

Use of flavonoids in complex treatment of women with genital inflammatory diseases of viral and bacterial aetiology

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Summary

The clinical and laboratory studies (bacteriological, bacterioscopic, virological, enzyme immunoassay, PCR methods) were performed in 68 women from 18 to 40 years of age with chronic genital inflammatory conditions caused by viral and Chlamydia infection. At the cervical mucus enzyme immunoassay a special attention was paid to the local immunity condition. A comprehensive anti-inflammatory treatment was performed for this group of women using antibiotic, antifungal, desensitizing, vitamin therapy, hepatoprotectors, and Proteflazid. It was found that the combined treatment of chronic genital inflammatory condition caused by viral chlamydial infection using Proteflazid shows a significant improvement in the clinical presentation of the disease, normalization of the main factors of the cervical mucus protective function, reduction in the genital tract seeding with microflora, which in general favorably affects the condition of the of female genital tract local immunity.

Key words: chronic genital inflammatory condition, viral chlamydial infections, blood immunoglobulins, protective factors, complex treatment, Proteflazid.

Introduction

In the last decade, the prediction, prevention, and treatment of the sexually transmitted genital inflammatory condition became one of the topical issues in modern gynaecology.

If up to the present moment the issue was of a purely medical nature, then, now it acquired the status of an important socio-demographic issue, since these diseases take a leading place among the causes of the impaired reproductive health of the Ukrainian population. This is evidenced by the Decree approved by the President of Ukraine and adopted for implementation National Program "Reproductive Health 2001 – 2005" of 26.03.2001 under the number 203/2001, which aims to improve the demographic and socio-economic situation in the state, as well as the population reproductive health [1, 2,3].

Infectious-inflammatory diseases of the female genitalia hold a special place in the overall morbidity structure and account for 50% - 78% of the gynecological patients [2, 3]. Their incidence is increasing alarmingly due to the increased sexual activity at a young age, sexual intercourse instability, environmental deterioration, uncontrolled use of drugs, namely, antibiotics [4, 5].

According to many authors the urogenital infections are almost always polymicrobial and caused by bacterial (gonococcus, chlamydia, pathogenic staphylococcus, streptococcus, mycoplasma, ureoplazma, anaerobes) and viral flora (papillomatosis, herpes simplex, and cytomegalovirus) [4, 6].

The literature analysis suggests that of the most importance in terms of incidence, variety and severity of consequences are the following STD causative agents: Chlamydia trachomatis, Herpes simplex virus, Mycoplasma hominis (especially M.genitalis), Ureaplasma urealiticum, Trichomonas vaginalis, which are often combined together [8, 9].

The presence of microbial associations contributes not only to the best intracellular parasitizing adaptation, but also enhances the pathogenic properties of each of this association member, which leads to a greater microorganisms' resistance to the external negative effect, including antibiotics.

Associated urogenital infections cause serious changes in both cellular and humoral immunities, creating conditions under which the conventional antibiotic therapy becomes ineffective.

Therefore, our work's objective is to develop a combined complex therapy using Proteflazid drug aimed not only at microbial association, but also at the phagocytic response normalization in the affected area and patient's body.

Study materials and methods

68 women with chronic genital inflammatory conditions of chlamydia viral aetiology were examined. 34 women were diagnosed with herpes infection (HSV-2), 34 had herpes infection combined with genital chlamydiosis.

All patients stayed in the Department of Planned Parenthood and Sexual Development of Young Women and Adolescent at the Institute of Pediatrics, Obstetrics, and Gynecology of the Academy of Medical Sciences of Ukraine. All women were of an active reproductive age (18 - 40 years), the mean age was 27.1 ± 3.4 years. The duration of the disease ranged from 2 to 10 years with frequent relapses, especially in autumn and spring period (an average of 6.6 ± 0.9 years). 74.5% of women complained of intermittent lower abdominal and sacral pain, 20.6% of patients complained of persistent pain. Only 14.7% of patients associated pain with periods. 58.8% of patients noted whites of a permanent nature with unpleasant smell, 26.5% – itching of the external genitalia. 11.8% of women had menstrual disorders (5.9% – oligomenorrhea, 5.9% – algodismenorrhea). Infertility occurred in 52.9% of patients (38.2% – primary, 8.8% – secondary). 59.9% of women associated disease with the sexual activity onset, 8.8% – with hypothermia, 8.8% – with the first abortion.

Only 41.2% of women had a history of anti-inflammatory therapy. Of these, 79.4% did not observe the improvement of the general state after treatment. 23.5% of women were unmarried, 55.9% were in the first and 20.6% in the second marriage.

