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Relation between HPV-16 serology and clinico-pathological data in cervical carcinoma patients: prognostic value of anti-E6 and/or anti-E7 antibodies

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Abstract To investigate the clinical significance of the enhanced sensitivity of antibody detection by radio immunoprecipitation assays (RIPA), using in vitro translated HPV-16 E6 and E7 proteins, over synthetic-peptide enzyme-linked immunosorbent assay (ELISA), RIPA for HPV-16 E6 and E7 were performed. The results obtained with E6 and E7 RIPA were related to clinico-pathological data from cervical carcinoma patients positive for HPV type 16 DNA in their primary tumour. The data obtained with E6 and E7 RIPA were then compared to the results obtained using the E7/6-35 synthetic-peptide ELISA. The prevalence of antibodies to E6, E7, E6 and/or E7 and E6 and E7, as determined by RIPA, was significantly higher in cervical cancer patients than in both controls and cervical intraepithelial neoplasia patients. Odds ratios, calculated for cervical carcinoma patients versus controls, ranged from 7.4 to 15.4. Antibodies to E6 and/or E7 were largely restricted to patients with HPV DNA in their primary tumour. Analysis of the relation between prevalence of antibodies to E6 and E7 and clinico-pathological parameters was limited to 85 patients positive for HPV-16 DNA. The strongest relation with clinico-pathological data, such as lesion size, lymph node involvement, and prognosis, was found for E7 synthetic-peptide ELISA, whereas E6 and E7 RIPA did not reach significance. The significance of these findings is discussed.

Key words Cervical cancer · Human papillomavirus · Serology · Prognostic markers

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Introduction

The strong association between the presence of mucosal human papillomavirus types (predominantly types 16, 18, 31 and 33) and the development of cervical cancer [12] has boosted interest in human papillomavirus serology to investigate its possible use in epidemiology and as a diagnostic or prognostic marker. Human papillomavirus (HPV) type 16 is most frequently found in squamous-cell cervical carcinomas, and the viral oncoproteins E6 and E7 are consistently transcribed in HPV-positive cervical cancer cell lines and cervical neoplasias [14, 17, 22]. Therefore, serology has focused for a large part on these two major transforming proteins of HPV type 16. Using synthetic peptides and fusion proteins, several authors have reported a significantly higher prevalence of antibodies to HPV-16 E6 [6, 11] and E7 [1, 7, 9–11, 13] in cervical carcinoma patients than in controls. Neither synthetic peptides, nor fusion proteins are ideal antigens in serological assays, as only non-conformational epitopes will be presented. Recently a number of studies have been performed using native proteins derived from in vitro translation [3, 11, 18–20] or baculovirus expression [15, 16]. These studies report increases in sensitivity and, especially, specificity compared to serological tests using antigens with non-conformational epitopes only. In order to validate the clinical significance of the enhanced sensitivity of antibody detection by radioimmunoprecipitation assays (RIPA) of native proteins over synthetic-peptide enzyme-linked immunosorbent assay (ELISA), we have performed RIPA for HPV-16 E6 and E7 and analysed whether the results relate to clinico-pathological data from cervical carcinoma pa-

tients. The findings were then compared to those obtained previously [1], using synthetic-peptide ELISA.

Materials and methods

Patients and controls

Pretreatment sera from 392 women with squamous-cell cervical carcinoma were obtained from the Department of Obstetrics and Gynaecology, University Hospital Groningen. The mean age of these patients was 50.7 years (SD 16.0 years). HPV DNA detection has been performed for 137 of the 392 cervical cancer patients on paraffin-embedded tissues, as described elsewhere [2]. The control group consisted of 197 healthy women with a similar age distribution (50.2 years SD 16.7). The control group for cervical cancer patients consisted of sera sent in for serology for infectious diseases unrelated to those sexually transmitted. No data for cytology or HPV DNA typing were available from the control group. In addition, sera from a group of 111 women with cervical intraepithelial neoplasia (CIN) lesions were obtained (age 32.5 years SD 7.9; CIN 1, $n = 13$; CIN 2, $n = 23$; CIN 3, $n = 75$).

Clinico-pathological parameters of patients with cervical carcinoma

The staging of the 392 patients with cervical carcinoma was in accordance with the recommendations of the International Federation of Gynaecology and Obstetrics (FIGO). Clinico-pathological data other than stage were available from 300 patients. The drawing of blood samples after exconization was a criterion for exclusion from the analysis. Examination of the patients was performed under general anaesthesia. During this procedure, the lesion size (largest diameter) was estimated routinely and expressed in centimetres for the large majority of patients.

