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Integrated human papillomavirus types 52 and 58 are infrequently found in cervical cancer, and high viral loads predict risk of cervical cancer

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Abstract

Objective. The aim of this prospective study was to analyze whether integration or high viral loads of human papillomavirus (HPV) is essential for malignant transformation of HPV types 52 and 58 as well as types 16 and 18.

Methods. Cervical swabs from 178 consecutive patients, including 81 with invasive cervical cancers and 97 with cervical intraepithelial neoplasias (CIN) II–III, were collected and examined to determine the physical status and viral load of HPV types 16, 18, 52 and 58 DNA using genechip and real-time PCR (polymerase chain reaction) analysis.

Results. In cervical cancer patients, the integrated form of HPV 52 and 58 DNA was found in 25.0% and 12.5% of swabs, respectively; while HPV16 and 18 DNA was found in 82.6% and 100% of swabs, respectively ($P < 0.01$, for pair-wise comparison of types 16, 18 versus types 52, 58).The viral loads reflected by the amount of E6 for HPV 16, 18, or 52 were significantly increased in invasive cervical cancer compared to CINII–III ($P = 0.022$ for type 16, $P = 0.003$ for type18, and $P = 0.001$ for type 52, respectively). Area under the receiver operating characteristic (ROC) curve for cervical cancer versus CIN II–III was 73.8%, 92.9%, and 88.5% for HPV 16, 18, and 52, respectively, indicating that real-time PCR had good diagnostic value in differentiating cervical cancer from CIN II–III.

Conclusions. Infrequent integration of HPV 52 and 58 DNA in cervical cancer suggests that it is not prerequisite for progression to cervical cancer. High viral loads (E6) of HPV 16, 18, and 52 DNA may be predictive of the transition of CIN II–III to cervical cancer. Our results indicate that both viral DNA physical status and viral loads of HPV are important factors in the carcinogenesis of different HPV types.

Keywords: HPV; Cervical intraepithelial neoplasia; Viral load; Integration; Polymerase chain reaction

Introduction

HPV is known to play an important role in the carcinogenesis of cervical cancer [1,2]. The most common oncogenic type of HPV in cervical cancer is type 16, which is detectable in more than 50% of cases [3]. The most important viral oncogenes involved in cellular immortalization are E6 and E7. Expression of both HPV oncoproteins, E6 and E7, is necessary for cancer cell division. Continuous expression of E6 and E7 is required for the induction and maintenance of neoplastic phenotype of cervical cancer cells [4]. Previous studies suggested that benign HPV lesions and CIN I lesions mostly contain the viral sequences only as episomes [5,6]. In contrast, viral DNA is integrated into the host genome in virtually all cases of cervical carcinomas and their derivate cell lines [7,8]. Recent study revealed that integrated HPV type 16 is frequently found in cervical cancer precursors, and rapid progression of the CIN lesions was closely associated with a heavy load of integrated HPV 16 [9]. HPV 16 DNA integration has been associated with malignant transformation, with increased frequency from CIN to invasive cancers [10]. In contrast, a significant proportion of advanced cancers containing only the episomal form of HPV DNA have demonstrated

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that HPV16 integration might not be essential for malignant transformation [11,12]. On the other hand, the presence of HPV 18 may be associated with an aggressive phenotype which more rapidly progresses from dysplasia to invasive cancer. One very important finding is that 100% of HPV 18 positive cervical carcinomas contain integrated viral DNA only [11].

In addition to integration, heavy viral loads in CIN lesions have recently been shown to increase the risk of carcinoma development in situ by at least 60-fold [13]. However, whether viral load or integration status of HPV is a risk factor for cervical cancer progression remains unclear due to the conflicting results obtained using different methodologies in previous studies.

Cervical cancer is the leading gynecologic cancer of women in Taiwan. In a study of the association between HPV infection and cervical cancer progression in Taiwanese women, Liaw et al. reported that 10% of low-grade and 33% of high-grade cervical neoplasia could be explained by infection with HPV subtypes 52 and/or 58 [14]. Huang et al. also reported that HPV 52 and 58 were as prevalent as HPV types 16 and 18, which are associated with high risk for cervical cancer in Chinese women [15]. In our previous study, HPV 52 DNA was commonly detected in both blood and swab specimens of women with cervical cancer in Taiwan [16].

