Using Proteflazid as a part of complex treatment of menstrual irregularities caused by a viral infection

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A high-profile position among urogenital infections belongs to viral disease. Pelvic inflammatory disease (PID) is the most prevalent gynecological disease and a frequent cause of problems with female reproductive health. In Ukraine, inflammatory disease occurs in 30–35 % women of reproductive age. PID has an adverse influence upon female reproductive organs, with sequelae including chronic pelvic pain syndrome (24 %), tubal or endocrine infertility (30 %), miscarriages (35 %) and ectopic pregnancy (3–4 %).

While microbial invasion has been historically recognized as a trigger of inflammation, the profile of causative agents of PID has dramatically changed over the last 20 years. At present, the leading PID initiators include the following microbial associations: **non-spore-forming Gram-negative** bacteria (such as *Bacteroides*, *Prevotella* and *Fusobacteria*), **Gram-positive anaerobic** organisms (e.g. *Peptostreptocci* and *Clostridia),* **Gram-negative anaerobes** *(e.g. E. coli*, *Klebsiella*, *Proteus* and *Enterobacteriaceae*) and **Gram-positive** bacteria *(e.g. Enterococci*, *Staphylococci* and *Streptococci*).

Since 80–90s, a leading role in the genesis of PID is assumed by sexually transmitted infections, such as Chlamydia, Mycoplasma, Ureaplasma, viruses and *Candida*.

One of the current characteristic aspects of genital inflammatory disease in females is the associative pattern of multiple causative agents (i.e. *Chlamydia, Mycoplasma, Ureaplasma* and viruses), all of which have a high degree of affinity to the cells of the cylindrical and transitional epithelium. Associations of candidiasis with a viral infection are by and large the most frequent. Thus, the so-called recurrent vaginal thrush or candidiasis is a marker of occult viral infection; this fungal infection is often resistant to traditional treatments. In addition to that, based on the investigators' experience, it should be emphasized that infection is not always transmitted via a sexual route. Compromised immune defenses and/or modifications of bacteria and viruses typically causing upper respiratory or gastrointestinal infections, etc., may furnish such pathogens with a tropism to genital tissues.

The following viruses are pathogenic in humans:

1. Herpes simplex virus 1 (HSV-1).
2. Herpes simplex virus 2 (HSV-2).
3. Varicella-zoster virus (VZV or HHV-3).
4. Epstein-Barr virus (EBV or HHV-4).
5. Cytomegalovirus (CMV or HHV-5).
6. Human herpesvirus 6 (HHV-6).
7. Human herpesvirus 7 (HHV-7).
8. Human herpesvirus 8 (HHV-8).
9. Human papillomavirus (HPV).

Clinical manifestations of viral infection are quite characteristic. Thus, in case of herpetic infection, a typical finding in a setting of general malaise is papulovesicular rash in the genital area and in the adjacent areas of skin.

The following clinical and morphological types are distinguished for genital herpes (GH):

1) Initial clinical episode of primary GH.
2) Recurrent GH.
3) Asymptomatic (atypical) GH.

The severity of GH is determined according to the course of the disease. The following data are assessed: the number of relapses per year (1–3, 4–5, 6 and more), duration of remission (1–2 months, 3–6 months, more than 1 year), degree of immunosuppression and location and extent of lesions.

The types of genital lesions caused by human papillomavirus (HPV):

1. Clinical forms (visible to the naked eye):
   - exophytic condylomas (typical pointed, papillary and popular condylomas);
2. Subclinical forms (invisible to the naked eye or asymptomatic; detectable only with colposcopy and/or with cytological and histological testing):
   - flat condylomas (a typical structure with large numbers of koilocytes);
   - minor forms (various lesions of stratified squamous epithelium and metaplastic epithelium with isolated koilocytes);
   - condylomatous cervicitis;/vaginitis;
   - inverted condylomas (located in the crypts).
3. Latent forms (absence of clinical, morphological or histological changes in a positive test for HPV DNA);
4. Cervical intraepithelial neoplasia (CIN) or squamous cell intraepithelial lesion (SIL):
   - CIN I — mild dysplasia ± koilocytosis, dyskeratosis;
   - CIN II — moderate dysplasia ± koilocytosis, dyskeratosis;
   - CIN III — severe dysplasia or carcinoma *in situ* ± koilocytosis, dyskeratosis;
   - microinvasive squamous cell carcinoma.

