Treatment of Cervical Intraepithelial Neoplasia by 5-Fluorouracil, Interferon or Albothyl in the Presence/Absence of High-Risk of Human Papillomavirus

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Abstract

Objectives: The primary objective of this preliminary study was to test the suitability of 5-fluorouracil ointment; interferon ointment and albothyl concentrate as alternative therapies for the treatment of cervical intraepithelial neoplasia (CIN) in the presence/absence of high risk human papilloma virus (hrHPV), as well as to compare the three treatment approaches. The secondary objective included the determination of causative factors of the genital infections and their correlation with various predictor variables.

Patients and Methods: 45 patients aged 18-64 years diagnosed with CIN were included in the study. 15 patients without hrHPV were treated with albothyl. The other 30 patients were randomly divided into two subgroups and treated either by 5-fluorouracil or by interferon. The results were evaluated by canonical correlation analysis, multiple regression, general regression model and odds ratio.

Results and Conclusion: Age, the presence of bacterial infection and number of births were the variables which showed the highest correlation with CIN and hrHPV. After a month-long treatment, total clearance was observed in 10 patients (22.2%) and lower grade lesions were observed in 22 of them (48.9%). Total clearance of hrHPV was found in 93.3% of the patients. There was no significant difference among the three therapeutic approaches. To the contrary, the type of contraception during the treatment had a significant influence on the outcome of the therapy. The obtained results confirmed that all the three selected approaches may be used successfully for the treatment of CIN lesions and represent a suitable alternative to surgical treatment.

Keywords

Bacterial infection; Non-invasive methods; Treatment modalities; Viral infection; Yeast infections

Introduction

The available treatment procedures for cervical intraepithelial neoplasia (CIN) in the presence or absence of high-risk human papillomavirus (hrHPV) may be invasive and non-invasive. Invasive treatment approaches include either ablative or excision techniques. Among ablative techniques, electrocaogulation [1], cryotherapy [2-4], and laser ablation [5,6] were in widespread use. Excisional techniques included either cold- or hot-knife conization [7,8] or a large loop excision of the transformation zone [9-11].

Non-invasive treatments include the application of cidofovir [12-14], vidarabine [15], 5-fluorouracil (5FU) [13-15], trichloroacetic acid [13], podophyllin [13], podophylotoxin [13], and interferons α, β, γ [13,16-20].

Although 5FU is commonly used as an efficient chemotherapeutic agent, its toxicity is also well-documented. Its toxicity, known to have resulted in a lethal outcome, was connected with the deficiency of one of the three critical enzymes involved in the catabolism of 5FU [21-25]. 5FU is catabolized by three consecutive enzymes of the pyrimidine degradation pathway. Dihydropyrimidine dehydrogenase (DPD) catalyzes the conversion of 5FU to FUH₂, which is further degraded to fluoro-β-ureidopropionate (FUPA) by dihydropyrimidinase (DHP), and finally to fluoro-β-alanine (FRAL) by β-ureidopropionase enzyme.

The aim of this preliminary research was to establish alternative therapies for the treatment of cervical intraepithelial neoplasia in patients who refused surgical treatment. Three agents were considered to be used for that purpose: 5-fluorouracil ointment, interferon ointment or albothyl concentrate. Their efficiency was compared and the influence of various causative factors on the outcome of the therapy was also examined. Furthermore, in order to determine the most significant factors that may promote the development of CIN and hrHPV, the incidence rates of CIN and hrHPV were correlated with the following variables: age, type of contraception, number of miscarriages, number of births and the presence of bacterial and yeast infection.

Patients and Methods

Patients

240 patients diagnosed with CIN lesions (grade I to III) or hrHPV were selected for the study. The number of patients was determined by power analysis (based on three groups, 80% strength). All the patients agreed to participate in the study by signing the written informed consent form. The study was approved by the Ethics Committee of the clinic. All the patients who experienced spontaneous regression of CIN I lesions were excluded from further study. The patients in whom no spontaneous regression of CIN I was observed over a six-month period (confirmed by two examinations every three months) remained in the study. The patients with CIN II and III who chose surgical treatment were also excluded from the study and directed to the appropriate clinic. All the above-mentioned exclusions having been made, only 45 patients aged 18-64 years remained in the study. The patients were divided into three groups and subjected to only one of the three selected therapy procedures. Since the aim of this study was to compare the three treatment approaches, the control group was not included.