Data on the physical state of HPV 52 and 58 DNA in patients with CIN II–III and invasive cervical cancer have not been reported. This study investigated whether the viral load of HPV DNA represented by copy number of E6 viral oncoprotein encoding sequence or physical status of viral DNA including types 16, 18, 52, and 58 detected by real-time PCR in cervical swabs is a risk indicator for cervical cancer.

Materials and methods

The study protocol was reviewed and approved by the Institutional Review Board and Research Grant Committee of Cathay General Hospital (CGH). This prospective study was conducted to determine the prevalence, physical status, and viral load of the HPV types 16, 18, 52, and 58 DNA in the cervical swabs of patients with CIN II–III and cervical cancer using genechips and real-time PCR analysis. Between January 2003 and March 2004, cervical swabs were collected from 81 consecutive Taiwanese patients at the time of diagnosis of cervical carcinoma and from 97 consecutive Taiwanese patients at the time of diagnosis of CIN II–III at the gynecologic cancer center of CGH. Informed consent for participation was obtained from each patient. The cervical swab was collected with an Ayre spatula and agitated into 3 ml of Tris–HCl (pH 8.3), 0.2% Triton X-100, then stored at −20°C. All patients with CIN II–III or cervical cancer were examined by colposcopy, and the diagnosis was confirmed by biopsy. All patients underwent complete physical and gynecologic examination, and the cervical cancer was staged according to the guidelines of the International Federation of Gynecology and Obstetrics (FIGO). All patients were followed up after primary treatment at our department at 3-month intervals for the first 2 years or until death. Follow up including physical examination, cervical cytology, blood chemistry profile, and tumor marker detection such as SCC or CA-125, and further work up such as computer tomography (CT) scans of the pelvis, bone scan, chest radiograph, or biopsy of any suspected lesions, were performed when clinically indicated. Associations were evaluated between HPV status and viral load in swabs and clinical parameters such as tumor size, clinical staging, tumor marker, lymph node metastasis, lymphovascular space involvement (LVSI), histological type, adjuvant therapy, and clinical outcome.

The real-time PCR methods used in this study were developed based on the following assumptions: (1) preferential disruption of E2 will cause the absence of E2 gene sequences in the PCR product following integration, (2) copy numbers of both genes (E2 and E6) should be equal when viral DNA presents in episomal forms, and (3) E2 gene copy number will be smaller than that for E6 when viral DNA presents in concomitant form.

DNA was extracted with the QIAamp DNA Blood Mini Kit (QIAGEN Catalog No.51106) according to manufacturer's instructions. Extracted DNA was eluted with 100 μl AE buffer (10 mM Tris, pH8.5) and stored at −20°C until analysis. The frequency of HPV DNA and genotypes were determined by a polymerase chain reaction (PCR)-based genechip method with HPV L1 gene MY11/Gp6+ consensus primers as previously described [17]. The MY11/GP6+ consensus primers were used to amplify a fragment of 190 bp in the L1 open-reading frame.

DNA amplifications were carried out in a 96-well plate in an ABI Prism 5700 Sequence Detection System (Applied Biosystems). Both E2 and E6 real-time polymerase chain reactions were carried out in triplicate for each sample. Amplification and quantification of the E2 and E6 genes were carried out simultaneously in separate reactions in a 96-well PCR plate. Additionally, one sibling control sample, which had been quantified previously, was included in each reaction to serve as quality assurance of the quantification system. Numbers of the threshold cycle (Ct) obtained from E2 PCR and those from E6 PCR were regressed to the standard curve to obtain the HPV copy number. For evaluation of triplicate data, the mean value and standard deviation (SD) were calculated. Data between the ranges of mean ± 1.96× SD were considered acceptable. Multiple negative water blanks were included in every analysis.

