HPV L1 capsid protein expression in squamous intraepithelial lesions of cervix uteri and its relevance to disease outcome

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Abstract

Purpose The purpose of this study was to investigate the usefulness of immunocytochemical detection of HPV L1 capsid protein expression in predicting the course of cervical intraepithelial neoplasia.

Background It is known that most of the low grade dysplastic lesions of cervix uteri regress spontaneously and only some will progress to high grade dysplastic lesions. HPV L1 capsid protein represents about 90% of the total protein on the surface of the virus and can be detected in mild to moderate dysplasia and rarely in severe dysplasia.

Methods Pap smears from 65 women, in whom diagnoses of LSIL (n = 43) and HSIL (n = 22) were made on cytology and histology specimens, were immunocytochemically stained using antibody against HPV L1 capsid protein. The results of immunocytochemical analysis were correlated with the outcome during the 24-month follow-up. p value < 0.05 was considered significant.

Results The immunostaining reaction for L1 capsid protein was positive in 28 cases (65.1%) of LSIL while 15 (34.9%) cases of LSIL and all of the 22 cases of HSIL were negative (p < 0.001). After 24 months of follow-up, among the 28 L1-positive LSIL cases, we found a 60.7% (17/28) spontaneous regression rate, whereas in the 15 L1-negative LSIL patients, the regression rate was 33.3% (5/15). Out of the 22 HSIL cases, 13.6% (3/22) had regression.

Conclusion Our data support that immunocytocytochemical detection of HPV-L1 protein could present prognostic information about the evolution of early dysplastic cervical lesions and can be useful in predicting their biologic potential.

Keywords L1 capsid protein · Cervical intraepithelial neoplasia · Human papilloma virus · Pap smear

Abbreviations

CIN Cervical intraepithelial neoplasia
HPV Human papilloma virus
HSIL High-grade squamous intraepithelial lesion
LSIL Low-grade squamous intraepithelial lesion
NPV Negative predictive value
PPV Positive predictive value
SCC Squamous cell carcinoma

Introduction

Cervical cancer is the second most common cancer affecting women worldwide [1, 2]. The frequency of cervical cancer has decreased markedly in developed countries which mostly results from early diagnosis through effective screening programs and management of precursor lesions rather than a significant progress in management of cervical cancer itself [3–6]. The human papilloma virus (HPV) infection is a well thought-out necessary intermediary step in the development of cervical cancer, in which this virus is present in more than 99% of cases. However, not all HPV infections give rise to cervical dysplasia and promote its development to carcinoma [7–11]. Therefore, it
would be most useful to have prognostic markers that can differentiate between patients who will experience a transition from a precursor condition to cancer and those who will not [12]. A range of possible prognostic markers of cervical intraepithelial neoplasia (CIN) have been suggested, such as L1 capsid and P16 proteins [13–23]. The L1 capsid protein of HPV which represents about 90% of total protein on the surface of the virus is typically evident during the reproductive phase of the infection of all HPVs, the highest frequency of humoral immune responses being noticed for the L1 capsid protein [16, 23, 24]. While expression of HPV-L1 capsid protein is usually negative in normal Pap smears, it can be detected in mild to moderate dysplasia. Furthermore, this marker would be positive in only rare cases of severe dysplasia and in none of the carcinoma cases. In 2004 Griesser et al. showed on routinely performed Pap smears that high-risk HPV is associated with low to moderate dysplastic squamous lesions without immunochemically detectable HPV-L1 capsid protein are significantly more likely to progress (76.4%) than L1-positive cases (23.6%) [14]. The aim of our study was to evaluate the prognostic significance of HPV-L1 capsid protein on routinely performed Pap smears with LSIL or HSIL diagnosis. The results were interrelated with the pathologic diagnosis of each patient’s lesion in order to determine the value of the test for predicting progression or regression in LSILs or HSILs.

Materials and methods

Case selection

In this retrospective study, 65 conventional cervical Pap smears with diagnoses of HSIL (n = 43) or LSIL (n = 22), from women who had been referred to the colposcopy clinic at the Women Hospital, Tehran, Iran between 1999 and 2009, were incorporated. The patients had been followed up by gynecology oncologist in colposcopy clinic with check-up every 6 month for at least 2 years. Cytological diagnosis was carried out according to the 2001 Bethesda reporting system [25] by board-certified gynecological pathologist. All diagnoses were confirmed in histology.

