

Role of a medical device for intra-vaginal use in improving the quality of the colposcopic examination and the anatomical/pathological reading of the cytological test and biopsy

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Summary. To evaluate the efficacy of a vaginal suppository in overcoming unsatisfactory colposcopy and/or possible change in terms of diagnosis in women who have had abnormal cervical cytology, 98 females were enrolled and treated for 10 days with Kramegin[®], a vaginal product in tablets containing *Lactobacillus acidophilus*, lactic acid and *Krameria triandra* extract. Seventy-eight of 98 females completed the study. Seventy-five of 78 volunteers served for the statistical analysis. According to the results got, diagnoses were modified in almost 62% of cases; cervical conization was reduced in 58% of cases; regression for HSIL (to LSIL) was 35% higher than expected; need of follow-up decreased by 36% and treatment was well tolerated in about 96% of cases. Use of Kramegin[®] before colposcopy can be considered a good tool to increase the possibility of turning unsatisfactory colposcopy into satisfactory and seems to indicate a possible role in reducing cervical lesion progression. Deeper clinical investigations are mandatory to confirm this possible result. (www.actabiomedica.it)

Key words: *Krameria triandra*, rhatany, colposcopy, vaginal suppository, *L. acidophilus*

Introduction

Use of medical devices in the form of intra-vaginal suppositories for the prevention and treatment of vaginitis and vaginosis has become increasingly widespread in recent years (1). Most of these devices contain lactobacilli and/or lactic acid and/or boric acid (2-4). The rationale for use of these formulations is based on the following considerations: when lactobacilli are introduced in the vagina, they compete with pathogens in terms of receptors and are lethal to the latter because of the release of bacteriocins, lactic acid and hydrogen peroxide; lactic acid and boric acid acidify the environment thus contrasting the growth of non-acidophilic forms; in addition, boric acid possesses marked antimicrobial properties acting in particular against fungal

species (5-9). Obviously even the anti-bacterial and/or anti-mycotic activities of some plant extracts can be exploited to contrast the growth of vaginal pathogens (10-11). A vegetable derivative recently used in gynaecology corresponds to *Krameria triandra* extract, also known as rhatany (12). The latter is chemically characterized by the presence of a neolignan fraction, overall amounting to 15% of the extract and possessing both anti-bacterial and anti-fungal properties (13-14). Formulations containing rhatany extract, associated with lactobacilli and lactic acid, were the subjects of clinical investigations that demonstrated their anti-inflammatory, anti-Candida, anti-Gardnerella and anti-*Trichomonas* (15) activities. These results on the anti-vaginitis and anti-vaginosis activities of the preparation, as well as the hypothesis that sub-clinical in-

flammation/infection forms may contribute, at least in part, to the difficult interpretation of both a gynaecological colposcopy situation and anatomical-pathological readings of a cytological test and biopsy performed during colposcopy (16), led us to investigate how use of these preparations might modify the reading and interpretation of colposcopic examinations. Colposcopy has been used since 1920 for early detection of cervix tumours (17). In spite of its huge success, about 10 to 15% of colposcopic readings are unsatisfactory (18-19). The objective of our work was to assess the efficacy of a vaginal suppository – generally used to prevent vaginitis and vaginosis events, endowed with local anti-inflammatory properties and capable of restoring the eubiotic vaginal flora balance – in reducing unsatisfactory colposcopic examinations and/or modifying colposcopic readings in females with a diagnosis of cytological cervix anomalies.

Materials and Methods

Clinical trial. The trial was conducted in the Colposcopy Clinic, Second Prevention Level, of the Local Health Agency TO 4, Chivasso (Turin, Italy). The trial was conducted in accordance with the Helsinki Declaration, with the approval of the local Ethical Committee and after each participant had been informed of the study procedures and objectives and had signed both their intention to take part in the trial (informed consent) and the form for personal data handling in accordance with the privacy law in force.

Inclusion criteria. The study enrolled 98 patients (from 29 to 71 years of age) coming to the clinic for the following diagnoses: double unreadable smear, ASCUS, LSIL, HSIL, AGC, phlogosis. The study also enrolled participants serving as control (with negative cytological test).

Exclusion criteria. Any individual not falling within the above inclusion criteria, patients refusing participation or follow up, underage patients (below 18 years old), patients intolerant to rhatany and those who refused to sign the informed consent and/or privacy form were excluded from enrolment.