The reaction was performed in a 25 μl mixture containing 1× reaction buffer (HPTM HotStart Taq SYBR Green Kit Cat No.PTM767B, Protech) and 100 nM of primers for both E2 and E6 regions. Fifty nanograms of total DNA was added to the reaction mixture. The primer sequences of E2 and E6 for HPV types 16, 18, 52, and 58 were as shown in Table 1. The amplification conditions were as follows: 10 min at 95°C, a two-step cycle at 95°C for 10 s, and 60°C for 1 min for a total of 45 cycles. The specificity was verified by the additional dissociation curve and followed by 2% agarose gel electrophoresis. Two standard curves were obtained by amplification of serial dilutions (ranging from 10 to 10,000,000 copies per μl) of cloned plasmid containing either partial HPV 16 (from base 28 to base 3890), HPV 18 (from base 45 to base 3993), HPV 52 (from base 95 to base 3895), or HPV 58 (from base 45 to base 3994) DNA which included equivalent amounts of E2 and E6 genes in pGEM T-Easy vector (Promega). The number of threshold cycles for E2 PCR and E6 PCR was equivalent in each run. A linear plot of the log of the copy number versus number of threshold cycles was consistent for both genes, and the correlation coefficient was between 0.995 and 1.00 in each run.

Concentrations of HPV DNA were expressed as copies of HPV genome in 50 ng of cellular DNA. Ratios of E2 to E6 less than 1 indicated the presence of both integrated and episomal forms. The amount of integrated E6 was calculated by subtracting the copy numbers of E2 (episomal). The ratio of E2 to integrated E6 represented the amount of the episomal form in relation to the integrated form.

Table 1
Primer sequences of E2 and E6 in HPV type 16, 18, 52 and 58

Oligo name	Sequence (5′ →3′)	Site in HPV genome
16E2-F	AATTATTAGGCAGCACTTGGCCA	3381–3403
16E2-R	ATCTTGGTCGCTGGATAGTCGTCT	3481–3458
16E6-F	GAGCGACCCAGAAAGTTACCAC	122–243
16E6-R	ACCTCACGTCGCAGTAACTGTTG	228–206
18E2-F	CCGCTACTCAGCTTGTTAAACAGCT	3454–3478
18E2-R	GCCGACGTCTGGCCGTAG	3550–3533
18E6-F	CGGCGACCCTACAAGCTACC	129–148
18E6-R	ACCTCTGTAAGTTCCAATACTGTCTTGC	235–208
52E2-F	ACTGAAACTGCTGTCCACCTATGC	3361–3384
52E2-R	TGACGTCTGGTCGTCGTCG	3454–3436
52E6-F	ACACGACCCCGGACCCT	120–136
52E6-R	CTTGTATACCTCTCTTCGTTGTAGCTCTTT	233–204
58E2-F	CCACTACTGAAACTGCTGACCCAA	3366–3389
58E2-R	GGGTGTTGTCTCTGGAGTCTGGTAA	3473–3449
58E6-F	GGAGAAACCACGGACATTGCA	127–147
58E6-R	ACCTCAGATCGCTGCAAAGTC	234–214

