

# Cells with dyskeratotic inclusions in smears from cervix uteri infected by human papillomavirus

*Celulas con inclusiones disqueratoticas en muestras de cervix uterinos infectados por virus de papiloma humano*

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## Summary

An additional feature has been reported in cervical smears from patients with human papillomavirus (HPV) infection: the "cell inside another cell", that shows inclusion bodies in addition to a nuclear smudging (cell with dyskeratotic inclusions: CDI).

We observed smears from 129 patients with diagnosis of HPV infection of the cervix uteri, stained by Papanicolaou technique. Koilocytes were observed in 48,8% of the patients, and CDI in 30,2% of them.

CDI was presented by 19,3% of the patients.

The CDI could not be related to a special type of HPV, therefore it is not a parameter for estimation of the oncogenic risk. Further studies are needed to clarify if CDI could be other indirect feature suggestive of HPV infection.

**Keywords:** cell with dyskeratotic inclusions; koilocytes; cervical condyloma

## Introduction

The cytologic patterns of condyloma of the cervix uteri have been extensively described in the literature <sup>(1-3)</sup>.

infection by HPV, but the squamous cells of the uterine cervix can develop other changes by the cytopathic action of the virus.

The koilocyte is the pathognomonic cell of the

The cytoplasm stain irregularly, either

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amphophilic or orangeophilic, nuclei are often double, sometimes multiple, with chromatin smudged or pyknotic (indirect signs of the infection by HPV).

Another cell usually seen in the smears is the dyskeratocyte. This small cell has a dense cytoplasm stained with the orange of the Papanicolaou technique, and a nucleus often pyknotic or pre-pyknotic.

In the last years, an additional feature has been described in the HPV infection: the "cell inside another cell" <sup>(4)</sup>. These peculiar cells show inclusion bodies (dyskeratocytes) in their cytoplasm, in addition to the nuclear changes characteristic of HPV action.

The purpose of the present paper is to establish the value of the CDI in the smears from cervix uteri of patients infected by HPV.

**Materials and Methods**

Smears from cervix uteri were obtained from 129 patients with diagnosis of HPV infection established by cervical biopsies <sup>(5)</sup>. The smears were stained with the Papanicolaou technique, and the following cytological features were recorded:

- a) Koilocytosis;
- b) Indirect signs of HPV infection: smudged or pyknotic chromatin, irregular stain of cytoplasm, binucleation, multinucleation, dyskeratosis, karyorrhexis;
- c) Cells with inclusion bodies in their cytoplasm and nuclear smudging (CDI).  
In situ hybridization was carried out in cervical biopsies. For HPV typing, the specific DNA probes were used: a) Low risk: 6,1; b) Moderate risk: 31,33,35; c) High risk: 16,18 (ENZO Pathogene).

The specimens were hybridized for 10 minutes on a 95°C heating block, and for 30 minutes on a 37° C heating block. Specific hybridization between the HPV DNA probes and DNA in the specimens was determined by the detection of biotin (incubation with streptavidin-biotinylated horseradish peroxidase complex 37° C, 15 minutes; post-incubation with 3-amine-9 ethylcarbazole with hydrogen peroxide 37° C, 15 minutes) <sup>(6)</sup>

Papanicolaou smears from the group with CDI were processed by in situ hybridization. After

removal of the coverslips in xylene, the specimens were destained (1% HCl in 70% Ethyl alcohol) and treated with Proteinase K in PBS for 5 minutes, washed with NaCl / EDTA pH 7,2 and covered with Hydrogen peroxide (0,05%) in Methyl alcohol for 10 minutes at 37° C. A mixture of biotinylated HPV DNA probes from 6/11, 16/18 and 31/33/35 types (each one: 25 ul) were added.

