Detection of HPV infection by analyzing the changes in structure of peripheral blood lymphocytes specifically induced by HPV E7 antigen

K. C. Chao, *M.D.*; **P. H. Wang,** *M.D., Ph. D.*; **M. S. Yen,** *M.D.*; **C. C. Chang,** *M.S.*

Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, and National Yang-Ming University School of Medicine, Taipei (Taiwan)

Summary

Purpose: Detection of human papilloma virus (HPV) infection in clinical practice was examined based on the observation that peripheral blood lymphocytes exposed in vitro to antigenic or mitogenic stimulation change their intracellular structures as measured by polarization of fluorescent light emitted by labeled cells.

Materials and Methods: A total of 47 women were enrolled in this study. They were classified into four groups based on the results of HPV-DNA detection in cervical tissues by the Hybrid Capture II kit (Digene, Gaithersburg, MD, USA) and pathological examination. Ten women with no HPV-DNA detection were used as a normal control group. Fifteen women without pathological diagnosis in the cervical tissues had HPV-DNA detection. Ten women with CIN lesions had 80% HPV-DNA detection. Twelve women with invasive squamous cell carcinoma had 100% HPV detection. Peripheral blood lymphocytes derived from all women were collected and then exposed to HPV-E7 antigen and PHA mitogen.

Results: The positive response rate of HPV-E7 antigen was ten percent (1/10) in the normal control group, 73.3% (11/15) in the HPV infectious women, 50% (5/10) in the CIN women, and 91.7% (11/12) in the cervical cancer patients. The overall sensitivity rate of blood tests was 77.1% and the specificity rate was 57.8% when the Hybrid Capture II HPV Test kit was used as the standard detection method for cervical tissue.

Conclusions: The results showed that peripheral blood lymphocytes derived from patients with cervical lesions might be another choice to be used as a screening method to detect HPV infection compared with conventional methods.

Key words: HPV; CIN; Cervical cancer.

Introduction

Detection of human papilomavirus (HPV) infection was examined based on the observation that cells exposed in vitro to antigenic or mitogenic stimulation change their intracellular structure as measured by polarization of fluorescent light emitted by labeled cells [1, 2]. Conventionally, HPV infection has been detected and confirmed by specific HPV typing primers for polymerase chain reaction [3, 4] or screened by commercial kits, for example, the Hybrid Capture II HPV test kit (Digene Corp., Gaithersburg, MD, USA) either from Pap smear cells or from cervical tissue biopsies with/without colposcopic guidance [5, 6]. Although Pap smears have been encouraged for all women who are sexually active, undoubtedly a relatively high percentage of women refuse to undergo Pap smear examination [7-9]. In Taiwan, only 30% of women have an annual Pap smear [10] which has resulted in a relatively high prevalence of cervix squamous cell carcinoma with an incidence of 22 per 100,000 women. The very close correlation between HPV and cervical cancer has been noted for years [5, 6], so the use of blood tests to detect the high risk of HPV infection would be a more acceptable screening method in these women. Some strategies could be used as a tool to detect HPV infection in blood. Different serum antibodies to HPV infection have been successfully used as a tool to detect HPV infection in women with cervical cancer [11, 12]. Moreover, the antibody to HPV infection could be used. HPV detection was determined by observing the differences in lymphocyte activation between individuals with and without virus infection.

The CellScan (Medis-El, Israel) is a highly precise static cytometry system that records changes in intensity and polarization of fluorescent light emitted by FDA (fluorescein diacetate) labeled cells [13, 14]. This apparatus facilitates polarization measurements and can analyze cells stimulated by a specific antigen. In this study we used the CellScan system to detect HPV infection and to evaluate its sensitivity and specificity.

