Revista de Chimie
https://revistadechimie.ro
https://doi.org/10.37358/Rev.Chim.1949

Biochemical Mechanisms of Action of Natural Ingredients from the Vaginal Applicators for the Management of Preinvasive Cervical Lesions

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Abstract: *A successfully implemented screening program for cervical cancer is essential for detecting cervical intraepithelial lesions which offer the possibility of appropriate management and monitoring, implicitly the limitation of the progression to an invasive form of cancer. For different grades of squamous intraepithelial lesions, the use of vaginal tablets or applicators based on various natural substances as hyaluronic acid, beta-glucan and Coriolus Versicolor is possible with the purpose of expectative management or prior and post excisional intervention. We present the incidence trend of abnormal Pap smear results for the last five years in the University Emergency Hospital of Bucharest, with a summary of the literature regarding the biochemical mechanism of the main natural components from vaginal tablets used in the management of precancerous cervical lesions HPV induced and we share our personal experience with three clinical cases that benefited from this therapy. As there is a therapeutic window between the Pap-smear result, colposcopy and preparing for intervention, local treatment with natural components is a suitable choice as it is easy to use, inexpensive and efficient. The conclusion we reached is that the vaginal treatment with natural substances can be useful in terms of improving the local inflammatory process on the one hand and lubricating and regenerating tissues with the improvement of subsequent procedural performance on the other.*

Keywords: *precancerous cervical lesions, hyaluronic acid, beta-glucan, Coriolus Versicolor*

1.Introduction

In the context in which we have an efficient prevention opportunity for cervical cancer, this pathology remains of a high incidence worldwide. In Romania, in 2020, 7.5% of all types of cancers in women, were cervix uteri cancers, meaning 3 380 new cases (1), being ranked second in Europe, after Montenegro, with an estimated age-standardized incidence rate of 22.6/100 000. A successfully implemented screening program for cervical cancer is essential for detecting cervical intraepithelial lesions which offer the possibility of appropriate management and monitoring, implicitly the limitation of the progression to an invasive form of cancer.

Nowadays, the role of hrHPV (high-risk Human Papilloma Virus) infection as a high-risk factor for cervical cancer is well known, so the sensibility and specificity of the cytological screening can be enhanced through HPV/Pap testing. According to the U.S. Preventive Services Task Force 2018 - is recommended the beginning of screening for cervical cancer at 21 years by performing Pap smear alone at every three years and co-testing every five yearsfor women aged 30-65 years alternatively to Pap

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smear every three years and HPV testing alone (3). The principle beyond these recommendations is that through co-testing the highest number of CIN 3 (cervical intraepithelial neoplasia) can be detected but also it associates the highest percentage of false-positive rate (4).

The cytology results are offered in Bethesda system, including squamous cells abnormalities (Atypical squamous cells of undetermined significance ASC-US, Atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion ASC-H, Low-grade squamous intraepithelial lesions L-SIL, High-grade squamous intraepithelial lesions H-SIL, Carcinoma in situ and Squamous cell carcinoma) and glandular cell abnormalities. Depending on the Papsmear results, the Romanian Society of Obstetrics and Gynecology published in 2019 the recommended management of precancerous lesions (5) in concordance with the American Society of Colposcopy and Cervical Pathology guidelines (6). One important point fromthese guidelines is that the treatment should always be patient-individualized and the immediate excisional treatment should be the preferred option only if the immediate CIN 3+ risk exceedsor is equal to 4% (6).

For different grades of squamous intraepithelial lesions, the use of vaginal tablets or applicators based on various natural substances as hyaluronic acid, beta-glucan and Coriolus Versicolor is possible with the purpose of expectative management or prior and post excisional intervention.

In our study, we aimed to analyze the incidence trend of abnormal Pap smear results for the last five years in the University Emergency Hospital of Bucharest and summarize the literature data regarding the biochemical mechanism implied in the action of the main naturalcomponents from vaginal tablets and applicators used in the management of selected cases ofcervical dysplasia. In addition, we will share our personal experience with a series of patients,the management of which included vaginal use of various natural components.

