EVALUATION OF COMPREHENSIVE TREATMENT OF CERVICAL PATHOLOGY ASSOCIATED WITH HPV

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A comprehensive examination and treatment using the drug inosine pranobex (Isoprinosine), was performed on 123 patients with cervical pathology associated with human pathology virus (HPV). The study of the structure of the microbial landscape in patients with cervical pathology showed that among types of HPV with high statistical significance the HPV type 16 prevailed, the second highest rate spread was HPV 18. Inclusion of isoprinosine complex therapy in patients with CIN I (LSIL, at virus load of 3 Lg/10x5) was not required to further degrade the pathological process of the cervix, in 96.23±2.62% of cases, and contributed to the positive effect in total of 91.06% of patients with pathology of the cervix, which is statistically significant.

Key words: pathology of the cervix associated with human papillomavirus, inosine pranobex, Isoprinosine

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Cervical cancer is one of the most serious medical and social concerns of the female reproductive health in economically developed countries. Cervical cancer in Russia ranks third following breast and corpus uteri cancer in the incidences of malignant neoplastic of reproduction system [1].

Human papilloma virus (HPV) is a proven factor affecting the development of cervical cancer.

Despite the current recommendations of World Health Organization (WHO), no programs regarding the prevention of cervical cancer are being applied in any country including Russia; the main reason behind it is the socio-economic problems and the need for expenses regarding the whole screening and vaccination of the female population [2, 3].

Centers for Disease Control and Prevention (CDC) estimates that 60% of sexually active American young women are infected with one or more HPV type(s) within 5 years after they become sexually active. The risk of infection increases according to the number of sexual partners the partners have as well as the number of sexual partners [4].

The rate of papilloma virus infection among asymptomatic women is 2 to 44% pursuant to age, geographical region, and socio-economic status [5].

According to WHO, 2,5-3 million HPV - cases are diagnosed each year globally and such cases cause a series of diseases including the following: mild cervical intraepithelial neoplasia (CIN I), moderate to severe CIN (CIN II, CIN III), condylomas and cervical cancer. Despite the current methods regarding the diagnosis and treatment of precancerous cervical patients, 470.000 new cases of cervical cancer occur each year globally, 233,000 of which are fatal [6-8].

There is no effective medicine treating HPV selectively. The methods of treatment generally aim to eliminate the background and precancerous lesions caused by HPV. In addition to destructive treatments, antiviral and immunoregulator medicines are prescribed more and more to eliminate the virus and reduce the number of recurrences of cervical dysplasia caused by HPV lesions (9, 10).

Based on the foregoing, the purpose of our treatment was to evaluate the efficiency of the comprehensive treatment of cervical pathology against the HPV background with the use of Inosine pranobex (Isoprinosine).

Materials and Methods

To meet the purpose of the study, we performed a comprehensive examination and treatment using Isoprinosine (Inosine pranobex) on 123 patients with cervical pathology associated with HPV.
The verification of the diagnosis in women was based on the results of the following tests:
-Clinical-visual method
-Vaginal smear microscopy
-Bacteriological method
-Extended colposcopy (ECS)
-Cytological method (Pap-Test)
-Verification of HPV DNA in the cervical canal through a polymerase chain reaction in epithelial cell scratches
-Determining the viral load through the method of hybrid capture – HPV Digene test
-Detecting chlamydia, myco-ureaplasma, cytomegalovirus infection through polymerase chain reaction
-Morphological examination.

The analysis of the clinical and laboratory data obtained during the study was performed by using Statistical 6.0 statistical processing software packet. The distribution of the feature in statistical populations was evaluated, the matched Student t-test was used in a normal distribution, and the non-parametric chi-square test was measured in other cases.

The formula derived from binomial distribution law was applied to compare the data obtained from the example with the alternative variation in the features: $m=\sqrt{Pxq/n}$, m used here is the relative error of arithmetic mean; P is the rate of the evaluated feature; q=100-P – rate of the opposite indicator; n – total number of study, if the n value is less than 30, the total number of study is shown as n-1.

After the definite verification of the diagnosis, the patients were treated with a 3-phase treatment regimen.
Stage I includes conservative treatment with antimicrobial/antimycotic agents (according to the etiology of pathology) and Isoprinosine (Inosin pranobex); Stage II includes the elimination of the pathological process of the cervix (the ablation of pathological areas with radio wave treatment); the application of Isoprinosine continued in Stage III (postoperative period).

