IMMUNOHISTOCHEMICAL IDENTIFICATION OF HUMAN PAPILLOMA VIRUS HIGH-RISK TYPE L1 MAJOR CAPSID PROTEINS IN ATYPICAL GLANDULAR CELLS

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Abstract: Endocervical lesions may be revealed by the identifying of atypical glandular cells (AGCs), limited by the involvement of the lining columnar epithelium of the cervical canal. The aim of this study was to perform a retrospective and prospective investigation of the AGCs observed on conventional cervical smears, stained with Papanicolaou staining, with subsequent histopathological diagnosis obtained on cervical biopsies and their correlations with immunohistochemical aspect of HPV high-risk type L1 major capsid proteins. We retrieved 87 cases of conventional cervical smears from our files, with AGC suspicions and correlated with their paraffin-embedded and routinely stained cervical biopsies, endocervical and endometrial curettages, during a 3 years follow-up period. Immunohistochemical staining, using high risk antibody VAHP, was performed in a standardized protocol. Biopsy findings of cervical smears were as follows: 62.06 % miscellaneous benign lesions, 22.98 % malignancies: cervical adenocarcinoma *in situ*; n= 6, cervical adenocarcinoma; n= 10, and cervical adenosquamous carcinomas; n= 4, and 14.94 % squamous intraepithelial lesion: LGSIL; n= 8 and HG- SIL; n= 5. HPV high-risk type L1 major capsid proteins were identified in 47% of AGC cases associated with adenosquamous carcinomas (2) and SILs (4 LSILs and 2 HSILs). In conclusion, biopsy follow-up of conventional cervical smears with diagnose suspicion of AGCs revealed *in situ* and invasive malignant glandular lesions, and squamous cervical intraepithelial lesions in 37.93 % of investigated cases were malignant type and precursors lesions. HPV infection could be incriminated in 47% of cases diagnosed with AGCs which were associated with low grade and high grade squamous intraepithelial lesions (LSILs and HSILs) and adenosquamous carcinomas, according to HPV high-risk type L1 capsid proteins immunohistochemical positivity of squamous epithelial cells.

INTRODUCTION

Carcinogenic human papillomavirus (HPV) infection is necessary for the development of cervical cancer (IARC, 2007), the second most common cancer in women worldwide (Parkin et al., 2005).

Although carcinogenic HPV types are found in virtually all invasive cervical cancer, with types 16 and 18 being found in approximately 70 percent of cases (Smith et al., 2007), HPV infection is common among young women (Koshiol et al., 2008). Persistent high-risk human papillomavirus (HPV), especially high-risk (oncogenic) types, infection is incriminated in the development of invasive cervical cancer. HPV infection is highly prevalent and is usually transient. It is effectively cleared by the immune system 90% of the time, suggesting that other biological, environmental and viral-specific factors must trigger the malignant transformation. HPV infection has a defined role in the aetiology of squamous intra-epithelial changes. 10% of HPV infections are considered to become persistent and, together with other co-factors, 3-4% to progress to intraepithelial lesions, of which 0.7-1% may develop high-grade lesions and 0.1% cancer (IARC, 2007). Consequently a cytologic screening, by Pap test, may detect early stages of HPV infection. Endocervical adenocarcinoma is responsible for 18-27% of all cervical carcinomas and constitutes 5-10% of all carcinoma arising in the cervix in developing countries (Chew et al., 2005).

The coexistence of cervical adenocarcinoma with squamous carcinoma suggests that they have a common ethiology and pathogenesis; both are associated with the same risk factors (Balan et al., 2009).

The Pap test was not designed as a screening test for either endometrial or endocervical adenocarcinoma and its sensitivity in the diagnosis of these entities and their glandular precursors is relatively low. The term ”atypical glandular cells of undetermined significance (AGUS)” was introduced at the Bethesda Conference in 1998 and defined as morphologic changes in glandular cells beyond those that are suggestive of the benign reactive process, but insufficient for the diagnosis of adenocarcinoma *in situ* and then revised in 2001 as ”atypical glandular cells” (AGCs) with the following subclassifications: not otherwise specified (NOS), favor neoplasia, endocervical AIS, and adenocarcinoma (Solomon et al., 2002) to encompass morphologic changes in glandular cells that were felt to be beyond normal changes, but non-diagnostic for adenocarcinoma *in situ* or invasive adenocarcinoma, and as a hope that the correct use of the AGC terminology would lead to an increased detection of glandular lesions by cervical cytology. The risks of premalignant or malignant disease associated with the AGC favor neoplasia category are substantially higher than in the AGC NOS category (96% vs. 9-41%, respectively) (Levine et al., 2003). The diagnosis of AGC strongly suggests underlying glandular lesions of either endocervical or endometrial origin. These patients are also at high risk for concurrent squamous dysplasia, as the HPV origin is common and concomitant.