Adverse effects of viral infection toxicity are seen in virtually all organs and systems in the body.

Concerning the central nervous system (CNS), it should be noted that morphological changes in infection-affected organs create abnormal impulses (abnormal afferentation) to those CNS sections, which regulate the hypothalamic-pituitary-ovarian system. Such signaling creates abnormal responses and regulation of ovarian endocrine function becomes uncoordinated; this, in turn, results in impaired synthesis of steroid hormones. At the initial stages of the disease, there is an increased synthesis of follicle-stimulating hormone (FSH) in a setting of relatively normal concentrations of

luteinizing hormone (LH). The damage to the ovarian receptor apparatus usually advances with time (in a chronic course of infection or in frequently relapsing forms thereof). This is confirmed by substantial (3-fold and greater) increases of LH relative to FSH; this occurs in a setting of clinically apparent signs of anovulation. Persistence of viral infection leads to even more profound changes in the hypothalamic-pituitary link, which manifests as a reduced synthesis of both FSH and LH (this is an evidence of suppressed function of this component of neurohumoral regulation).

The damage inflicted by viral infection primarily involves adrenal and ovarian function. The toxic effects of viral infection in the adrenal glands are manifest by reduced adaptation mechanisms. Viral infection may cause intracellular damage of the receptor apparatus in the genital epithelium, leading to destructive cellular changes and changes in production levels of estrogen and progesterone. The most frequent sequelae of such impact include reduced synthesis of estrogen and gestagens, that is, Phase 1 and Phase 2 insufficiency, up to anovulation. Unlike a viral infection, in PID of microbial etiology, the suppression involves mainly the luteal phase. Moreover, morphological changes in endometrial tissues and reduced receptor counts upset the very governing principles of neurohumoral regulation, such as direct interrelationship and feedback. Initially, a hormonal imbalance is a typical post-viral infection, including hypoestrogenemia and luteal phase insufficiency. If no adequate therapy is used, the impaired synthesis of steroids at this phase aggravates as a result of straining the functions of the adaptive system. This may result in such hormonal imbalances as hyperandrogenism, relative hyperestrogenism, anovulation and hypoestrogenism (amenorrhea).

Clinical manifestations of these changes include ovulatory premenstrual bleeding, postmenstrual bleeding and intermenstrual bleeding. The acme of the aforementioned abnormal processes includes anovulation and amenorrhea. Destructive changes in the ovarian receptor apparatus in a setting of viral infection are accompanied by impaired synthesis of hormones.

In case of viral infection, immune functions have certain specific characteristics. Reduced interferon activity and lower activity of natural killer cells, suppression of cellular immunity (T-cell deficiency), impaired immune response and increased production of B-cells: all of these factors contribute to malignant disease.

The presentation of the viral genital disease can be subclinical, oligosymptomatic or clinically overt (symptomatic); this disease is prone to recurrences, which are triggered by endogenous and exogenous factors (low and high temperatures, ultraviolet irradiation, emotional stress, physical illness, etc.).

The viruses with tropism to genital mucous membranes will affect vulva, vagina, cervix, endometrium and tubal epithelium. At the moment, the scholars identify several groups of viruses causing PID directly: herpes simplex virus (HSV-1 and HSV-2), cytomegalovirus (CMV) and papillomavirus (the types with high and low cancerogenic risk). All other viruses impact reproductive system indirectly.

Clinical forms of the viral disease include the following:

1. GH: manifest, abortive, atypical and subclinical.

2. CMV: latent and generalized (with the involvement of nervous system and visceral organs). Some of the researchers see CMV as a virus of special significance, pointing out that its prevalence may adversely affect the intellectual potential of the nation. In gynecological practice, endocervix-located CMV is seen increasingly more often; this condition is manifested by an endocervicitis refractory to traditional methods of treatment.