Table 1: Social-status features of the patients in the survey group

Parameters	Number of absolute observations (n=123)	Number of relative observations (P±m),	Level of reliability, p
Age categories			
Below 20	15	12,20±2,95	-
Between 21-30	52	42,28±4,45	<0,001
Between 31-40	33	26,83±4,00	<0,01
Between 41-50	21	17,07±3,39	-
Between 60-70	1	0,81	-
Age 80 (asymptomatic HPV 16 carrier)	1	0,81	-
Educational Level			
Higher education	68	55,28±4,48	<0,001
Secondary private technical education	24	19,51 ±3,53	-
Secondary education	18	14,63±3,19	-
Unfinished higher education	5	4,06±1,78	-
Unfinished secondary education	8	6,50±2,22	-
Marital Status			

Married	80	65,04±4,3	<0,001
Single	20	16,26±3,33	-
Divorced	23	18,70±3,52	-
Number of sexual partners			
1	23	18,70±3,52	
2	33	26,83±4,00	
3	50	40,65±4,43	<0,05; <0,001
>4	17	13,82±3,11	

Table 2. Features of the microbial landscape in the study group

Parameters	Number of absolute observations (n=123)	Number of relative observations (P±m), %	Level of reliability, p
HPV Types			
HPV 16	68	55,28±4,48	<0,001
HPV 18	23	18,70±3,52	<0,05
HPV 16, 18	11	8,94±2,57	-
HPV 52	6	4,88±1,94	-
HPV 58	6	4,88±1,94	-
HPV 45	3	2,44	-
HPV 33	2	1,63	
HPV 34	1	0,81	
HPV 31	2	1,63	-
HPV 35	1	0,81	-
Structure of the co-existing microbial agents			
Representatives of mollicutes class			
M. genitalium / M. hominis	33	26,83±4,00	
U. urealiticum	50	40,65±4,43	<0,001
Intracellular pathogens			
C. trachomatis	12	9,76±2,68	
Aerobic flora			
Escherichia coli	27	21,95±3,73	
Staphylococcus aureus/ Staphylococcus epidermidis	20	16,26±3,33	
Streptococcus agalactiae	15	12,20±2,95	
Viruses			
HSV-1 and HSV-2	17	13,82±3,11	
CMV	52	42,28±4,45	<0,001
Fungal flora			
Candida albicans	23	18,70±3,52	

Pharmacological Features and Effect Mechanism

Inosine pranobex is a synthetic complex purine derivative having antiviral and immunomodulator activity. The antiviral activity is determined through the effect of viral replication and immune response over the modulation. The direct antiviral effect of Inosine pranobex is associated with the inhibition of viral RNA, inhibition of viral translation processes and the increase of lymphocyte matrix RNA synthesis suppressed/reduced by viral infection. The direct antiviral effect of Pranobex inosine is associated with the inhibition of viral RNA, pressuring of viral translation processes and the increase of lymphocyte matrix RNA synthesis suppressed /reduced by viral infection. Such reactions occur due to the changes in the stereochemical structure of polyribosome, the prevention of polyadenylic acid binding to viral matrix RNA, molecular re-regulation of intramembranous particulates of lymphocytic cell plasma membranes and the three times increase of their density. The indirect antiviral effect is determined through the induction of interferon forming (interferon having antiviral features increases the production of (IFN)-a, IFN-g lymphocytes).

Antiviral effect mechanism is associated with the inhibition of viral RNA and dihydroperoxide synthetase enzyme which takes part in the replication of certain viruses; it increases the viral suppression of matrix RNA synthesis of lymphocytes through suppressing the translation of viral RNA biosynthesis and viral proteins and increases the lymphocyte production of IFN-a, IFN-g having antiviral features. When combined, it increases the efficiency of IFN-a which are antiviral agents [10].

The application of Isoprinosine (inosine pranobex) is as follows: 50 mg/kg daily (for body weight, 500 mg per every 10 kg), frequency of application; it was applied three times a day for 10 days, then a 10-day break and then 10-days treatment was repeated for two times.

