IMMUNOCYTOCHEMICAL EXPRESSION OF HUMAN PAPILLOMAVIRUS (HPV) HIGH RISK TYPE L1 CAPSID PROTEINS IN LSIL AND HSIL

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Abstract: The genesis of uterine cervix carcinoma has as central etiology the active infection of HPV, especially high risk subtypes. At present, the proportion of cervical carcinomas attributed to HPV infection is estimated at 99%. The aim was to investigate the prevalence of HPV L1 capsid proteins in HPV infected HSIL and LSIL. HPV L1 capsid proteins are considered to be a major target of cellular immune response in cervical intraepithelial squamous lesions. Cervical conventional smears from 82 women with cytologically and histologically confirmed LSIL (n=54) and HSIL (n=28) were collected retrospectively to detect HPV L1capsid proteins by immunocytochemistry using the monoclonal antibodies (High Risk Antibody VAHP) in a standardized protocol. HPV L1-capsid proteins were detected in HSIL and LSIL for HPV high risk in 28% and 40% of the specimens, respectively. Expression of L1-capsid proteins was significantly reduced for HPV positive HSIL. In HPV positive LSIL, no significant reduction of L1 capsid protein expression could be demonstrated. These data support the statement that failure to detect L1 capsid protein in smears and biopsies correlates with progression of the lesion. Immunocytochemical detection of HPV L1 capsid protein has prognostic value for the follow up of early dysplastic cervical lesions.

INTRODUCTION

The genesis of uterine cervix carcinoma has as central etiology the active infection of HPV, especially high risk subtypes. However, this is a necessary, but not sufficient cause of virtually all cases of cervical cancer worldwide. At present, the proportion of cervical carcinomas attributed to HPV infection is estimated at 99%. Human papillomaviruses (HPVs or “wart viruses”) have been suspected for many years as the cause of ordinary skin warts and of the common wart-like lesions known as venereal warts or condylomata acuminata, often simply designated as “condylomas” (Koss et al, 2006). The viral origin of condylomata acuminata was strongly approved when viral particles were observed in the nuclei of squamous epithelial cells by electron microscopy (Dunn and Ogilvie, 1968; Oriel and Alameida, 1970). The earliest study documenting the presence of HPVs in a neoplastic lesion of the cervix was based on electron microscopy of biopsies of the cervix, cited above, and extended to corresponding cells in cervico-vaginal smears by Meisels et al (1984). By this technique, only the mature virions of unknown type can be demonstrated in the nuclei of the affected cells. Other techniques, which were developed, could reveal only mature virions which were generally limited to the nuclei of cells in the upper layers of the squamous epithelium, notably the nuclei of koilocytes. A positive reaction with the nuclei of cells of the basal layer was exceptional. They could not provide informations on latent infection (Jean Gupta et al, 1983). To identify latent infection and to determine the relationship of specific viral types to human disease, molecular hybridization techniques were required. The presence of high-risk HPVs has been documented in nearly all invasive cancers and in 50% to 90% of precancerous squamous lesions (Lorincz et al, 1992; zur Hausen, 1987; Bosch et al, 1992; Howley, 1991; Shah and Howley, 1992; Kleter et al, 1998; Lazo, 1999; Burk, 1999; Munoz et al, 2003). The follow-up of the patients, with or without cytologic abnormalities, suggested that HPV infected women, especially of the high-risk type, are at risk for developing intraepithelial precursors lesions, some of which are high-grade (de Villiers et al, 1992; Koutsky et al, 1992; Schlecht et al, 2001). Several follow-up studies, notably by Ho et al (1995); Walboomers et al (1999); Chua et Hjerpe (1996); and Wallin et al (1999), presented strong evidence that women with persisting infection of high-risk HPV were at risk for developing high-grade squamous intraepithelial lesions and, further on, invasive cancer of the cervix.
The aim of our study was to investigate the prevalence of HPV L1 capsid proteins in HPV infected high grade squamous intraepithelial lesions (HSILs) and low grade squamous intraepithelial lesions (LSILs). HPV L1 capsid proteins are considered to be a major target of cellular immune response in cervical intraepithelial squamous lesions (White et al, 1998).

