Human Papillomavirus DNA in Plasma of Patients with HPV16 DNA-positive Uterine Cervical Cancer

Takako Shimada1*, Naohiro Yamaguchi2, Noriyuki Nishida2, Kentaro Yamasaki1, Kiyonori Miura1, Shigeru Katamine3 and Hideaki Masuzaki1

1Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, 2Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Medicine and 3Nagasaki University Graduate School, Nagasaki, Japan

*For reprints and all correspondence: Takako Shimada, Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
E-mail: shimachan-ngs@umin.ac.jp

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Objectives: The squamous cell carcinoma antigen is considered the most accurate serologic tumor marker for uterine cervical carcinoma. However, serum squamous cell carcinoma antigen levels were found to correlate significantly with clinical severity of atopic dermatitis and chronic renal failure. The present study was conducted in patients with human papillomavirus 16 DNA-positive uterine cervical cancer to determine the plasma level of human papillomavirus 16 DNA and the diagnostic values of plasma human papillomavirus DNA in these patients.

Methods: Forty-three human papillomavirus 16-positive patients with cervical intraepithelial neoplasia or uterine cervical squamous cell carcinoma were recruited in this study. The diagnosis was cervical cancer in 20 patients, high-grade squamous intraepithelial lesions in 21, low-grade squamous intraepithelial lesions in 1 and negative for intraepithelial lesion or malignancy in 3 patients. Before any treatment, blood samples were collected from all patients. For analysis of human papillomavirus DNA in plasma of patients with cervical cancer, quantitative polymerase chain reaction fluorescent assay for human papillomavirus 16 was performed using human papillomavirus 16 primers and SYBR Green dye using the LightCycler 480 SW1.5 apparatus.

Results: Plasma human papillomavirus 16 DNA was detected in only 30.0% of the patients with human papillomavirus 16-positive cervical cancer and in none of normal controls. The copy number of plasma human papillomavirus 16 DNA was higher in patients with invasive cancer than in those with cervical intraepithelial neoplasia (CIN3), micro-invasive cancer and in normal individuals.

Conclusions: These results indicated that the plasma human papillomavirus DNA level could be potentially used as a marker of low-invasive cervical cancer tumors in patients with normal squamous cell carcinoma antigen levels before treatment.

Key words: gynecol-basic — genetics-cancer genetics — diagnosis

INTRODUCTION

The squamous cell carcinoma antigen (SCCA), an SCC tumor-associated protein, was first discovered in uterine cervical SCC by Kato and Torigoe (1) in 1977. SCCA is expressed not only in SCC but also in normal squamous epithelium (2) and considered the most accurate serological marker of uterine cervical carcinoma. However, serum levels of SCCA have been reported also to correlate with the clinical severity of atopic dermatitis, chronic hypertension and chronic renal failure (3).

Epidemiological studies have provided data on the incidence of human papillomavirus (HPV) infection and the risk factors for HPV infection and genital precancerous lesions (4–6). The long duration of HPV infection is attributed to the ability of the virus to subvert innate immune responses (7,8). Persistent
infection with HPV confers a strong risk for the development of subsequent neoplasia (9,10). Several groups have examined the prevalence of HPV DNA in plasma of cervical cancer patients, although there is discrepancy in the results of these studies (11–14). The different prevalence rates could be due to the different populations examined, different HPV types and different methods used for the detection.

The present study was designed to determine the prevalence of HPV16 DNA in plasma of patients with HPV16 DNA-positive uterine cervical cancer and its diagnostic and prognostic value in these patients.

PATIENTS AND METHODS

PATIENTS AND SAMPLE COLLECTION

This study was approved by the Human Ethics Committee of Nagasaki University Hospital. Forty-three HPV16-positive patients with cervical intraepithelial neoplasia (CIN) or uterine cervical SCC were recruited in this study. All patients were examined and/or treated at Nagasaki University Hospital between December 2007 and June 2008. Each patient underwent pelvic examination followed by conventional cervical cytology and determination of the serum SCCA level (see below). After obtaining informed consent, specimens for HPV typing were harvested (SurePath and CytoRoch, MBL) and evaluated for HPV DNA by polymerase chain reaction (PCR).

Table 1 shows the stage of cervical cancer and treatment modalities. The diagnosis was cervical cancer in 20 patients, high-grade squamous intraepithelial lesions in 21, low-grade squamous intraepithelial lesions in 1 and negative for intraepithelial lesion or malignancy (NILM) in 3 patients. The stage of cervical cancer was CC Ia1 in 4 patients, CC Ia2 in 1, CC Ib1 in 2, CC Ib2 in 2, CC IIa in 4, CC IIb in 4, CC IIIb in 1, CC IVa in 1 and CC IVb in 1, according to the International Federation of Gynecology and Obstetrics (FIGO) staging system. We also recruited 20 normal individuals who had no history of cervical dysplasia or neoplasia, as negative controls. Subjects of the control group were confirmed by cervical cytology to be free of cancer or intraepithelial lesions and by hybrid Capture II test to be HPV DNA-negative. Before any treatment, 7 ml blood samples were collected from all patients and placed immediately into tubes containing ethylenediaminetetraacetic acid.

SCCA ASSAY

Serum SCCA levels were assayed by radioimmunoassay (RIA, SRL, Tokyo). The normal value of SCCA in our hospital is ≤1.5 ng/ml.

ISOLATION OF DNA

Blood samples were centrifuged at 3000 rpm for 5 min at room temperature. The plasma was saved and stored at −20°C until analysis. The DNA was extracted from 200 μl of plasma using a commercial kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany) and the DNA was eluted with 50 μl of MiliQ.

