Herpes Genitalis and its Relationship to Cervical Cancer

Raymond H. Kaufman, M.D. and William E. Rawls, M.D.

A considerable volume of literature has accumulated in the past decade, suggesting an association between herpes simplex virus type 2 (HSV-2) infections of the genital tract and the development of cervical carcinoma. The following article reviews the clinical features of herpes genitalis and evaluates whether this virus plays a role in the genesis of human cancer.

Clinical Features

The incidence of herpes genitalis is far greater than generally suspected. Approximately nine percent of patients seen in private gynecologic practice and 22 percent of those treated in gynecologic clinics of city-county hospitals have serologic evidence of prior herpesvirus type 2 infection. These figures are even higher in the Health Department's Social Hygiene Clinics. Recent studies also confirm the venereal nature of herpes genitalis and its frequent association with Haemophilus vaginalis vaginitis, trichomoniasis and condyloma acuminatum. The majority of patients with primary herpes infections are teenage girls and unmarried women.

Infection with the herpes simplex virus can present as either a primary or recurrent disease; our experience shows that primary infections are more frequently encountered. Additionally, despite the general impression that all genital herpes infections are caused by the type 2 virus, we have found that approximately 13 percent are caused by the type 1 strain. Herpesvirus type 1, which causes infections of the oral-pharyngeal area, is closely related to the type 2 virus, but has different biologic and epidemiologic characteristics. Nahmias et al. have also noted a smaller, but still significant, number of patients with type 1 infections involving the genitalia.

Primary Herpes Genitalis

Primary herpesvirus type 2 is usually acquired through sexual contact. It may produce symptoms generally within three to seven days after exposure, although symptoms have occasionally occurred in less than 24 hours. Before the lesion becomes visible, the patient may experience mild paresthesia and a burning sensation. Once the lesion develops, headache, generalized malaise associated with low-grade fever are common. The patient may also complain of rather severe vulvar pain and tenderness, as well as discomfort due to inguinal lymphadenopathy. Urination may be exceedingly painful and urinary
retention can develop. In most cases, these symptoms are due to herpesvirus type 2 infections of the urethra and bladder. Severe herpes cervicitis often produces a profuse watery discharge.

On the other hand, primary herpes genitalis may be relatively mild or even completely asymptomatic, as evidenced by the number of patients with antibodies against the type 2 virus who revealed no past history of infection. This may possibly be explained by the fact that individuals who have had prior type 1 herpes infections may be somewhat protected against subsequent type 2 infections.

Primary herpes infections usually present as extensive lesions, involving large areas of the vulva, perineum, perianal region, vagina or cervix. Multiple vesicles form early in the course of disease and rapidly develop into shallow, moist ulcerations, often surrounded by a red areola. Indurated papules with surface ulcerations may be noted. Herpes lesions can coalesce, producing bullae and larger ulcerations, possibly involving the vulva and perianal skin. Extensive vulvar involvement is usually associated with erythema and varying degrees of edema, particularly of the labia minora and foreskin of the clitoris. Superficial ulcerations may also be found on the ectocervix and vagina and generally exhibit a grayish membrane of collapsed epithelium. On rare occasions, a fungating, necrotic-appearing mass covering all or portions of the ectocervix can be easily confused with cervical carcinoma. Primary herpes genitalis persists for three to six weeks with no residual scaling or ulceration after healing.

Recurrent Herpes Genitalis

Some patients with primary herpes genitalis never develop recurrent episodes, while others have “flare-ups” for many years. Although factors such as fever, emotional disturbances, premenstrual tension or severe systemic disease were thought to be associated with recurrent herpesvirus, they are probably unrelated to the development of this infection.