All the available biopsy or cone material of 300 patients was carefully reviewed. Tumours were classified into well (grade 1), moderately (grade 2) or poorly (grade 3) differentiated or undifferentiated (grade 4) squamous-cell carcinoma, in accordance with the criteria laid down by Ferenczy and Winkler [5].

From 367 patients with cervical carcinoma, consecutively admitted and treated at the Department of Gynaecological Oncology at the University Hospital Groningen, follow-up data were available. Of these 367 patients, 106 died of cancer.

In vitro translation

pGEM1 plasmids containing the open reading frames for E6 and E7 were kindly provided by Dr P. Howley, (described in [4] and [21]). A 1-μg sample of plasmid DNA was used for combined in vitro transcription and translation using the TnT coupled Reticulocyte lysate system (Promega, Leiden, The Netherlands), in the presence of 40–50 μCi [^{35}S]-methionine and 40 units ribonuclease inhibitor (RNA guard, Pharmacia, Woerden, The Netherlands). After incubation for 2 h at 30 °C, the lysate was run over a Sephadex G25 column (Pharmacia), fractions were collected, and the size of the labelled protein was confirmed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis.

Detection of antibodies by RIPA

The fraction containing the labelled protein was diluted to approximately 30 000 cpm/100 μl in RIPA buffer (10 mM TRIS-HCl pH 8.0, 140 mM NaCl, 2.5 mg/100 ml NaN_3, 0.1% Nonidet P-40). A 2-μl sample of serum and 100 μl labelled protein were mixed and incubated at 4 °C for 16 h. A 0.45 μm Multiscreen microtitre plate (Millipore, Molsheim, France) was prewetted with RIPA buffer and loaded with protein-A–Sepharose (Sigma, St. Louis, Mo., USA, 0.15 g/plate) in RIPA buffer. Serum and lysate were transferred to the Multiscreen microtitre plate and incubated at 4 °C for 2 h, whilst shaking. After seven washes with RIPA buffer, the protein-A–Sepharose was transferred to a fresh Multiscreen microtitre plate, and washed three more times. The filters were punched out, transferred to scintillation vials containing 1.5 ml InstaGel Plus (Packard, Groningen, The Netherlands), and radioactivities (cpm) were read in a scintillation counter (Packard Tri-carb 460 CD). Each serum was tested in triplicate. In each plate, positive and negative controls were included, as well as wells without serum (background control).

Statistical methods

Odds ratios were calculated to compare the antibody prevalence between cervical cancer patients and controls. The association between anti-E6 and/or anti-E7 positivity in pretreatment sera from cervical carcinoma patients and tumour-related variables was investigated using bivariate techniques (Pearson χ^2 test) and multivariate logistic regression analysis. The prognostic value of anti-E6 and/or anti-E7 antibodies and other possible risk factors was determined in a Cox' regression model. The data used for analysis of the cervical carcinoma patient group included stage of disease (FIGO stage), lesion size, tumour grade, lymph node status, vascular invasion, depth of infiltration and the presence of antibodies against E6 and/or E7 protein.

Results

Prevalence of antibodies to HPV-16 E6 and E7 in patient groups and controls

Seroreactivities to the various combinations of antigens in both patient groups and controls are shown in Table 1 and Fig. 1. Cut-off values were calculated on the basis of results for the controls (mean + 3×SD) and were 188 cpm for E6 and 116 cpm for E7. High values for RIPA E6 (mean + 5×SD) were exclusively found in cervical cancer patients, whereas one control had a high antibody level in RIPA E7. The prevalences of antibodies to E6, E7, E6 and/

Table 1 Prevalence of antibodies to human papilloma virus (HPV) 16 E6 and E7 in patients with cervical carcinoma (*CC*) cervical intraepithelial neoplasia (*CIN*) patients and controls by radio-immunoprecipitation assay (*RIPA*). Odds ratios were calculated for cervical carcinoma patients versus controls. *OR* odds ratio, *CI* confidence interval