As AGC interpretation continues to be a problematic diagnostic entity, usually mandates some form of immediate surgical biopsy (Cangiarella et al., 2003).
The aim of this study was to perform a retrospective and prospective investigation of the atypical glandular cells (AGC) observed on conventional cervical smears, stained with Papanicolaou staining, with subsequent histopathological diagnosis obtained on cervical biopsies and their correlations with immunohistochemical aspect of HPV high-risk type L1 capsid proteins.

MATERIAL AND METHODS

We retrieved 87 cases of conventional cervical smears from our files, with AGC suspicions and correlated with their paraffin-embedded and routinely stained cervical biopsies, endocervical and endometrial curettages, during a 3 years follow-up period. Immunohistochemical staining, using high risk antibody VAHP, was performed in a standardized protocol.

RESULTS AND DISCUSSIONS

In countries with an organised cervical screening, a steady decline in the overall incidence of cervical cancer was noted. Cervical screening has enabled identification and treatment of squamous epithelial changes in its pre-malignant state, leading to a noted decline in the incidence of squamous cell carcinoma in all age groups (Chew et al., 2005). Conversely, there is a concomitant rise in the incidence of cervical adenocarcinoma. This is largely due to a relative increase, but there is evidence of an absolute increase in the incidence in more recent birth cohorts; particularly in women aged less than 35 years old. Recent reports indicated that the adenocarcinoma accounted for 20–25% in uterine cervical cancer compared with only 5–15% in the past (Smith et al., 2000).
HPV infection is necessary for the development of cervical cancer (Ferguson et al., 1998; Walboomers et al., 1999). While the role of HPV in the pathogenesis of cervical adenocarcinoma is less well understood, the association of HPV infection in endocervical lesions have been reported (Xavier-Bosch et al., 1995). The incidence of concurrent intraepithelial changes in the presence of cervical adenocarcinoma is quoted as high as 40% (IARC, 2007).
The epidemiologic risk for cervical adenocarcinoma is similar to those for invasive squamous cell carcinoma, such as multiple sexual partners and the early onset of sexual intercourse, which are related to the risk factors of human papilloma virus (HPV) infection (An et al., 2005). Whereas the role of HPV infection in the development of cervical squamous cell carcinoma is well established, the pathogenetic role remains unclear. The previously reported prevalence of HPV infection in cervical adenocarcinoma varies significantly from study to study (Skyldberg et al., 1999). It has been generally believed that the HPV 18 is more frequently associated with cervical adenocarcinoma in contrast to HPV 16 in cervical squamous cell carcinoma (Munoz et al., 2003; An et al., 2005).
Nonetheless, the causal linkage of HPV infection to the cervical adenocarcinoma has not been considered as strong as it is for the squamous cell carcinoma of the cervix. However, the recent more sensitive techniques have made it possible to identify the higher rate of HPV infection in adenocarcinoma with a frequency of 85% or more (Zielinski et al., 2003). A poorer 5-year survivals of cervical adenocarcinoma compared to squamous cell carcinoma with the same stages is reported by several investigators (Smith et al., 2000).
AGCs were identified in cervico-vaginal smears, exhibiting nuclear enlargement, hypercromasia, prominent nucleoli and pleomorphism (fig. 1).