Recurrent herpes lesions are often inconspicuous and difficult to identify; on casual inspection they may be entirely overlooked. Recurrent lesions of the cervix and vagina appear similar to those of a primary infection. Lesions of the vulva vary from 1 to 5 mm. in diameter. They are vesiculo-ulcerative in nature, have an erythematous base and frequently develop in small localized patches. Vesicles rapidly rupture within 24-48 hours, forming superficial ulcers. Several lesions may coalesce, producing larger vesicles or bullae and, subsequently, larger ulcerations. Healing usually takes place in seven to 10 days and leaves the vulva with a completely normal appearance. However, secondary bacterial infection may delay healing and produce inguinal lymphadenopathy. Patients with recurrent herpes infections complain of vulvar pain as well as burning on urination, but these symptoms are much less severe than those experienced with a primary infection.

Most patients with recurrent herpes genitalis have involvement of the cervix. A recent study revealed that 80 percent of women with vulvar lesions had positive cervical cultures, but many had no clinical evidence of cervical infection.

In a study of 81 patients, an excellent correlation was noted between the clinical diagnosis of herpesvirus and laboratory confirmation. We also found an accurate correlation between clinical and laboratory diagnosis of primary and recurrent infection by relating serial changes in the serum antibody titer to type 1 and 2 herpes simplex viruses. This correlation was confirmed in over 90 percent of cases studied.

During pregnancy, herpes genitalis presents a special problem to the clini-
Treatment

Until recently, treatment for herpesvirus infections consisted of hot sitz baths, applications of an antibiotic cream to prevent secondary infection and analgesics. The use of gauze sponges soaked in ether and applied to the lesion has also been of some benefit. We are now treating patients with 0.1 percent proflavine followed by immediate and repeat exposure in 18-24 hours to incandescent or fluorescent light at a distance of six inches. The tricyclic dyes, proflavine and neutral red, are incorporated into the virus during replication so that exposure to light results in inactivation. In 1973 we reported the results of this treatment in 48 patients. (Table 1.) Of 20 patients with primary disease, eight had immediate relief of symptoms; of those with recurrent infections, 15 had immediate relief.

While treating primary infections with photoinactivation, we noted that although many patients reported rapid relief of symptoms, the objective clinical course of the disease did not seem to be altered. Ulcers were persistent and in most women continued to develop despite treatment. As these new lesions were treated, symptoms were again relieved. Whereas in recurrent infections, once the symptoms were relieved, the ulcers rapidly dried up and disappeared.

The above study was performed using a single application of dye followed by exposure to light. If the patients had been given a second treatment with light, approximately 18 hours following the application of dye, more favorable results could have been achieved. Immediate exposure to light inactivates the preformed virus which has taken up the dye; 18 hours later any new infectious virus, even if located intracellularly.
would also have the dye incorporated into its structure and would therefore be destroyed by light. Rapp has raised the possibility of a theoretical hazard using photodynamic inactivation of the virus. He points out that although it sharply reduces the activity of herpesviruses types 1 and 2, the noninfective and probably defective viruses are still able to "transform" normal mammalian cells into cells with heritable characteristics and a loss of contact inhibition—properties often associated with malignant potential. Rapp postulated that elimination of viral infectivity may reveal the oncogenicity of herpesvirus and other DNA viruses that possibly cause cervical and other cancers. Melnick and Rawls have disputed Rapp's theory by questioning whether the untreated infections are more hazardous than those treated by photoinactivation. They feel that continued and repeated assault of the same area with an abundance of defective viruses, produced under natural conditions, may well constitute a greater risk of oncogenesis than infections in which the replication of the virus is shortened by photoinactivation. They also question whether current experimental studies performed on hamster kidney cells grown in tissue culture can be applied to humans.