The smears were covered with coverslips and placed on a 95°C heating block for 5 minutes. Then, they were incubated at 37°C for 3 hours. After washing with NaCl / EDTA, the slides were treated with buffered NaCl and formamide for 10 minutes. They were rinsed with NaCl / EDTA and incubated with streptavidin-biotinylated horseradish peroxidase complex at 37° C for 15 minutes. The detection was performed using Hydrogen peroxide in acetate buffer plus 2% 3-amine-9 Ethyl carbazole in solvent (37° C, 15 minutes).

The development of a red nuclear staining identified the presence of HPV DNA from at least one of the probes used <sup>(7)</sup>.

**Results**

30,2% of the smears showed CDI (Figure 1). Typical koilocytes were seen in 48,8% out of the cases. Most of the smears presented indirect signs of HPV infection (96,9%) (Table 1).

**Table 1 - Cytological features of the cervix**

Koilocytes		Smears CDI		Indirect signs of HPV infection	
n	%	n	%	n	%
63	48,8	39	30,2	135	96,9

In 18 biopsies from patients with CDI in their cytological smears, dyskeratotic inclusions were observed in the upper layers (Figure 2). CDI was accompanying koilocytes in 14 patients. 49 smears did not show CDI, but these groups showed typical koilocytes. The presence of CDI without koilocytes was certified in 25 out of the cases (Table 2).

**Table 2 - Relation between koilocytes and CDI**

Smears koilocytes (without CDI)		Smears showing CDI (without koilocytes)		Smears showing CDI + koilocytes	
n	%	n	%	n	%
49	37,9	25	19,3	14	10,8



The three groups of HPV types were identified by in situ hybridization in the cervical biopsies (Figure 3).

A few cases were negative to the probes used (Table 3).

The sub-group of 25 cervical biopsies taken from women which only presented CDI in their cervical smears showed all the spectrum of viral types, with the exception of two specimens negative for the probes used (Table 4).

**Table 3 - HPV types in cervical biopsies by in situ hybridization**

Probes: HPV types	Number of positive specimens
6, 11	75
31, 33, 35	20
16, 18	26
No HPV *	8

\* Negative specimens for the probes used

**Table 4 - HPV types in a sub-group of biopsies with CDI in the cytological smears (in situ hybridization)**

Probes: HPV types	Number of positive specimens
6, 11	11
31, 33, 35	5
16, 18	7
No HPV *	2

\* Negative specimens for the probes used

68% out of the Papanicolaou-stained cervical smears showed positive cells for the cocktail of probes for HPV used; 7 cases exhibited the red nuclear staining in the CDI (Figure 4) (Table 5).

**Table 5 - In situ hybridization of Papanicolau-stained cytologic smears from patients with CDI (N=25)**

Positive Smears #						Negative Smears	
Positive in CDI and no-CDI		Positive in CDI (only)		Positive in no-CDI			
n	%	n	%	n	%	n	%
7	28	0	0	10	40	8	32

# Positive for the HPV DNA cocktail.

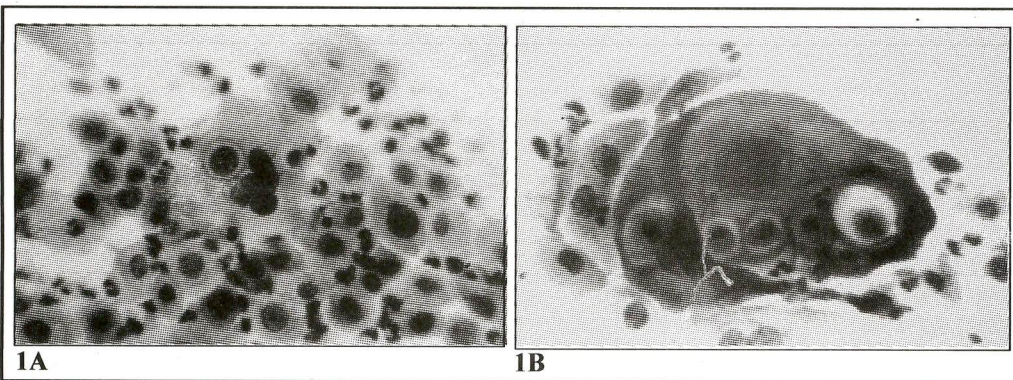


Figure 1: Cells with dyskeratotic inclusions in cervical smears: (A) Multinucleated cell with one dyskeratotic inclusion. (B) Big cell with several dyskeratotic inclusions. (Papanicolaou stain x 500)