In 85.3% of the women from studied group the chronic bilateral salpingitis was combined with the pelvic organs adhesive process, in 8.8% – with polycystic ovary syndrome, 8.8% – with a fibroid uterus, 17.6% – with endometriosis. The speculum examination showed vaginal mucous membrane hyperemia in 58.8% of women, grainy and spotted in 8.8%, endocervite in 11.8%, cervical erosion in 17.6%.

An objective examination in 55.9% of women showed enlarged tender appendages, uterine displacement in one or another direction, short uterosacral ligaments.

In order to determine the causative agent the PCR diagnostics, enzyme immunoassay, bacteriological, bacterioscopic methods in vagina, cervical canal, urethra scrapings were used. The composition of the anaerobic microflora was evaluated according to the methodical recommendations of "Laboratory diagnosis of chronic inflammatory diseases caused by asporous anaerobic microorganisms" (Kharkiv, 1985).

The genitalia mucus inoculation was performed using a number of diagnostic and differentiated media: egg yolk high salt agar culture medium, blood agar, chocolate agar, Endo, Ploskirev's, Saburo, thioglycolic medium, MRS medium for lactobacilli. The microbial content level was determined by secretory inoculation of medium allowing identifying the maximum possible range of aerobic and anaerobic bacteria.

Identification of lactic acid bacteria, Peptostreptococcus, bacteroides, peptococcus was performed by cultural, morphological, and tinctorial properties. After colonies count the data obtained were converted into logarithms of the microbial markers count.

Diagnosis of chlamydiosis was performed using enzyme immunoassay (Chlamybest test-system, Novosibirsk) and bacterioscopy method. Ureaplasmas were detected in culture by material inoculation in the placenta medium including urease test.

Specific antiherpetic HSV-2 Ig and M in the blood serum were determined using Novum diagnostica (Germany) test systems on strip enzyme-immunoassay analyzer Star fax 300 (USA) at a wavelength of 450 nm.

In addition, we have studied the local immunity by determining the levels of G, A, M immunoglobulins, secretory Ig, lysozyme, and C3 complement component in the cervical mucus.

All patients received conventional complex anti-inflammatory with antibiotic therapy (taking into account the selected microflora and microorganisms' sensitivity to antibiotics), antimycotic, desensitizing, vitamin therapy, hepatoprotectors, and Proteflazid.

Proteflazid was administered in women with inflammatory diseases of the genitalia of herpes virus aetiology from 1st day of antibiotic therapy. Drops applied to a piece of sugar (or food starch) were administered per os according to the scheme: first week – 5 drops three times a day, second and third weeks – 10 drops three times a day and fourth week – 8 drops three times a day. Duration of the first course of Proteflazid combined with anti-inflammatory therapy was 28 days.

In parallel with oral drug administration a local treatment with solution was given composed of Proteflazid. To prepare the vaginal tampons, Proteflazid 3ml was dissolved in 20 ml of saline. A gauze plug soaked in the prepared solution was administered into vagina twice daily for 10 days.

The simultaneous treatment was performed in case of herpesviral infection in a sexual partner.

Study results and discussion

The efficacy evaluation of administered therapy was performed in 2 weeks and 1 month after treatment based on clinical data (absence of disease recurrence and bacteriological, smear, virological and immunological studies results).

85.3% of patients observed on the 2 – 3rd day of the prescribed therapy an increase in body temperature up to 37.2 – 37.4°C, increased lower abdominal pain and vaginal discharge. This is indicative of an exacerbation of the inflammatory condition, which in turn positively affects the efficacy of antibiotic therapy.

The pain resolved on the 4 – 5th day of treatment, body temperature returned to normal, general state improved, patients reported a decrease in vaginal discharge, reduced itching and odor.

After treatment in 86.7% of patients no *Staphylococcus aureus* was observed, the number of conditionally pathogenic staphylococci inoculation was reduced significantly (80.88%), which does not reach diagnostic titres. The bacteriological test results are given in Table 1.

Table 1

Microbiocenosis of patients with chronic genitalia inflammatory condition of viral and chlamydial aetiology

