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# THE USE OF FLAVONOID GLYCOSIDES IN THE THERAPY OF PAPILLOMAVIRUS GENITAL INFECTION IN WOMEN

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**Abstract**. Zaporozhan V. N., Marichereda V. G., Dimcheva L. I. **THE USE OF FLAVONOID FLAVONOIDGLYCOSIDES IN THE THERAPY OF PAPILLOMAVIRUS GENITAL INFECTION IN WOMEN -** *Odessa National Medical University, Odessa, Ukraine.* The aim of the work was to assess the clinical efficacy of flavonoid glycoside derivatives in the therapy of benign and precancerous cervical disease. The study has been performed in Oblast Teaching Hospital of Odessa in 2010–2013. The study enrolled 80 women of reproductive age (mean age 28.4 ± 0.2 years) with confirmed mild to moderate cervical epithelial neoplasia (the main group) and 30 apparently healthy women assessed during prophylactic health check-ups. Human papillomavirus has been quantified in fixed preparations using a standard technique of real-time PCR. Immunohistochemical testing for CD1a was performed with murine monoclonal antibodies diluted 1:25. All of the female patients had dysplasia of squamous cervical epithelium, including CIN I in 27 (54.0 %) cases, CIN II in 15 (30.0 %) cases and CIN III in 8 (16.0 %) cases. In a setting of dysplasia, some of the patients had other genital disease. In 84.5 % of cases, human papillomavirus

belonged to the A9 group (types 16, 31, 33, 35, 52 and 58). Using Proteflazid as a part of multimodality treatment had reduced the viral burden of genital papillomavirus infection (by 3.0 lg D50) and facilitated the increase in numbers of antigen-presenting cells. In terms of virustatic and anti-relapse effects, standard therapy was inferior to multimodality regimen with Proteflazid.

**Key words**: flavone glycoside, benign cervical disease, precancerous cervical disease.

Cervical cancer is second to breast cancer in terms of incidence of female cancers in the developed countries of the world [1]. According to the National Cancer Registry of Ukraine, the prevalence of cervical cancer in 2011 was 15.4 per 100,000 of the population; the respective mortality rate was 5.5 per 100,000 of the population [2].

The leading role in etiology and pathogenesis of preneoplastic cervical disease and cervical cancer is played by human papillomavirus [3–5]. Since 85 % of cervical areas with dysplasia of various degrees are seen in proximity to areas of pre-invasive cancer, early diagnosis and treatment of this disease is becoming a high-priority objective [6].

The progress of epithelial dysplasia is a dynamic process determined by a number of clinical and biological parameters, such as the activity of the immune system, aggressiveness of the HPV type, the age of the patient, the duration of the disease and the presence of concomitant infections. According to literature, up to

57 % cases of mild dysplasia, 44 % cases of moderate dysplasia and 32 % cases of severe dysplasia reverse spontaneously [1, 3, 5]. The causes for this phenomenon are, however, unknown.

In the recent years, it has been demonstrated that two distinctive stages can be identified within the infectious process of HPV: the stage of reproductive infection, when viral DNA is in a free state, and the stage of integrative infection, when viral DNA incorporates into the genome of infected cells. The first stage is reversible and many infected individuals may develop remission. The stage of integrative infection is the first step to neoplastic transformation of the cell, very frequently resulting in carcinoma [1, 3, 4, 6]. Neoplastic transformation is more likely when HPV interacts with other carcinogens or infectious agents. Integration of the virus into the genome is accompanied by increased synthesis of E6 and E7 proteins, which, through a number of reactions, may lead to an unchecked cellular proliferation. This proliferation is constrained by the P16 ink - 4a protein, whose synthesis is dramatically increased in the integrative type of HPV. In addition to that and according to various researchers, one of the pathways to malignant transformation of HPV-infected cells is the ability of the virus to alter cellular metabolism in such a way that the cell acquires the capacity to convert estradiol mainly into 16a-hydroxysterone (16a -OH), the latter being a direct activator of expression of the E7 gene, which is in turn responsible for neoplastic transformation of the cells [5, 6]. The concomitantly produced E7 oncoprotein actuates the mechanisms responsible for abnormal cellular proliferation on the one hand and blocks the mechanisms of immunological protection on the other hand, the latter having their specific peculiar aspects in human papillomavirus infection (HPVI) [7, 8]. As in the case with other chronic disease with prolonged viral persistence, HPVI naturally results in immunodeficiencies, which are viewed as secondary immunodeficiencies due to the failure of various components within the immune system [8]. It should be noted that HPV particles are unable to infect antigen-presenting dendritic cells, which explains the lack of a direct pathway for