Treatment. The medical device was administered in the evenings for 10 days, between 30 and 20 days prior to the examination.

The product. The product (Kramegin®) that has been clinically assessed in this work is a class II medical device, registered at the Italian Ministry of Health, and containing the following active ingredients: *Lactobacillus acidophilus* (1×10^9 CFU/dose), lactic acid (60% pure; 15 mg/dose) and standardized *Krameria trandra* extract (titre in neolignans 15%, 1 mg/dose). The device is produced by S.I.I.T. (Trezzano S/N, Milan, Italy) and marketed in Italy by PharmExtracta (Pontenure, Piacenza, Italy) and in Europe by Pharma Vinci (Copenhagen, Denmark).

Study pattern. In agreement with the above inclusion and exclusion criteria, the study enrolled 98 females who had come to the II prevention level clinic and underwent colposcopy, Papanicolaou (smear) test and biopsy. For all subjects a liquid phase Pap-test has been used. The 98 participants were then invited to come back to the clinic six month later for a new colposcopic examination (with smear test and biopsy) and instructed on how to use the product, which was to be applied between 30 and 20 days prior to the examination. Seventy-five patients were included in the analysis of the results since 3 cases were considered non-analysable (see Statistical Analysis) while 20 participants were excluded because they missed the control examination (15 women) or did not perform the treatment correctly (5 women). To avoid possible bias, always the same colposcopist and always the same cytologist, both blinded to whether the patients received the medication, evaluated all patients and samples. According to the protocol study, biopsies were performed only in those patients who had a colposcopy frame of white epithelium, mosaic or punctuation.

Sampling. It was performed between 14th and 21st day of the menstrual cycle and at least 10 days later the use of the tested product and at least 3 days later a possible sexual intercourse.

Diagnosis of the 98 participants. Atypical Squamous Cells Undetermined Significance (ASCUS), 5

cases; Low Grade Squamous Intraepithelial Lesion (LSIL), 20 cases; High Grade Squamous Intraepithelial Lesion (HSIL), 7 cases; Atypical Glandular Cells (AGC), 1 cases; double unreadable smear (the cell sample taken from the cervix is insufficient or the cells are qualitatively incomplete), 4 cases; phlogosis, 48 cases; negative, 13 cases.

Statistical analysis. Analysis of variance was used to verify independence with respect to the age parameter of the individuals grouped by improved, worsened or unchanged diagnosis. The comparison among the groups of individuals with a diagnosis at the baseline and after 6 months was made using the non-parametric Wilcoxon test. The need for a correct statistical analysis of the data, in the absence of a control group, was solved by assigning a progressive score to the lesions allowing the determination of their progression (0=negative; 1=phlogosis; 2=ASCUS; 3=LSIL; 4=HSIL) and by performing the statistical analysis on the relevant individuals. This approach helped analyse the data of 75 of the 78 individuals who completed the study. Three cases were considered non-analysable as their diagnoses at T=0 were reported as "inadequate" (2 cases) or AGC (1 case) and, therefore, impossible to be staged.

Results

In agreement with the hypothesis that sub-clinical inflammations/infections may contribute, at least in part, to the difficulties encountered in colposcopy and the anatomical-pathological reading of cytological examinations and biopsies, we decided to investigate how the use of an intra-vaginal medical device with anti-inflammatory, anti-bacterial and anti-mycotic properties might modify the diagnostic report of individuals coming to a gynaecological clinic of second prevention level.

As shown in Table 1, the analysis of variance ($p=0.3943$) in the 5 stage groups of the whole sample (referred to 75 of 78 individuals) showed that the differences in age were not statistically significant. As can be seen in Table 2, the diagnosis was modified in 48 of 78 cases (61.5%). Of the 7 HSIL cases, which would

Table 1. Age of the participants on the basis of the lesion stages

Stage	Age of participants (N=75)		
	Mean	SD*	Frequency
0	38.5	10.3	8
1	39.7	8.7	39
2	45.0	9.2	5
3	42.8	12.3	16
4	45.7	9.7	7
Total	41.1	9.9	75