Antigen	CC patients			Controls			CIN patients			OR (95% CI)
	n	Pos.	(%)	*n*	Pos.	(%)	*n*	Pos.	(%)	
E6 RIPA	383	102	26.6	195	8	4.1	111	3	2.7	8.5 (4.0–17.8)
E7 RIPA	388	121	31.2	195	9	4.6	111	3	2.7	9.4 (4.6–18.9)
E6 or E7	378	131	34.7	195	13	6.7	111	3	2.7	7.4 (4.1–13.6)
E6 and E7	378	92	24.3	195	4	2.1	111	3	2.7	15.4 (5.6–42.5)

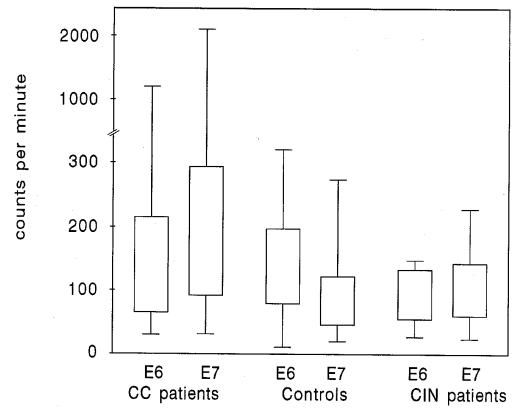

Fig. 1 Distribution of radioactivities (cpm) of sera as determined by E6 and E7 radio-immunoprecipitation assays (RIPA). *x*-axis studied groups; *y*-axis antibody level (cpm). The length of the box corresponds to the interquartile range, with the upper boundary representing the 75th, and the lower boundary the 25th percentile. The vertical line links the minimum and maximum values

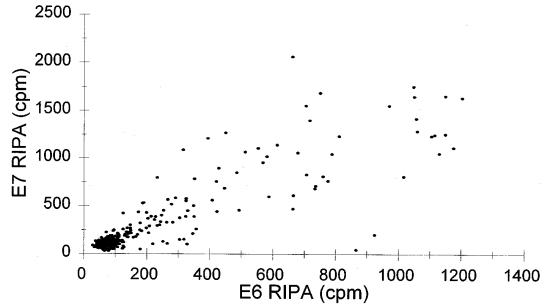

Fig. 2 Correlation between E6 and E7 antibody levels as determined by RIPA. *x*-axis anti-E6 antibody level (cpm); *y*-axis anti-E7 antibody level (cpm). Spearman's $r = 0.71$, $P < 0.001$

or E7 and E6 and E7 was significantly higher in cervical cancer patients than in either controls and CIN patients ($P < 0.001$). Odds ratios, calculated for cervical carcinoma patients versus controls, ranged from 7.4 to 15.4. There was a strong tendency for patients to have antibodies to both E6 and E7 simultaneously (Spearman's $r = 0.71$, $P < 0.001$, Fig. 2).

HPV type specificity of anti-E6 and anti-E7 antibodies

From 137 of the 392 patients, the HPV type in the tumour could be determined by the polymerase chain reaction (PCR) on paraffin-embedded tissue. We analysed whether the presence of antibodies against E6 and/or E7 was related to the HPV type in the primary lesion. We compared the findings to those with antibodies detected by E7 synthetic peptide ELISA (E7 ELISA, Table 2). The seroprevalence of antibodies to E6 and/or E7, as detected by RIPA, was significantly higher in the HPV-16-positive patients than in the non-HPV group (E6 RIPA, $P < 0.025$; E7 RIPA, $P < 0.05$). Furthermore, in this group of HPV-16-positive patients, antibody positivity was higher by E6 and/or E7 RIPA than by E7 ELISA. Finally, RIPA antibody positivity was found significantly more frequently in HPV-16-positive patients than in patients positive for other HPV types (E6 RIPA, $P < 0.01$; E7 RIPA, $P < 0.025$).

Analysis of the relation between E6 and E7 antibodies in pretreatment sera from HPV-16-DNA-positive patients with cervical carcinoma and clinico-pathological parameters

On basis of the higher prevalence of E6 and/or E7 antibodies, the analysis was limited to 85 HPV-16-DNA-positive patients. No relation was found with differentiation grade, vascular invasion and depth of invasion in any of the tests. Table 3 shows the prevalence of anti- E6 and/or E7 antibodies as determined with RIPA and ELISA in relation to tumour volume, lymph node metastasis and prognosis. Of the different serological assays, only positivity for antibodies against E7, as detected by ELISA, showed a significant relation to tumour volume ($P = 0.003$). Neither E6 or E7 RIPA positivity nor positivity for both E6 and E7 by RIPA showed a significant relation with tumour size. A significant relation with lymph node metastasis was obtained for patients positive for antibodies against both E6 and E7 as detected by RIPA. All serological assays showed a relation close to significance ($P = 0.05-0.07$) with the clinical outcome of patients.