activation of immunity in HPVI. A prolonged persistence of HPV is facilitated by the ability of the virus to ‘evade’ systemic immune surveillance, which has been established in a number of research studies. Although HPV chiefly infects basal cells, replication of the virus and assembly of viral particles occur in the differentiated cells in the superficial layer of the epithelium, the latter cells subject to subsequent apoptosis. This process is not accompanied by any signs of inflammation and the immune system virtually neglects it [8, 9]. Nevertheless, the effector cells of the immune system produce a number of cytokines (including α, β and γ-interferons), which reduce the transcription of E6 and E7 genes in type 16, 18 and 33 HPV within virus-transformed cells. The imbalance of cellular immunity in HPVI is confirmed by such data as combination of spontaneous regression of hyperplastic manifestations of HPVI with infiltration of the surrounding tissues with lymphocytes and macrophages, as well as by the results of some research studies, which provide evidence of the relationship between HPV elimination from the body and responses of T-helper cells to the C-terminus domain of the E2 viral protein [8].

One of the most promising trends of management and secondary prevention of viral infections is the use of flavonoid glycosides, which are potent immunomodulating agents and are capable of influencing the activity of apoptosis. Finally, due to their direct impact upon the activity of virus-specific enzymes (such as thymidine kinase, DNA polymerase, reverse transcriptase [10, 11] and DNA-dependent RNA polymerase), the use of this group of medicinal agents exerts a pronounced virustatic effect [10]. Nevertheless, the efficacy of using flavonoid glycoside derivatives in HPVI has not been evaluated.

**The aim of the study** includes assessment of clinical efficacy of flavonoid glycoside derivatives in the management of benign and precancerous cervical disease.

**Materials and methods**.

This research study has been performed at the site of Regional Clinical Hospital (Odessa, Ukraine) in 2010–2013. This study has enrolled 80 women of reproductive age with verified mild to moderate cervical intraepithelial neoplasia (the main group) and 30 apparently healthy women assessed during prophylactic health check-ups.

All of the patients enrolled for participation in the study underwent comprehensive assessment according to the current clinical protocols, specified by the Orders of the Ministry of Health (MoH) of Ukraine (Orders No. 582 dated 15.12.2003, No. 676 dated 31.12.2004 and No. 624 dated 03.11.2008 [12]).

The HPV in the fixed preparations was assessed with quantification using a standard methodology of real-time PCR.

For the immunohistochemical assay of the CD1a marker (the marker of dendritic cells), the investigators have used murine monoclonal antibodies to CD1a (Clone O10, Cat. No.: 1590), diluted 1:25 (manufactured by Immunotech, France). The analysis of microscopic photographs of the resulting preparations was performed using Image J 1.48d software (NIH, USA). The CD207 antigen (Type II transmembrane lectin or langerin, with specificity to mannose and function as an endocytosis receptor) was quantified by immunoblotting (Langerin Antibody (E - 17), manufactured by Santa Kruz Biotechnology, USA). As secondary antibodies, the investigators have used Cruz Marker™ IgG-HRP antimurine antibodies: sc-2031 (at 1:2,000–1:5,000 dilution). Additionally used reagents included Cruz Marker ™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent sc-2333 i Western Blotting Luminol Reagent: sc-2048 (USA).

The patients of the main group were randomized into clinical subgroups (depending on the methods of treatment used in these patients). Patients in Group I (n = 40) received standard drug therapy and patients in Group II (n = 40) received Proteflazid, an antiviral drug with direct immunomodulating action. Proteflazid was used orally, as drops, and locally, as vaginal tampons (2 treatment courses

14 days each with a 10-day interval; for local use, Proteflazid was diluted 1:4 with normal saline).