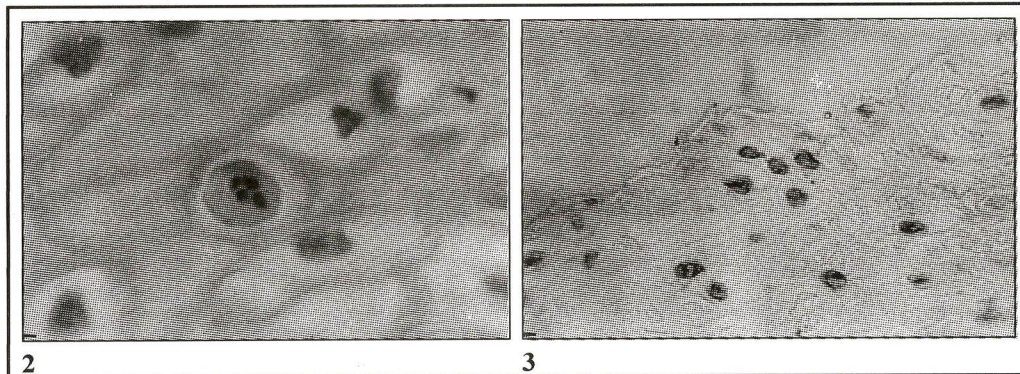


Figure 2: Dyskeratotic inclusions in the upper layers of cervical epithelium infected by human papillomavirus (H&E X 500)

Figure 3: In situ hybridization of cervical condyloma. HPV-positive nuclei (6/11) within superficial epithelial cells (X 500)

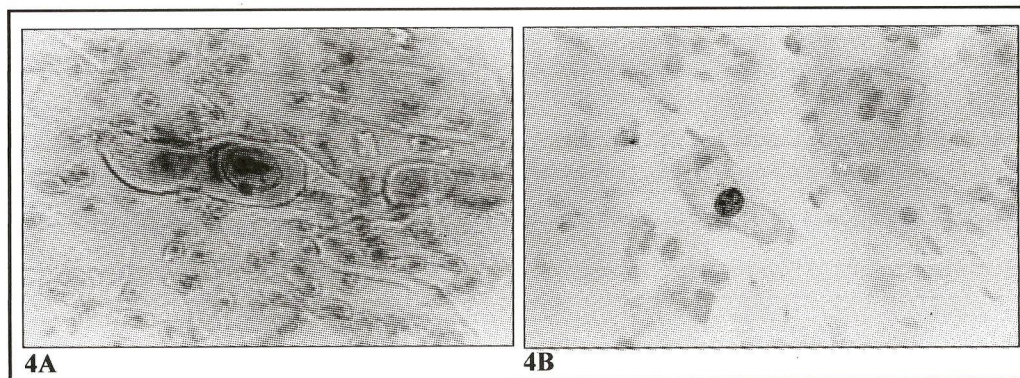


Figure 4: CERVICAL SMEARS: (A) CDI showing a positive nuclear reaction to the HPV DNA cocktail by in situ hybridization. (B) No-CDI showing viral DNA by in situ hybridization (X 500)

### Discussion

The concourse of Folic acid and HPV infection in the etiopathogenesis of the “cell within a cell” has been suggested<sup>(8)</sup>

The deficiency of Folic acid leads to a nuclear-cytoplasmic asynchrony, that produces macrocytosis and multinucleation, and this alteration has been designed as abortive cellular division<sup>(9)</sup>. However, our group of patients had no antecedent of megaloblastic anemia that could justify the deficiency of Folic acid.