2. Material and methods

We analyzed retrospectively the pap smear results from the Anatomic Pathology Department of the University Emergency Hospital of Bucharest for a period of five years (2016-2021), according to the Bethesda categories along with the trend of the age of the affected patients.

Three natural substances that are most frequently found in vaginal products offered to womenwith HPV infection and cervical precancerous lesions were chosen for specialized literature review regarding the local cervical biochemical mechanism.

The series of cases consists of patients with abnormal Pap smear results who underwent colposcopy at the point of diagnosis (point 0) and received subsequent local treatment with the natural substances mentioned above.

3. Results and discussions

During the last five years, the Anatomic-Pathology Department of the University Emergency Hospital of Bucharest processed about 15 500 Pap smears, from this 1625 (10.4%) were abnormal. The mean age for ASC-US results was 42 years, 48 years for ASC-H, 38 years for L-SIL and 49 years for H-SIL (Figure 1).

There were no significant differences between incidences of the studied types of cervical dysplasia during the analyzed period (Table 1).

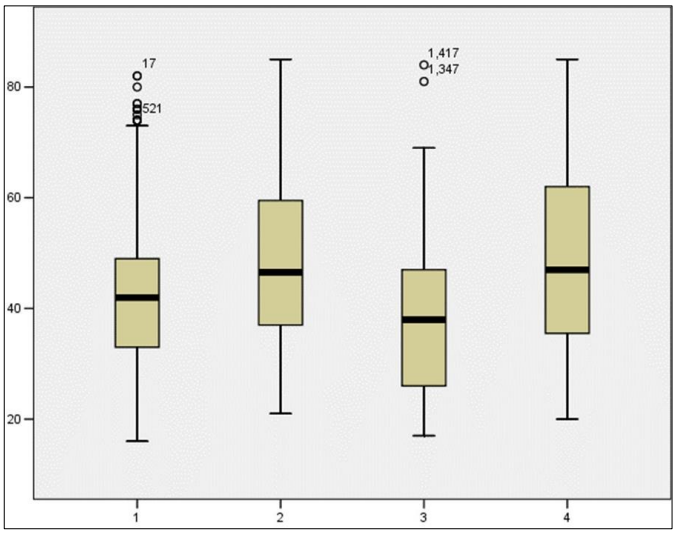

Figure 1. Mean age and extremities (vertical axis) for ASC-US-1, ASC-H-2, L-SIL-3 and H-SIL-4 (horizontal axis)

Table 1. Incidence per year for ASC-US-1, ASC-H-2, L-SIL-3 and H-SIL-4, with no statistical significance between incidences per year

	Cytology result							
	1		2		3		4	
	Count	Row %	Count	Row %	Count	Row %	Count	Row %
2020	114	72.2%	11	7.0%	17	10.8%	16	10.1%
2021	78	72.9%	7	6.5%	16	15.0%	6	5.6%
2016	127	73.0%	15	8.6%	18	10.3%	14	8.0%
2017	225	71.7%	31	9.9%	30	9.6%	28	8.9%
2018	334	76.3%	25	5.7%	47	10.7%	32	7.3%
2019	333	76.9%	35	8.1%	36	8.3%	29	6.7%

