

IDENTIFICATION OF CHLAMYDIA TRACHOMATIS, HERPES SIMPLEX VIRUS AND HUMAN PAPILOMAVIRUS IN IRRADIATED UTERINE-CERVIX: CRITICAL ANALYSIS OF POTENTIAL PROBLEMS IN PAPANICOLAOU SMEARS ROUTINE*

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ABSTRACT: This review present a critical analysis of the morphologic patterns and immunocytochemical reactions of three infections of irradiated uterine cervix: Chlamydia trachomatis, Herpes virus and Papillomavirus. The effect of ionizing radiation on squamous epithelium leads to some alterations of the cytoplasm and nucleus which could difficult the identification of these etiologic agents. Frequently, radiation can induce cytoplasmic vacuolation, multinucleation and bizarre cells features which in some instances can mimich Chlamydia, Herpes or Papilloma infections.

DESCRIPTORS: Chlamydia trachomatis, Herpes simplex virus, Human Papillomavirus, Post radiation smears, Cytology, Immunocytochemistry.

INTRODUCTION

Morphologic alterations inducec by ionizing radiation have been well recognized since the pioneer studies of the first decade of the century as reported by Rubin and Casarett³² about the uterine cervix cancer in biopsies specimens. Later on these patterns were also studied in smears by Graham in 1947⁶, who established cytlogic parameters to the evaluation on the morphologic changes due to radiotherapy(Rxt).

Cytological analysis of gynaecologic smears after Rxt has been a very useful non-invasive method for the follow up of these patients. However, radiation itself can induce several morphologic effects which should be differentiated from residual or recurrent neoplasia^{9,11,13,21,27}.

The most frequent ionizing alterations found in the cytologic smears is cytoplasmic vacuolation. In

addition, there was found multinucleation, anisonucleosis and bizarre cell features^{16,24,26,27,38,41}. These alterations may be found since the first weeks post Rxt till many years later.

These morphologic alterations can difficult the identification of some infections of uterine cervix in irradiated patients. In our personal experience, three infections havebeen recently demostrated in patients treated of cervical cancer with radiotherapy: Chlamydia trachomatis²⁰, Herpes simplex virus¹⁵ and Human Papillomavirus. Epidemiological studies have demonstrated that cervical dysplasia and cancer are associated with factors related to sexual transmission, as these three infections mentioned; so, to identify them is a very important point in the cytologic routine. We would like to present the characteristic morphologic pattern of those infections in the cervical smears of irradiated patients.

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Chlamydia trachomatis(Ct):

The first specific infection described in post Rxt smears was that caused by Chlamydia trachomatis²⁰. The real meaning of Ct infection in cervical dysplasia and cancer is still not clearly defined, but its association is important³⁴.

Radiation induces cytoplasmic vacuolation, which should be carefully distinguished from Ct infection. Even in post radiation smears, we can recognize all the classical criteria described by Gupta et alii⁷ as well as the "moth-eaten" features, reported by Gupta et alii in 1988, for Ct.

The identification of Ct infection in post radiation smears is mainly important due to two factors: first, the possibility of Ct infect other cells than those usually described as columnar and metaplastic endocervical cells; since the majority of patients studied until now had an expressive vaginal stenosis due to radiation effects, Ct infected cells belonged to the squamous epithelium of vagina²⁰. The second important fact reported by Maeda et alii²⁰ is the higher Ct frequency in irradiated (4,1%) than that in non-irradiated cancer patients (0,3%) or in cervical screening clinics (0,13 to 0,3%)²⁰.

The morphologic confirmation of Ct infection can be done usually by immunofluorescence^{19,20}, immunocytochemistry^{33,36} in situ hybridization^{5,10} or polymerase chain reaction³¹. All of these methods have a high specificity and sensitivity, and their use in laboratorial routine substituting the culture which require time and costs, is a progressive tendency.

Recently, Iwen et alii¹⁰ analysed DNA probe, direct fluorescent-antibody(DFA) and cell culture for detecting Chlamydia trachomatis in cervical specimens, and reported the similar specificity of both DNA-probe and DFA (98% and 99%, respectively) when compared to the cell culture; and the best sensitivity of DNA-probe (93%) versus DEA.

Ratti et alii³⁰, compared the use of polymerase chain reaction with cell culture and obtained sensitivity and specificity of 100% and 96,8%, respectively.

Ghirardini et alii⁵ reported a control study by in situ hybridization applied in Papanicolaou-stained cervical smears, for the use of laboratorial routine: the sensitivity and specificity of Pap test compared with in situ hybridization were 95% and 89%, respectively.