Antibodies and Immunocytochemistry

To evaluate the presence of HPV-L1 capsid protein by immunocytochemistry using the monoclonal antibody, coverslip was detached from Pap stained cervical smears by placing the slides in xylene. The slides were rehydrated in decreasing concentration of alcohol. Microwave antigen retrieval was then performed. The slides were incubated in room temperature with one droplet of CYTOACTIV® antibody (Cytoimmune diagnostic, GMBH, Pirmasens Germany) for 30 min, then with one drop of detection reagent for 10 min, and finally with one droplet of chromogen solution for 5 min. They were then counterstained with Mayer hematoxyline, cleaned and mounted. The slides were studied by light microscopy for the immunocytochemical reaction and classified as positive when an apparent nuclear staining was detected. The L1 capsid protein is a nuclear protein, therefore only a nuclear staining is specific. The positive control provided in the CYTOACTIV® antibody kit showed nuclear staining with or without cytoplasmic staining (30–50% of all cells on slide).

Statistical analysis

The usefulness of the antibody for the demonstration of prognostic significance of HPV-L1 capsid protein detection found on routinely performed Pap smears with LSIL or HSIL diagnosis was evaluated by Statistical Package for Social Sciences v 18.0 (SPSS Inc., Chicago, IL, USA) software for Windows using Chi square and Fisher’s exact test. p value <0.05 was considered significant.

Results

In this study, we analyzed 65 cervical conventional smears including 43 LSIL and 22 HSILs. The mean age of patients was 38.9 years, with standard deviation (SD) of 10.7 years. In the HSIL group, 19 and 3 patients were, respectively, older and younger than 30 years old. In the LSIL category, 31 patients were older and 12 patients were younger than the age of 30. All out of the 22 smears with diagnosis of HSIL were negative regarding the presence of HPV-L1 protein. Out of the 43 LSIL cases, HPV-L1 protein was positive in 28 (65.1%) and negative in 15 (43.9%) (p < 0.001). Positive reaction was characterized by the strong staining of the whole nucleus, surrounded by a cytoplasm with no background (Fig. 1). In most cases, positive reaction for HPV-L1 was seen in typical koiocytes or in dyskeratinocytes, presenting nuclear characteristics for LSIL. The observation period was 24 months for all cases. The follow-up of the 22 cases in HSIL group revealed remission in 3 (13.6%) and persistence in 6 (27.3%) cases. Thirteen (59.1%) patients underwent hysterectomy at some time during the 24-month follow-up period (p < 0.013). Among the 28 L1-positive LSILs, 17 (60.7%) patients went into remission, 9 (32.1%) had persistent disease and 2 (7.1%) underwent hysterectomy in 24 months follow up. It was noticed that, among the 15 HPV-L1-negative LSIL patients, remission and progression
of the disease occurred, respectively, in 5 (33.3%) and 4 (26.7%) cases, while 6 (40%) cases were stable in disease during the 24-month observation period. The relationship between positive HPV L1 immunocytochemical staining and lack of disease progression in LSILs was statistically significant with 70.2% sensitivity, 100% specificity, 100% positive predictive value (PPV) and 26.6% negative predictive value (NPV).

Women under the age of 30 had significantly more frequency of positive L1 staining than those over this age. In the 12 LSILs under the age of 30, 9 cases were positive and three cases were negative for L1 protein; whereas in the 31 LSILs over the age of 30, 19 cases were positive and 12 were negative for HPV-L1 protein. The outcome in the two diagnostic groups and in patients older and younger than 30 years old is summarized in Table 1. In the current study, disease status in all patients was evaluated every 6 months. The results are summarized in Table 2.

**Discussion**

Recently, testing for HPV has been adopted for management of uncertain abnormal Pap tests. Nevertheless, it cannot differentiate between latent, subclinical and clinically important infections. The majority of acute productive HPV infections spontaneously resolve within 24 months, thus rendering more follow-up procedures-including repeat Pap testing and colposcopy-unnecessary [26]. HPV-L1 encoded protein is the major capsid protein and is highly preserved with papillomavirus from all species [27]. The presence of L1 capsid protein within the dysplastic cells, as evidenced by L1 capsid protein positive immunocytochemical reaction, is the sign of a complete life cycle. L1-capsid-protein-negative cases, however, have lost the ability to produce virions depending on squamous epithelial cell differentiation. This also explains the higher L1 capsid positive rate in LSILs than in HSILs and cancers.

**Table 1** The outcome at 24 months of follow-up in patients over and under 30 years of age

<table>
<thead>
<tr>
<th>SIL</th>
<th>Age</th>
<th>24-month follow-up</th>
<th>Total (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Regression (%)</td>
<td>Persistant (%)</td>
</tr>
<tr>
<td>HSIL</td>
<td>≤30</td>
<td>IHC</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>0</td>
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<tr>
<td></td>
<td>&gt;30</td>
<td>IHC</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>15.8</td>
</tr>
<tr>
<td>LSIL</td>
<td>≤30</td>
<td>IHC</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>66.7</td>
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<tr>
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<td></td>
<td>Total</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td>&gt;30</td>
<td>IHC</td>
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<tr>
<td></td>
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<td>57.9</td>
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<tr>
<td></td>
<td></td>
<td>Total</td>
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</tr>
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</table>

