the production of bacteriocins, lactic acid, and maintenance of low vaginal pH[10,11].

Data on the impact of LNG-IUS on the cervicovaginal epithelium and women's susceptibility to genital infections are controversial. Some investigators have shown a general risk to develop a genital infection after LNG-IUS insertion[12,13].

A study using 16SrRNA gene technology to characterize the bacterial profiles of vaginal microflora in women using LNG-IUS showed that bacteria typically associated with the dysbiotic vaginal environment were significantly more abundant than in controls[6]. However, other studies have failed to demonstrate an association between such contraceptive method and genital infections or changes in the vaginal microbiota[7-9].

Another factor that protects the cervicovaginal epithelium is the secreted cervical mucus, which is subject to hormone-induced physical or biochemical alterations, thus affecting the risk of genital infections[14]. Moreover, the study group has shown that changes in the profile of inflammatory cells in the vaginal cavity are an important marker of infections, justifying the investigation of such parameter in LNG-IUS users[15].

Cellular alterations in the Pap smear, vaginal bleeding, altered vaginal pH, inflammatory cellular infiltrate in the vagina and cervicitis are common adverse effects that need to be better investigated in LNG-IUS. This study aims to evaluate, in the short term, biochemical, microbiological, and clinical modifications in the endocervix and vagina after LNG-IUS insertion. We hypothesized that the LNG-IUS insertion can diminish vaginal discharge due to the progestagenic effects on the vaginal epithelium and endocervical glands

METHODS

This quasi-experimental study involving 60 women was conducted at the Family Planning Clinic of CAISM-UNICAMP, Campinas, Brasil, between May 2016 and December 2016. The study was approved by the Research Ethics Committee at the University of Campinas, CAAE nº 46001315.7.0000.5404, research project nº 1.208.156, and written informed consent was obtained from all participants. Inclusion criteria were: women of reproductive age (between 18 and 45 years), with regular menstrual cycles, sexually active and willing to use long-term contraception. As exclusion criteria, we considered: use of vaginal or systemic antibiotics, or vaginal douching in the 30 previous days, current hormonal therapy, vulvovaginitis or genitourinary pain symptoms, pregnancy, sexual intercourse less than 6 hours prior to sample collection, uterine anomalies, unexplained bleeding, contraindication to hormonal treatment and history of breast cancer.

The insertion of the LNG-IUS was performed in the first phase of the menstrual cycle, shortly after the end of menstruation (6th to 9th day), without any indication of menstrual bleeding. The insertion procedure was guided by a pelvic examination, which consisted in holding the cervix by a tenaculum and passing the uterine sound in order to measure the depth of the uterus; next, the IUS was inserted, leaving out approximately 3 centimeters of the string, and the women were kept at rest. Analgesics, anesthetics, or anti-inflammatory drugs (NSAIDs) were not necessary before the insertion. Previously, all women were submitted to gynecological examination, and samples were collected from the vaginal wall and endocervix by sterile swabs. The collected samples were submitted to bacterioscopy (Gram-stain) and cytological analysis by Pap smear to characterize the type of vaginal flora, inflammatory process, presence or absence of pathogens, vaginal epithelium lysis, and bacterial vaginosis. Evaluations of vaginal and endocervical pH, cervical ectopy, the appearance of cervical mucus, and appearance and amount of vaginal discharge were also performed. To characterize the vaginal pH, a 4.5 cut-off was assumed, considering that normal vaginal range varies from 3.8 to 4.5[11].

In order to quantify the leukocytes both in the endocervix and in the ectocervix, we used Gram-stained smears observed at 1000x magnification, scoring the slides as follows: 1) absent or discrete frequency: less

than 4 leukocytes per field; 2) moderate frequency: five to nine neutrophils per field; and 3) accentuated frequency: more than 10 leukocytes per field. This same scoring system was used for the quantification of squamous cells in the Pap smear, but at 400x magnification. Regarding cytolysis, we used as a criterion on the quantification of the number of nude nuclei of intermediate cells in the Pap smear, also using the same scoring system described above.