Methods

All the patients were subjected to Pap test, punch biopsy and
cervical screening tests for the presence of aerobic bacteria, yeasts, *Ureaplasma urealyticum*, *Chlamydia trachomatis*, Mycoplasma, as well as hrHPV DNA typing. HPV DNA detection and typing were performed by polymerase chain reaction (PCR) according to the protocol developed by Fujinaga et al. [26].

Microbiological examination of the uterine cervix swabs was performed at the Department of Microbiology of Dr. Andrija Štampar Institute of Public Health. Two separate swab samples were used to perform bacterial and mycotic examinations. One swab sample was used for Gram stain so as to determine the number of polymorhonuclear leukocytes and the presence of multicellular yeast forms. The other swab sample was immersed into Amies transport medium and used for the cultivation of possible causative agents.

Yeasts were grown on Sabouraud Glucose Agar and Candida ID 2 chromogenic agar medium (BioMerieux, France). Nutrient agar plates were incubated at 35°C for 24 hours and at room temperature for the next 48 hours. *Candida* species were identified by a germination test, the colony appearance on chromogenic and sweet corn agar, and using a standard yeast identification system (API-Candida, BioMerieux, France). *C. dubliniensis* was identified at the Croatian Ministry of Health and Social Welfare Reference Centre for Mycological Diagnosis of Systemic and Disseminated Infections within the Croatian National Institute of Public Health. The susceptibility of candida isolates to five systemic antifungal agents (5-flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole) was determined using the standardized microdilution technique (ATB FUNGUS 3, BioMerieux, France).

All microorganisms cultured from the cervical smears were identified by routine laboratory methods, including the API test (Bio-Merieux). The diagnosis of genital mycoplasmas was confirmed using semi-quantitative tests for cultivation and Mycofast and MacoIST (Bio-Merieux), while molecular detection was performed using real-time polymerase chain reaction (PCR) (Applied Biosystem).

Bacterial vaginosis was confirmed by the standard microbiological treatment of the cervical smear samples.

Pap smears were evaluated by Bethesda system.

The patients with bacterial and/or yeast infection were treated with antibiotics and local therapy. Following the antimicrobial and antifungal therapy the patients were once again subjected to cervical screening tests and DNA typing, both of which confirmed their total clearance from bacterial/fungal infections. After that the patients were subjected to treatment with 5-fluorouracil, interferon or albothyl.

15 patients free of hrHPV DNA were treated locally with albothyl concentrate (Policresulen; Altana pharma AG, Konstanz, Germany). The other 30 patients were randomly divided into two groups and treated locally with 5-fluorouracil ointment (Efudix; Valenat pharmaceuticals, Eschborn, Germany) or interferon ointment (Interferoni unguentum 3 g, Num. Reg.: 07-3831/1-82, Immunological Institute, Zagreb, Croatia). In all the three groups the patients were treated for a month, three times per week. The therapy was applied with a swab around the uterine cervix.

Three months after the therapy all the patients underwent repeated pap smears, punch biopsy and DNA typing in order to confirm the therapy outcome.

For statistical evaluation, canonical correlation analysis, multiple regression, general regression model expressed in the form of Pareto charts of t-values and odds ratio were used. For that purpose Statistica 6.0 and MedCalc software packages were employed. Statistical significance was set at p<0.05.

**Results**

**Description of the tested population**

The tested patients ranged in age from 18 to 64 years (median age 29 years). Summary results of the variables including age, type of contraception, number of births, number of miscarriages, type of lesions before therapy, the presence of bacterial infection, the presence of yeast infection, the presence of hrHPV, type of therapy, type of lesions after therapy/hrHPV and response to therapy for the tested population are presented in Table 1.

At the first check-up 23 out of 45 individuals (51.1%) showed no bacterial infection, while in the other 22 (48.9%) individuals *Ureaplasma urealyticum*, *Chlamydia trachomatis*, *Escherichia coli*, *Beta hemolytic streptococcus* and *Gardnerella vaginalis* were determined. 24 out of 45 patients (53.3%) also suffered from persistent yeast infections.