In our experience, immunofluorescence and immunocytochemistry provide good results to confirm Ct infection morphologically reported in metaplastic-like cells with fine vacuolation called as "moth-eaten"^{20,33}, or in presence of the "nebulous" type (second phase of the cycle) appearance³⁶.

Both methods can also exhibit negative reactions in morphologically positive cases, namely by immunocytochemical reaction. These findings are frequently credited to the different phases of the Ct cycle that can hide natural reactivity of the infection^{20,33,36}.

Recently, cytobrush an accurate device of material collection of squamous-collunar junction, have yielded improved results to the identification of Chlamydia trachomatis in screening clinics routine through the morphological and immunofluorescent methods¹⁹.

Further studies in post radiation patients without severe vaginal stenosis could bring new information about the incidence of Ct in this group with this new efficient collection device.

Herpes simplex virus(HSV):

For years, several studies have associated HSV infection and cervical carcinoma²⁹; so, the finding of HSV infection in post-radiation cervical smears revealed new perspectives of this association, since the future studies can research the presence of HSV in cervical biopsies before and after Rxt. In recent study, we failed to identify HSV in biopsies prior Rxt, even though we have used in situ hybridization method¹⁵.

Papanicolaou stained smears detect HSV patterns with high accuracy, since the first description of Stern and Longo in 1963³⁹; others studies have confirmed the cellular features of HSV infection³⁷ morphologically or through the viral isolation¹⁴, immunoperoxidase^{15,40} or in situ hybridization techniques^{15,40}; in our personal experience we have used only the last two proceedings.

The multinucleation due to ionizing alterations can simulate HSV cytopathic effect; besides that, the cases we have recognized in cytologic follow-up routine, shows the classical signs of HSV infection, such as multinucleation with molding of nuclei and a ground-glass appearance of the chromatin. The cytoplasm was in general, abundant, pale and finely granular in some cells; others had only a narrow perinuclear rim of cytoplasm; bizarre giant forms are frequently seen¹⁵.

Immunocytochemical research with HSV antigen can present positivity in cells with or without morphological signs of HSV infection¹⁵.

Based on our previous experience¹⁵, three hypotheses could be raised regarding major factors which affect defense mechanisms and facilitate viral infections: 1) the nature of the epithelium that replaces neoplastic cells; 2) the environmental alterations induced by radiation or 3) a hypothetical local immunodepression.

Human Papillomavirus (HPV):

The association of HPV infection and cervical cancer has been exhaustively studied^{23,28}.

Its occurrence in post Rxt smears require further epidemiologic investigations in this group, in order to clarify whether HPV acts in such condition, as an opportunistic agent to the aggressive Rxt or it has been present in this cervix since early events of the development of cervical cancer.

The first cases we described of HPV infection in post Rxt smears were composed by epithelial cells with scanty or absent alterations of ionizing effects: this fact led us to think that HPV infection could be related with the eutrophic appearance of the cells. However, afterwards, we have found classical signs of HPV infection, even in the smears of atrophic appearance which frequently reveals a good response to the treatment^{16,24,26}.

Pap smears can present many signs suggestive of HPV infection that proved a high index of agreement with the specific means to recognize the virus^{3,4,22,35}, such as immunocytochemistry¹⁸, in situ hybridization^{1,25,35} or the recent polymerase chain reaction^{2,25}.

In our experience, the major morphological sign detected as suggestive of HPV infection in post radiation smears, was the classical koilocyte, in general associated with nuclear hyperchromasia and dyskeratosis^{12,22}. Additional features of HPV infection such as "bi or multinucleation", "mild koilocytosis", "hyperchromatic nuclei", "clear cytoplasm", "keratohyalin granules", "perinuclear halos", "condensed filaments", and "spindle cells"^{3,17,35} could not help in this special setting due to the similar effects caused by the ioniz-

ing radiation. We have noticed no Rxt effect mimicking koilocyte.

We have found out (unpublished data), that positive immunocytochemical reactions were found in cells with HPV classical signs and in others with no morphological suggestion of the HPV infection. All reactivity was observed in nuclei. All biopsy specimens previous to radiation examined by in situ hybridization with highly sensitive biotin-probes, were negative. The results led us to think in morbidity post-treatment, although the hypothesis of HPV DNA degradation in the formalin-fixed paraffin embedded cancer specimens cannot be ruled out²⁵.

FUTURE PERSPECTIVES

To improve new means to evaluate the real importance of these infections, further studies which assort, beyond the cytological and immunocytochemical proceedings and a complete follow-up of the patients, prior and after treatment by radiation, are required. To answer the question about the hypothetical resistance of these etiologic agents to the radiation, several efforts should be done to exclude the probable morbidity postherapeutic. Epidemiological view requires also an explanation about the frequency of these infections in irradiated patients, in order to obtain information important enough to the knowledge of the special cellular habitat; and more, a question raised: do the patients who present these infections, mainly by HPV, present a higher risk to neoplastic recurrence?

