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The detection of human papilloma virus 16 L1 capsid protein and p16 in cervical lesions

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Abstract

Background: Cervical cancer is the second most common type of cancer in women worldwide and also the second most common female malignancy in Mongolia. The association of p16 and hr-HPV in Asia, and in particular in Mongolia, are still relatively unexplored. So we aimed to detect the immunohistochemistry expression of p16 and L1 protein of HPV16 and to investigate the combined expression of these markers in cervical lesions.

Methods: A total of 96 cases were selected from the records of Pathology services, National Cancer center of Mongolia. There were 50 cases diagnosed as LSIL and 46 cases diagnosed as HSIL. The immunohistochemical staining with p16 and HPV 16 L1 were done on all cases.

Results: The positive rate of HPV 16 L1 capsid protein was identified 74% in LSIL cases and 52% in HSIL cases. There were a significant difference for HPV16 in HSIL and LSIL groups. Immunohistochemistry of p16 staining shows 76% in LSIL cases and 72% in HSIL cases. There was not a statistically significant difference for p16 in HSIL and LSIL groups. A chi square test was used to analyze the result and the obtained p value was <0.05.

Conclusion: The combination between hr-HPV and p16 is considered to be more useful, having a higher accuracy than hr-HPV or p16 alone. There is still critical need to find other molecular surrogate markers, which can provide accurate information about which precursor lesions would progress toward cancer.

Keywords: Immunohistochemistry, Human papillomavirus, Cervical cancer, p16.

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Introduction

Cervical cancer is the second most common type of cancer in women worldwide [1] and also the second most common female malignancy in Mongolia [2]. Human papillomavirus (HPV) infection is a well-known prerequisite for the development of cervical cancer. Moreover, the integration of high risk HPV (hr-HPV) is a key step in cervical neoplastic progression [3,4].

HPVL1 capsid protein comprises 90% of HPV viral surface proteins and is typically expressed during the late phase of viral replication [5-9]. Thus, so far most immunochemical studies on the HPVL1 capsid protein have investigated the predictive role of the protein in squamous cell intraepithelial lesions (SIL) [6-11].

P16 is a cell cycle inhibitor that binds to the cyclin dependant kinase 4 (cdk4)/cdk6 and prevents the phosphorylation and subsequent inactivation of the retinoblastoma protein (pRb). Thus p16 overexpression in cervical neoplasia is a surrogate marker of hr-HPV E7 mediated pRb catabolism reflecting disruption of mechanisms that control cell proliferation and indicating persistent infection with high risk of development of neoplasia [12-13].

The association of p16 and hr-HPV in Asia and particularly in Mongolia are still relatively unexplored. So we aimed to detect the immunohistochemistry expression of p16 and L1 protein of HPV16, which is the most dominant HPV subtype in Mongolia [14] and to investigate the combined expression of these markers in cervical lesions.

Material and Methods:

A total of 96 cases were obtained from the National Cancer Center of Mongolia, from January until December 2013. The study was approved by the Medical Research Ethics Committee of Mongolian National University of Health and Sciences and the informed consent was obtained from all patients. There were 50 cases diagnosed as LSIL and 46 cases diagnosed as HSIL. All biopsy specimens were examined by two experimental pathologists. Paraffin-embedded cervical tissues were processed at the time of diagnosis and immunohistochemistry was done at the Laboratory of Pathology Department of Mongolian National University of Health and Sciences. All the 96 cases were submitted for IHC with anti-p16 antibody (Mouse monoclonal [2S9A12] to CDKN2A/p16INK4a, Abcam ab 54210) and anti-HPV16 L1 antibody (Concentrated Mouse monoclonal [camvir-1] to HPV16 L1, Biocare medical).

From paraffin-embedded tissues 4-µm sections were cut, mounted on glass pretreated with 2% 3-aminopropyltriethoxysilane (Sigma), and air-dried overnight at 37°C. Sections were deparafinized in xylene, hydrated in a graded alcohol series and subsequently washed with distilled water. Briefly, sections were incubated for 15 minutes in boiling citrate buffer, cooled down in buffer for 20 minutes, and subsequently incubated overnight at RT with the first

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antibody. Binding of the mAb was detected using an anti-mouse ABC system. Antibody binding was revealed using biotinylated secondary antibodies, avidin-peroxidase and diaminobenzidine substrate. Slides were counterstained with haematoxylin, dehydrated before mounting and observed with a microscope equipped with bright-field illumination. Histology for all sections was analyzed by two pathologists independently. A positive and a negative control were run with every batch of IHC staining done. For IHC staining of the cervical tissue was implemented to allow semi-quantitative analysis on the IHC stained slides. Staining density for each antibody was semi-quantified into three main categories based on the percentage of cells being stained positive: (-) = no cells stained positive or = <10% of the cells stained positive, (+) = 10-50% stained positive, (++) = 50-90% stained positive

Statistical analysis was performed using SPSS 17.0. Descriptive analysis was performed by Chi-Square test. A probability value of < 0.05 was considered to indicate statistical significance.