Microorganism	Total, % n = 50	Including by number,		
		10 ²	10 ¹	10 ⁰
<i>Staphylococcus: epidermidis</i>	61,33	24,0	28,0	9,33
<i>saprophyticus</i>	6,67	6,67	-	-
<i>aureus</i>	13,33	1,33	5,33	6,67
<i>Candida albicans</i>	34,67	21,33	6,67	6,67
<i>Streptococcus: viridans</i>	12,0	8,0	2,67	1,33
<i>faecalis</i>	9,33	5,33	2,67	1,33
(P-hematolytic) <i>agalactiae</i>	5,33	2,67	-	2,67
<i>Corinebacterium xerosis</i>	13,33	5,33	6,67	1,33
<i>Escherichia coli</i>	29,33	9,33	13,3	6,67
<i>Enterobacter cloace</i>	5,33	1,33	2,67	1,33
<i>Klebsiella spp.</i>	5,33	5,33	-	-
<i>Acintobacter spp.</i>	1,33	-	1,33	-
<i>Peptostreptococcus:</i>	10,67	5,33	2,67	2,67
	1,33	-	-	1,33
	1,33	1,33	-	-
	4,0	1,33	2,67	-
<i>Bacteroides: melamin</i>	2,67	-	1,33	1,33
<i>fragilis</i>	6,67	-	4,0	2,67
<i>Enterobacter spp.</i>	1,33	-	-	1,33
<i>Pseudomonas</i>	1,33	-	1,33	-
<i>alcalig. Lactobacillus spp.</i>	37,33	9,33	13,33	14,67

Status data of cervical mucus specific protection factors are given in Table 2.

Table 2

**Immunoglobulins cervical mucus concentration in women examined
prior and after treatment, g/L**

Group of patients	n	SIgA	IgG	IgA	IgM
Prior treatment	50	1.20±0.16	0.31 ±0,04	0.05±0.01	-
After treatment	50	0,74±0,14	0.15±0.03	0.10±0.02	-
Healthy women	20	0.62±0.12	0.17±0.02	0.12±0.02	-
Reliability	p	p ₁₋₂ <0,05	p ₁₋₂ <0,05	P ₁₋₂ <0,05	-

These data suggest that during the prolonged course of chronic inflammatory condition of viral chlamydial aetiology there is no Ig production and significant increase is observed in the local synthesis of G class sIg secretory immunoglobulin. Thus, in a female group prior treatment the sIg level was 1.20 ± 0.16 g/L, Ig – 0.31 ± 0.04 g/L, which, in turn, reflects the occurrence of chronic disease. At that, some deficit is preserved in Ig production, level of which is significantly lower compared to the same period after treatment.

As a result of Proteflazid administration the protective function of cervical mucosa recovers, as evidenced by normalization in studied local protection factors.

Table 3

**Lysozyme and C3 complement component concentration in the cervical mucus
prior and after treatment, g/L**

Group of patients		Lysozyme	C3 complement component
Female patients (prior treatment)	15	0,080±0,020	0,290±0,040
Female patients (after treatment)	15	0,140±0,040	0,080±0,010
Healthy women	20	0,17±0,03	0,050±0,012
Difference accuracy	P	P ₁₋₂ < 0,001	P ₁₋₂ < 0,001

Thus, the secretory A immunoglobulin and G immunoglobulin levels in the group of women after treatment is reduced compared to their level prior treatment (Table. 2). In addition, the local IgA synthesis recovery is observed, the level of which increases significantly as a result of therapeutic interventions.

The established fact is that the cervical mucus has expressed proteolytic and bactericidal activity due to the presence therein of soluble proteins such as lysozyme and complement. Data on the content of these local protection factors are given in Table 3. The acquired data indicate that the chronic course of genital sphere diseases among examined women is characterized by decreased lysozyme levels in the cervical mucus prior treatment compared to the results after treatment using Proteflazid (0.080 ± 0.020 g/L and 0.170 ± 0.030 g/L, respectively, $p < 0.001$); C3 complement component increase is possible (0.290 ± 0.040 g/L vs. 0.050 ± 0.012 g/L, respectively, $p < 0.001$).

1 month after treatment 88.2% of women treated with Proteflazid, according to Ig enzyme immunoassay results, microscopy, and RLP methods had no chlamydiosis diagnosed. In 41.2% had Ig determined in blood serum (that is a sign of previous chlamydial infection), which remained at non-significant level (+), (++)). The use of enzyme immunoassay of cervical canal and urethra scraping also indicates the absence of Chlamydia antigen in the test material.

As for the genital herpes, 85.3% of women, according to PLR results, had no virus observed; in 91.2% Ig was absent. It should be noted that a small percentage of treatment failure accounted for patients with a combined herpetic and chlamydial infection with a prolonged, often recurrent course of disease.

Conclusions

The studies conducted indicate the high efficacy of Proteflazid in the complex therapy of chronic genitalia inflammatory conditions of viral chlamydial etiology. This is evidenced by a significant improvement in the disease clinical presentation, normalization of the main factors of the

cervical mucus protective function (sIg, immunoglobulins, lysozyme and C3 complement component), decrease (according to the clinical and bacteriological analyzes) in the genital tract inoculation with microflora (as evidenced by the reduction in Ig levels) which positively affects the local protection status of the female genital tract as a whole.

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