Materials and Methods

A total of 47 women with or without HPV infection were enrolled in this study. They were classified into four groups based on the results of HPV-DNA detection in the cervical tissue by the Hybrid Capture II HPV test kit (Digene, Gaithersburg, MD, USA) and pathological examination. Ten women without HPV-DNA detection were used as a normal control group. Fifteen women without pathological diagnosis in the cervical tissues who had positive HPV-DNA detection were classified as the HPV infection group. Eight of the ten women with pathologically proven cervical intraepithelial neoplasm (CIN)

Revised manuscript accepted for publication August 21, 2002

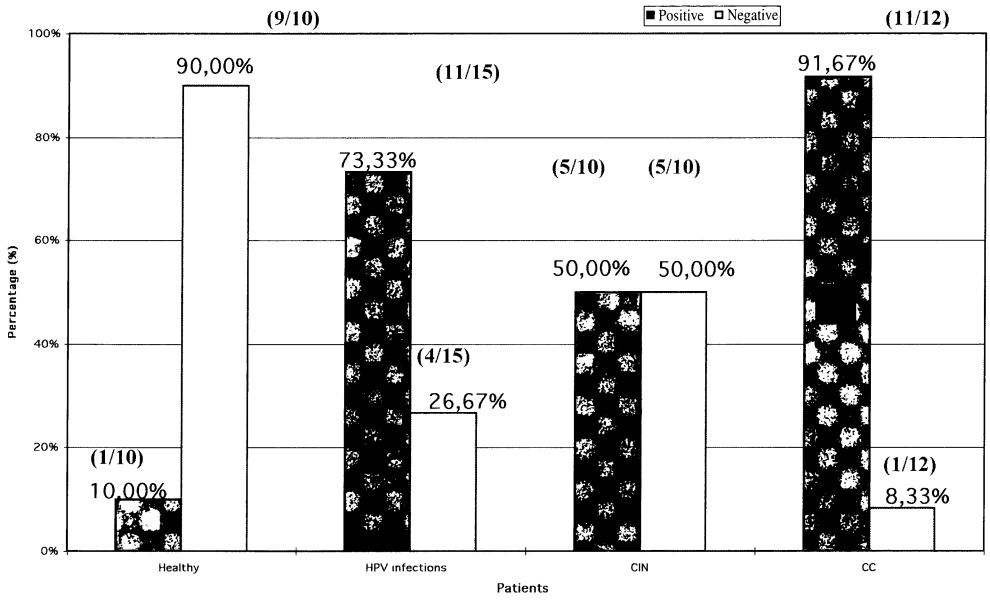

Figure 1. — Results of CellScan.

lesions were detected to have HPV infection (80%). All 12 women with invasive squamous cell carcinoma were found to have 100% HPV infection. All patients signed consent forms according to the guidelines of the Human Ethics Committee in the Department of Obstetrics and Gynecology, Taipei Veterans General Hospital (the National Medical Center of Taiwan) for study and received peripheral blood examinations.

To process cells exposed in vitro to antigenic or mitogenic stimulation and observe the change of their intracellular structures, two technically demanding steps were done including lymphocyte separation and a series of fluorescence polarization measurements. Other blood cells and control lymphocytes were separated from 20 ml heparinized blood following 30 minutes' incubation with carbonyl iron (0.1g) (Sigma) and separation on a step Ficoll-Hypaque (Pharmacia) gradient. Lymphocytes adjusted to 6 x 10^6 cells per ml were left overnight.

Of each sample 90 µl, with 10 µl of glucose (1 mM), were incubated separately for 30 min with 10 µl of E7-HPV (1 ng/ml) antigen, and 10 µl of PHA (0.9 mg/ml). Base-line samples (P0) were incubated in parallel with 10 µl of PBS. Then 30 µl of FDA (5 uM in final concentration) were then added to each sample. The samples were scanned on the CellScan system [13] following a 5-minute incubation at room temperature.

Three repeat scans were done and variability did not exceed 0.5%.

Results

Forty-seven patients were examined in this study. We found that nine of ten (90%) healthy women showed a negative response to E7-HPV antigen and only one (10%) was positive. We found a 73.33% (11/15) positive rate in tissue-proven HPV infected patients. In the patients with CIN (n = 10) in contrast, we had only a 50% positive detection rate. This result showed a 62.5% sensitivity (5/8) and 100% specificity (5/5). The patients with cervical cancer expressed high sensitivity to E7-HPV antigen stimulation with a 91.7% sensitivity rate (11/12) and 100% specificity rate. In this study the overall sensitivity of all patients was 77.1% (27/35) and the specificity was 91.7% (11/12) when we used the Hybrid Capture II HPV test kit method as a standard control. The positive predictive value was 96.4% (27/28) and the negative predictive value was 57.9% (11/19). All data are shown in Figure 1.