Some authors have pointed out inclusion bodies in squamous epithelial biopsies. These bodies can be related to a process of cell's death, like in treatments by cytostatic drugs (apoptosis)<sup>(10)</sup>

In the present paper, the dyskeratotic cells or the dense round bodies seen inside another cells could be a consequence of the damage by HPV infection. It could be related to an apoptotic mechanism, since one of the steps of this phenomenon is the development of cellular keratinizant fragments with picnotic nuclei (phase 3 of apoptosis)<sup>(11,12)</sup>

The elevated content of cytokeratines in the “cell within the cells” was detected by electron microscopy, as electron-dense masses composed of intermediate filaments of approximately 10 nm diameter<sup>(13)</sup>

This paper attempts to establish the value of the CDI for the cytodiagnosis of HPV infection in cervical smears.

The pathognomonic cell of HPV, the koilocyte, was observed in 63 cases (48,8%), while the CDI were observed in 39 cases (30,2%).

37,9% of the patients had koilocytes without CDI, and 10,8% had both cellular types in the same smear.

Although the percentage of CDI is less than that of koilocytes, 19,3% of the smears only showed CDI. In these cases, this peculiar alteration, together with the other indirect signs of viral infection, were the clue to the diagnosis of HPV. The presence of HPV DNA in 93,8% out of the biopsies detected by in situ hybridization, confirmed the viral etiology of the lesions



studied. It can not be ruled out the presence of HPV DNA in the negative smears using this method, since it can be due to viral types other than the probes used, or to a low number of HPV DNA copies.

Although the sensitivity of in situ hybridization (2,5-12 copies per cell)<sup>(14)</sup> is lower than the technique of PCR, we have preferred the use of the former because this method can distinguish between latent and subclinical HPV infection<sup>(15)</sup>

The group of 25 biopsies from patients who only showed CDI (without koilocytes) in their cervical smears, presented all the spectrum of HPV types (low, moderate and high oncogenic risk), indicating that the presence of CDI can not be related to any special type of virus. The

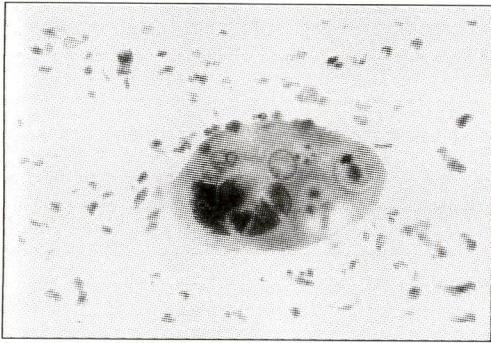


Figure 5: Cell displaying nuclear atypies and dyskeratotic inclusion. It was detected HPV 16/18 by in situ hybridization. (Papanicolaou stain X 500)

morphological findings, in general, do not provide accuracy for distinguishing among viral types<sup>(16)</sup>. Only when marked atypies are associated with HPV, we can think of the possibility of a high risk viral type, as in the case showed in the Figure 5.

When in situ hybridization was carried out in cells (Papanicolaou smears) from patients who only showed CDI, the number of positive cases, for HPV DNA was smaller than those of the respective biopsies (68% versus 96%); of them, 7 cases presented viral DNA in their CDI. The lack of signal for viral sequences could be due to a low number of HPV DNA copies, to the origin of exfoliated cells (grade of epithelial differentiation) or to a low sensitivity of in situ hybridization in cytologic smears<sup>(7)</sup>.

By now, there is not a clear explanation for the presence of dyskeratotic cells in the interior of other epithelial cells, but having in mind the morphological features of apoptosis: cell shrinkage, loss of cell-to-cell contacts, aggregation of the chromatin into dense masses under the nuclear membrane and the possibility of phagocytosis by neighboring cells, is possible to relate CDI with apoptosis. Experiences in course are trying to confirm the existence of programmed cell death using in situ end-labeling of DNA<sup>(17)</sup>.