Table 3: Verification of diagnosis in women with cervical pathology associated with HPV

Parameters	Number of absolute observations (n=123)	Number of relative observations (P±m), %	Level of reliability, p
Morphological variants of the detected cervical dysplasia			
CIN (LSIL*)	74	60,16±4,41	<0,001
CIN II (HSIL**	43	34,96±4,30	<0,001
CIN III (HSIL)	5	4,07±1,78	-
Cervical cancer in situ	1	0,87	-
Morphological results of the combination of cervical dysplasia and other cervical abnormalities with carrying HPV			
Lympholeukocyte cervicitis, stromal angiomatosis	37	30,08±4,14	<0,05; <0,001
Serous lymphocytic cervicitis with micropapillomatosis and polyposis	25	20,33±3,63	-
Viral-fungal cervicitis	23	18,70±3,52	<0,05
DNA viral cervicitis	12	9,76±2,68	<0,001
Chronic productive DNA viral cervicitis	6	4,88±1,94	<0,001
Leukoplakia	2	1,63	-
Epidermal glandular pseudo-erosion of cervix with inflammation	18	14,63±3,19	<0,001

**LSIL – low-grade squamous intraepithelial lesions
**HSIL – high-grade squamous intraepithelial lesions

The monitoring of the treatment in Stage I included extended colposcopy, determination of viral load and deciding on the necessity of inosine pranobex treatment and the elimination of pathological process of the cervix.

Results and Discussion

We have analyzed the social status of the patients with cervical pathology associated with HPV during their follow-up. The details of the women in the study group are listed in Table 1. According to the data in Table 1, while the rate of patients between 21-30 was statistically significant in comparison with women below 20 ($p<0,001$); between 31-40 ($p<0,01$); and over 41-50 ($p<0,001$), the rate of patients between 31-40 were significantly high in comparison with patients below 20 ($p<0,01$).

The patients in the study group were mostly those who received higher education ($p<0,001$), were married ($p<0,001$) and had three sexual partners ($p<0,05$; $p<0,001$) (see Table 1). The study of the structure of the microbial landscape in patients in study group showed the following: Among the HPV types, HPV type 16 with statistical significance prevailed ($p<0,001$), HPV type 18 was the second most common HPV type in comparison with other HPV types ($p<0.05$) (Table 2).

When the co-existing microbial agents were evaluated, it was seen that 103 (83,74%±3,33) women ($p<0,001$) had microbial agents (bacterial, viral, fungal) in addition to HPV (See Table 2).

As can be seen from the data listed in Table 2, it was detected that representatives of Mollicutes class (Mycoplasma genitalium/Mycoplasma hominis; Ureaplasma urealiticum) – 67,48±4,22% ($p<0,001$) prevailed in the co-existing microbial flora. In terms of frequency of spread, the viruses (cytomegalovirus - CMV, herpes simplex virus - HSV type 1 and 2) -% 56.10±4.47 ranked second and aerobic flora (50,41±4,51%) ranked third.

U. urealiticum ($p<0,001$) and CMV ($p<0,001$) were considerably more common;(see Table 2). A diagnosis verification was performed on the patients in the observation group based on the clinical, laboratory and diagnostic examination (Table 3).

As can be seen from the data stated in Table 3, CIN (LSIL) was more common in the patients in the study group with statistical significance in comparison with CIN II (HSIL) and CIN III (HSIL) ($p<0,001$). Also, CIN II (HSIL) was more common in comparison with CIN III (HSIL) ($p<0,001$).

The comparative analysis of morphological findings of other cervical pathologies based on HPV carrying showed that lympholeukocyte cervicitis and stromal angiomatosis were significantly more common (see Table 3).

In 53 patients with CIN I (LSIL), the viral load was seen up to 3 Lg/10×5, this shows that the risk of cervical cancer is low and indicates the existence of a temporary lesion. A comprehensive treatment including 50 mg/kg inosine pranobex daily for 10 days, and for (three times) 10 days later was performed before the biopsy.

After the diagnosis was verified morphologically, the treatment was repeated at intervals of two months according to the same regimen.

The follow-up of the treatment results included extended colposcopy and viral load measurements every 6 months within a year. Recovery was observed in 51 patients (96,23±2,62%) with Stage II (the elimination of pathological process of cervix) and who did not require treatment.

As a result, the application of Isoprinosine supported regression without destructive treatment (CIN I with viral load up to 3 Lg/10x5) in 96,23±2,62% of cases.