The cervical ectopy diameter was measured and was considered discrete when it occupied less than half the diameter of the cervix and as moderate or accentuated when it occupied more than half the diameter of the uterine cervix.

All patients were evaluated again two months after the device insertion (± 3 days variation) to avoid menstrual cycle variation. Vaginal microbiological analysis and characterization of bacterial vaginosis were performed based on the Gram-staining method and according to the Nugent score[16]. The existence of vulvovaginal candidiasis was indicated by the presence of yeasts and hyphae, white and lumpy vaginal discharge and inflammation in the vaginal wall. Normal flora (Grade I) was defined by the absence of pathogens in the analysis of the vaginal smear and presence of 80% or more of lactobacilli[17]. Grade III flora was defined when Lactobacilli was substituted by coccobacilli or cocci flora (Gram-negative and/or anaerobic flora). Grade II was considered an intermediate flora.

The evaluation of cellular cytolysis, cellular inflammatory alterations, genital infections, and vaginal microbiota was performed both in the Gram and Pap samples of the cervix and vagina, relating these factors to the vaginal and endocervical pH before and after LNG-IUS insertion.

The mean of the intermediate cells in the vaginal mucosa was used to calculate the sample size (lowest expected variation and more conservative or representative for this study). A 10% beta error, a significance level of 5%, and a supposed estimated incidence of 40% of intermediate cells were considered in the patients submitted to the insertion of the LNG-IUS. A total sample was estimated in 58 cases. The 9.2 version SAS System for Windows (Statistical Analysis System), SAS Institute Inc, 2002-2008, Cary, NC, USA, was used for the statistical analysis. The LNG-IUS used was manufactured by Bayer Oy (PO Box 415, Fl-20101 Turku, Finland) and the insertion followed the label recommendations.

RESULTS

The mean age of the study participants was 32 ± 7 years (only one patient over 45 years old, aged 52), data not shown, of which 44 (73%) were white women; further socio-demographic information from study participants are shown in Table 1.

Clinical, cellular, and microbiological effects on the cervix and vagina before and two months after insertion of the LNG-IUS are shown in Table 2. The number of cases with endocervical pH ≥ 4.5 increased significantly after insertion ($p < 0.05$) and the number of cases with vaginal pH ≥ 4.5 also increased after insertion of the device, although with no statistical significance (73% vs. 60%, $p = 0.116$). The presence of neutrophil cells was not altered in the vagina ($p = 0.317$); however, it showed a significant increase in the endocervix in the presence of LNG-IUS ($p < 0.0001$). Vaginal discharge reported by patients changed from moderate/accentuated to absent/discrete after LNG-IUS insertion, with a marked decrease in intensity ($p = 0.011$). There were no changes in the appear-

TABLE 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF 60 WOMEN PARTICIPATING IN THE STUDY.

Characteristic		N/60	%
Race			
	White	44	73
	Black	5	8
	Brown	11	18
Educational level			
	Primary school	5	8
	High school	21	35
	University	34	56
Number of gestations*			
	0	12	20
	1 to 2	40	66
	≥ 3	8	13
Parity			
	0	12	20
	1 to 2	43	71
	≥ 3	5	8
Number of miscarriages			
	0	55	91
	1 to 2	5	8
	≥ 3	0	0

* Including parity and miscarriage

TABLE 2: CLINICAL, CELLULAR, AND MICROBIOLOGICAL ASPECTS RELATED TO THE CERVIX AND VAGINA BEFORE AND TWO MONTHS AFTER INSERTION OF THE LNG-IUS.