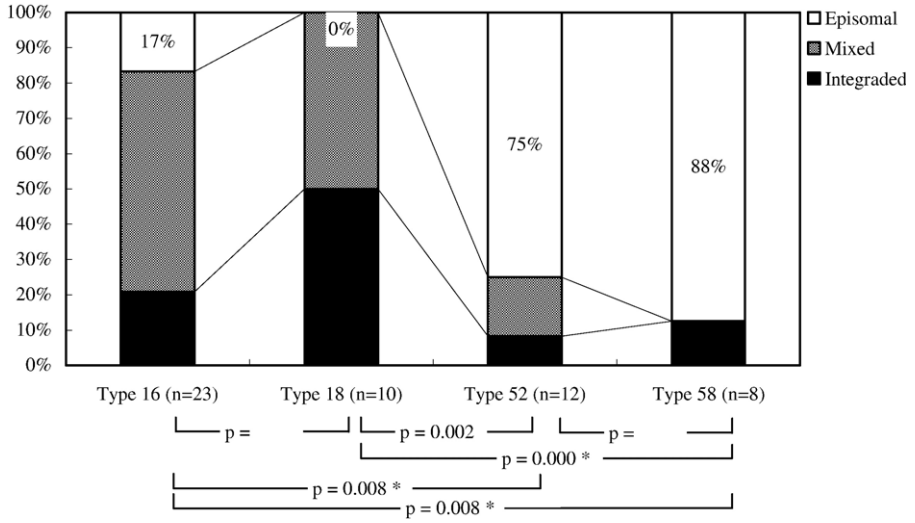

Fig. 1. Differences in the prevalence of the physical status of HPV 16, 18, 52, and 58 DNA detected in the swabs of cervical cancer patients. The segments (white, stippled, and black) represent ratios of specimens harboring HPV 16, 18, 52, and 58 in pure episomal, mixed, and pure integrated forms, respectively. $P < 0.05$ represents a significant difference.

Statistical analysis was mainly performed using SAS 9.1.3 software. The viral load was analyzed using Wilcoxon rank sum test based on log transformed data ($y = \log(x + 1)$) to compare differences between groups. The frequency distribution of physical status was analyzed by Fisher's Exact Test. A diagnostic test based on log transformation of E6 viral loads was used to perform receiver operating characteristic (ROC) curve analysis.

Results

Cervical swabs were obtained from 178 patients including 81 with cervical cancers and 97 with CIN II–III lesions. Among the 81 cervical cancer patients, disease was stage IA in 9, IB in 35, IIA in 3, IIB in 24, IIIA in 1, IIIB in 6, and IV in 3. The median age of patients with cervical cancer was 56.5 years (range, 30–85 years) and of patients with CIN II–III was 44 years (range, 25–84 years) ($P < 0.001$). Histological diagnoses included squamous cell carcinoma ($n = 67$), adenocarcinoma ($n = 11$), and adenosquamous ($n = 3$). Genechip analysis detected HPV DNA sequences in 87.7% (71/81) of the cervical cancer swabs, among which 76.1% (54/71) harbored a single type and 23.4% (17/71) contained multiple types. HPV 16 was detected in 30.9% (25/71), followed by type 52 in 16.1% (13/71), type 18 in 14.8% (12/71), and type 58 in 12.4% (10/71). In the swab samples from patients with CINII–III, HPV DNA was detected in 83.5% (81/97), among which 64.2% (52/81) harbored a single viral type while 35.8% (29/81) harbored multiple types. The prevalences of HPV types 16, 18, 52, and 58 in CIN II–III were as follows: 19.6% (19/81) for HPV type 58, followed by 18.6% (18/81) for HPV type 16, 18.6% (18/81) for HPV type 52, and 7.2% (7/81) for HPV type 18. All swabs that were positive for HPV 16, 18, 52, and 58 detected in genechips were quantified by real-time PCR. The discrepancy between HPV detection in cervical cancer using genechips versus real-time PCR analysis

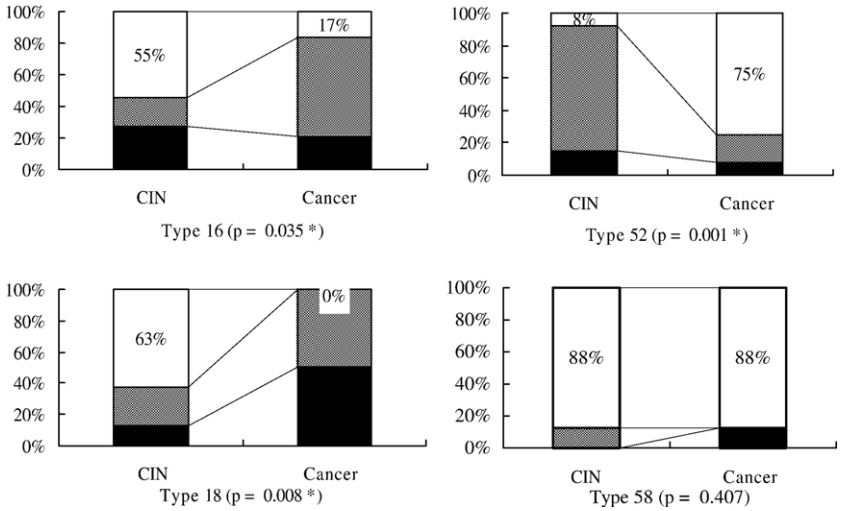

Fig. 2. Differences in the prevalence of physical status of HPV 16, 18, 52, and 58 DNA between swabs of patients with cervical cancer and CIN II–III. The segments (white, stippled, and black) represent ratios of specimens harboring HPV 16, 18, 52, and 58 in pure episomal, mixed, and pure integrated forms, respectively. $P < 0.05$ represents a significant difference.