MATERIALS AND METHODS

Cervical conventional smears from 82 women with cytologically and histologically confirmed LSIL (n=54) and HSIL (n=28) were collected retrospectively to detect HPV L1capsid proteins by immunocytochemistry using the monoclonal antibodies (High Risk Antibody VAHP) in a standardized protocol. Epithelial cells with positive nuclear staining were

scored as positive. The cervical biopsies were paraffin-embedded and routinely stained and the cervico-vaginal smears were fixed and stained with Papanicolaou method. HPV infection was detected by the presence of cytopatic HPV effect (koilocytes) in the smears and biopsies.

RESULTS AND DISCUSSIONS

Human papillomaviruses (HPVs) represent the etiological agents of several genital cancers, including cancer of the uterine cervix. It is considered that the detection of HPV infection in genital samples can increase the sensitivity of primary and secondary screenings of cervical cancer. HPV testing may also improve the specificity of screening programs, resulting in the avoidance of overtreatment and cost savings for confirmatory procedures. The studies revealed, as major determinants of clinical progression of HPV infection, the persistence of HPV infection, involvement of high-risk HPV types, high HPV viral load, integration of viral DNA and presence of several potential cofactors (Coutlee et al, 2005).

L1 capsid protein is expressed in the active phase of HPV infection and is necessary in viral cellular cycle completion. Consequently, viral protein detection, by immunohistochemical reaction is an evidence of active HPV infection in examined tissue (Gu et al, 2007). LSIL and moderate SIL without immunohistochemically detected L1 are correlated, in more than 80% of cases, with dysplasia progression. Fiedler et al (2006) certify these aspects, evidentiating that minor and moderate lesions without L1 capsid protein expression are significantly more exposed to a progression in comparison to L1 positive cases (Griesser et al, 2004). Most probably, the lack of HPV antigen is determined by a weak protein synthesis, under immunohistochemical test minimum level.

Our study contained specimens from 82 women, 54 with LSIL and 28 women with HSIL. In all women, the diagnosis was confirmed by cytology (Papanicolaou stained smears) and histology (cervical biopsies). Mean age of all patients was 34 years. Mean age of the LSIL patients was 34.7 years; mean age of the HSIL patients was 34.3 years. Out of 28 HSIL cases submitted to the High-Risk HPV L1 protein (HR- HPV L1), 8 (28%) were positive, 20 (72%) were negative. Positive reaction was characterized by the strong staining of the whole nucleus, surrounded by a cytoplasm with no background (figure 3). In most cases, positive reaction for HR-HPV L1 was positive in typical koilocytes or in dyskeratocytes, presenting nuclear characteristics for HSIL (CIN 2 or CIN 3) (figures 2 and 4). Out of 54 LSIL cases submitted to the HR- HPV L1 protein, 22 (40%) were positive, 32 (60%) were negative. In these cases, the positivity of the nuclei was presented only in typical koilocytes (figure 1).

In our study, patients' mean age was 34 years. This fact is in concordance with the literature data, where it has been demonstrated that the prevalence of HPV infection varies with age and geographical region, reaching highest rates below 35 years of age (Cox, 1999).

The evidentiation of positively stained nuclei of squamous epithelial cells correlated with the clinical course. Expression of L1-capsid proteins was significantly reduced for HPV positive HSIL. In HPV positive LSIL, no significant reduction of L1 capsid protein expression could be demonstrated. Mild and moderate dysplastic cervical lesions without immunohistochemical positive reaction of HPV L1 capsid protein are more likely to progress as compared to positive cases (Griesser et al, 2004). After 6 months of follow-up, the progressive disease occurred in L1-positive cases. It was noticed that, among the 22 LSIL L1-positive cases, 13 presented remission, while from 32 LSIL L1-negative patients, 17 presented the progression of the lesion. These data support the theory that failure to detect L1 protein in cervico-vaginal smears correlates with progression of the lesion, even in cases of LSIL (Melsheimer et al, 2003). Researches consider

that lack of detectable HPV antigen in the Pap smears is due to low protein synthesis in squamous epithelial cells below the limit of the immunocytochemical test. The loss of L1 capsid protein immunoexpression can be due to the integration of the viral DNA into the human genome. Although most of cervical carcinomas show integration of viral DNA, it is detectable only in a small proportion of LSIL and HSIL (Klaes et al, 1999). As was already reported in the literature (Melsheimer et al, 2003), there are perhaps additional control mechanisms which lead to the L1-negative immunoexpression in the mentioned cases.