QUANTITATIVE REAL-TIME PCR ANALYSIS OF HPV DNA

For analysis of HPV DNA in plasma of patients with cervical cancer, quantitative real-time PCR (qPCR) fluorescent assay for HPV16 was performed using HPV16 primers and SYBR Green dye using the LightCycler 480 SW1.5 apparatus (Roche Molecular Biochemicals). HPV16 primers were as follows. (i) HPV16E6E7 forward primer: 5'-ATC ATC...
DETERMINATION OF VIRAL LOAD

To prepare plasmids containing HPV16E6 and/or HPV16E7 sequences for standard curves, we used the HPV16-positive cervical cancer cell line (Caski cell). Total cellular DNA was isolated by using Genomic DNA purification kit (Promega, Madison, WI, USA) according to the instructions provided by the manufacturer. HPV16E6 and/or HPV16E7 were re-cloned in PCR2.1-TOPO for standard curves. From the known molecular weights of the recombinant plasmids, the amount of plasmid DNA equivalent to 5 × 10^10 copies of HPV16E6 and/or HPV16E7 was first determined. Standard curves were generated automatically by plotting the threshold cycle values against the logarithm of the copy numbers of the recombinant plasmids (serial 10-fold dilution from 5 × 10^10 to 0.5 copies of HPV16E6 and/or HPV16E7). The copy numbers of each sample were calculated from the standard curve. The concentration of plasma HPV DNA was expressed as copies of HPV genome per milliliter of plasma. The results are expressed as mean ± SD (n = 3).

STATISTICAL ANALYSIS

The Mann–Whitney test was used to compare the HPV DNA copy numbers among cancers of different stages. Probability values <0.05 were regarded as statistically significant.

RESULTS

Quantitative PCR was highly sensitive in detection of HPV16 DNA with as little as 50 copies/μl. Plasma HPV16E6E7 DNA was detected in 6 of 20 (30.0%) patients with HPV16-positive invasive cervical cancer, but in none of those with CIN3 and normal controls (Table 2). The copy numbers of plasma HPV16E6E7 DNA in patients with uterine cervical cancer remains controversial.

DISCUSSION

The major finding of the present study was the detection of HPV16 DNA in 60% of primary cervical cancers and in plasma samples of 30.0% of the patients with HPV16 DNA-positive primary tumors. The detection rate of HPV DNA in plasma of patients with uterine cervical cancer remains controversial.
Pornthanakasem et al. (11) detected HPV DNA in the plasma of 12% of their HPV-positive patients with cervical cancer, whereas the same rate was 70% in another study (12). Furthermore, Yang et al. (13) reported detection of HPV16 DNA in plasma samples of 50% of their patients with cervical cancer. In this context, Capone et al. (15) reported that they could not detect HPV16 DNA in the plasma of 65 patients with nasopharyngeal SCC using L1 primer but detected the same DNA in the plasma of 2 of their 65 patients by real-time PCR using E7 primer. These differences in the detection rate may be due to differences in sample numbers and method of analysis.

In the present study, plasma HPV16E6E7 DNA was detected in 6 of 20 (30.0%) HPV16-positive patients with invasive cervical cancer but in none of the normal controls. The copy number of plasma HPV16 DNA in patients with invasive cervical cancer was higher than in CIN3 and micro-invasive cancer and in normal individuals. On the other hand, the copy number of plasma HPV16 E6E7 DNA in cervical cancer stage IVa was higher than that of cervical cancer stage IVb. More sample collection and further studies are required to determine the relationship between the detection level of HPV DNA in plasma and clinical stage of uterine cervical cancer.

The source of plasma HPV DNA remains unclear. However, the DNA level is probably related to tumor size, stage and presence or absence of metastasis. In this regard, Pornthanakasem et al. (11) reported that the plasma level of HPV DNA in metastatic patients was three times higher than that of patients without metastasis. Their results suggested that the amount of plasma HPV DNA could be a useful tumor marker for prediction of disease progression and clinical outcome after treatment of patients with cervical cancer.

In our study, only 30.8% of the patients with high levels of serum SCCA were plasma HPV16 DNA-positive. And detection rate of plasma HPV16 DNA was different based on differences in primers’ position (Table 3). In this context, the form of HPV DNA in the plasma of cervical cancer patients is not clear. It is possible that various HPV DNA fragments are present in the peripheral circulation since the HPV DNA detection rates in the plasma varied with the use of different primers in the same patients with cervical cancer.

What is the source of HPV DNA in the peripheral circulation? While the exact source is not clear at this stage, circulating HPV DNA levels could represent lysis of circulating cancer cells or micrometastasis shed from the tumor (16,17). Others have suggested that circulating tumor DNA in plasma might reflect tumor cell metastasis because of the high in vitro transforming activity (18,19).

The results of the present study indicated that HPV16E6E7 DNA in plasma might not be a sensitive marker of cervical cancer recurrence because plasma HPV16E6E7 DNA before operation was detected in only 4 out of 13 (30.8%) patients with high serum SCCA levels. However, HPV DNA could be potentially used as a marker of low-invasive cervical cancer tumors in patients with normal SCCA levels before treatment because two out of seven (28.6%) patients with normal serum SCCA levels had detectable levels of plasma HPV16E6E7 DNA before operation. We recommend the use of several HPV DNA primers to detect various HPV DNA fragments in the peripheral circulation in patients with cervical cancer.

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Conflict of interest statement
None declared.

References