Discussion

HPV infection was closely related to cervical cancer development which is well known in the literature [5, 6, 11, 12, 15-21]. In this study we used another strategy – by the detection of changes in the structure of lymphocytes specifically induced by HPV-E7 antigen to evaluate the sensitivity and specificity in detecting HPV infection. As a result, overall there were 40.4% of patients (19/47) whose lymphocytes did not respond to HPV-E7 stimulation. In the group of patients with HPV tissue infection, 22.9% (8/35) showed a negative response to HPV-E7 antigen stimulation. In contrast, in the group of patients without HPV tissue infection, 91.7% (11/12) showed a negative response to HPV-E7 antigen stimulation. Our study showed a relatively high correlation between HPV tissue infection and peripheral blood lymphocytes responding to HPV-E7 antigen stimulation. In patients with HPV tissue infection we were able to use this strategy to observe a positive change, up to 77.1%, which was not inferior to other methods reported in the literature. In fact, there was a 46% to 55.5% positive rate of HPV infection detected in the CIN patients [15-20]. More specifically, in the patients with cervical cancer, up to 90% were found to have peripheral blood lymphocytes showing a positive response to

HPV-E7 antigen stimulation which was equally valuable when compared to other reported methods in the literature [19, 21].

In this study we used a more convenient and acceptable method to detect the possibility of HPV infection, although it could not distinguish high-risk HPV groups from lower risk patients. However, in patients with a high risk of future cervical cancer, it could be used as a routine check-up to evaluate the status of HPV infection. This approach is also suitable to screen HPV infection in cervical cancer patients.

References

[1] Cercek L., Cercek B.: "Application of the phenomenon of changes in the structuredness of cytoplasmic matrix (SCM) in the diagnosis of malignant disorders: a review." *Eur. J. Cancer*, 1977, *13*, 903.

[2] Deutsch M., Ron I., Weinreb A., Tirosh R., Chaitchik S.: "Lymphocyte fluorescence polarization measurements with the CellScan system: application to the SCM cancer test". *Cytometry*, 1996, *23*, 159.

[3] Kwasniewska A., Gozdzicka-Jozefiak A., Postawski K., Miturski R.: "Evaluation of DNA mismatch repair system in cervical dysplasias and invasive carcinomas related to HPV infection". *Eur. J. Gynaecol. Oncol.*, 2002, *23*, 231.

[4] Bekkers R. L. M., Melchers W. J. G., Bulten J., Boonstra H., Quint W. G. V., Hanselaar A. G. J. M., Massuger L. F. A. G.: "Localized distribution of human papillomavirus genotypes in the uterine cervix". *Eur. J. Gynaecol. Oncol.*, 2002, *23*, 203.

[5] Law K. S., Chang T. C., Hsueh S., Jung S. M., Tseng C. J., Lai C. H.: "High prevalence of high grade squamous intraepithelial lesions and microinvasive carcinoma in women with a cytologic diagnosis of low grade squamous intraepithelial lesions". *J. Reprod. Med.*, 2001, *46*, 61.

[6] Lin C. T., Tseng C. J., Lai C. H., Hsueh S., Huang H. J., Law K. S.: "High-risk HPV DNA detection by Hybrid Capture II. An adjunctive test for mildly abnormal cytologic smears in women > or = 50 years of age". *J. Reprod. Med.*, 2000, *45*, 345.

[7] Juang C. M., Wang P. H., Yen M. S., Lai C. R., Ng H. T., Yuan C. C.: "Application of tumor markers CEA, TPA, and SCC-Ag in patients with low-risk FIGO stage IB and IIA squamous cell carcinoma of the uterine cervix". *Gynecol. Oncol.*, 2000, *76*, 103.

