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LYSOZYME ACTIVITY AS A CRITERION OF TREATMENT EFFICACY IN WOMEN WITH UNDERLYING CERVICAL DISEASE, ASSOCIATED WITH VIRAL INFECTIONS

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INTRODUCTION

Medical professionals keep the problem of viral infection in the spotlight due to its high prevalence, the significant carcinogenic risk of cervical disease and an adverse effect in the female reproductive system.

According to J.J. Danelly (1996), DNA of human papillomavirus is found in the tissues of the genital tract of 11–46 % sexually active women [9,10].

However, there is a number of papers (I.B. Manukhin et al. (1995), A.T. Loginus et. al. (1992), according to which human papillomavirus can be diagnosed in 79.3 % of subjects with cervical disease. It is to be noted that herpetic genital infection (as reported by V.I. Kozlova) is diagnosed in 83.6 % of patients with refractory recurrent colpitis, leukoplakia and ectopy [4,6,11].

A well-known fact is that a large number of subclinical and atypical cases of viral infection complicate diagnosis and adequate management.

Viral infection has a tropism to the stratified squamous epithelium and mostly affects the mucous membrane of the exocervix. Generalization of viral replication and dissemination during the period of primary infection is counteracted by a number of factors of general/nonspecific antiviral resistance of the body. These factors include the capacity of the stratified squamous epithelium of cervical mucosa for ceaseless exfoliation and to regeneration, the humoral factors in the mucus of the cervical canal and cellular activity of mononuclear cells and phagocytes in the inflammation zone.

It is generally known that lysozyme is a substance with enzymatic activity, which participates in specific and non-specific (general) immune responses. Important properties of lysozyme include impairing membrane permeability and metabolism of pathogens and its influence on cell growth and differentiation of immune and non-immune cells.

Accordingly, the investigations into such a powerful protective barrier factor as lysozyme may provide criteria for evaluation of therapeutic quality and efficacy in women with the underlying cervical disease.

The aim of this study was to evaluate changes with time of lysozyme levels in cervical contents and serum in course of multimodality therapy of underlying cervical disease, associated with viral infections.

MATERIALS AND METHODS OF STUDY

Sixty (60) women with the underlying cervical disease have been assessed. Twenty-eight (28) women have been diagnosed with a viral infection (HSV type 1,2 and HPV type 6–11, type 16–35 and type 18–59); in 16 women viral infection was associated with chlamydial infection, ureaplasmosis or mycoplasmosis (mixed infection). All women were of reproductive age (19–42 years), mean age was 26.2 years.

Schedule of assessments in the patients included history, pelvic exam, bacterioscopic and bacteriological testing of the vaginal and cervical microbiome, cytological assessment and simple and extended colposcopy.

Diagnosis of viral infection was performed using the methods of enzyme-linked immunoassay and polymerase chain reaction. Detection of mixed infection (*Chlamydia, Ureaplasma* and *Mycoplasma*) was performed with a direct immunofluorescence method in scrapings from mucous membranes of the cervical canal and the urethra, with parallel immunoenzyme serum tests in women to identify type-specific IgG antibodies against viruses.

Depending on the identification of genital infections, the women were divided into two groups: Group 1 included women with a viral infection and Group 2 included subjects with viral infection combined with other genital infections. As a part of their multimodality treatment, the first group of women received an antiviral drug, Proteflazid (manufactured by Scientific & Manufacturing Company Ecopharm Ltd., Kyiv, Ukraine); the second group of women received Cycloferon (manufactured by Polysan Scientific & Technological Pharmaceutical Company Ltd., St. Petersburg, Russia). Proteflazid was administered orally according to the following regimen: Week 1: 5 drops daily 3 times daily; Week 2–3: 10 drops daily 3 times daily; Week 4: 8 drops daily 3 times daily. The product was also used locally as vaginal tampons soaked in a 1:4 solution of Proteflazid (diluted with 0.9 % NaCl) 2 times daily, daily for 10 days.

Cycloferon solution for injections 12.5 % was administered intramuscularly according to a basic regimen; Cycloferon liniment was also used locally as vaginal tampons 2 times daily, daily for 10 days.

Assessment of lysozyme activity in cervical contents and in the serum was performed using O.V. Bukharin technique (1974).

Assay principle is based on decreasing the optical density of bacterial suspension incubated with a lysozyme-containing biological sample. This research has used standardized washes from a 1-day old culture of *Micrococcus Lisodeicticus* on a phosphate buffer (pH = 6.2). The study was performed using a KFK-3 photometer. Computations were performed using calibration charts.

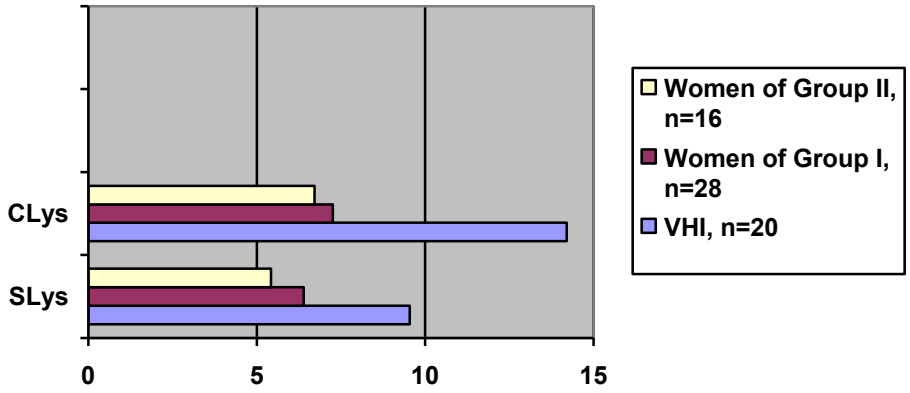
Study material included serum and cervical contents. Assessment of lysozyme activity was performed before and after treatment.