Statistical analysis was performed using the methods of dispersion and correlation analysis with the Statistica 10.0 software (manufactured by StatSoft Inc., USA) [13].

**Study results**.

The mean age of study subjects was $28.4 \pm 0.2$ years. All patients were diagnosed with dysplasia of cervical squamous epithelium, including CIN I in 27 (54.0 %) cases, CIN II in 15 (30.0 %) cases and CIN III in 8 (16.0 %) cases. In a setting of cervical neoplasia, some patients had various genital inflammatory diseases, such as vulvovaginitis (6.0 %), bacterial vaginosis (12.0 %), cervicitis (6.0 %), candidiasis (4.0 %) and chronic endometritis (10.0 %).

The patterns of extragenital disease were dominated by ENT conditions (nasopharyngitis [6.0 %], tonsillitis [4.0 %], sinusitis [2.0 %]), upper respiratory infections (bronchitis [4.0 %]), urinary system (chronic pyelonephritis [4.0 %], glomerulonephritis [2.0 %], cystitis [4.0 %]), gastrointestinal conditions (gastritis [4.0 %], gastroduodenitis [4.0 %], biliary dyskinesia [2.0 %]), neuro-circulatory dystonia (10.0 %) and allergy (allergic rhinitis, atopic dermatitis, etc.). Fourteen (28.0 %) patients had frequent colds (four and more upper respiratory infections per year). The HPV infection was verified in 82.0 % of study patients. The quantitative values of HPV control were within the range of 1,000–6,628 copies/pairs. It has been established that in most cases (78.0 %) the levels of viral burden in the patients did not exceed 5 lg per $10^5$ cells. In women of the control group, subclinical HPVI was seen in 13.3 % of cases, with viral burdens at the threshold of clinical significance (2–3 lg per $10^5$ cells). In the vast majority of cases (85.4 %), group A9 human papillomaviruses were detected (the group including types 16, 31, 33, 35, 52 and 58). There was one report of high (6.5 lg per $10^5$ cells) titer of group A5/A6 HPV (the group includes types 51 and 56). There were no cases of detection of group A7 HPV (types 18, 39, 45 and 59).

All patients with cervical neoplasia were found to have reduced populations of CD1a+ and CD83+ cells in their cervical epithelium. At that, the mean number of immature CD1a+ dendritic cells in patients with cervical intraepithelial neoplasia was $2.7 \pm 0.1$ per power field, and the number of mature CD83+ dendritic cells was $0.02 \pm 0.002$ per power field, while in healthy women these findings were $3.1 \pm 0.1$ CD1a + per power field and $0.04 \pm 0.003$ CD83 + per power field, respectively.

Further research has shown that standard therapy was inferior to multimodality regimen with Proteflazid in terms of virustatic and anti-relapse effects. Thus, the post-treatment HPV viral burden was reduced by 3.0 lg D50 (see Fig. 1)