3.1. Hyaluronic acid (HA, Sodium hyaluronate) on cervical dysplasia

Hyaluronic acid is a polysaccharide, physiologically found in the organism with the highest levels in soft connective tissues (7). There are some particularities of this substance like its filter effect, exclusion properties and osmotic pressure which made it suitable for medical use. Mainly, the physicochemical characteristics are to count when choosing to use this un-sulfated glycosaminoglycan as a treatment option. These properties include a high capacity for holding water, specific viscosity and elasticity and high lubrication (8). Via its receptors which are RHAMM (Receptor for Hyaluronan Mediated Motility) and CD44, HA has functions in the production of cytokines, PGE2, matrix metalloproteinases and is implicated in various cellular phenomena such as cell proliferation, inter-cell interaction, cell differentiation and cell motility. These are the particularities that are responsible for the use ofHA in the control of HPV infection and its effects on tissues. HA-noisome, obtained when HA is esterified with monostearate, acts as an endo-cellular danger signal and as a regulator of the inflammatory process related to the viral infection. Endogenously, HA complexes have the capacity to bound into larger particles, cable-like, at the inflammation site, where the immunity cells, like monocytes, adhere and dissolve these structures resulting in portions of HA matrix that are subsequently internalized and regulate the inflammatory process. Association of HA with pro-inflammatory cells, macrophages, inducetheir activation and synthesis of nitric oxide synthase and plasminogen activator inhibitor (9).To summarize, the local cervical effect of Hyaluronic acid, a component of vaginal tablets prescribed for the management of low-grade intraepithelial cervical lesions, is epithelial regeneration and attenuation of the local inflammatory reaction.

3.2. Beta-glucans mechanism of action in cervical dysplasia

Beta-glucans are groups of polysaccharides formed of D-glucose monomers linked through beta-glycosidic bonds (1,3; 1,4; 1,6). These substances are naturally found in bacterial, fungal or cereals algae cells membranes. Being intensively studied in terms of presumed immunological properties (10), studies have found that beta-glucans stimulate the immune system, modulate cellular immunity and hormonal activity and are efficient in fighting bacterial, viral, fungal and parasitic infections (11,12). Beta-glucans activate macrophages, T cells, natural killer cells, complement, reduce blood sugar and cholesterol. The four different glucan`s receptors (complement receptor 3 with function in cellular adhesion, cytotoxic reactions and phagocytosis; lactosylceramide involved in the induction of the inflammatory proteins; selected scavenger receptors that are a recognition receptor and beta-glucan receptor with function in recognizing pathogens) are expressed on the membrane of natural killer (NK) cells, macrophages and neutrophils.

The beta-glucans anti-tumoral activity lies in their role in the modulation of adaptive and innate immunity. Two research were conducted on analyzing the impact of beta-glucans low grade intra-epithelial cervical lesions and the common conclusion was that these natural substances can eradicate cervical lesions resulted from HPV infection and also have a significant effect on the infection's amplitude (13,14).

3.3. Coriolus versicolor in cervical dysplasia

Different natural extracts are frequently used as adjuvant therapy. Coriolus Versicolor is a type of mushroom defined as a biological response modifier and it is believed to have antibacterial, antiviral and antitumor effects. The stimulatory effect on the immune system is attributed to the complex sugars from its structures like polysaccharide peptide and polysaccharide resin. In Japan, coriolus versicolor extract is approved for use as an immunotherapeutic agent for different types of neoplasms (15). In vitro and in vivo studies showed that Coriolus versicolor's component- polysaccharide peptide- increases the expression of tumor necrosis factor (TNF)-α which is known to have a crucial role in apoptosis and possesses an important tumoricidal activity (16). Subsequently, the change produced by Coriolus Versicolor is the augmentation of NK cells cytotoxic activity and proliferation of these cells (17).

We present the next, three suggestive cases from our experience where the local treatment with vaginal tablets containing hyaluronic acid, beta-glucan and Coriolus versicolor was prescribed. The chosen cases presented a HPV 16 infection and an abnormal Pap smear result, respectively ASC-H. For one-month same regimen was followed by each of the three patients, respectively 1 vaginal application per day for 21 days. What we have noticed, is that there was no proper lesion regression, but the follow-up examination revealed that the local inflammation associated with the lesion dissolute, with a better individualization of it and with the subsequent facilitation of the successful excision.

Case 1. 29-year-old women with abnormal Pap smear ASC-H and HPV 16,51 and 52 infections. On colposcopy we noticed a type I transformation zone (TZ) with opaque aceto- white epithelium and distinct yellow on iodine staining (Figure 2). The overall Swede scoreat moment 0 was 6 and the colposcopically directed biopsy result was CIN II/H-SIL. Considering the obstetrical history, respectively women without any children and the infection with three hrHPV types, the large loop excision of the transformation zone (LLETZ)was decided and performed. The histopathological result was areas of CIN 2 alternating with CIN 1 and with clean generating limits of the excised samples.