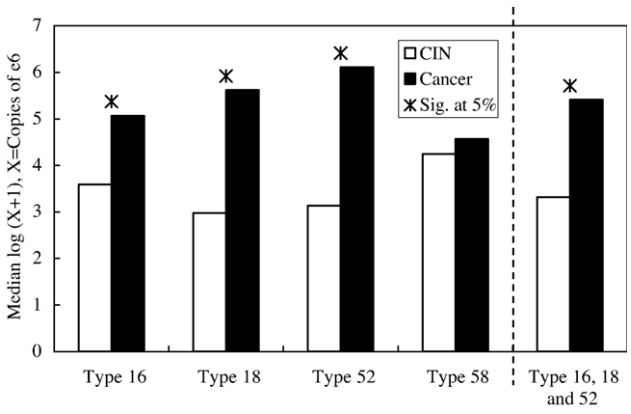

Fig. 3. Differences in the median Log of E6 viral loads of HPV DNA types 16, 18, 52, and pooled data between CIN II–III and cervical cancer. $P < 0.05$ represents significant.

(E6 level undetectable) for each of the different DNA types was as follows: 8% (2/25) for type 16, 0% (0/12) for type 18, 7.7% (1/13) for type 52, and 20% (2/10) for type 58.

Differences in the prevalence of the physical status of HPV 16, 18, 52, and 58 DNA detected in the swabs of cervical cancer patients were shown in Fig. 1. The prevalence of integrated HPV types 16, 18, 52, and 58 DNA detected in swabs from patients with CIN II–III was as follows: 92.3% for HPV type 52, 45.5% for HPV type 16, 42.9% for HPV type 18, and 12.5% for HPV 58, respectively. A significant difference in the physical status of HPV16, 18, and 52 DNA was found between patients with CIN II–III and cervical cancer ($P = 0.022$ for type 16, $P = 0.022$ for type 18, and $P < 0.001$ for type 52). However, integrated viral DNA of types 16 and 18 was more prevalent in cervical cancer compared to CIN II–III while cervical cancer swabs showed less prevalent HPV type 52 and similar prevalence of HPV type 58 (Fig. 2).

Differences in the median Log of E6 viral loads of HPV DNA types 16, 18, 52, and pooled data between CIN II–III and cervical cancer were shown in Fig. 3. The median E2 and E6 viral loads for pooled data of HPV 16, 18, 52, and 58 DNA showed either a significant or marginally significant differences between patients with early cervical cancer and patients with advanced cervical cancer ($P = 0.029$ and $P = 0.054$, respectively).

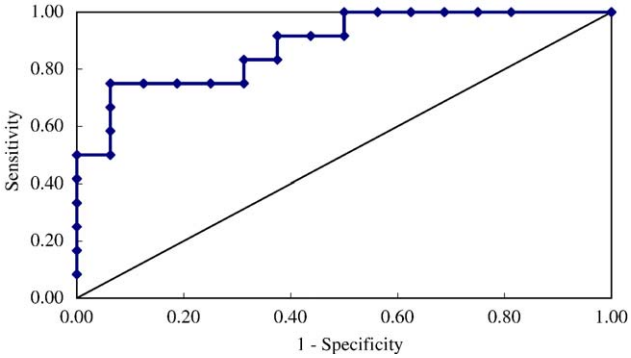

Fig. 4. Prediction of CIN II–III or cervical cancer using the ROC curve of the median Log of E6 viral loads of HPV 52 DNA.