Taking into account the prevalence of genital viral infections and insufficient efficiency of available treatments, the investigators find it appropriate to continue improving therapeutic interventions in this disease. The existing traditional methods of treatment include large amounts of medications with adverse effects in the CNS, the vascular system and the kidneys. In addition to that, such therapies are often associated with high economic burden. Therefore, when developing the method of treatment for women with a viral infection, the investigators undertook to make this method more accessible and cost-effective for the patient. The treatment has been developed according to traditional principles of treatment used in inflammation of viral etiology and included antiviral, desensitizing, detoxification, immunomodulating and symptomatic interventions. The effects of Proteflazid, the study drug, include antiviral action and immunological correction.

Proteflazid is a combination antiviral drug, a liquid alcoholic extract of wild graminaceous plants (*Deschampsia caespitosa L.* and *Calamagrostis epigeios L.*). Its principal active ingredients include quercetin/rutin-like flavonoids. The active substances are different from rutin in terms of degree of glycosylation, the adjoining sites of carbohydrate moieties and configuration of glycoside bonds. The difference of Proteflazid flavonoids from quercetin includes different radicals in the aromatic part of the molecule. Proteflazid has a high antiviral activity, as well as immunomodulatory, apoptosis-modulating and antioxidant effects. The direct antiviral action of Proteflazid occurs as a result of inhibition of virus-specific enzymes, such as thymidine kinase and DNA-polymerase in virus-infected cells, which facilitates reduction or complete block of viral replication. The immunomodulatory effect of Proteflazid is implemented through reversing IFN-alpha and IFN-gamma levels to normal, thereby improving the parameters of cellular and antibody-dependent immunity. In addition to that, study drug exhibits an apoptosis-modulating effect, accelerating the apoptosis of virus-infected cells and promoting their faster elimination from the body. The aforementioned effects of study drug play a considerable role within the mechanisms of its antiviral and antibacterial activity; in the investigator's opinion, these mechanisms can be used as a part of therapy for herpetic infection.

During the period from 2000 to 2006, the investigator has assessed 100 women of reproductive age (18–35 years) with herpetic infection (HI) and 50 women with HPV treated with the proposed multicomponent method below:

1. Proteflazid, systemic regimen: 3 days of 3 drops three times daily (t.i.d), followed by 3 days of 5 drops t.i.d, followed by 3 days of 7 drops t.i.d; then 8–10 drops t.i.d for 3 months. This regimen was followed by 2 to 4 months of maintenance therapy (5 drops three times daily on alternate days (q2d).

2. Local therapy: Viferon rectal suppositories containing 500,000 IU each q.d for 10 days.

3. Antihistamines for 10 to 14 days.

4. Vitamins: 10 I.M. doses of Vit. B1 (1.0) on alternate days; 10 I.M. doses of Vit. B6 (1.0) on alternate days; 5 I.M. doses of B12

(500 μg) on alternate days; 15 doses of Vit. E (0.2) and 10 IM doses of Vit. C (0.5) in a cyclic mode.

5. Eubiotic products (after completion of antiviral therapy).
6. Hepatoprotectors for 30 to 60 days.
7. Antifungal therapy (systemic products, e.g. fluconazole).
8. Hormonal correction (as required).

The treatment program was started on Day 1 of the menstrual cycle and continued for a total of 3 to 6 months. Then, as a therapy to prevent exacerbations, the patients were transferred to the following regimen: Proteflazid in repeated courses 3 and 6 months after the main regimen (a repeated course included oral administration according to the above scheme for 1 month); Difluzol® (fluconazole) at 0.15 g daily every month at Day 3 of the menstrual cycle for 3 consecutive cycles. In case of menstrual irregularities (at initial stages), one of the following products was used: Remens®, Mulimen, Dysmenorm, etc.

The diagnosis was based on microbiological, virological, serological, immunological and instrumental tests; assessment of endocrine functions of the reproductive system was also performed.

Microbiological testing included bacterioscopy of vaginal smears, bacteriological cultures of cervical discharge, bacterioscopy of vaginal smears, bacteriological tests of vaginal secretions (using conventional methods) with cultures for bacteria and fungi of various taxonomic groups. Levels of specific antibodies (IgG and IgM) were assessed with serologic methods, using enzyme-linked immunoassay test systems. Virological methods were used to assess cervical material (PCR testing for viral genome at Day 5–Day 8 of the menstrual cycle). In addition to type 1 and type 2 HSV, the PCR method was used to test for the following pathogens: *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Toxoplasma gondii* (using scrapings from the cervical canal).