*IHC* Immunohistochemistry
It has been suggested that integration of HPV DNA into the cellular genome of the host cells may cause loss of L1 expression, as occurs in 5–15% of CIN1 and CIN2 lesions, and—according to various studies—in 16% up to 50–94% of CIN3 lesions [28, 29]. However, some other investigations have shown apparently different results; for example, study of Hoshikawa et al. [7] on the relationship between L1 capsid and p16 expression in CIN showed that the L1-negative/p16-negative pattern was significantly associated with the regressive nature in CIN1 and 2. Also, some believe that combined expression pattern of p16 and HPV L1 may serve as a valuable index for predicting the prognosis and guiding the follow-up of cervical dysplastic lesions [22, 30]. Nevertheless, it can be argued that the L1-negative/p16-negative lesions in these studies might have been reactive and independent of HPV infection from the beginning. This is supported by the observation that in some specimens the dysplasia had been lost in the subsequent sections for immunohistochemistry and in others the dysplasia had been mimicked by marked inflammatory changes [22, 30].

In the current study, we observed a regression rate of 60.17% in the 28 L1-positive LSIL patients that is close to what Grisser et al. [27] observed (a regression rate of 69% for mild or moderate dysplasia). Our results suggest that mild dysplastic lesions without immunochemically detectable HPV-L1 capsid protein are significantly more likely to progress as compared to L1-positive cases. These data support the theory that failure to detect L1 protein correlates with chance of progression of the lesions, even in cases of LSIL [16]. According to data presented in Table 2 about clinical course of disease evaluated every 6 months during the 24-month follow-up period, in the first 6 months only 8 (28.6%) L1-positive LSIL cases have shown regression; while after 24 months, a regression rate of 69% was observed. Moreover, in patients with L1-negative LSIL after 6 months of follow-up, no regression was seen; however, at 12, 18 and 24 months of follow-up, respectively, 1, 3 and 5 instances of regressions were observed. Based on the present study it can be suggested that the exact rate of regression or progression in HPV infected cases is not clear. This rate can be influenced by many factors, such as immune status of the individual, nutrition, time of HPV contamination in each patient and other factors such as viral types. In addition, as shown in Table 1, regression rate was 66.7% in the HPV-L1-positive patients under 30 years of age, but 57.9% in those over this age. These findings propose that there is a reduced risk for progression in L1-positive patients in younger-than-30-year-old group, even though the differences from age dichotomization were minor. It would be particularly valuable if the rate of unnecessary surgical intervention could be reduced in the group of patients under the age of

![Table 2](image-url)}
30 years, in whom childbearing is often an important concern. Our study demonstrates that the expression of L1 capsid proteins is significantly reduced in HSIL (100%) (p < 0.013). Melsheimer et al. [16] had also recognized that expression of L1 capsid protein is significantly reduced in HPV16 and in other high risk HPV-type positive HSILs. These findings are consistent with our present data that demonstrate expression of L1 capsid protein in 65.1% of LSILs and 0% of HSILs, consequently representing that L1 capsid protein expression tends to turn down with increasing severity of lesions. As presented in Table 2, during the 24-month follow-up in HSIL group, in the first 6 months only one case showed regression while nine cases persisted and one case progressed to SCC. At 12, 18 and 24 months regression occurred in 1, 3 and 3 cases and persistence was observed in 8, 6 and 6 cases, respectively. In the present study hysterectomy was done in 13 cases with high-grade lesions, for whom we have no clue about their outcome.

We observed a statistically significant relationship between positive HPV L1 immunocytochemical staining and lack of disease progression in LSILs. In our study, positive HPV L1 immunocytochemistry in Pap smear could predict remission/persistence of LSIL with 70.2% sensitivity and 100% specificity. Furthermore, among the LSILs, a positive HPV L1 test would predict remission/persistence in 100% of the cases (PPV = 100%); while with a negative test, 26.6% would be expected to progress (NPV = 26.6%).

It is worth mentioning that some studies such as that of Galgano et al. [21] showed no utility for L1 immunostaining in distinguishing between CIN and non-CIN. However, L1 negativity is by no means sufficient evidence for association of the dysplastic alteration with HPV, since HPV-L1 capsid protein staining is not positive in all dysplastic squamous cells with HPV features. As the final point, the HPV-L1 capsid protein detection seems to be a promising new tool to predict the behavior of early dysplastic lesions and may be particularly useful in assessing those lesions that are still in the productive phase of carcinogenesis. Immunocytochemical detection of L1 capsid protein on Pap smears may indicate the protection status locally induced on HPV infection and may offer prognostic information in LSIL lesions.

**Conflict of interest** The authors declare that they have no conflict of interests.

**References**


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