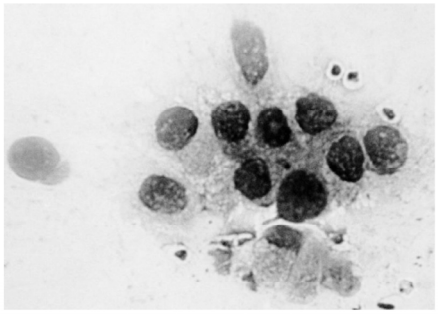

Fig. 1 Group of AGCs

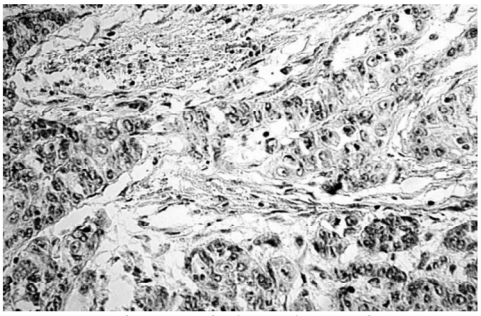

Fig. 2 Cervical G1 adenocarcinoma

Biopsy findings of cervical smears were as follows: 62.06 % miscellaneous benign lesions (chronic cervicitis and endometritis, endocervical, endometrial, and mixed polyps, irregular proliferative endometrium, adenomyosis, simple and complex endometrial hyperplasia, tubal and squamous metaplasia, and cervical endometriosis), 22.98 % malignancies: cervical adenocarcinoma *in situ*; n= 6, cervical adenocarcinoma (fig. 2); n= 10, and cervical adenosquamous carcinomas (fig. 3); n= 4, and 14.94 % squamous intraepithelial lesion: LGSIL (fig. 4); n= 8 and HG- SIL (fig. 5); n= 5.

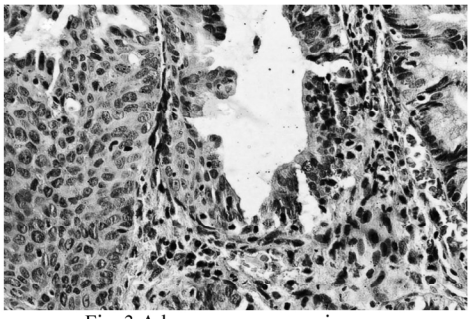

Fig. 3 Adenosquamous carcinoma

Fig. 4 LGSIL with evident HPV infection (koilocytosis)

Adenocarcinoma *in situ* (AIS) is defined as a lesion in which normally situated glands are partially or wholly replaced by cytologically malignant epithelial cells and is regarded as a precursor of adenocarcinoma. Approximately 90% of AIS cases are infected with HPV, mostly high-risk HPV-18 and HPV-16. Glandular dysplasia is defined as a lesion that closely resembles AIS but differs in that the nuclei are not cytologically malignant and that mitotic figures are less numerous but its malignant potential has not been clarified (Ueda et al., 2008).

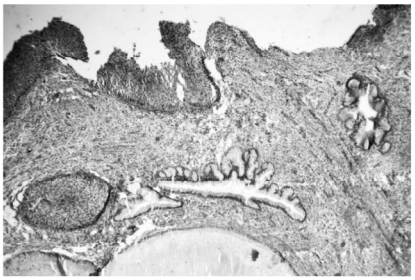

Fig. 5 HGSIL in the transformation zone

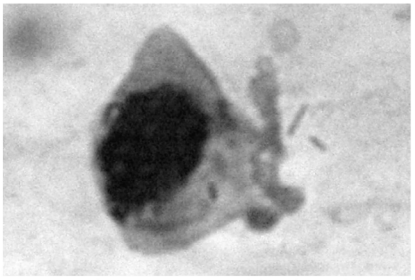

Fig. 6 HPV L1 positive (x 100)

Similarly to literature data, concurrent intraepithelial lesions in the presence of endocervical lesions were demonstrated in 14.94% of the investigated cases.

The cervical carcinoma heterogeneity was illustrated in the investigated cases by the identification of adenocarcinoma *in situ* (n=6), mucinous histological type (n=10) and adenosquamous carcinoma (n=4). The data from literature adds other histological types: intestinal mucinous, signet ring cell, and a minor proportion of endometrioid, minimal deviation, villoglandular, clear cell, serous and mesonephric, and a mixture of subtypes (An et al., 2005).

Approximately 10% of uterine cervical carcinomas are of the adenosquamous carcinoma type, which are, by definition, composed of a mixture of malignant glandular and squamous epithelial elements (Amalinei et al., 2005). As in the adenomatous lesions, high-risk HPVs have also been detected in 42% to 95% of cervical adenosquamous carcinomas (Ueda et al., 2008). Until now, several researchers have been studying whether adenosquamous carcinoma of the uterine cervix is a combination tumor derived from a single cell or a collision tumor of an adenocarcinoma and a squamous cell carcinoma. Moreover, the character of the precursor lesion of adenosquamous carcinoma is still unclear, whereas CIN is widely regarded as the precursor lesion of squamous cell carcinoma.