Logistic regression analysis was performed to predict the presence of lymph node metastasis. Pretreatment variables included FIGO stage, lesion size, differentiation grade, vascular invasion and invasion depth, as well as E6 and E7 RIPA and E7 ELISA. It was shown that four variables contributed significantly to the prediction of lymph node metastasis: FIGO stage, lesion size, vascular invasion and E7 RIPA ($\chi^2 = 23.03$; $df = 6$; $P = 0.0008$; sensitivity 62.5%; specificity 91.11%).

Similarly, logistic regression analysis for the prognosis of the patient, based on the same pretreatment variables, was performed. This showed that three variables contrib-

Table 2 Prevalence of antibodies to HPV 16 E6 and E7 in cervical cancer patients with a known HPV status. *Other types* include HPV types 6, 18, 31, 33, 34, 45 and 52. *ELISA* enzyme-linked immunosorbent assay

Antigen	HPV-negative			HPV type 16			HPV other types		
	n	Pos.	(%)	*n*	Pos.	(%)	*n*	Pos.	(%)
E7 ELISA	19	1	5.3	85	22	25.9	33	7	21.2
E6 RIPA	19	1	5.3	85	33	38.8	31	4	12.9
E7 RIPA	19	2	10.6	85	33	38.8	33	5	15.2
E6 or E7	19	2	10.6	85	37	43.5	31	7	22.6
E6 and E7	19	1	5.3	85	29	34.1	31	2	6.5

Table 3 Correlation of the presence antibodies to HPV 16 E6 and E7 in cervical carcinoma patients positive for HPV 16 in their primary tumour with clinico-pathological parameters. *NS* not significant, values below 0.1 are given in full; *NED* no evidence of disease; *DOD* dead of disease

	E7 ELISA		RIPA E6		RIPA E7		RIPA E6/E7		RIPA E6+E7	
	+/*n*	*P*	+/*n*	*P*	+/*n*	*P*	+/*n*	*P*	+/*n*	*P*
Tumour volume										
<4 cm	8/50		18/50		17/50		21/50		14/50	
≥4 cm	13/27	0.003	13/27	NS[1]	14/27	NS	14/27	NS	13/27	0.077
Lymph nodes										
Negative	9/48		15/48		15/48		18/48		12/48	
Positive	11/27	0.039	14/27	0.079	14/27	0.079	15/27	NS	13/27	0.041
Patient outcome										
NED	14/66		22/66		22/66		25/66		19/66	
DOD	8/19	0.070	11/19	0.053	11/19	0.053	12/19	0.050	10/19	0.053

uted significantly to the prediction of patient outcome; FIGO stage, differentiation grade and E6 RIPA (χ^2 = 16.64; d = 6; P = 0.0107; sensitivity 27.78%; specificity 94.92%). The other parameters did not have any additional value for the estimation of patient survival.

Discussion

Early HPV serology has shown that the sensitivity and specificity of synthetic peptide ELISA and fusion-protein immunoblotting are too low to be of clinical significance. Therefore, interest has shifted to the use of native proteins in various assays. Although a baculovirus expression system has been developed for both E6 and E7 [15, 16] most studies report the use of in vitro transcription/translation of E6 and E7 [3, 11, 18–20]. Radio-immunoprecipitation of the proteins generated by in vitro translation has shown a remarkable increase in both sensitivity and specificity. In the present study, investigating 392 patients with squamous-cell carcinoma of the uterine cervix in E6 and E7 RIPA, we found both an increase in sensitivity when compared to ELISA with E7 synthetic peptide (E7 RIPA 31.2%, E7 ELISA 17.7% [1]), and an increase of the specificity (E7 RIPA 95.4%, E7 ELISA 89% [1]). Furthermore, a slightly higher cut-off (mean + 5×SD) increased the specificity to nearly 100%. For E7 RIPA this is in accordance with other studies (sensitivity 19%–43%, specificity 95%–96% [3, 18, 19]). For E6 RIPA a sensitivity of 26.6%, and a specificity of 95.9% was found. A slightly higher sensitivity has been reported (37%–56% [3, 18, 19]). We found that most patients have antibodies to E6 and E7 simultaneously, in contrast with the results of other investigators [18, 19]. This discrepancy is striking, since it has been shown that E6 and E7 are co-expressed in cervical cancer cells and cancer-derived cell lines [14, 17, 22], suggesting simultaneous exposure to the immune system.