[8] Chao H. T., Wang P. H., Tseng J. Y., Lai C. R., Chiang S. C., Yuan C. C.: "Lymphocyte infiltrated on FIGO stage IIB squamous cell carcinoma of the cervix is a prominent factor for disease-free survival". *Eur. J. Gynecol. Oncol.*, 1999, *20*, 136.

[9] Wang P. H., Li Y. F., Juang C. M., Lee Y. R., Chao H. T., Tsai Y. C., Yuan C. C.: "Altered mRNA expression of sialyltransferase in squamous cell carcinomas of the cervix". *Gynecol. Oncol.*, 2001, *83*, 121.

[10] Yuan C. C., Wang P. H., Lai C. R., Yen M. S., Chen C. Y., Juang C. M.: "Prognosis-predicting system based on factors related to survival of cervical carcinoma". *Int. J. Gynecol. Obstet.*, 1998, *63*, 163.

[11] Heim K., Widschwendter A., Pirschner G., Wieland U., Awerkiew S., Christensen N. D. *et al.*: "Antibodies to human papillomavirus 16 L1 virus-like particles as an independent prognostic marker in cervical cancer". *Am. J. Obstet. Gynecol.*, 2002, *186*, 705.

[12] Silins I., Avall-Lundqvist E., Tadesse A., Jansen K. U., Stendahl U., Lenner P. *et al.*: "Evaluation of antibodies to human papillomavirus as prognostic markers in cervical cancer patients". *Gynecol. Oncol.*, 2002, *85*, 333.

[13] Merimsky O., Kaplan B., Deutsch M., Tirosh R., Chaitchik W. A.: "Detection of melanoma by monitoring the intracellular fluorescein fluorescence polarization changes in lymphocytes". *Cancer Det. Prev.*, 1996, *21*, 167.

[14] Klein O., Lin S., Embon O., Sazbon A., Zidan J., Kook A. I.: "An approach for high sensitivity detection of prostate cancer by analysis of changes in structure of the cytoplasmic matrix of lymphocytes specifically induced by psa-act". *J. Urol.*, 1999, *161*, 1994.

[15] Hennig E. M., Di Lonardo A., Venuti A., Holm R., Marcante M. L., Nesland J. M.: "HPV 16 in multiple neoplastic lesions in women with CIN III". *J. Exper. Clin. Cancer Res.*, 1999, *18*, 369.

[16] Lertworapreecha M., Bhattarakosol P., Niruthisard S.: "Detection and typing of human papillomavirus in cervical intraepithelial neoplasia grade III in Thailand women". *Southeast Asian J. Tropical Med. Public Health*, 1998, *29*, 507.

[17] Konno R., Paez C., Sato S., Yajima A., Fukao A.: "HPV, histologic grade and age. Risk factors for the progression of cervical intraepithelial neoplasia". *J. Reprod. Med.*, 1998, *43*, 561.

[18] Takac I., Marin J., Gorisek B.: "Human papillomavirus 16 and 18 infection of the uterine cervix in women with different grades of cervical intraepithelial neoplasia (CIN)". *Int. J. Gynecol. Obstet.*, 1998, *61*, 269.

[19] Arends M. J., Buckley C. H., Wells M.: "A etiology, pathogenesis, and pathology of cervical neoplasia". *J. Clin. Pathol.*, 1998, *51*, 96.

[20] Siritantikorn S., Laiwejpithaya S., Siripanyaphinyo U., Auewarakul P., Yenchitsomanus P., Thakernpol K., Wasi C.: "Detection and typing of human papilloma virus DNAs in normal cervix, intraepithelial neoplasia and cervical cancer in Bangkok". *S.E. Asian J. Tropical Med. Pub. Health*, 1997, *28*, 707.

[21] Klaes R., Woerner S. M., Ridder R., Wentzensen N., Duerst M., Schneider A. *et al.*: "Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes". *Cancer Res.*, 1999, *59*, 6132.

Address reprint requests to:
Kuan-Chong CHAO, M.D.
Chief of Gynecology,
Department of Obstetrics
and Gynecology,
Taipei Veterans General Hospital, 201,
Section 2, Shih-Pai Road, Taipei 112,
(Taiwan)