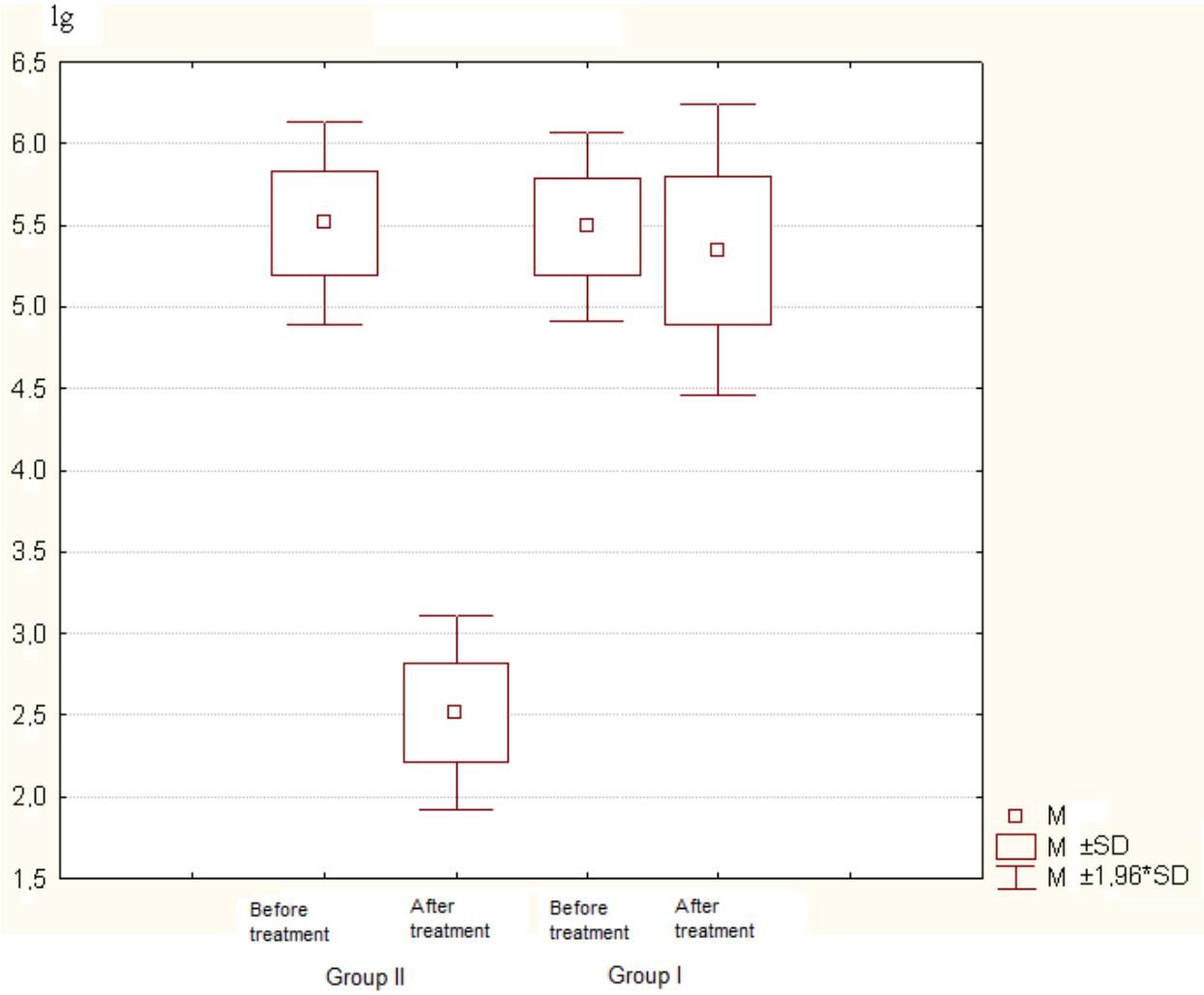


Fig. 1 — Changes of HPV viral burden with time in study groups

As seen from the figure above, the viral burden in patients treated with conventional regimen remained virtually unchanged (with a substantially increasing the variance of the parameter), while Group II was characterized by a substantial decrease of viral counts (by 3.0 lg D50).

At the same time, there was an increase in the quantity of dendritic cells, which is an evidence of positive effects of flavonoid glycosides in cellular immunity. Therefore, the influence of study treatment has significantly increased the numbers of immature dendritic cells (DCs), with CD1a being their marker (see Fig. 2).