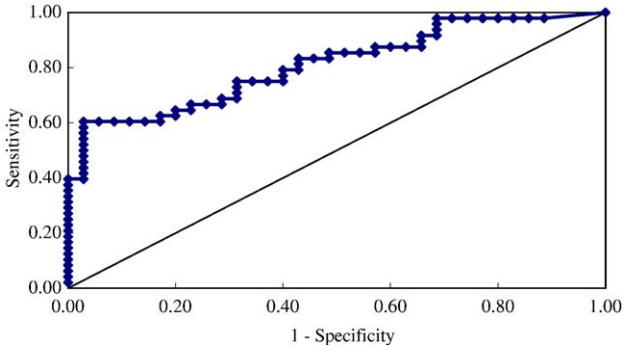

Fig. 5. Prediction of CIN II–III or cervical cancer using the ROC curve of the median Log of E6 viral loads of pooled data of HPV 16, 18, and 52 DNA.

When the median Log of E6 viral loads was used to predict which patients had cervical cancer, the cut-off value of 4.15 for the median Log of viral load of HPV 16 achieved 62.5% sensitivity with a 25% false positive rate. In contrast, the cut-off value of 3.76 of the median Log of viral load of HPV 18 achieved 83.3% sensitivity with 0% false positive rate, and that of 4.92 of the median Log of the viral load of HPV 52 achieved 75% sensitivity and 6.3% false positive rate. The ROC curve analyses indicated that the area under ROC curve was 73.8%, 92.9%, 88.5%, suggesting that the model could accurately predict the assignment to CIN II–III or cervical cancer groups in 73.8%, 92.9%, 88.5%, of patients with positive HPV types 16, 18, and 52 DNA, respectively. The ROC curve of the median value of the Log of E6 viral loads for each type or pooled data of HPV 16, 18, and 52 DNA could predict progression of disease from CIN II–III to cervical cancer ($P \leq 0.002$ for all tests of AUC > 0.5). These ROC curves are shown in Figs. 4, 5, 6, and 7.

Discussion

The integration of HPV DNA into the host genome is thought to occur early in cancer development and to be an important event in malignant transformation of cervical cancer [7,8,10]. However, most studies on the integration of HPV DNA focused on type16 and a few on type18 or other oncogenic types such as 31 and 35 [5–12,18]. While HPV types 52 and 58 are oncogenic types with relatively low prevalence in cervical cancer in the Americas, Europe, Africa,

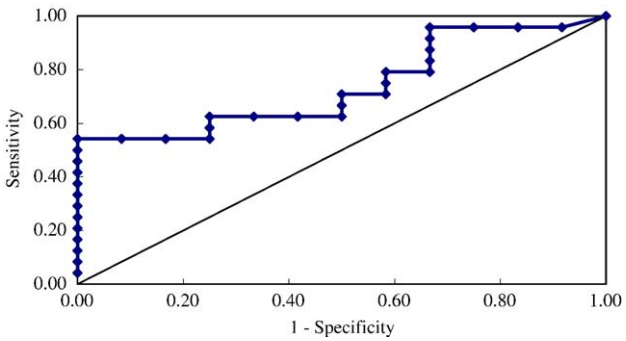

Fig. 6. Prediction of CIN II–III or cervical cancer using the ROC curve of the median Log of E6 viral loads of HPV 16 DNA.

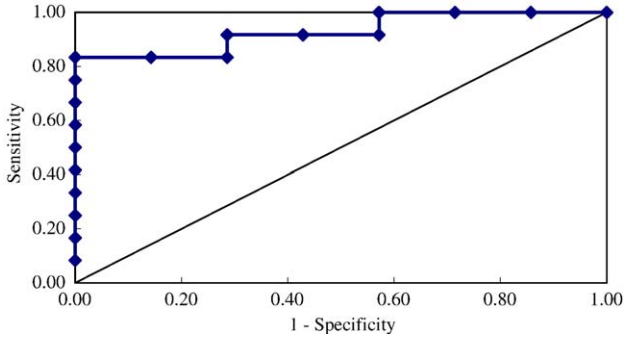

Fig. 7. Prediction of CIN II–III or cervical cancer using the ROC curve of the median Log of the E6 viral load of HPV 18 DNA.