Variable	Before LNG-IUS	After LNG-IUS	P value*
	N (%)	N (%)	
Endocervical pH			0.0253
≥ 4.5	53 (88)	58 (97)	
< 4.5	07 (12)	02 (2)	
Vaginal pH			0.1167
≥ 4.5	36 (60)	44 (73)	
< 4.5	24 (40)	16 (27)	
Neutrophils in endocervix			< 0.0001
Moderate/accentuated**	22 (37)	44 (73)	
Absent/discrete	38 (63)	16 (27)	
Appearance of endocervical mucus			0.1573
Cloudy	1 (2)	3 (5)	
Limpid	59 (98)	57 (95)	
Neutrophils in vagina			0.3173
Moderate/accentuated	1 (2)	3 (5)	
Absent/discrete	59 (98)	57 (95)	
Intensity of vaginal discharge			0.0116
Moderate/accentuated	20 (33)	9 (15)	
Absent/discrete	40 (67)	51 (85)	
Aspect of vaginal discharge			0.1797
Cloudy	1 (2)	4 (7)	
Clear-appearing	59 (98)	56 (93)	
Endocervical ectopy			0.2568
Moderate/accentuated	6 (10)	3 (5)	
Absent/discrete	54 (90)	57 (95)	
Squamous cells predominance***			0.7389
Intermediate/parabasal	47 (78)	48 (63)	
Superficial	11 (18)	7 (12)	
Cytolysis			0.0455
Moderate/accentuated	7 (12)	15 (25)	
Absent/discrete	53 (88)	45 (75)	
Bacterial vaginosis			0.2059
Positive	6 (10)	10 (17)	
Negative	54 (90)	50 (83)	
Vulvovaginal Candidiasis			1.0000
Positive	3 (5)	3 (5)	
Negative	57 (95)	57 (95)	
Microflora grading			0.5433
I	48 (80)	45 (75)	
II	6 (10)	5 (8)	
III	6 (10)	10 (17)	

*McNemar or Symmetry test. OR= Odds ratio. CI 95%: Odds ratio 95% confidence interval. ** Moderate/accentuated: presence of more than 10 neutrophils in high magnification field (1000 x). *** The lower n value in this analysis is due to a lower number of Pap-smears available for analysis of cell dominance (two missing cases before and five missing cases after LNG-IUS insertion).

ance of vaginal discharge or cervical mucus (p = 0.150), and the clear-looking mucus was predominant in both study phases. The predominance of

TABLE 3: COMPARISON OF ENDOCERVICAL AND VAGINAL PH, AND NUGENT SCORE BEFORE AND TWO MONTHS AFTER LNG-IUS INSERTION.

Parameter	Mean ± SE		P value*	95% CI	
	Before insertion	After insertion		Before insertion	After insertion
Nugent score	2.14 ± 0.28	2.58 ± 0.35	0.496	1.59-2.7	1.87-3.28
Endocervical pH	6.44 ± 0.13	6.71 ± 0.81	0.083	6.17-6.71	6.55-6.87
Vaginal pH	4.69 ± 0.62	4.74 ± 0.71	0.792	4.57-4.82	4.60-4.88

*Wilcoxon test

parabasal/intermediate vaginal squamous cells increased from 47 (81%) to 51 (85%), while the superficial cells decreased from 11 (19%) to 7 (15%), without statistical significance (p = 0.738). There was a slight increase in vaginal cytolysis (12% vs. 25%, p = 0.045) and bacterial vaginosis (10% vs. 17%, p = 0.205) at the end of the two-month period.

Table 3 shows that the mean Nugent score was slightly higher post-insertion (2.14 ± 0.28 vs. 2.58 ± 0.35; p = 0.496). The mean value of the endocervical and vaginal pH after insertion was not statistically different from the pre-insertion period (Table 3).

Moreover, a case-by-case analysis showed that 20 (33%) of the women had some degree of increase in Nugent score values after LNG-IUS insertion. The ectopy of the transformation zone increased in size in 3 (5%) of the women, decreased in 9 (15%), and remained unchanged in 48 (80%). Infection by *Candida* sp. was diagnosed in three women before insertion of the LNG-IUS; in one of them, the infection was maintained after insertion of the LNG-IUS, but without clinical symptoms. Two new cases of *Candida* sp. were detected after insertion of the LNG-IUS.