N=numerosity; *Standard deviation

Table 2. Second level diagnosis (N=78) 6 months apart from each other

Diagnosis at T=0	Diagnosis at T=6 months
Unreadable (2)	ASCUS (2)
Phlogosis (38)	Negative (17), ASCUS (3), LSIL (5), phlogosis (13)
ASCUS (5)	negative (2), phlogosis (2), HSIL (1)
HSIL (7)	LSIL (4), phlogosis (1), HSIL (2)
LSIL (16)	negative (5), phlogosis (1), ASCUS (1), LSIL (9)
AGC (1)	negative (1)
Negative (9)	phlogosis (3), negative (6)

* the numbers in brackets indicate the volunteers with that diagnosis

have required cone biopsy, 5 cases changed and were sent to the follow-up, with surgery being performed only in 2 cases. Of the 5 ASCUS cases, 4 cases (80%) regressed, with 1 becoming HSIL and being sent to conization. Of the 16 LSIL cases, the diagnosis was confirmed only in 9 cases (56%), while 5 cases became negative. The only AGC case (diagnosis of AGC was made with a endocervical sampling) became negative, and the 2 situations of anatomical-pathological illegibility were actually found to be ASCUS cases. Of the 38 inflammatory situations, the diagnosis of phlogosis remained for 13 of them, while 17 became negative, 3 were found to be ASCUS and 5 were found to be LSIL. On the basis of the results obtained, and without considering the situations of illegibility or regressions from phlogosis to a normal situation, the device determined important regressions in the diagnosed lesions. These were then compared with spontaneous regressions reported in literature (20). As described in section Materials and Methods, the whole sample

(N=78) described in Table 2 was reduced, for an adequate statistical assessment, by 3 cases which could not be subject to a statistical analysis as they did not fall within the established stages. The 75 participants were then subdivided into three groups – those who improved, those who remained unchanged and those who worsened in comparison with the established stage. It was ascertained, with the analysis of variance ($p=0.9984$), that there was no difference in age among the three groups, which were therefore compatible with one another in age (Table 3). It follows that, according to the available data, age does not affect the results. The comparison of baseline diagnoses with those made after 80 days was made using the non-parametric Wilcoxon test and indicates that the same results (32 improvements vs 13 worsening) are significantly different ($p=0.028$). This demonstrates a positive effect, in terms of diagnostic evolution, due to treatment with the vaginal suppository. Even the comparison made on the whole sample (N=78) to assess spontaneous progression or regression after 6 months, fully agrees with the statistical significance obtained from the assessment of the 75 cases that were considered analysable. As shown in Table 4, use of the intra-vaginal medical device doubled the regression index relevant to an HSIL diagnosis. The device also determined some slight improvement of the regression indices of lesions ASCUS and AGC, while keeping LSIL lesions un-

Table 3. Comparison by age (N=75) on the basis of the frequency of the diagnostic evolution after 6 months

Diagnosis	Age of participants		Frequency
	Mean	SD	
Improved	41.1	9.6	32
Unchanged	41.2	10.8	30
Worsened	41.1	9.0	13

N= numerosity; *Standard deviation

Table 4. Regression rate of the lesions according to guidelines and following use of the intra-vaginal device (N=78)

According to guidelines	Following Kramegin®
ASCUS 70%	ASCUS 80%
LSIL 50%	LSIL 50%
HSIL 35%	HSIL 70%
AGC 70-80%	AGC 100%

Table 5. Progression rate of the lesions according to guidelines and following use of the intra-vaginal device (N=78)

According to guidelines	Following Kramegin®
ASCUS 2-7%	ASCUS 20%
LSIL 6-20%	LSIL 0%
HSIL 7-23%	HSIL 0%
AGC 22-30%	AGC 0%

Table 6. Tolerability, compliance, side effects (N=78)

	Excellent	Good	Fairly good	Poor
Tolerability	70	5	3	0
Compliance	78	0	0	0

	Type	Number of cases
Side effects	Burning	1
	Swelling	1
	Itch	1

changed. Table 5 shows the lesion progression indices according to literature (20) and following resort to the device. It appears from the percentage analysis that the device resulted in the increase of lesions with an ASCUS diagnosis. Five of 6 of these diagnoses came from illegibility situations (2 cases) or phlogosis situations (3 cases). It may be probably more correct to hypothesize a better anatomical-pathological reading, owing to reduced phlogosis, rather than a progression of lesions. The analysis of the data also shows a clear tendency to the reduction of LSIL, HSIL and AGC diagnoses. Finally, the device proved to be very well tolerated. Only three patients (3,8%) reported the onset of side effects, but the symptoms (burning sensation, hitch, swelling) were quite modest and did not cause the patients to leave the trial (Table 6).