There were 3,5-5 Lg/10×5 viral loads showing the existence of HPV persistence in 21 patients with CIN I (LSIL), therefore they were treated with the same Isoprinosine (phase I) to reduce the viral production before the biopsy. Phase II treatment included the ablation of pathological areas by using radio wave therapy.

In the postoperational period, the treatment with Isoprinosine (phase III) was performed three times (for 10 days every two months). The control of the efficiency of the treatment included extended colposcopy, HPV test and determining the viral load within the next 6 months-1 year. A recovery was recorded in 20 patients with CIN I (95,24±4,76%).

The viral load was more than 5 Lg/10×5 in 48 women with CIN II-III (NSIL) and this indicated that the risk of cervical cancer had increased (the area of transformation – type 2,3). Such patients received Isoprinosine (phase I) as part of a combination treatment of 50 mg/kg daily for 24 days before the biopsy. The treatment with Isoprinosine was repeated one month later as the positive dynamic was not observed.

After the CIN II-III (HSIL) was verified histologically in the biopsy material, the treatment with Isoprinosine continued according to the following scheme: for 10 days – 1000 mg three times a

day, 10 days break; then the cure was repeated for 3 times as the preparation stage of radio wave loop excision. The final histological response of 14 patients with CIN II showed lower-grade dysplasia or was not verified.

This can be explained with the technique of correct biopsy under the control of colposcopy by taking out the lesion completely as well as the positive effects of Isoprinosine.

The patients were performed Isoprinosine in different ways in postoperational period (phase III) to prevent recurrence. In CIN II patients, the treatment was performed on a 10-day regimen of 1000 mg three times a day for two months, with extended colposcopy, cytological monitoring and viral load measurements for the first year. 36 patients (75,00±6,25%) were recorded to have recovered.

Inosine pranobex was applied to six women with CIN III and in situ cervical cancer for 10 days within two months – 1000 mg three times a day within the first year after the elimination of cervical pathological process.

The efficiency of the treatment was followed through cytological examination and extended colposcopy within the three-year follow-up period. Patients with CIN III and cervical in situ cancer were followed by an oncogynecologist. 5 patients (10.42 ±% 4.41) were recorded to have recovered. Therefore, the analysis of the efficiency of the comprehensive treatment of cervical pathology associated with HPV shows that the inclusion of Isoprinosine (Inosine pranobex) in the treatment program contributed to the positive effect (HPV elimination and complete recovery) in 112 patients (91,06±2,57%) which is statically significant (p<0,001).

Also, when the period required for efficient treatment is considered, it should be stated that especially the oral tablet form of Isoprinosine was well-received by the women. The affordability of the medicine enables the patients to take Isoprinosine commonly without disrupting their normal lifestyles [11, 12] and contributes to the high compliance with the treatment suggested by a physician.

Findings

1. The existence of HPV was detected in patients between 21-30 with statistical significance.
2. The study on the structure of the microbial landscape in women with cervical pathology showed the following: among types of HPV, the HPV type 16 prevailed with high statistical significance (p<0,001), the second highest one was HPV 18 in comparison with other HPV types (p<0.05).
3. It was detected that representatives of Mollicutes class prevailed in the co-existing microbial flora in patients with cervical pathology associated with HPV. In terms of frequency of spread, the viruses (cytomegalovirus - CMV, herpes simplex virus - HSV type 1 and 2) - 56.10±4.47% ranked second and aerobic flora (50,41±4,51%) ranked third.
4. In comparison with other microbial representatives, *U. urealiticum* (p<0,001) and CMV (p<0,001) were considerably more common.
5. CIN I (LSIL) was more common with statistical significance in women with cervical pathology associated with HPV in comparison with CIN II (HSIL) and CIN III (HSIL) (p<0,001).
6. The comparative analysis of morphological findings of other cervical pathologies based on HPV carrying showed that lympholeukocyte cervicitis and stromal angiomatosis were significantly more common.
7. Inclusion of Isoprinosine in the treatment program of 51 patients with CIN I (41,46±4,44%) (LSIL, at virus load of 3 Lg/10x5) was not required to eliminate the pathological process of the cervix in 96,23%±2,62 of cases.
8. It shows that the inclusion of Isoprinosine in the treatment program contributed to the positive effect (HPV elimination and complete recovery) in 112 patients (91,06%±2,57) which is statically significant (p<0,001).

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