Case 1.29-year-old women

Figure 2. Colposcopy image, CIN II/III, swede score-6, image before medical and surgical intervention

Case 2. 33-year-old woman with HPV 16 infection in her history and abnormal Pap smear- ASC-H. Colposcopy examination reveals type II TZ and an approximatively 10 mm lesion in quadrant 3, with atypical vascularization, irregular and fragile with dense aceto-white epithelium with rapid appearance (Figure 3). The Swede score offered was 8 and the chosen procedure was conization of the cervix. The patient followed local and general immunostimulatory therapy one month before the procedure. The histopathological result was endo-cervical adenocarcinoma, FIGO T1B1.

Figure 3. Colposcopy image. Cervical lesion atypical vascularization, irregular and fragile

Case 3. 49-year-old woman with modified cytology- ASC-H and HPV16 infection. Colposcopy examination reveals normal vaginal discharge, a type 3 TZ, aceto-white epithelium in quadrant 1 and at the level of squamo-columnar junction a coarse mosaic and punctuation with the distinct yellow coloring of this lesion on iodine staining (Figure 4). The case was appreciated with a total Swede score of 7 and regarding the age and the complete familial planning, the conization of the cervix was preferred and performed after one month oflocal treatment with natural substances. The histopathological result was H-SIL, CIN II/III with appropriate safety limits.

Figure 4. Colposcopy image of a cervical lesion with coarse mosaic and punctuation and distinct yellow coloring on iodine staining

4. Conclusions

Precancerous cervical lesions are conditions that can evolve into cancer, but also these lesionsare treatable with a correct management protocol applied. We focused on the ASC-H cytological category, mainly because it represents a diagnostic and management challenging for each practitioner. In our study, the incidence of ASC-H among abnormal Pap smear results varied between 6.5 and 9.9%; this is a high proportion compared to that reported in the literature, where the ratevaries between 0 and 2% (19). Current recommendations include immediate colposcopy for ASC-H results, regardless of HPV infection status (6), but some studies showed that HPV negative patients have a better outcome and HPV triage can guide the subsequent management (20). We agree with this strategy and the presence of hr-HPV infection was a criterion for a more aggressive follow-up and easy acceptance by the patient of the excisional procedure.

Our accent on HPV 16 presence, was because is a highly carcinogenic type with an estimated risk of developing CIN III at 5 years of 40% (21) and all available anti-HPV vaccines offer protection against this viral strain.

Cervical cancer is an international important public health matter. In Romania, the cervical cancer screening program conducted in 2012-2017 was an absolute failure due to lack of information, insufficient allocated funds and insufficient specialized staff members involved in the program. In November 2020, the World Health Organization published a proposed strategy for 10 years (2020-2030) to eliminate cervical cancer. This includes as follows: a limit of 4 cases/100 000 per year for cervical cancer to disappear as a public health problem; 90% of suitable girls by the age 15 completely vaccinated; minimally two highly sensitive screening tests performed by the age 45, with the age limit 35 for the first one; 90% of patients with cervical dysplasia treated and90% of women with cervical cancer correctly managed (22).

As there is a therapeutic window between the Pap-smear result, colposcopy and preparing for intervention, local treatment with natural components is a suitable choice as it is easy to use, inexpensive and efficient (23). The study of Serrano et al (24) showed that Coriolus Versicolor applied vaginally led to clearing hr-HPV infection in 63% of cases of ASC-US and L-SIL.

What we observed with the described strategy above is that the vaginal treatment with natural substance can be useful in terms of improving the local inflammatory process on the one hand and lubricating and regenerating tissues with the improvement of subsequent proceduralperformance on the other.

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Manuscript received: 27.08.2021