**Herpesvirus and Cervical Cancer**

Cervical cancer is more prevalent in women from lower socioeconomic groups, and those who begin heterosexual activity early in life, marry early and have many sexual partners. Although many of these attributes are interrelated, age at first intercourse and the number of sexual partners appear to correlate with the highest risk of developing cancer.

| Study Area             | Relative Risk of Invasive Cancer | Relative Risk of Carcinoma in situ | Relative Risk of Dysplasia |
|------------------------|----------------------------------|-----------------------------------|---------------------------|
| Brussels, Belgium      | 10.3                             | -                                 | -                         |
| Copenhagen, Denmark    | 6.1                              | -                                 | -                         |
| Chicago, Illinois      | 4.5                              | -                                 | -                         |
| Atlanta, Georgia       | 9.0                              | 7.0                               | 5.8                       |
| Uganda                 | 4.7                              | -                                 | -                         |
| Prague, Czechoslovakia | 3.8                              | 4.3                               | 5.0                       |
| Houston, Texas**       | 3.4                              | -                                 | -                         |
| Houston, Texas***      | 2.8                              | 4.1                               | 2.7                       |
| Yugoslavia             | 1.9                              | -                                 | -                         |
| Auckland, New Zealand  | 1.5                              | -                                 | -                         |
| Boston, Massachusetts  | -                                | 3.8                               | -                         |

| Mean Values            | 4.8                              | 4.7                               | 4.5                       |

*Relative risks from selected studies are shown, in order to avoid distortions, two studies with high relative risks and three studies with low relative risks are not included.
**White patients and controls.
***Black patients and controls.
Since the factors associated with a high risk of cervical cancer are similar to those associated with an increased risk of venereal disease, it is postulated that cervical cancer may be caused by a venereally transmitted virus.

Cytologic screening of cervical secretions in patients with cervical cancer revealed cells with intranuclear inclusions characteristic of herpesvirus infections. Other efforts to isolate viruses from genital secretions and tissues have predominantly yielded herpesviruses, mainly herpesvirus type 2.

Like other venereally transmitted diseases, herpesvirus type 2 occurs most frequently in patients in the second and third decades of life. Cervical cancer is generally found in women in the fourth and fifth decades of life. These observations are consistent with the theory that viral infections may initiate changes in the cervical epithelium which manifest as invasive cancer after a latent period of approximately 20 years.

Retrospective case-controlled studies have also shown that significantly more neutralizing antibodies to herpesvirus type 2 were found in women with invasive cancer than in controls. Indeed, it has been reported that at least one-third and possibly all patients with cervical cancer have antibodies to the herpesvirus, while 20-80 percent of controls had antibodies. It must be remembered that current antibody assay techniques are still in their infancy, and a number of women may have antibodies to herpesviruses that cannot be detected by present assay methods.

Based on information available, however limited, it is interesting to compare the risk of cervical cancer in relation to the presence of herpesvirus antibodies. (Table 2.) Patients with antibodies to herpesvirus type 2 were as much as 10 times more likely to have invasive cancer than women without antibodies to the virus. Furthermore, women with herpesvirus antibodies were up to seven times more likely to have carcinoma in situ, and as much as six times more likely to have severe dysplasia than women without antibodies to the herpesvirus.

If infection with herpesvirus type 2 in the second and third decades of life does initiate cancer, an association between premalignant cervical lesions and antibodies to the virus would also be expected. In fact, the mean values of the estimated relative risk for premalignant and invasive cancers are quite similar—4.8 for invasive cancer, 4.7 for carcinoma in situ and 4.5 for severe dysplasia. Thus, while there is variation in the results of seroepidemiologic studies, most show an association between genital herpesvirus and the development of cervical cancer.

Is this association between herpesvirus and cervical cancer due to similar sexual patterns, or is the herpesvirus an etiologic agent in the development of cervical cancer? Two approaches were used to evaluate the role of sexual behavior. One studied the incidence of venereal diseases in women with cancer and in controls. No differences were found in the incidence of trichomoniasis or syphilis, but marked differences were found in the occurrence of herpesvirus type 2 between the two groups. The second approach examined the influence of sexual promiscuity on the distribution of antibodies to herpesvirus type 2 in cancer patients and controls. Differences in antibody activity between the two groups could not be accounted for solely in terms of sexual behavior. Adam et al. found a relationship between early age at first intercourse, multiple sexual partners, and the development of cervical cancer. Early age at first intercourse seems to be a more significant factor than multiple sexual partners. (Table 3.) However, as shown in Table 4, the risk of cervical cancer is linked more closely to the presence of herpesvirus antibodies than to either age
at first intercourse or number of sexual partners. Such studies suggest that antibodies to herpesvirus type 2 and cervical cancer are not simply covariates of sexual promiscuity.