Results:

IHC staining for p16 and HPV16 L1 were done for all 96 cases (100%). Positive staining for p16 and HPV16 L1 were observed within both the nuclear and cytoplasmic subcellular regions (Figure 1-2).

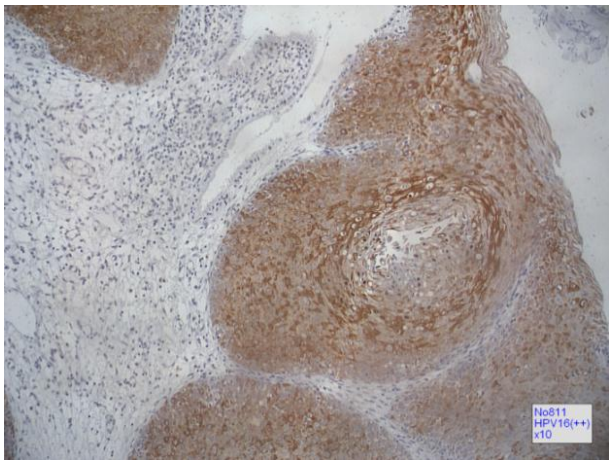

Figure1. HPV16 L1 (++) positive staining in LSIL specimen.

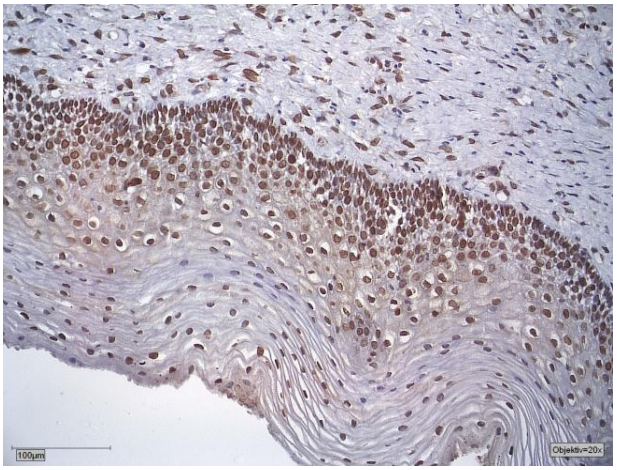

Figure 2. P16 (++) positive staining in LSIL specimen.

A positive result for p16 was found in 74% (71/ 96) from all these cases, namely 76% from LSIL cases (38/50) and 72% from HSIL cases (33/46). There was not a statistically significant difference for p16 in HSIL and LSIL groups ($X^2 = 0.23$, $P > 0.05$).

Table 1. *The P16 expression in cervical lesions*

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	Staining density			Total
	-	+	++	
LSIL	12	5	33	50
HSIL	13	12	21	46
Total	25	17	54	96

The positive rate of HPV 16 L1 capsid protein was identified in 64% (61/96) of cases HPV 16 L1 capsid protein was expressed in 37 of the 50 LSIL cases (74%) and 24 of the 46 HSIL cases (52%). There was a significant difference for HPV16 in HSIL and LSIL groups($X^2 = 4.93$, $P < 0.05$)

Table 2. *The expression of HPV16 L1 in cervical lesions*

	Staining density			Total
	-	+	++	
LSIL	13	19	18	50
HSIL	22	9	15	46
Total	35	28	33	96

In our study HPV16 L1-/p16+ represented 22% (21/96) of all cases. This pattern was found in 16% of LSIL cases and 26% of HSIL cases. L1+/p16- represented 11% (11/96) of all cases. This pattern was found in 14% of LSIL cases and 8% of HSIL cases (Table 3).

Table 3. *Combination of HPV16 L1 and p16 expression*

	L1-/p16+	L1-/p16-	L1+/p16+	L1+/p16-
LSIL	8	5	30	7
HSIL	13	9	20	4
Total	21	14	50	11

Discussion:

The incidence of cervical cancer is increasing gradually in the developing countries, and early examination and early diagnosis cervical cancer and precancerous lesions play an important role in clinical practice.

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Increasing attention has been paid to the role of HPVL1 capsid protein in the early diagnosis of cervical cancer [15-18]. Recently some reports showed that L1 capsid protein can be a new powerful and useful marker for revealing the status of productive and/or active HPV infections [19-21]. HPVL1 capsid protein, the major structural protein of HPV, is expressed in the early productive phase of cervical cancer but is gradually lost in the later proliferative phase when HPV DNA is integrated into the host DNA [21]. A number of recent studies have evaluated the expression of major capsid protein L1 in cytologic specimens. It has been shown that 30%-75% of LSILs and 33%-40% of HSILs express L1 in cytology specimens [22-28]. As a result of immunohistochemical studies, the positive rate of HPVL1 protein is increasing while the pathologic grade is decreasing. In the present study, the positive rate of HPV16 L1 decreased with the increase in histological grade. In LSIL patients, the HPV16 L1 capsid protein expression was higher than that in patients with HSIL. Our result for HPV16 L1 has a concordance with results from other similar study.