Molecular researches which used cellular and tissue cultures gave an understanding of mechanism through which HPV transform the cervical epithelium. High risk HPV types, as HPV 16 and 18, produce three proteins with growth-stimulating and transforming proteins, E5, E6, and E7. E5 is not essential for transformation as the E5 region is frequently deleted in cervical carcinoma cells (Schwarz et al, 1985).

The development of viral capsid antigen L1 depends upon transcriptional factors which only can be expressed during maturation process from basal epithelial cell to superficial epithelial cell (McMurray et al, 2001). In HSIL, the natural structure as well

as maturation of the epithelium are disturbed, thus the dysplastic basal squamous cells represent the predominant cell type with reduced L1 capsid protein expression.

Viral L1 capsid antigen is considered to be a major target of cellular immune response. Thus, a reduction or loss of capsid antigen production might result in a reduction of cellular immune response (Hagensee et al, 2000). This explains the role of HPV 16 as the most oncogenic HPV type, causing higher rates of progression and persistence of SILs than other HPV types (Melsheimer et al, 2001).

Because L1 protein is the major target of the cellular immune response (White et al, 1998), its loss at early stages of the transformation process can lead to an inappropriate stimulation of the immune response, promoting further transformation of immature epithelial cells (McMurray et al, 2001).

Other studies revealed the same observation - as L1 represents the major target of the immune cellular response (Melsheimer et al, 2003; Steele et al, 2002), its deficient translation may result in an inefficient comb out of the infected cells, promoting viral DNA integration in host cellular genome and the transformation of immature epithelial cells. The notification that the decrease of the HPV16 capsid positivity in cervical cancer patients serum is an indicator of a poor prognosis sustains the importance of a specific immune response. Immunohistochemical detection of L1 capsid, on conventional Papanicolaou smears, may consequently indicate the defence status locally induced on HPV infection and may offer prognosis information in LSIL lesions.

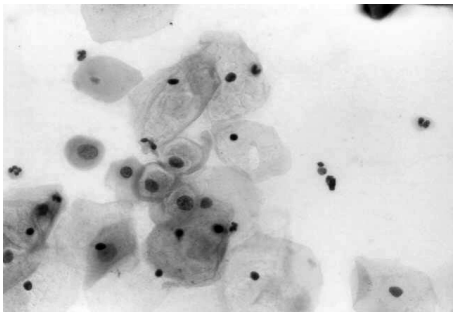

Fig. 1 LSIL Conventional smear, Pap x 20

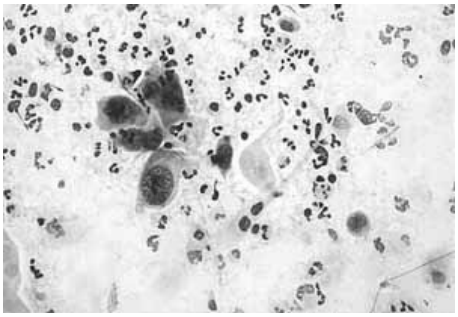

Fig. 2 HSIL Conventional smear, Pap x 40

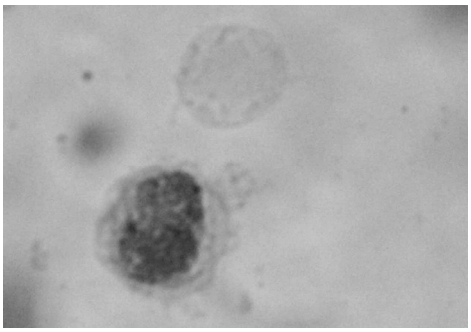

Fig. 3 HPV L1 positive (x100)

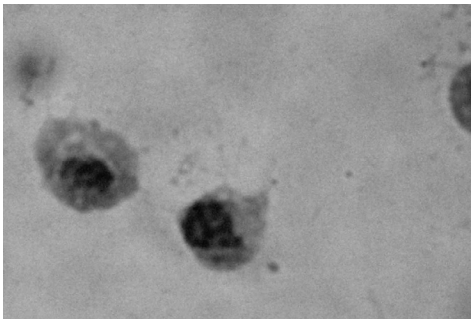

Fig. 4 HPV L1 positive (x100)

CONCLUSIONS

These data support the statement that failure to detect L1 capsid protein in smears and biopsies correlates with progression of the lesion. Because L1 is the major target of the immune response, its loss at early stages of the transformation process may lead to inefficient stimulation of the immune response and promote further transformation of immature epithelial cells. Immunocytochemical detection of HPV L1 capsid protein has prognostic value for the follow up of early dysplastic cervical lesions.