Extended colposcopy was performed according to the conventional method. Immunological testing included assessment of cellular and antibody-mediated immunity. Assessment of interferon status included the following: circulating levels of serum interferon (IFN); production of IFN-α by leukocytes of peripheral blood under in vitro induction with virus inducers (Newcastle disease virus, Kansas strain) and the level of IFN-γ production by lymphocytes of peripheral blood under in vitro induction with a mitogenic agent (phytohemagglutinin). IFN measurement (in IU/ml) was performed using a standard methodology. The phagocytic capacity of monocytes and neutrophils in peripheral blood was studied in a microscopic test (*Staphylococcus aureus* test culture, strain 209). Phagocytic index (PI, %) and phagocytic number (PN, units) were assessed. Identification of surface structures on T-cells and B-cell, as well as T-lymphocytic subpopulations, was performed using kits of Leu series monoclonal antibodies in a direct immunofluorescence test. The investigators assessed T-cells (CD3+), T-helper cells (CD4+), T-killer cells (CD8+), natural killer cells (CD56+) and B-cells (CD19+) in peripheral blood. Assessment of serum immunoglobulin (IgA, IgM, IgG) levels (in g/l) was performed using a method of radial immunodiffusion in gel (after Mancini). Serum levels of circulating immune complexes (CIC) (measured in absorbance units, AUs) were assessed by precipitation with 3.5 % solution of polyethylene glycol.

During assessment of serum endocrine parameters with a radioimmunoassay technique, the investigators assessed for levels of LH, FSH, estradiol, testosterone and prolactin at Day 6–7 of the menstrual cycle and levels of LH, FSH and progesterone at Day 17–19 of the menstrual cycle (MTM diagnostic laboratory) using the test kits produced by 'CEA-IRE Sory' (France). The investigators have assessed both absolute levels of hormones and the ratios of gonadotropic (LH, FSH) and gonadotropic and ovarian hormones, namely: E_2/FSH, E_2/LH, progesterone/LH and E_2/progesterone. The resulting data were compared with the results of cyclic ultrasonography assessment. In addition, to rule out thyroid disease and related menstrual irregularities (MIR), thyroid ultrasound was appended with testing for TSH, T_3, T_4 and anti-thyroid antibodies regardless of the phase of the menstrual cycle.

Clinical, laboratory and instrumental investigations were performed before the onset of rehabilitation therapy and as a follow-up 3 months and 1 year after the onset of treatment. Treatment efficacy was evaluated in 1 year as a number of GH relapses and absence of HPV on PCR. In a setting of HI, MIR were seen more frequently in married women. The history of sexually transmitted disease was dominated by gardnerellosis and HI (60.0 % and 57.1 %, respectively).

The specter of clinical signs included dyspareunia in 25.7 % patients, genital itching in 51.4 %, excessive discharge in 34.2 % and lower abdominal pain in 31.4 % patients.

The data found in gynecological assessment are evident of damage to genital mucous membranes in women with MIR in a setting of viral infection: cervical dysplasia (25.71 %), colpitis (22.0 %), cervicitis (42.85 %), abnormal (mostly cheese-like) discharge (28.5 %), foul-smelling discharge (34.2 %) and pelvic adhesions (20.0 %).

Assessment of cellular composition of vaginal smears has shown high leukocyte counts per power field (28.9 ± 0.4) and clue cells (34.7 ± 1.6). Bacterioscopic assessment has shown a high incidence of lysis (60.0 %) and the predominance of mixed microbiome (68.6 %) with high levels of yeast cells (60.0 %). Assessment of vaginal microbiome informed the prevalence of *G. vaginalis* (in 24 [68.5 %] patients, with a concentration of 3.56 ± 0.16 CFU/ml); *E. coli* (haem-) (in 17 [48.5 %] patients, with a concentration of 1.06 ± 0.01 CFU/ml); *Staph. epidermidis* (in 14 [40.0 %] patients, with a concentration of 2.54 ± 0.05 CFU/ml); *Lactobacillus* (in 13 [37.1 %] patients, with a concentration of 4.64 ± 0.26 CFU/ml), and *Candida* (in 19 [54.2 %] patients, with a concentration of 2.37 ± 0.03 CFU/ml).