In cervical lesions, overexpression of p16 is observed and it is thought to be resulted from the increasing level of E2F transcription factor which is released from pRB after binding to HPV E7 oncoprotein [14]. Therefore it is a sensitive surrogate marker for such HPV infections, specifically for integration of viral E6 and E7 into squamous epithelial cell genome. Only the hr-HPV subtypes have the ability to integrate into the replicating basal and parabasal epithelial stem cell genome resulting in overexpression of p16 protein from the basal layers up, and perhaps reflecting malignant transformation. Therefore, the evaluation of hr-HPV infection as well as p16 immunoreactivity, could be useful tools of particular clinical value in identifying cases with a higher possibility to progress to high grade lesions.

In a meta-analyze result by Tsoumpou et al [29], p16 positivity was shown in only 2% of normal biopsies and 38% of CIN1 in comparision to 68% of CIN2 and 82% of CIN3. In our study, P16 positivity was approximately 76% in LSIL and 72% in HSIL cases. The IHC expression of p16 as a marker of progression risk in LSIL of the cervix uteri was evaluated in four studies [30-33]. Negri and colleagues [31] assessed the role of p16 in predicting CIN1 lesions that were likely to progress to CIN3 in a four year follow-up. The authors concluded that although p16 may be expressed in CIN1 that underwent spontaneous regression, cases with diffuse staining had a significantly higher tendency to progress to a HSIL than p16 negative cases. Overall, 71.4% and 37.8% of p16 negative and diffusely positive CIN1 regressed at follow-up, respectively. Whereas 28.6% and 62.2% negative and diffusely positive CIN1 lesions progressed to CIN3, respectively [29]. Hariri et al collected 100 CIN1, 50 CIN 2/3 and 50 non-dysplastic lesions and performed a 5-7 years follow-up [33]. All p16 negative CIN1 cases showed regression during follow-up while 45% of the p16 positive CIN1 cases progressed or had persistent CIN1. Based on the studies published so far, to better evaluated the potential of p16 as a cervical marker for LSIL, it is important to assess findings from cytology relative to follow-up histology data. In our results, p16 positivity was 76% and 74% respectively for LSIL and HSIL. A possible reason for the lower expression of p16 in HSIL may be that a certain percentage is thought to be caused by low risk HPV types. This is because the affinity of the E7 protein of low risk HPV for

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pRb is much lower than that of high risk HPV types, and there would not be any overexpression of p16.

As expressed in different phases of the cervical carcinogenesis, HPV L1 capsid protein and p16 are potentially promising markers of progression risk of cervical lesions. HPV L1 capsid protein is expressed in the early, productive phase of cervical carcinogenesis and is progressively lost in the later phases, when p16 become overexpressed. Yoshida et all [34]have demonstrated that the combination of p16 and L1 capsid protein immunostaining is a very useful and powerful test as a prognostic marker. The L1(-)/p16(+) results of their study represented 74.6% of all lesions, including 45%, 88%, and 100% of LSILs, HSILs, and SCCs, respectively. In our study, the L1(-)/p16(+) pattern was found in 21 of all cases, including eight LSIL and thirteen HSIL, respectively. The L1(-)/p16(+) pattern showed the integration of HPV DNA into the host genome with alteration of the cell cycle. This pattern might be defined as “high-risk”pattern, which is typically found also in HSIL of the cervix. The L1(+)/p16(-) pattern was found in 11 cases, including: seven LSIL and four HSIL, respectively. This pattern showed that viral DNA is present as a productive state without alteration of the cell cycle, which indicates a low risk of developing HSIL lesion. There is need of follow-up within a short time interval, because this pattern means a productive status of HPV infection that may produce a high-grade lesion in the future. L1(+)/p16(+) with the following distribution: thirty LSIL and twenty HSIL . This pattern showed a productive status of HPV infection and is associated with an alteration of the cell cycle. It indicates that the lesion is in a virus-producing state with immediately risk of progression. Cases with L1(-)/p16(-) pattern can be followed with a longer time interval, because this pattern means that the lesion is in a latent state.

The combination between hr-HPV and p16 is considered to be more useful, having a higher accuracy than hr-HPV or p16 alone. There are also opinions for the relevance of p16 expression in cervical squamous and glandular epithelium, as a marker of dysplasia or malignancy irrespective of the HPV infection status. Thus will increase the chance for infection to persist and for the intraepithelial squamous lesion to progress.

Conclusions:

In conclusion, there is still critical need to find other molecular surrogate markers, which can provide accurate information about which precursor lesions would progress toward cancer.

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