and Southeast Asia, they are as prevalent as the known high-risk (for cervical cancer) HPV types 16 and 18 in Taiwan and other Asian countries [3,14–16,19]. To our knowledge, the prevalence of integrated HPV 52 and 58 DNA in patients with CIN II–III and cervical cancer has not been reported. In this study, the prevalence of integration of HPV types 52 and 58 in patients with cervical cancer was unexpectedly low, while the prevalence of integration of HPV type 52 in patients with CIN II–III was very high (92.3%). These results indicate that integration of HPV type 52 is not required for progression from CIN II–III to cervical cancer. On the contrary, detection of a high E6 viral load of HPV type 52 detected in cervical cancer patients was a good marker of invasiveness. This finding reflects that unknown factors other than integration are likely to be involved in the induction or maintenance of E6 viral load leading to the expression of E6 oncoprotein in the carcinogenesis of cervical cancer. The high prevalence of intact HPV episomal E2 DNA supports that disruption of ORF is not required for increased oncogene expression in patients with HPV types 52 and 58. This study highlights the need for investigating alternative mechanisms of oncogene expression during the oncogenesis of HPV 52 and 58.

There are several possible explanations for the presence of episomal HPV DNA 52 and 58 in most of the swab samples of cervical cancer in this study. These include extensive sequence variations present in the HPV 52 and 58 genes, mutations in the long control region of which may have served as an alternative mechanism for increasing the expression of viral oncogenes, and epigenetic events such as methylation of viral DNA. A significant proportion (10–30%) of invasive cervical cancers containing only episomal HPV DNA have been reported, demonstrating that HPV16 integration might not be essential for malignant transformation [11,12]. In this study, 17.5% of invasive cervical cancers contained only HPV type 16-episomal HPV DNA, a finding which is compatible with previous studies [11,12]. Elimination of the expression of HPV 16 E2 protein may be due to integration or to up-regulation of the transcription of the E6 and E7 oncogenes resulting from YY1 mutation, possibly providing a selective growth advantage for the infected cell [20,21]. This may contribute to an increase in cell proliferation and genomic instability, leading to further genetic alterations [22]. The E6 and E7 oncoproteins interfere with the normal cell cycle by targeting the p53 and pRb tumor suppressor proteins, respectively. These effects are important steps in cervical carcinogenesis, resulting in the loss of cell cycle control [23,24].

Identification of viral and host factors that contribute to the delineation of women at the highest risk of HPV progression to cervical neoplasia is an essential step in determining the candidate markers of potential use for diagnostic or prognostic purposes. The potential utility of viral load measurements as etiologic risk factors as well as diagnostic and prognostic tools for cervical cancer in clinical practice warrants their continued attention in research. Previous cross-sectional epidemiologic studies [13,25] and one longitudinal study [26] have demonstrated an association between increased HPV viral load and risk of carcinoma in situ (CIS) and cervical cancer. However, its predictive utility remains unclear, and a model establishing a useful cut-off value for identifying the subgroup of patients with cervical cancer has yet to be determined. Viral load has been suggested as a marker for non-transient infection, and high HPV load in smears with normal cytology has been associated with increased risk of developing dysplasia and CIS [13,25]. However, it remains unclear whether high viral load is also predictive of invasive cervical cancer, as past studies have pooled the outcomes of invasive cancers together with earlier stages of the disease, and data are limited for HPV types other than 16 and 18 [27–29]. Because most studies have focused on HPV 16 or overall (i.e., non-type-specific) viral load measures, extremely little is known about the relationship of viral load to viral types other than HPV type 16 such as HPV types 52 and 58, and their relationship to cervical neoplasia. Moberg et al. reported that high loads of HPV types 16 and 18/45 are type-dependent risk markers for invasive cancer [26]. By contrast, whether high loads of HPV 52 and 58 increase the risk of cervical cancer has not been previously reported. As a consequence, the association between viral load and development of CIS may not automatically imply a strong association of high viral load and progression into invasive carcinoma. Our results indicate that elevated viral loads of HPV 16, 18, and 52 in cervical swabs are associated with increased risk of invasive cervical cancer. Our data also indicated that the median E2 and E6 viral loads for pooled data of all subtypes of HPV 16, 18, 52, and 58 showed either significant or marginally significant differences between patients with early cervical cancer and patients with advanced cervical cancer ($P = 0.029$ and $P = 0.054$, respectively). Potential clinical applications of viral load measurements include use as an indicator of a missed cervical cancer lesion among CIN II–III lesions with margin involvement or an undetected advanced cervical cancer needs more aggressive therapy. High viral loads of HPV in cervical swabs may be indicative of a HLA class II-mediated immune response to HPV, reduced immune surveillance against HPV infection at mucosa, and subsequent development of cervical cancer [30]. Longitudinal studies including both physical state and load of the virus are needed to determine their relative importance in the development of cervical cancer.