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RESUMO: A presente revisão faz análise crítica de padrões morfológicos e imunocitoquímicos de três distintas infecções em colo uterino irradiado: Chlamydia trachomatis, Herpes simplex virus e Papilomavirus. Os efeitos ionizantes sobre os epitélios escamosos, levam a algumas alterações núcleo-citoplasmáticas, multinucleação e às formas celulares bizarras as quais, em algumas circunstâncias, podem mimetizar as infecções por Chlamydia, Herpes ou Papilomavirus.

DESCRITORES: Chlamydia trachomatis, Herpes simplex virus, Papilomavirus humano, esfregaços pós-irradiação, citologia, imunocitoquímica.

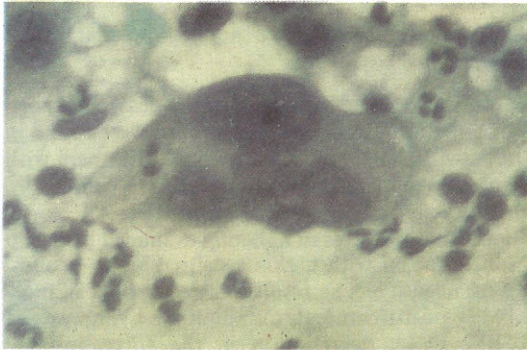


FIGURE 1

Giant cell with marked Effects of radiation as nonspecific multinucleation with anisonucleosis of cytoplasm. (x 500 Papanicolaou)

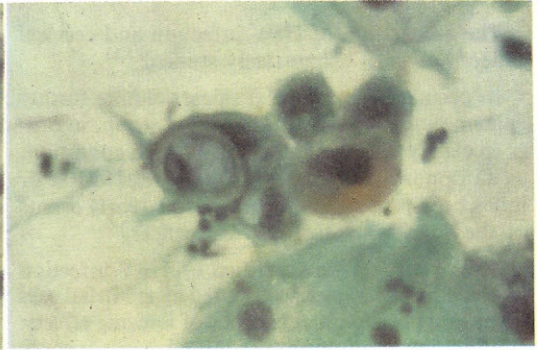


FIGURE 2

Major effects of radiation include marked nonspecific intracytoplasmic vacuoles (x 500 Papanicolaou).

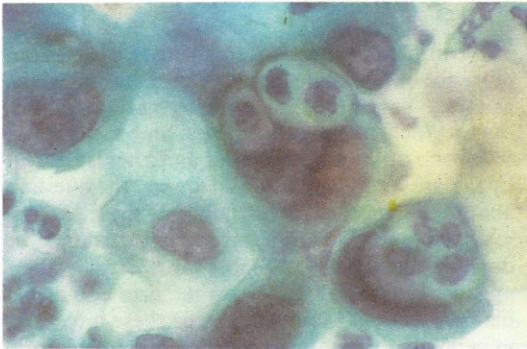


FIGURE 3

Other effects of radiation on squamous cells include intracytoplasmic inclusion of neutrophils and mild vacuolation (x 1250 Papanicolaou).



FIGURE 4

Bizarre findings after radiation on squamous cells as aberrant vacuolation with peculiar "cell within a cell feature" separating two hyperchromatic nuclei. (x 1250 Papanicolaou).

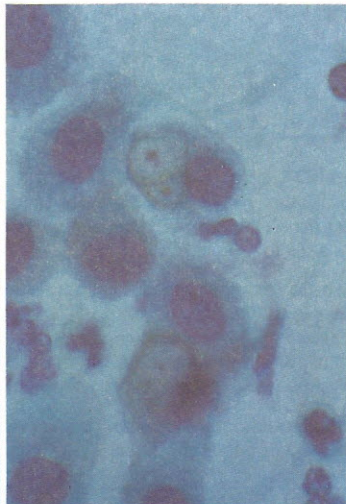


FIGURE 5 e 6

Chlamydia-infected cells after radiation with typical vacuolated cells show intracytoplasmic vacuoles with multiple cocoid bodies and central target formation (compare these pictures with Fig.2) (x 800 Papanicolaou)

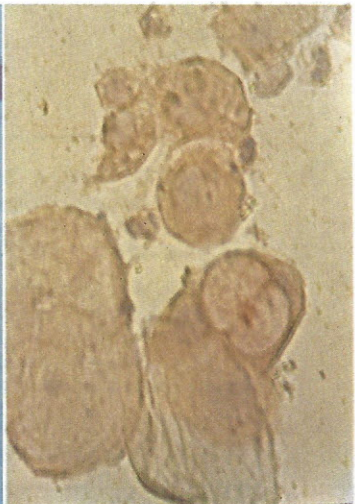


FIGURE 7

Positive immunoperoxidase reaction to Chlamydia trachomatis on irradiated squamous cell (x 500 Papanicolaou).