Antibody prevalence is related to the specific HPV type in the primary tumour. Because of the source of material, formaldehyde-fixed paraffin-embedded tissue, it is possible that patients negative in PCR do harbour low amounts of HPV DNA. Recently the antagonistic effect of formaldehyde fixation causing DNA modification has been described [8]. In fact, one patient negative in HPV PCR was strongly positive in all three serological tests, suggesting this patient had an HPV-16 infection.

Because of the higher prevalence of antibodies in the E6 and E7 RIPA, analysis of the prevalence of antibodies against E6 and E7 to clinico-pathological parameters was limited to 85 HPV-16-positive patients. This inevitably leads to a reduction in statistical power. Nevertheless, in agreement with our previous finding [1], a significant relation between E7 ELISA and tumour volume (P = 0.003), as well as lymph node involvement (P = 0.039), was found. No significant relation was found between RIPA and the clinicopathological parameters. However, in nearly all comparisons the probability under the null hypothesis was 0.05–0.08. In our opinion this finding indicates a relationship between antibodies against E6 and/or E7 and clinico-pathological parameters. Logistic regression analysis, including all three serological assays, showed the strongest relation between anti-E7 antibody positivity and lymph node involvement, and anti-E6 antibody positivity and survival. Analysis of all three serological assays separately, however, showed that there were only marginal differences between the predictive values of E6 and E7 positivity for both lymph node involvement and survival.

Since both E6 and E7 proteins are consistently and simultaneously expressed in cancer cells [14, 17, 22], a similar course of antibody expression for E6 and E7 can be expected in cervical cancer. To obtain a humoral immune response, E6 and E7 protein, or fragments thereof, should be recognized by B cells. For this to occur, E6 and E7 protein must be released from the HPV-infected cells. This can be achieved either by necrosis of tumour tissue, or by a cellular immune attack on HPV-infected cells. Our results suggest that the chance that E6 and E7 are released from HPV-infected tumour cells increases with tumour load. The observed correlation between anti-E6 and anti-E7 positivity with lymph node involvement suggests that extension of the tumour beyond a certain barrier increases the chance of antibody production. The cancer metastasis to the lymph nodes, as immunocompetent centres, may especially enhance a humoral immune response.

In conclusion, the results of this study underline our previous finding that antibody levels against the transforming proteins E7 and also E6 are related to tumour load and lymph node involvement. Further research is warranted to elucidate the immune response to HPV in cervical cancer as this may be of key importance in the immune response to the tumour.