Measurement of HPV DNA load has been suggested as having positive predictive value for detecting CIN III and

cancer based on the assumption that higher load values are more strongly associated with severe disease [13,28,31]. HPV load determined using the Hybrid Capture 2 DNA test has been shown to be linear over a broad dynamic range and well correlated well with quantitative PCR measurements [28,31]. However, the Hybrid Capture 2 does not adjust load determinations for specimen cellularity and cannot distinguish between infections with single or multiple types of HPV. The value of viral load estimates based on summary measurements of HPV, such as those generated by the HC2 assay, may be limited by not distinguishing HPV types or multiple infections. Within any specific grade of CIN, infections with multiple oncogenic types were associated with significantly higher HPV loads using Hybrid Capture 2 than infections with single types. However, after adjusting for number of HPV types detected, HPV load would not have specifically identified patients with CIN3 [28]. Several studies have noted differences in viral load pattern between HPV 16 and other HPV types [25,28]. Gravitt et al. found an increased odds ratio (OR) of HSIL/cancer with HPV 16 load but did not find a similar trend for HPV 18 [28]. These observations led us to investigate the risk of cervical disease associated the individual high-risk HPV types.

Approximately, 35% of CINII–III lesions in our study were infected with multiple oncogenic HPV types. We examined the relationship between viral load and CIN II–III or cervical cancer for high-risk HPV types 16, 18, 52, and 58, which accounted for about 70% HPV types. The E6 viral loads of both single and multiple infections with HPV 16, 18, and 52 DNA showed a strong positive relationship with cervical cancer. However, HPV 58 load appeared to have a weak relationship with risk of cervical cancer.

Real-time PCR is a more recent addition to the molecular diagnostic methods used to detect HPV infection. Real-time PCR has the advantage of being highly specific, reproducible, and capable of detecting an HPV viral load at up to eight orders of magnitude in a linear range. These qualities make real-time PCR attractive for use in epidemiological studies and as a potential diagnostic test. Several studies reported the predictive value of HPV viral burden was demonstrated using real-time PCR. These studies, however, only focused on the quantitation of HPV type 16 viral load [28,32]. Due to wide variation in viral load among patients, a model for measuring viral load with cut-off values to predict severity of disease remains to be established. In this study, we successfully used the median Log of viral loads of HPV 16, 18, and 52 DNA to predict the presence of cervical cancer. The selected cut-off values of median Log of viral loads in HPV 16, 18, and 52 DNA achieved 62.5–75% sensitivity and 0–25% false positive rate in predicting the presence of cervical cancer. The ROC curve analyses indicated that the model could accurately predict the diagnostic group of CIN II–III or cervical cancer in 73.8%, 92.9%, 88.5% of patients with positive HPV 16, 18, and 52, respectively.

There were a number of limitations in the present study. First, the sample size was small, and the study design was cross-sectional. A longitudinal study involving a greater number of patients is required before definite conclusions can be reached regarding usefulness of HPV DNA viral loads to diagnose and predict the risk of cervical cancer. This study showed that quantification of E6 HPV 16, 18, and 52 DNA can serve as a prognostic tool for identifying women who are at increased risk of developing cervical cancer. Further longitudinal studies are needed to establish the predictive significance of viral load measurements for the different oncogenic HPV types.

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