Discussion

Use of vaginal suppositories, whether in tablets or ovules, containing lactobacilli and/or lactic acid and/or boric acid and/or botanical extracts, finds a valid application in treatment and, in particular, prevention of vaginitis and vaginosis. This seems particularly important for individuals with a diagnosis of recurrence, when prophylactic treatment would help reduce the

risk of the onset of the disease. These suppositories are endowed with both anti-microbial and anti-inflammatory activities. These properties may be exploited to reduce sub-clinical infections and inflammatory conditions, which are potential contributory factors to the illegibility of the cytological test and biopsy of colposcopy. Our study demonstrated that use of these suppositories actually modified the colposcopy results. In particular, use of Kramegin® in our study: 1) modified the diagnosis in 61.5% of cases; 2) reduced conizations in 58% of cases (from 7 HSIL at the second level examination to 2 HSIL + 1 ASCUS at colposcopy performed following use of the product); 3) increased HSIL regression rates by 35%; 4) increased ASCUS and AGC regression rates by 10% and 20-30% respectively; 5) finally, reduced the need for a follow-up in 36% of cases; in fact, 25 of 69 patients were able to leave routine checks, thus reducing costs for the National Health Service and giving the patient a clear psychological benefit, also in terms of much fewer leaves from work. Moreover the preparation, well tolerated by 96.2% of cases, also exhibited a high degree of safety. By classifying the 78 individuals who completed the study on the basis of their lesion stages and then performing the statistical analysis on 75 patients, we have been able to demonstrate that these diagnostic modifications are statistically significant. We also chose to conduct the entire trial by resorting to a single gynaecologist for the enrolment, medical examinations, colposcopy and drawing of samples (smear test and biopsy) and to a single pathologist for the reading of cytological and histological samples, so as to limit any variables affecting the procedure and ascribable to different operators. It is also relevant to highlight the rate of agreement/disagreement between the previous cytological and histological diagnosis and the histological diagnosis on cone biopsy samples performed in our work. Due to positive colposcopic report, biopsies have been done in 50% of the ASCUS, in 70% of the LSIL and in 100% of the HSIL diagnosis; and the percentage of agreement between pre-cone biopsies and histological (II level) reports was 85%. However, this study has some limits: first of all the absence of a placebo group; then the difficulty to understand to what extent the diagnostic change should be ascribed to a conceivable inflammation-reducing effect (which

would simply result in a clinical situation of an improved reading of lesion) or to what extent it should instead be ascribed to a regressive effect on a certain type of lesions, as, for example, is evident in the case of HSIL diagnoses. This second hypothesis, in our opinion, is more improbable and would require really important numbers to be verified again and demonstrated in a highly significant manner. Cervical cancer is a neoplasm papilloma virus-driven (21). Therefore the HSIL incidence reduction has to be taken with caution since demonstration of anti-virus activity of the tested product still misses. The idea that an inflammation-reducing effect may facilitate or improve the interpretation of the results is obviously not so exciting as a real regressive effect on lesions, but the fact that it was obtained with a preparation with a low potential in terms of side effects makes it nonetheless interesting. Our paper is based on the recent Bethesda system of classification (22). In that document the ASCUS category has been divided in two categories: ASC-US that accounts for 95% and ASC-H that represents 5-10% only respectively, with different meaning and implications. Indeed, in the ASC-US categories is easier to have as histological equivalent a negative/inflammatory or CIN I rather than a CIN II/III finding. On the other hand, in the ASC-H categories is easier to have as histological equivalent CIN II/III, a negative/inflammatory or CIN I rather than carcinoma finding. ASCUS is then a container in which end different questionable diagnosis. Therefore, to correctly speak of regression of ASCUS and to better validate the results so far obtained, larger number of patients with anomalous cytological readings of the uterine cervix are presently being enrolled for a new verification of the results demonstrated in this work in terms of regression of gynaecological lesions. In this subsequent pilot trial, 50% of the patients will be treated with a placebo suppository to try to demonstrate whether, even in a controlled pattern, the overcome of colposcopy result illegibility and the regression cases observed in this study are a statistically significant event. A parallel study will be also organised to validate a possible role played by a suppository only containing lactic acid and *L. acidophilus*, in order to confirm, or not, a possible pharmacological role just linked to the presence of rhatany extract.

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