DISCUSSION

In this paper, we have shown that there was no increase of vaginal candidiasis or bacterial vaginosis, as well as no significant changes on cervical ectopy size or vaginal discharge after two months of the LNG-IUS insertion. Although cervical and vaginal pH had slight increases and a significant amount of inflammatory cells was observed, such factors do not seem to be relevant as causes of disease or complaints.

Taking into consideration that long-term use and/or microbiological findings after IUS insertion can be

confounded with many different risk factors (sexual activity, smoking, diet, medication intake, etc), we decided to check cervical and vaginal changes after only two months, in order to avoid bias. Indeed, the increase or decrease of vaginal candidiasis or bacterial vaginosis after one or two years of IUS insertion could be related to the patient's behavior rather than only IUS. The majority of published papers in this area are frequently focusing only on specific aspects, thus neglecting the overview of the cervical and vaginal environment as a whole. Certainly, the clinical correlation to microscopic findings presented herein is a different approach that provides excellent strength to our results.

The insertion of the LNG-IUS seems to promote changes in the vaginal and endocervical environment, without, however, presenting a relevant clinical adverse outcome. The presence of a foreign body in any biological cavity is a concern for clinicians, whether in the short or long term. In the case of intrauterine devices, this concern arises due to the possibility of complications such as missing strings, ascending infections, uterine perforation, and pelvic inflammatory disease[s18-20].

The use of IUDs has increased among young and sexually active women in recent years (particularly in nulliparous women, although not as much as the increase observed for women in general). In 2002, only around 0.5% of nulliparous women using contraception methods were using an IUD in the United States. This rate increased to 4.8% between 2011 and 2013[21]. In Brasil, the percentage of sexually active women using IUDs for contraception is around 3.0%. However, this number is also expected to rise in the next years despite problems, such as high cost and limited availability of the IUDs in the public health system that prevent a larger increase[22].

The numbers for younger and nulliparous women are still smaller than for multiparous women due to outdated beliefs and misconceptions about the safety of IUD use. Particular concerns such as the risk of pelvic inflammatory disease (PID), infertility, safety, and difficulty of insertion may still present as biases in the provision of this group of women. However, recent studies have shown that intrauterine devices are safe and effective for the majority of women, including those who are young and nulliparous and should be routinely included in the contraception options offered to them[21].

This study evaluated 60 women before and two months (in the same phase of the menstrual cycle) after the insertion of the LNG-IUS, which induced favorable modifications including a decrease of vaginal discharge and endocervical ectopy (p=0.256). On the other hand, we observed increased cytolysis and number of endocervical neutrophils, and endocervical pH (> 4.5). There were non-significant differences in bacterial vaginosis and vaginal candidiasis. This is consistent with the overall good acceptance of this contraceptive method in clinical practice.

Our results are in accordance with other researchers who also reported the absence of significant changes in the composition of vaginal microflora or in the frequency of bacterial vaginosis in LNG users, even after a long period of time[7,9,23,24]. Donders et al.[23] suggest that both hormonal and non-hormonal contraceptive methods have a greater tendency to present candidiasis, while our study showed a non-significant decrease in *Candida* sp infection after a short-term LNG-IUS insertion.

A decrease in cervical ectopy, as well as, a reduction in vaginal discharge, could be related to the local progestogenic effect of the LNG-IUS. It has been suggested that the possible mechanism responsible for the progestogenic and anti-estrogenic effect of LNG-IUS is the inhibition of the Insulin-like growth factor (IGF), which stimulates the proliferation and differentiation of cells that contain IGF membrane receptors, such as epithelial cells[25]. Hence, the decrease in endocervical ectopy found in our study could be related to this proliferative mechanism.