RESULTS AND DISCUSSION

A substantial portion of main patient complaints was represented by the mucopurulent genital discharge of different thickness. The patients also reported lower abdominal pain (56 %), pronounced premenstrual syndrome and painful periods (38 %) and vaginal itching/burning (68 %). Quite frequently, the patients reported neurotic disturbances: increased irritability, reduced performance in the workplace and headache. The vast majority of women (89 %) were diagnosed with III–IV relative degree of microbial contamination (vaginal cleanliness) and shift of vaginal pH to the alkaline side. Analysis of colposcopic findings has shown signs of inflammation, with a characteristic blurring between cylindrical and stratified squamous epithelium, hyperemia of the endocervix, dyshesion and swelling of cervical tissues and increased vasculature.

During the assessment of lysozyme activity in serum and cervical contents, apparent lysozyme deficiency was found.

In virtually healthy individuals (VHI), lysozyme was 9.54 ± 0.63 µg/ml in serum and 14.2 ± 0.41 µg/ml in cervical contents ($p < 0.05$). In the first group of women with underlying cervical disease in a setting of papillomavirus and herpes infection, lysozyme level was 6.39 ± 0.37 µg/ml and 7.25 ± 0.84 µg/ml in serum and in cervical contents, respectively ($p < 0.05$).



Notes: SLys = Serum lysozyme; CLys = Lysozyme in cervical swabs; Probability value $p < 0.05$

Fig. 1. Lysozyme levels in cervical swabs and in the serum of study subjects

In the second group of women, where the viral infection was diagnosed in associations with *Chlamydia*, *Ureaplasma* or *Mycoplasma*, lysozyme levels were significantly ($p < 0.05$) lower than in the first group of women: 5.42 ± 0.28 µg/ml in serum and 6.72 ± 0.36 µg/ml in cervical contents.

Table 1. Lysozyme levels (µg/ml) in cervical contents and in serum in course of treatment

Index	**VHI, n = 20**	**Proteflazid, n = 28**		**Cycloferon, n = 16**	
		Before treatment	After treatment	Before treatment	After treatment
Serum lysozyme	9.54 ± 0.63	6.39 ± 0.73	8.87 ± 0.35	5.42 ± 0.28	8.64 + 0.12
Lysozyme in cervical swabs	14.2 ± 0.41	7.25 ± 0.84	12.85 ± 0.2	6.72 ± 0,36	12.54 ± 0.18
Probability value	$p < 0.05$	$p < 0.05$		$p < 0.05$	

The resulting findings (presented in Fig. 1) demonstrate lysozyme deficiencies in women with the underlying cervical disease both in serum and in cervical contents. Of note, the depression of this parameter correlates with the degree of vaginal infectious contamination.

Analysis of data obtained during the use of Proteflazid and Cycloferon (see Table 1) has shown increased lysozyme levels both in serum and in cervical contents. In particular, post-treatment lysozyme levels have virtually approximated the respective levels in VHI. It should be noted that lysozyme activity in cervical contents in a setting of Proteflazid use in women of Group I was somewhat higher (12.85 ± 0.24 μg/ml) than in therapy with Cycloferon in women of Group II (12.54 ± 0.18 μg/ml); $p < 0.05$ for the difference. Evaluation of serum lysozyme has shown Proteflazid to be more effective in women of Group I (8.87 ± 0.35 μg/ml) than Cycloferon in women of Group II (8.64 ± 0.12 μg/ml).

The author may assume that regeneration of vaginal microbiome and restoration of barrier functions of cervical mucus secretions is related to virulence and the number of infectious factors.

During evaluation of standard clinical findings at Day 5–6 of treatment, the author has noted improvement of patient well-being, reduced or absent pain and reduction of vaginal discharge, burning and itching. Evaluation of bacterioscopic and colposcopic data has also demonstrated improvement over time.

Therefore, antiviral therapy with Proteflazid and Cycloferon increases lysozyme levels both in serum and in cervical contents.

In addition, Proteflazid, a domestic production containing flavonoid glycosides, is quite effective, easy to use and does not have any side effects.

CONCLUSIONS

1. Levels of lysozyme in serum and in cervical contents may serve as one of the diagnostic criteria in underlying cervical disease, associated with viral infections.
2. Development and implementation of therapeutic interventions in women with the underlying cervical disease, associated with viral infections must be accompanied by an assessment of lysozyme levels in serum and in the cervical contents; this is important to achieve not only clinical improvement but also a complete regeneration of local protective barriers.
3. Administration of Proteflazid and Cycloferon as a part of multimodality treatment of underlying cervical disease provides for a complete restoration of lysozyme activity both in serum and in cervical contents.

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