REFERENCES

Bosch MMC, Rietveld-Scheffers PEM, Boon ME, 1992. *Acta Cytol*, 36: 711-716.
Burk RD, 1999. *Hosp Pract*, 34: 103-111.
Chua KL, Hjerpe A, 1996. *Cancer*, 77 : 121-127.
Coutlée F, Rouleau D, Ferenczy A, Franco E., 2005. *Can J Infect Dis,* 16(2): 83–91.
Cox JT, 1999. *JAMA*, 17: 1645-1647.
de Villiers EM, Wagner D, Schneider A, et al, 1992. *Gynecol Oncol*, 44 : 33-39.
Dunn AEG, Ogilvie NM, 1968. *J Ultrastruct Res*, 22: 282-295.
Gupta JW, Gupta PK, Shah KV, Kelly DP, 1983. *Int J Gynecol Pathol*, 2: 160-170.
Fiedler M, Campo-Fernandez B, Laich A, Moser B, Stockl P, et al, *Purification 2006.* J Virol Methods, 134(1-2): 30-5.

Griesser H, Sander H, Hilfrich R, Moser B, Schenk U, 2004. *Analytical and Quantitative Cytology and Histology*, 26 (5): 241-245.
Gu W, Ding J, Wang X, de Kluyver RL, et al, 2007. *Nucleic Acids Res*, 35(14): 4820-32.
Hagensee ME, Koutsky LA, Lee SK, Grubert T, Kuypers J, Kiviat NB, Galloway DA, 2000. *J Infect Dis*, 181(4):1234-9.
Howley PM, 1991. *Cancer Res*, 51 (suppl): 5019-5022.
Ho GYF, Burk RD, Klein S, et al, 1995. *J Natl Cancer Inst*, 87: 1365-1371.
Klaes R, Woerner SM, Ridder R, Wentzensen N, Duerst M, Schneider A, et al, 1999. *Cancer Res*, 59: 6132-6136.
Kleter B, van Doorn L-J, ter Schegget J, et al, 1998. *Am J Pathol*, 153: 1731-1739.
Koutsky LA, Holmes KK, Critchlow CW, et al, 1992. *N Engl J Med*, 327 : 1272-1278.
Koss LG, Melamed MR, 2006. *Koss' Diagnostic Cytology and its Histopathologic Bases*, Lippincott Williams & Wilkins, Philadelphia.
Lazo PA, 1999. *Br J Cancer*, 80: 2008-2018.
Lorincz AT, Reid R, Jenson B, et al, 1992. Obstet Gynecol, 79:328-337.
McMurray HR, Nguyen D, Westbrook TF, Mcance DJ, 2001. *Int J Exp Path*, 82: 15-33.
Meisels A, Morin C, Casa-Cordero M, 1984. in Koss LG, Coleman DV (eds) *Advances in Clinical Cytology* 2: 1-31, Masson, New York.
Melsheimer P, Kaul S, Dobeck S, Bastert G, 2003. *Acta Cytol*, 47: 124-128.
Melsheimer P, Klaes R, von Knebel-Doeberitz M, Bastert G, 2001. *Cytometry*, 46(3):166-71.
Munoz N, Bosch FX, de Sanjose S, et al, 2003. *N Engl J Med*, 348: 518-527.
Oriel JD, Alameida JD, 1970. *Br J Vener Dis*, 46: 37-42.
Schlecht NF, Kulaga S, Robitaille J, et al, 2001. *JAMA*, 286 : 3106-3114.
Schwarz E, Freese UK, Gissman L, et al, 1985. *Nature* 314:111-114.
Shah KV, Howley PM, 1992 in: Lennette EH (ed). *Laboratory Diagnosis of Viral Infection*, 2nd ed., Marcel Dekker, New York.
Steele JC, Roberts S, Rookes MS, Gallimore PH, 2002. *J. Virol*, 76: 6027-6036.
Walboomers JMM, Jacobs MV, Manos MM, et al, 1999. *J Pathol*, 189: 12-19.
Wallin K-l, Wiklund F, Angstrom T, et al, 1999. *N Engl J Med*, 341: 1633-1638.
White WI, Wilson SD, Bonnez W, Rose RC, Koenig S, Suzich JA, 1998. *J Virol*, 72: 959-964.
zur Hausen H, 1987. *Appl Pathol*, 5: 19-24.

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