Furthermore, the higher frequency of cytolysis after LNG-IUS insertion found herein can be explained by the anti-estrogenic effects of LNG-IUS, leading to the predominance of intermediate cells rather than superficial cells. The intermediate cells are rich in glycogen and, therefore, more susceptible to the cytolysis by lactobacilli, since glycogen is an important factor in *Lactobacillus* growth[10,11].

Our study found an increase in the number of endocervical neutrophils after LNG-IUS insertion. This was probably a direct physical effect of the LNG-IUS string, which can lead to neutrophil chemotaxis and can modify the biochemical properties in this environment. In fact, it has already been demonstrated that LNG-IUS users present an increase in chemokines that promote leukocyte chemotaxis, such as Interleukin 8, in the endometrial epithelium[26]. Another study has also demonstrated that users of such IUS would be more susceptible to infection by microor-

ganisms that have an affinity for endocervical cells, such as *Chlamydia trachomatis*[27]. However, our study does not allow us to infer if these alterations could increase the susceptibility to endocervical inflammation. Therefore, longer follow-up studies focusing specifically on biochemical and microbiological changes in the endocervix are necessary to confirm this possible association.

This study aimed to evaluate practical modifications in the vaginal and endocervical environment in order to support the Gynecologists clinical decisions. Our study is particularly relevant because the data analysis was not limited to comparing average values, but it also presented a paired analysis with case-by-case follow-up. Nevertheless, new studies with larger populations and with a control group using Cu-IUS should be considered in the future.

CONCLUSION

The short-term LNG-ISU use mainly causes reactional changes in the vagina and endocervical microenvironment related to the decrease of the vaginal discharge and lysis of the vaginal epithelium, an increase of the pH and neutrophil amount in the endocervix. The use of this intrauterine device did not seem to be related to vaginal infection and dysbiosis in short-term uses.

Conflicts of interest

None of the authors have a conflict of interest to declare.

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RESUMO

OBJETIVO: *Avaliar as alterações do ambiente endocervical e vaginal em mulheres usuárias de sistema intrauterino liberador de levonorgestrel (SIU-LNG).*

MÉTODOS: *Um estudo quase-experimental incluiu 60 mulheres que inseriram o SIU-LNG na Clínica de Planejamento Familiar da UNICAMP entre abril e novembro de 2016. Mulheres em idade reprodutiva, não gestantes, sem uso de antibióticos e contraceptivos, em busca pela inserção do SIU-LNG, foram selecionadas para este estudo. Todas as mulheres foram avaliadas quanto ao pH vaginal e endocervical, bacterioscopia vaginal e endocervical por coloração de Gram, exame de Papanicolau antes e dois meses após a inserção de SIU-LNG. Aspectos clínicos como muco cervical, corrimento vaginal e ectopia cervical também foram observados.*

RESULTADOS: *Após a inserção do SIU-LNG houve aumento nos seguintes parâmetros: pH endocervical >4,5 (p=0,02), quantidade de neutrófilos endocervicais (p<0,0001), citolise vaginal (p=0,04). Houve diminuição do conteúdo vaginal (p=0,01). Não foram encontradas alterações estatisticamente significativas no pH vaginal, na quantidade de neutrófilos na mucosa vaginal, apecto do corrimento vaginal, candidíase vaginal, vaginose bacteriana, microbiota cocobacilar vaginal, aparência de muco cervical ou tamanho da ectopia cervical.*

CONCLUSÃO: *O uso do SIU-LNG em curto prazo não aumentou a candidíase vulvovaginal ou a vaginose bacteriana, levou à diminuição do conteúdo vaginal. No entanto, este dispositivo promoveu mudanças reacionais no ambiente vaginal e endocervical, sem modificação no tamanho da ectopia cervical.*

PALAVRAS-CHAVE: *Levanogestrel/efeitos adversos. Anticoncepcionais/adverse effects. Vaginose bacteriana. Descarga vaginal.*

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