We conclude that the presence of CDI in cervical smears has not yet a clear significance, and further studies, specially concerning their origin, are needed to clarify if they could be other indirect parameter suggestive of HPV infection.

## References

1. MEISELS A, FORTIN R. Condylomatous lesions of the cervix and vagina I: Cytologic patterns. *Acta Cytol*, 1976, 20: 506-509.
2. MEISELS A, ROY M, FORTIER M, MORIN C. Condylomatous lesions of the cervix: Morphologic and colposcopic diagnosis. *Am J Diagn Gynecol Obstet*, 1979, 1: 109-116.
3. MEISELS A, ROY M, FORTIER M ET AL. Human papillomavirus infection of the cervix: the atypical condyloma. *Acta Cytol*, 1981, 25: 7-16.
4. DE BORGES RJ, MIRALLES EP. Aspectos morfológicos y epidemiológicos de las llamadas "Atipias koilocíticas". *Rev Obstet Ginecol Venez*, 1982, 42: 1-5.
5. MEISELS A, FORTIN R, ROY M. Condylomatous lesions of the cervix II: Cytologic, colposcopic and histopathologic study. *Acta Cytol* 21: 379-390, 1977
6. CRUM CP, NAGAI N, LEVINE RU, SILVERSTEIN S. In situ hybridization analysis of the HPV 16 DNA sequences in early cervical neoplasia. *Am J Pathol*, 1986, 123: 174-182.
7. GHIRARDINI C, GHINOSI P, RAISI O, PORTOLANI M. Human papillomavirus DNA detection in Papanicolaou-stained cervical smears with a nonradioactive, in situ hybridization assay. *Acta Cytol*, 1992, 36: 183-188.
8. LUZZATTO R, RECKTENVALD M LAMAS PORTUGAL JP. Multinucleation and abortive cellular division in human papillomavirus infection. *Acta Cytol*, 1990, 2: 286-287.
9. VAN NIEKERK WA. Cervical cytological abnormalities caused by folic acid

- deficiency. *Acta Cytol*, 1966, 10: 67-73.
10. EL-LABBAN NG, OSORIO HERRERA E. Apoptotic bodies and abnormally dividing epithelial cells in squamous cell carcinoma. *Histopathology*, 1986 10: 921-931.
  11. ARENDS MJ, HARRISON DJ. Apoptosis: Molecular aspects and pathological perspective. In: John Crocker De , Molecular Biology in Histopathology. Baffins Lane, Chichester, West Sussex, England, 1994, 151-170.
  12. ARENS MJ, WYLLIE AH. Apoptosis: Mechanisms and roles in pathology. *Int Rev Exp Pathol*, 1991, 32: 223-254.
  13. DE BRGES RJ, GARCÍA TAMAYO J, ZAITZMAN M. Cytologic and ultrastructural findings of a peculiar alteration in cervical cells from patients with human papillomavirus infections. *Acta Cytol* 1989, 3: 314-318.
  14. HERRINGTON CS, GRAHAM AK, MC GEE JO'D. Interphase Cytonetics III: Enhanced sensitivity and flexibility of digoxigenin labelled probes for in situ hybridization. *J Clin Pathol* , 1991, 44: 33-38.
  15. HERRINGTON CS. Human Papillomaviruses (HPV) in gynaecological cytology: from molecular biology to clinical testing. *Cytopathology*, 1995, 6: 176-189, 1995
  16. TAKANA H, CHUA K-L, LINDH E, HJERPE A. Patients with various types of human papillomavirus: covariation and diagnostic relevance of cytological findings in Papanicolaou smears. *Cytopathology*, 1993, 4: 273-283.
  17. WIJSMAN JH, JONKER RR, KEIJZER R et al. A new method to detect apoptosis in paraffin sections: insitu end-labeling of fragmented DNA. *J Histochem Cytochem* , 1993, 41: 7-12, 1993