Another explanation for the association between herpesvirus type 2 and cervical cancer is that cancer increases the susceptibility of the cervical epithelium to infection by the virus. Catalano and Johnson collected serum samples from healthy women in order to monitor early changes in the cervical epithelium. Antibodies to herpesvirus type 2 were found in 36 percent of women who subsequently developed carcinoma in situ and in only seven percent of those who did not develop cervical abnormalities. In another study, 871 women with herpes genitalis (confirmed by virus isolation),
cervical cytology or serological tests) and 562 controls were followed from one to six years for the development of cervical anaplasia. Cervical dysplasia developed twice as often, and carcinoma in situ developed eight times as often, in women with herpes genitalis compared to controls. The results of these studies indicate that infection by the virus precedes neoplastic changes in the cervix.

If evidence of herpesvirus genetic information could be repeatedly demonstrated in cervical cancer cells, and if a relationship between virus and cancer cells, similar to that already demonstrated experimentally, could be induced, a very strong link between herpesvirus and the genesis of human cancer would be established.

Using immunofluorescence, herpesvirus antigens have been detected in cells exfoliated from premalignant lesions of the cervix, as well as carcinoma in situ. The virus was not apparent under normal culture conditions, but active viral replication occurred when the cells were held under alkaline conditions. Further support for the presence of the virus comes from studies in which a soluble antigen, extracted from cancers of the vagina, vulva and cervix, reacted with antiserum against semi-purified herpesvirus type 2. Finally, sequences of herpesvirus type 2 DNA were found by nucleic acid hybridization techniques in DNA extracted from cells of one case of cervical cancer.

Less direct evidence demonstrating herpesvirus type 2 genetic information in cervical cancer comes from analyses of antibody activity to "virion" (structural) and "nonvirion" (nonstructural) antigens induced by the virus. For example, neutralizing antibodies, thought to be directed against virion antigens, fluctuate depending on the disease state. Increasing titers of neutralizing antibody were found in women with persisting or progressing cervical dysplasia. Following therapy of preinvasive lesions, anti-body activity to herpesvirus type 2 decreased. Recently, women with invasive cervical cancer were found to have antibodies to nonvirion antigens. Since antibodies to the nonvirion antigens do not appear following an acute infection with herpesvirus, their presence implies that sufficient amounts of the antigens are present in the cancer tissue to bring about an immune response.

In addition, herpesvirus type 2 can transform hamster cells in vitro. The cells are able to produce malignant tumors when injected into the animal. Further studies have revealed that various strains of herpesvirus type 2 differ in their ability to produce such transformation. Even some strains of herpesvirus type 1 can transform hamster cells. These observations, as well as experimental tumor induction by other herpesviruses, support the concept that herpesvirus type 2 may be capable of inducing cancer in humans.

Summary

A review of the data suggests that there is some relationship between herpesvirus type 2 and the development of cervical cancer. Indeed, recent evidence points to an even more widespread association between herpesviruses and cancer. For example, herpesvirus particles have been visualized by electron-microscopy in prostate cancer cells; viral antigens could also be detected by immunofluorescence. Antibodies to herpesvirus "nonvirion" antigens found in the sera of patients with cervical cancer have also been found in patients with other types of cancer such as squamous cell carcinoma, especially of the head and neck. While these observations are preliminary, they support the concept that herpesvirus type 2 is an etiologic agent in the development of cervical cancer, and suggest that herpesviruses types 1 and 2 may also play a ubiquitous role in the genesis of a number of other human cancers.
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