FIGURE 8

Giant cell with multinucleated syncytial conglomerates with prominent inclusions on enlarged bizarre cytoplasm in post radiated smear with HSV pathognomonic cytologic appearance (X 320 Papanicolaou).

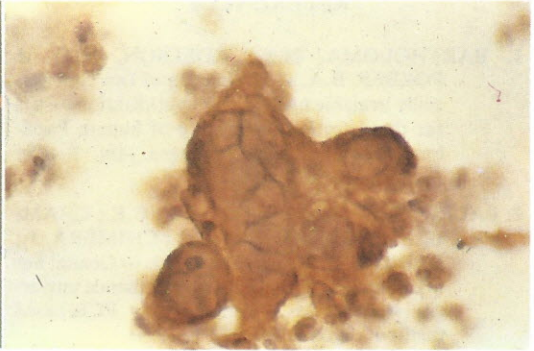


FIGURE 9

Positive staining for HSV antigen with multinucleated cell (x 500 Papanicolaou).

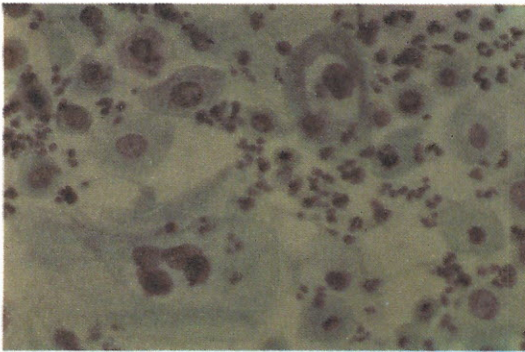


FIGURE 10

Multinucleation and koilocytosis suggestive of HPV infection in cell with marked effects of radiation injury and atrophic appearance (x 400 Papanicolaou).

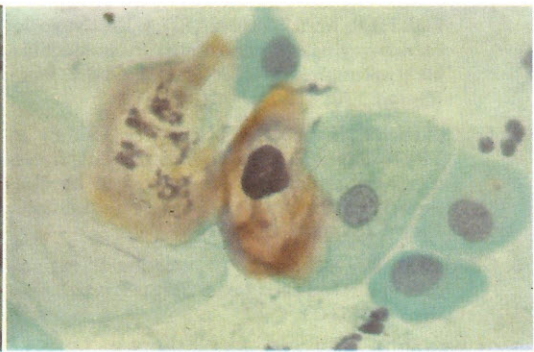


FIGURE 11

A single cell with koilocytosis suggestive of HPV infection beside a cell with marked degenerated signs of radiation (x 500 Papanicolaou).

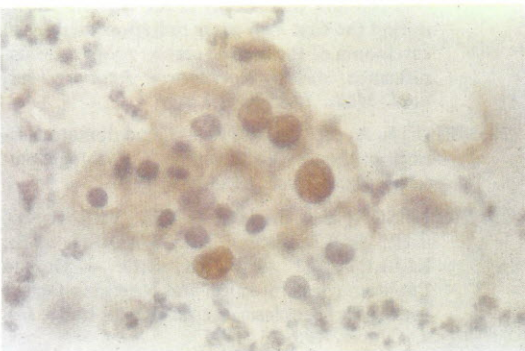


FIGURE 12

HPV antigen was found in many nuclei in a cluster of cells with radiation effect (x 400).

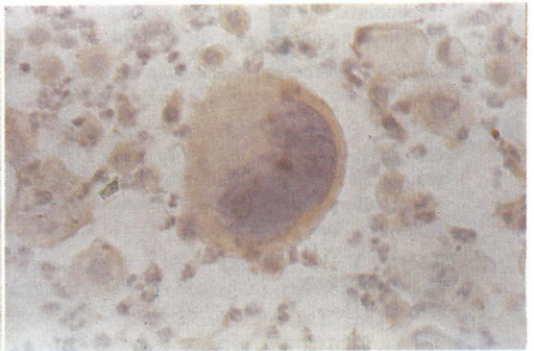


FIGURE 13

Nonspecific multinucleated giant cell of the same smear of Fig. 12, with negative reaction the HPV antigen (x 400 Papanicolaou).

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