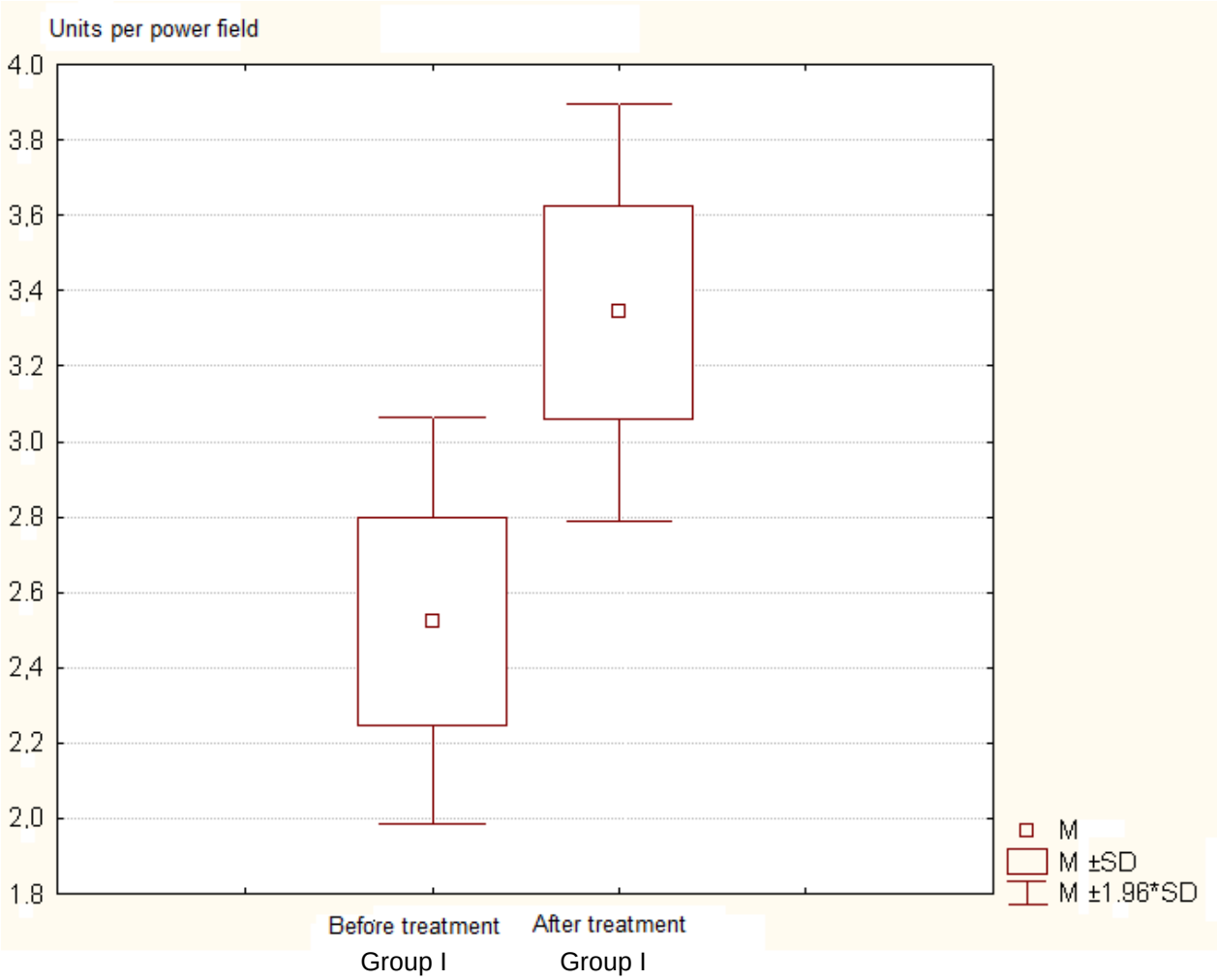


Fig. 2 — Changes with time in a population of immature dendritic cells in study subjects

A similar profile of changes with time was observed concerning the numbers of mature DCs, including the CD83+ (see Fig. 3).

Therefore, the clinical experience of the systemic and vaginal use of flavonoid glycosides presented herein supports the high efficacy of the study product. Standard therapy was inferior to multimodality regimen with Proteflazid in terms of virustatic and anti-relapse effects. Taking into consideration the 3.0 lg D50 reduction of HPVI viral burden and the increased counts of antigen-presenting cells with Proteflazid, it is apparently expedient to use this product as a part of multimodality therapy in all patients with HPVI.