Considering CIN lesions, the first check-up revealed the following types of the lesions: CIN I was diagnosed in 12 cases (26.7%) and CIN II in five (11.1%) cases. In 21 individuals (46.7%) CIN I + HPV were found. There were also six cases of CIN II + HPV (13.3%) and one case of CIN III + HPV (2.2%). hrHPV DNA was confirmed in 30 out of 45 patients (66.7%). Among them, nine patients (9.0%) suffered from bacterial and yeast infections. It was found that hrHPV DNA was associated equally with low and high grade lesions.

**The influence of different cofactors on the type of lesions and the presence of hrHPV**

The influence of different cofactors on the type of lesions and the presence of hrHPV was tested by canonical correlation analysis. The obtained results showed a good significant correlation (canonical R=0.759; χ²=37.5, p=0.0001) between two sets of variables. The first set included age, type of contraception, number of miscarriages, number of births, the presence of bacterial infection and the presence of yeast infection, while the second set included the type of lesions confirmed and the presence of hrHPV DNA.

According to the results of correlation analysis of the two sets of variables, the factor structure and the canonical weights, it seems that the types of lesions are highly correlated with age, the presence of bacterial infections and the presence of yeast infections, while the presence of hrHPV is highly correlated with age, the presence of bacterial infections and number of births.

**Response to therapy**

After the therapy, 28 out of 30 patients (93.3%) were negative for hrHPV DNA, while total clearance of the lesions and hrHPV DNA (nonexistence of the lesions or hrHPV DNA) was confirmed in 10 out of 45 patients (22.2%). Three of those ten patients were treated with 5FU, four with interferon and three with albothyl. The ten patients were diagnosed with CIN I lesions at the first check-up, while seven of them were also diagnosed with hrHPV. 8 out of the 10 patients who achieved total clearance used condoms as type of contraception during the therapy. Following the therapy, lower grade lesions were found in 22 out of 45 patients (48.9%), while the same grade was diagnosed in 13 (28.9%) of the total number of cases.

**5-fluorouracil:** Prior to therapy with 5FU the following lesions
Discussion

Statistical evaluation of our data confirmed that the presence of hrHPV was in high correlation with age, the presence of bacterial infection and number of births. 22 out of 30 (73.3%) hrHPV positive patients were in the 18-30 age group, with 13 patients being in the youngest age group (18-24 years). Our results were in agreement with the previously published data. Del Prete et al. [27] reported the presence of hrHPV was in high correlation with age, the presence of bacterial infection and number of births. The influence of different cofactors on the response to the therapy

According to the multiple regressions analysis, a good, statistically significant correlation was achieved ($R=0.52$; $p=0.0003$) among the following variables: age, type of contraception, type of lesions before therapy, bacterial infection, yeast infection, the presence of hrHPV and the type of therapy as the predictor variables on the one hand, and the response to the therapy as a dependent variable on the other. Among the seven predictor variables, type of contraception was the only variable with statistically significant influence on the response to the therapy ($\beta=0.35$; $p=0.0011$). A significantly better response to the therapy was obtained in the patients who used condom as type of contraception. The results of multiple regressions were in agreement with those obtained by general regression model presented as Pareto charts (Figure 1). When comparing 5FU and interferon therapy (Table 2), it was found that 5FU had 0.69 and 0.76 times lower odds of total clearance and lower grade lesions respectively, as well as 1.83 times higher odds of no response to the therapy. 95% confidence intervals revealed that none of those differences were statistically significant. When comparing albothyl and interferon (Table 2), albothyl had 0.69 times lower odds of total clearance and lower grade lesions as the treatment outcome, and 1.71 times higher odds of no response to the therapy. 95% confidence intervals revealed that none of those differences were statistically significant. When comparing albothyl and interferon (Table 2), albothyl had 0.69 times lower odds of total clearance and lower grade lesions as the treatment outcome, and 1.71 times higher odds of no response to the therapy. 95% confidence intervals revealed that none of those differences were statistically significant.
highest percentage (32.6%) of the HPV infected patients ranging from 20 to 30 years of age. Similar findings have been reported by Bardin et al. [28], who reported the highest prevalence of HPV (24.2%) in women ranging from 25 to 34 years of age. Bell et al. [29] found that the incidence of HPV infection was inversely correlated with age. In younger women (<24 years) HPV infection was significantly higher (41%, p<0.005) as compared to all the other age groups.