As informed by serology titers, positive titers of anti-HSV IgG antibodies (Ab) were found in all study subjects; in part, anti-HSV-1 Ab was seen in 31.4 % subjects and anti-HSV-2 Ab was seen in 51.4 % subjects; titers of IgG against HSV-1 and HSV-2 were seen in 17.1 % of female study subjects. A substantial proportion of women (60 %) had positive PCR to Type 1 and Type 2 HSV, which is evident of viral persistence in the cervix.

Women with the viral infection have substantial hormonal imbalances with a predominance of changes indicating anovulatory menstrual cycles, including elevated levels of gonadotropic hormones, hypoestrogenism, hypoprogestinemia and hyper-androgenism, as well as elevated LH/FSH ratio, which also informs anovulatory menstrual cycles.

As informed by the results of cellular immunity, women with MIR in a setting of viral infection have markedly reduced main

parameters of phagocytosis, which requires an additional adjustment.

The presence of viral infection leads to immunosuppression of both cellular and antibody-mediated immunity, as well as changes in the interferon system.

Therefore, the review of clinical characteristics demonstrates that viral infections lead to specific symptoms (both genital and extragenital physical symptoms). The presence of viral infection adversely affects immune defenses, causes endogenous toxemia and deteriorates ovarian endocrine function.

During the assessment of clinical efficacy of the proposed methodology of MIR improvement in women with a viral infection, the investigators have assessed principal clinical parameters. All women tolerated the treatment well; no complications and/or adverse effects were seen.

Treatment efficacy criteria included the following groups of parameters:

- clinical parameters: recovery of the normal menstrual cycle (regularity, the volume of bleeding, painlessness and no exacerbations of viral infection throughout the year;
- hormonal parameters: recovery of normal hormonal levels in 3 and in 6 months of treatment;
- immunological parameters: recovery of normal parameters of cellular and antibody-based immunity in 3 months of treatment;
- the findings of specific study methods: negative PCR test for HSV of cervical samples and no increase in specific anti-herpes antibodies;
- recovery of the normal vaginal microbiome.

It should be noted that the clinical efficacy of the proposed method was 82.8 % (recovery of normal menstrual cycles). No exacerbations of herpes-associated inflammation were seen during treatment.

Post-treatment assessment for viral genome in scrapings from the cervical canal have shown the following: absence of diagnosed Type 1 and Type 2 HSV antigen (according to PCR data) and increased titers of IgG against both HSV-1 and HSV-2 ($p < 0.05$). Thus, the use of investigator-proposed technique facilitates recovery of the normal genital microbiome. Lack of PCR identification of the causative agent in women with HPV was viewed as a result of effective treatment, which was found in 32 (64.0 %) of 50 female subjects in the study.

The lymphocytic link of systemic immunity is informative concerning the prognosis of potential systemic abnormalities. The resulting findings for evaluation of the effect exerted by the proposed methodology in the antibody-based immunity (changes with time in serum levels of main immunoglobulin classes (A, M, G), as well as recovery of normal interferon levels, including IFN-α and IFN-γ) provide evidence for improved immunogenesis. The results of immunological changes confirm the clinical efficacy of the proposed technique and explain the main findings of changes in the vaginal microbiome and immune status in patients with MIR in a setting of viral infection.

The review of clinical, ultrasonographic and laboratory follow-up presented herein allows making the following statements:

1. The investigator-proposed therapeutic regimen used in females of reproductive age in a setting of viral infection allows for a 5.3-fold reduction in the incidence of MIR (including a 2.2.-fold reduction in hypomenstrual syndrome and a 3.5-fold reduction of hypoestrogenism). This regimen restores hormonal balance, immunological status and genital microbiome.

2. The use of Proteflazid, an antiviral and immunomodulating drug, as a part of complex treatment allows reducing the number of relapses 2–3 times (in a herpes infection) and 1.5–2 times (in an HPV infection).

Therefore, a positive effect of fundamental antiviral therapy on the regeneration of reproductive system and immunogenesis can be noted.

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