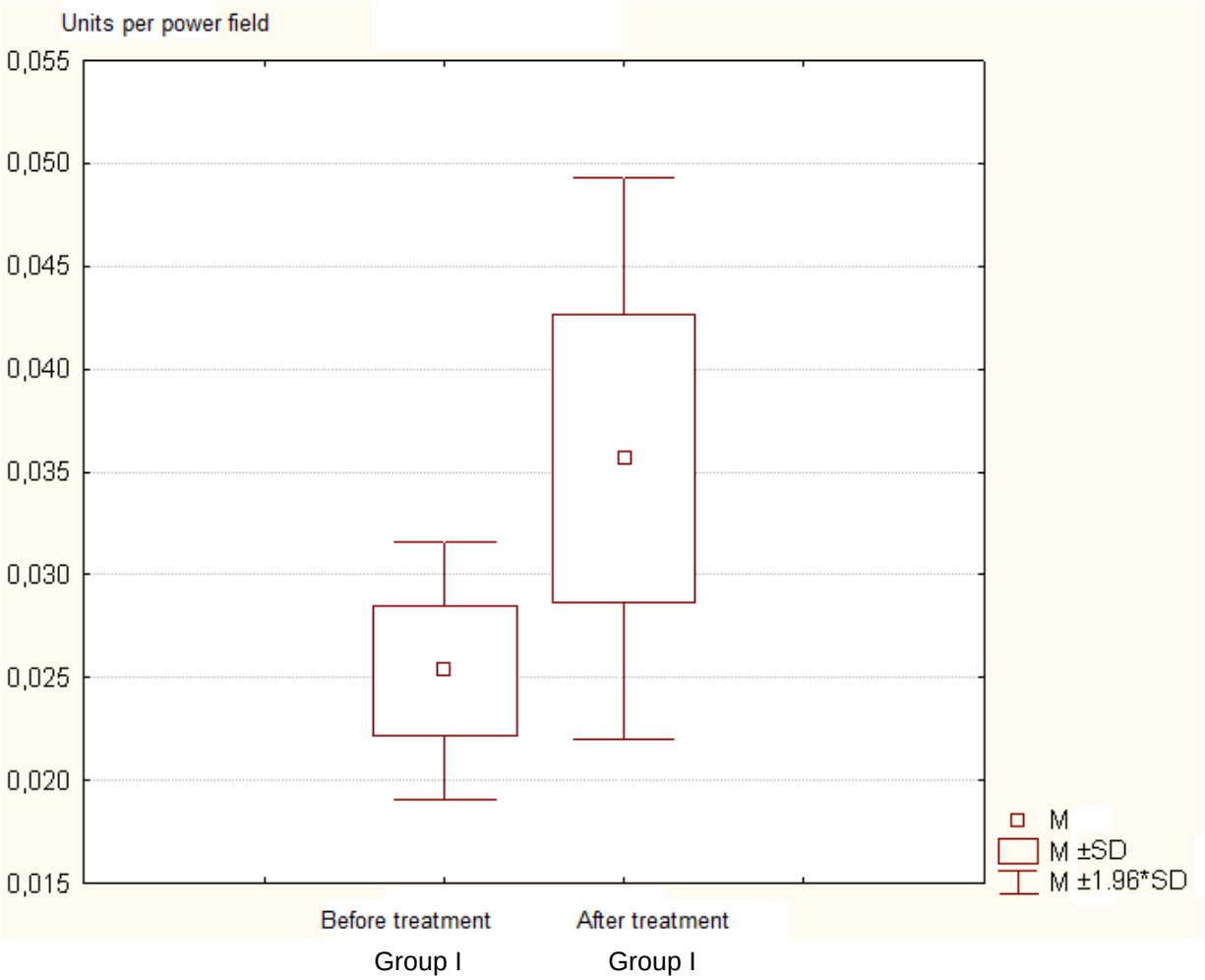


Fig. 3 — Changes with time in a population of mature dendritic cells in study subjects

Conclusions:

1. Using Proteflazid reduces HPVI viral burden by 3.0 lg D50.
2. Proteflazid facilitates the increase in antigen-presenting cells.
3. Standard therapy is inferior to multimodality regimen with Proteflazid in terms of virustatic and anti-relapse effects.

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