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References

1. Baay MFD, Duk JM, Burger MPM, Walboomers J, Schegget J ter, Groenier KH, Bruijn HWA de, Stolz E, Herbrink P (1995) Antibodies to human papillomavirus type 16 E7 related to clinicopathological data in patients with cervical carcinoma. J Clin Pathol 48:410
2. Baay MFD, Quint WGV, Koudstaal J, Hollema H, Duk JM, Burger MPM, Stolz E, Herbrink P (1996) A comprehensive study of several general and type-specific primer pairs for detection of human papillomavirus DNA by polymerase chain reaction in paraffin-embedded cervical carcinomas. J Clin Microbiol 34:745
3. Chee YH, Namkoong SE, Kim DH, Kim SJ, Park JS (1995) Immunologic diagnosis and monitoring of cervical cancers using in vitro translated HPV proteins. Gynecol Oncol 57:226
4. Dyson N, Howley PM, Munger K, Harlow E (1989) The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 243:934
5. Ferenczy A, Winkler B (1987) Carcinoma and metastatic tumours of the cervix. In: RJ Kurman (ed). Blaustein's pathology of the female tract. Springer, New York Berlin Heidelberg p 218
6. Ghosh AK, Smith NK, Stacey SN, Glew SS, Connor ME, Arrand JR, Stern PL (1993) Serological response to HPV 16 in cervical dysplasia and neoplasia: correlation of antibodies to E6 with cervical cancer. Int J Cancer 53:591
7. Jochmus-Kudielka I, Schneider A, Braun R, Kimmig R, Koldovsky U, Schneweis KE, Seedorf K, Gissmann L (1989) Antibodies against the human papillomavirus type 16 early proteins in human sera: correlation of anti-E7 reactivity with cervical cancer. J Natl Cancer Inst 81:1698
8. Karlsen FM, Kalantari M, Chitemerere M, Johansson B, Hagmar B (1994) Modifications of human and viral deoxyribonucleic acid by formaldehyde fixation. Lab Invest 71:604
9. Köchel HG, Monazahian M, Sievert K, Höhne M, Thomssen C, Teichmann A, Arendt P, Thomssen R (1991) Occurrence of antibodies to L1, L2, E4 and E7 gene products of human papillomavirus types 6b, 16 and 18 among cervical cancer patients and controls. Int J Cancer 48:682
10. Mann VM, Loo de Lao S, Brenes M, Brinton LA, Rawls JA, Green M, Reeves WC, Rawls WE (1990) Occurrence of IgA and IgG antibodies to select peptides representing human papillomavirus type 16 among cervical cancer cases and controls. Cancer Res 50:7815
11. Müller M, Viscidi RP, Sun Y, Guerrero E, Hill PM, Shah F, Bosch X, Muñoz N, Gissmann L, Shah KV (1992) Antibodies to HPV-16 E6 and E7 proteins as markers for HPV-16-associated invasive cervical cancer. Virology 187:508
12. Munoz N, Bosch FX, De Sanjose S, Tafur L, Izarzugaza I, Gili M, Viladiu P, Navarro C, Martos C, Ascunce N, Gonzalez LC, Kaldor JM, Guerrero E, Lorincz A, Santamaria M, Alonso de Ruiz P, Aristizabal N, Shah K (1992) The causal link between human papillomavirus and invasive cervical cancer: a population-based case-control study in Colombia and Spain. Int J Cancer 52:743
13. Onda T, Kanda T, Zanma S, Yasugi T, Watanabe S, Kawana T, Ueda K, Yoshikawa H, Taketani Y, Yoshiike K (1993) Association of the antibodies against human papillomavirus 16 E4 and E7 proteins with cervical cancer positive for human papillomavirus DNA. Int J Cancer 54:624
14. Sherman L, Alloul N, Golan I, Durst M, Baram A (1992) Expression and splicing patterns of human papillomavirus type-16 mRNAs in pre-cancerous lesions and carcinomas of the cervix, in human keratinocytes immortalized by HPV 16, and in cell lines established from cervical cancers. Int J Cancer 50:356
15. Stacey SN, Bartholomew JS, Ghosh A, Stern PL, Mackett M, Arrand JR (1992) Expression of human papillomavirus type 16 E6 protein by recombinant baculovirus and use for detection of anti-E6 antibodies in human sera. J Gen Virol 73:2337
16. Stacey SN, Ghosh A, Bartholomew JS, Tindle RW, Stern PL, Mackett M, Arrand JR (1993) Expression of human papillomavirus type 16 E7 protein by recombinant baculovirus and use for the detection of E7 antibodies in sera from cervical carcinoma patients. J Med Virol 40:14
17. Stoler MH, Rhodes CR, Whitbeck A, Wolinsky SM, Chow LT, Broker TR (1992) Human papillomavirus type 16 and 18 gene expression in cervical neoplasias. Hum Pathol 23:117
18. Sun Y, Shah KV, Muller M, Munoz N, Bosch XF, Viscidi RP (1994) Comparison of peptide enzyme-linked immunosorbent assay and radioimmunoprecipitation assay with in vitro-translated proteins for detection of serum antibodies to human papillomavirus type 16 E6 and E7 proteins. J Clin Microbiol 32:2216
19. Sun Y, Eluf-Neto J, Bosch XF, Munoz N, Booth M, Walboomers JNN, Shah KV, Viscidi RP (1994) Human papillomavirus-related serological markers of invasive cervical carcinoma in Brazil. Cancer Epidemiol Biomarker Prevention 3:341
20. Viscidi RP, Sun Y, Tsuzaki B, Bosch FX, Munoz N, Shah KV (1993) Serologic response in human papillomavirus-associated invasive cervical cancer. Int J Cancer 55:780
21. Werness BA, Levine AJ, Howley PM (1990) Association of human papillomavirus type 16 and 18 E6 proteins with p53. Science 243:76
22. Yee C, Krishnan-Hewlett I, Baker CC, Schlegel R, Howley PM (1985) Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. Am J Pathol 119:361