Our data also showed that both CIN and hrHPV were in high correlation with the presence of bacterial infections. A statistically significant correlation between the presence of bacterial and yeast infection and the appearance of CIN has been reported by Guijon et al. [30]. They suggested that CIN may be promoted by vaginal microorganisms in conjunction with human papillomavirus cervical infection. In order to find a possible correlation between different grades of CIN and the frequency of bacterial and yeast infection, Takac [31] carried out a research involving 578 patients with CIN. Bacterial or yeast infection was confirmed in 65.6% of them but its presence was not dependent on lesion grade.

Our results showed 93.3% clearance of hrHPV and 20% clearance of CIN in the patients treated by 5FU. No side effects were observed. Available data about the application of 5FU therapy were scarce and opportunistic. This antimetabolite is usually used as part of chemotherapy after the surgical treatment of human papillomavirus-associated lesions of the cervix or vagina [15,32-34].

Negative outcomes or no significant effects were reported by Holmes et al. [35]; Krebs and Helmkamp [36] and Husseinzadeh et al. [33]. Holmes et al. [35] found a lower rate of regression of cervical or vaginal HPV in patients treated with 5FU compared to placebo. Husseinzadeh et al. [33] showed that there was no significant difference in the clearance of HPV between patients who received no treatment after CO2 laser ablation and those who received 5FU treatment, while Krebs and Helmkamp [36] concluded that topical 5FU therapy, although efficient in the treatment of HPV, may lead to chronic mucosal ulcers persistent to conservative therapy.

The negative outcome reported by Krebs and Helmkamp [36] was probably connected with the toxicity of 5FU among the patients deficient in one of the three enzymes responsible for 5FU metabolism. Severe mucositis, thrombopenia, anemia, leukopenia, total alopecia, scaling and erythrodermia of the skin with lethal outcome were reported by van Kullenburg et al. [24,25].

Contrary to the abovementioned results, Kirwan and Naftalin [37] reported complete clearance of vaginal intraepithelial neoplasia in 13 out of 14 patients treated with 5FU for at least ten weeks (once weekly). Brodman et al. [32] reported 88% clearance of hrHPV and 59% clearance of CIN in the patients postoperatively treated by 5FU for eight weeks. Niwa et al. [15] found 50% clearance of hrHPV following the therapy with 5FU of the patients who have previously been unsuccessfully treated with vidarabine. Mainan et al. [34] showed that intra-vaginal application of 5FU therapy successfully reduced the recurrence of cervical intraepithelial neoplasia in HIV-infected women.

In our study 100% clearance of hrHPV DNA was observed after the therapy with the interferon ointment. Total clearance of the lesions was found in 26.7% of the patients, while 46.7% of them had partial response following the therapy. Gonzales-Sanchez et al. [20] reported a positive response to the therapy in 79% of the patients diagnosed with hrHPV following the therapy with interferon-β. Somewhat better results were observed in the patients also diagnosed with CIN. Subcutaneous injections of interferon-α [16] in the patients with persistent intraepithelial neoplasia of the vulva, vagina and cervix resulted in complete remission of the cervical lesions. Sikorski and Zrubek [18] reported complete clearance of the epithelial lesions and remission of HPV infections in 53% of the patients and partial response in 23.5% of them after the therapy with interferon gamma. Long-term efficiency of the therapy was also confirmed through a five-year follow-up study [19]. Iwasaka et al. [17] reported improvement of the lesions in all patients after the treatment with interferon γ, while total clearance was observed in 62.5% of the patients.

Our own results and the available literature data indicate that the highest incidence of hrHPV infection was found among young fertile women (age 18-30 years). The prevalence of CIN and hrHPV infection was in high correlation with (or possibly promoted) the presence of bacterial infection, especially in the presence of Ureaplasma urealyticum. Stress factors like birth and/or miscarriage also played a significant role in the development of CIN and hrHPV infections. Our preliminary results revealed that all the three applied treatment approaches may be used as alternative therapies for the management
of CIN instead of surgical treatment. Although some authors were concerned about the possibility of poor absorption of the interferon ointment, the concern proved unfounded, as our results confirmed the adequate absorption. There was no significant difference among 5FU, interferon and albothyl in the efficiency of CIN management. Our results concerning the outcome of the therapy were consistent with the relevant literature data.

References


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