The detection of the same subtype and physical status of HPV in adenocarcinoma and squamous cell carcinoma portions demonstrates that adenosquamous carcinoma is derived from a single stem cell, which has a pluripotency to differentiate into both squamous and glandular cells. Many concurrent glandular and squamous lesions are collision tumors formed separately by the same type of HPV and that adenosquamous carcinoma is possibly a combination tumor. These results may suggest that the glandular and squamous parts of concurrent lesions do not develop into the glandular and squamous components of adenosquamous carcinoma, respectively; however we cannot deny the possibility that adenosquamous carcinoma is derived from the glandular or squamous part of the concurrent glandular and squamous lesions. The stratified mucin-producing intraepithelial lesions are potential precursors for adenosquamous carcinomas but further investigation is necessary to clarify the entire mechanism of development of adenosquamous carcinoma of the uterine cervix.

Direct infection of columnar cells is the main route of HPV 16 infection, as only approximately a quarter of the HPV 16 positive cases have concurrent infected squamous epithelium (Chew et al, 2005). This lower prevalence on concurrent HPV infection in the squamous epithelium can be explained by clearance of HPV infection from the squamous epithelium. It is possible that the infected basal cells, of Stanley's immunobiological model of HPV infection of the squamous epithelium, could have differentiated into glandular epithelial cells and therefore the squamous cell will bear no evidence of HPV infection (Chew et al., 2005).

HPV high-risk type L1 major capsid proteins were identified in 47% of cases diagnosed as AGC, concurrent with low grade and high grade squamous intraepithelial lesions (4 cases of LSILs and 2 cases of HSILs) and adenosquamous carcinomas (2 cases), demonstrating the role of HPV infection in initiating the cervical lesions. The rate of AGC-associated cervical disease with concurrent HPV testing has been variably reported in literature (Derchain et al., 2004; Grieser et al., 2004; Oliveira et al., 2004; Costa et al., 2007; Rabelo-Santos et al., 2008; Schnatz et al., 2009; Sharpless et al., 2009), depending on the method of detection and histological diagnosis. Recent researches were mainly focused on exocervical lesion (Grieser et al., 2004), and correlated with prognosis (Rauber et al., 2008). Our results are as we expected, the expression of HPV L1 capsid protein in endocervical glandular cells, although morphological atypical, being negative. Positive reaction was characterized by the strong staining of the whole nucleus of dysplastic epithelial squamous cells, typical koilocytes or dyskeratocytes, surrounded by a cytoplasm with no background (figure 6). This evidence is in concordance with the literature data, which emphasize that the presence of mature virions is generally limited to the nuclei of cells from the upper layers of the squamous epithelium. In lower layers of the squamous epithelium or in other types of epithelia, HPVs do not achieve full maturity, thus it can be detected only as occult or latent infection, by their DNA (Koss et al, 2006).

CONCLUSIONS

Diagnosis of AGCs is difficult in current practice but is important as it may reveal a cervical malignancy or a precursor lesion.

Biopsy follow-up of conventional cervical smears with diagnose suspicion of atypical glandular cells (AGC) revealed *in situ* and invasive malignant glandular lesions, and squamous cervical intraepithelial lesions in 37.93 % of investigated cases.

The correlation of endocervical lesions with HPV infection could be immunohistochemically demonstrated in squamous epithelial cells, in 47% of cases diagnosed as AGC, concurrent with low grade and high grade squamous intraepithelial lesions and adenosquamous carcinomas.

The concurrence of squamous lesions with AGC was relatively high in the investigated cases, corresponding to the simultaneous HPV infection of the both types of epithelium (endo- and exocervical).

The HPV infection in AGC diagnosis identifies a group of women at higher risk for cervical malignant disease who should be closely followed-up, the risk being even higher in cases with concurrent SIL.

REFERENCES

Amalinei C, Balan R, Stolnicu S, Radulescu D, Boeru C, Cotutiu C, 2005. *Rev Med Chir Soc Med Nat Iasi*, 109.(2): 343-346.

An HJ, Kim KR, Kim IS, Kim DW, Park MH, Park IA, Suh KS, Seo EJ, Sung SH, Sohn JH, Yoon HK, Chang ED, Cho HI, Han JI, Hong SR, Ahn GH, 2005. *Modern Pathology*, 18: 528–534.

Balan R, Crauciuc E, Gheorghiţă V, Păvăleanu M, Toma O, Amălinei C, 2009. An *Stiint Univ AI I Cuza Iasi Sect II A Genetică şi Biologie Moleculară*, X (2): 77-83.

Chew GK, Cruickshank ME, Rooney PH, Miller ID, Parkin DE, Murray GI, 2005. *British Journal of Cancer*, 93: 1301 – 1304.

Costa S, Negri G, Sideri M, Santini D, Martinelli G, Venturoli S, Pelusi C, Syrjanen S, Syrjanen K, Pelusi G, 2007. *Gynecol Oncol*, 106(1):170-176.

Derchain SF, Rabelo-Santos SH, Sarian LO, Zeferino LC, de Oliveira Zambeli ER, do Amaral Westin MC, de Angelo Andrade LA, Syrjänen KJ. 2004. *Gynecol Oncol.*, 95(3): 618-623.

Ferguson AW, Svoboda-Newman SM, Frank TS. 1995. *Mod Pathol*, 11(1): 11–18.

Griesser H, Sander H, Hilfrich R, Moser B, Schenck U. 2004. *Anal Quant Cytol Histol*, 26(5): 241-5.

International Agency for Research on Cancer. Human papillomaviruses. (IARC monographs on the evaluation of carcinogenic risks to humans, vol 90). Lyon, France: International Agency for Research on Cancer, 2007.

Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, SmithJS. 2008. *Am J Epidemiol*, 168:123–137

Koss LG, Melamed MR, 2006. *Koss' Diagnostic Cytology and its Histopathologic Bases*, Lippincott Williams & Wilkins, Philadelphia.

Levine L, Lucci JA 3rd, Dinh TV, 2003.*Obstet Gynecol Surv*. 58(6): 399-406.

Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ, 2003. *N Engl J Med*, 348: 518–527.

Oliveira ER, Derchain SF, Rabelo-Santos SH, Westin MC, Zeferino LC, Campos EA, Syrjanen KJ, 2004. *Diagn Cytopathol,* .31(1):19-22.

Parkin DM, Bray F, Ferlay J, Pisani P, 2005. *CA Cancer J Clin*, 55:74-108.

Rabelo-Santos SH, Derchain SF, do Amaral Westin MC, Angelo-Andrade LA, Sarian LO, Oliveira ER, Morais SS, Zeferino LC, 2008. *Cytopathology*.,19(1): 34-43.

Rauber D, Mehlhorn G, Fasching PA, Beckmann MW, Ackermann S., 2008. *Eur J Obstet Gynecol Reprod Biol*, 140(2): 258-62.

Schnatz PF, Sharpless KE, O'Sullivan DM, 2009. *J Low Genit Tract Di*s, 13(2): 94-101.

Sharpless KE, O'Sullivan DM, Schnatz PF, 2009. *J Low Genit Tract Dis*., 13(2):72-78.

Skyldberg BM, Murray E, Lambkin H, et al, 1999. *Mod Pathol*, 12: 675–682.

Smith HO, Tiffany MF, Qualls CR, et al, 2000. *Gyn Oncol*, 78: 97–105.

Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM, 2007. *Int J Cancer*, 121: 621–32.

Solomon D, Schiffman M, Tarone R, 2002. *Obstet Gynecol*, 99(4): 671-674.

Ueda Y, Miyatake T, Okazawa M, Kimura T, Miyake T, Fujiwara K, Yoshino K, Nakashima R, Fujita M, Enomoto M, 2008. *Am J Clin Pathol*, 130: 389-400.

Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N, 1999. *J Pathol.* 189(1): 1–3

Xavier-Bosch F, Michele Manos M, Munoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV, 1995. *J National Cancer Institute,* 87(11): 796–802.

Zielinski GD, Snijders PJ, Rozendaal L, Daalmeijer NF, Risse EK, Voorhorst FJ, Jiwa NM, van der Linden HC, de Schipper FA, Runsink AP, Meijer CJ, 2003. *J Pathol* , 201: 535–543.

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