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Research Note

HUMAN PAPILLOMAVIRUS AND ANOGENITAL CANCERS IN NORTHERN BRAZIL

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The association between certain human papillomavirus (HPV) types with both benign and malignant proliferative lesions of adult anogenital tract epithelium has been well established by different authors (H. zur Hausen & A. Schneider, 1987, p. 245-263. In N. P. Salzman & P. M. Howley (eds). *The Papovaviridae,* Plenum Press, New York; L. A. Koutsky et al., 1988, *Epidemiol. Rev., 10:* 122-163). Types 6 and 11 are known to be the most frequent cause of the widespread venereal wart (condyloma acuminatum) and low-grade cervical intraepithelial neoplasia (CIN) (L. Gissmann et al., 1983, *Proc. Natl Acad. Sci. USA, 80:* 560-563), whereas types 16 and 18 are predominantly related to higher grade CIN lesions and invasive cervical carcinoma (M. Durst et al., 1983, *Proc. Natl Acad. Sci. USA, 80:* 3812-3815; M. Boshart et al., 1984, *EMBO, 3:* 1151-1157; S. Baudenon et al., 1986, *Nature, 321:* 246-248).

There is currently a growing interest in establishing the epidemiological features of HPV infection and cancer of anogenital tract throughout the world. J. H. Scholefield et al. (1991, *Gut, 32:* 674-676), for instance, found that the prevalence of HPV type 16 associated anal squamous cell carcinoma was significantly lower in tissue from India (3%) and South Africa (11%) than in Swiss (43%), Polish (35%) or São Paulo, Brazil (47%) samples. In a case-control study of incident invasive cervical cancer (ICC) in Panama, Costa Rica and Bogota, Colombia, W. C. Reeves et al. (1987, *Int. J. Cancer, 40:* 450-454) found percentages of either HPV 16 or 18 of 37%, 92% and 86%, respectively, among women with newly diagnosed ICC.

Despite the well characterized world's highest incidence of both cervical and penile carcinomas in Brazil, studies on the occurrence of HPV infection among cases of anogenital neoplasia in this country are limited. Two different investigations, carried out in the northeast region of the country (D. McCance et al., 1986, *Int. J. Cancer, 37:* 55-59) and São Paulo (L. L. Villa & A. Lopes, 1986, *Int. J. Cancer, 37:* 853-855) yielded HPV DNA detection in carcinomas of the penis. In northern Brazil, where anogenital cancer (particularly of the uterine cervix) seem to be highly prevalent (Instituto Ofir Loiola, 1990, Belém, Pará), data on the occurrence of HPV infection has not been available.

The present report deals with the first cases of cancer of anogenital tract from northern Brazil, in which HPV type 6/11 and 16/18 were detected.

Formalin-fixed, paraffin-embedded tissue blocks from ten patients suffering from invasive epidermoid carcinoma of anogenital region were examined, as shown in Table. Patients were treated at Instituto Ofir Loiola, Belém, Pará, and six of them attended public, official medical units in the rural area of Pará state. Tissue sections from biopsy and/or hysterectomy specimens of the latter group were sent to the Pathology Section of "Instituto Evandro Chagas", Belém, for diagnosis. All samples were examined by haematoxylin and eosin staining for the presence of residual tumour and subsequently processed by *in situ* hybridization, with the commercial kit *Viratype in situ,* Life Technologies, Inc., Gaithersburg, Maryland 20877, U. S. A.

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TABLE

Human papillomavirus types and associated lesions in ten patients suffering from invasive epidermoid carcinoma of anogenital region. Pará and Maranhão, Brazil – 1987-1991

Case	Date of collection	Locality	Age in years	Site of lesion	HPV type
1	Oct. 1987	Altamira, PA	27	Penis	N.D.
2	Apr. 1989	Mte. Dourado, PA	60	Uterine cervix	Neg.
3	May. 1989	Mte. Dourado, PA	42	Perianal[a]	6/11
4	Jun. 1989	Marabá, PA	65	Uterine cervix	Neg.
5	Jul. 1989	Mte. Dourado, PA	73	Penis	N.D.
6	Sep. 1990	Belém, PA	28	Uterine cervix	16/18
7	Sep. 1990	? MA	78	Penis	Neg.
8	Nov. 1990	Belém, PA	40	Uterine cervix[b]	6/11
9	Dec. 1990	Abaetetuba, PA	36	Penis	6/11
10	Apr. 1991	Itaituba, PA	?	Uterine cervix	16/18

a: female patient.
b: suspicious.
PA = Pará state.
MA = Maranhão state (unknown city).
N.D. = Not determined, uninterpretable results.

All samples were further examined at the Ludwig Institute for Cancer Research, São Paulo, Brazil, with the purpose of confirming our results, by using a previously described *in situ* hybridization procedure that employs ^{35}S-labelled HPV DNA probes (J. E. Levi et al., 1989, *Am. J. Pathol., 135:* 1179-1184).

All the biopsy specimens showed histological features of invasive epidermoid carcinoma. In five (50%) out the ten samples examined for the presence of HPV DNA positive results were yielded. Three specimens were found to be negative and, in two cases, conclusive interpretation of results could not be done, as preparations were not in optimal condition (ie. presence of excess residual blood). HPV type(s) 6/11 were found in cancers of penis, uterine cervix and perianal region, whereas HPV type(s) 16/18 were demonstrated in cases of uterine cervix cancers only.

Although preliminary, our results strongly suggest that a significant proportion of cases of anogenital cancer in our region is associated with HPV infection. This has been previously postulated by local oncologists (J. E. Macedo, personal communication), on the basis of both histological and citological observations. The current availability, however, of the specific *in situ* hybridization method, not only enhances our diagnostic sensitivity, but also allows the identification of HPV genotypes involved in the aetiology of malignancies. Studies conducted by Schneider et al. (1987, *Diagn. Cytopathol., 3:* 250-253) have shown that citological classical criteria for diagnosis of HPV infection, based on the detection of both koylocitosis and diskeratosis, has a sensitivity of only 15% if compared with methods that detect the presence of viral DNA such as *in situ* hybridization.

A conclusive diagnosis could be made in eight out of the ten paraffin-embedded human anogenital tissues examined by *in situ* hybridization, suggesting that this method may be useful regarding retrospective studies involving stored paraffin-blocked human tissues. However, for optimal performance, it is recommended that the *Viratype in situ* kit be used in tissues fixed no longer than 24h (McGadey, 1970, *Histochimie, 23:* 180-184). Interpretation of results was not possible in two specimens for technical reasons, particularly the presence of residual blood in excess in the preparation. An interesting finding in our investigation is the detection of HPV type(s) 6/11 in two out of five HPV DNA positive specimens and possibly a third one. Interestingly, in one of these cases the positive cells were restricted to a non-tumor epithelium. This seems to be in contrast with previous studies that associate these genotypes with the aetiology of venereal wart and low-grade cervical intraepithelial neoplasia, and not with invasive carcinomas (L. Gissmann, *loc. cit.*).

In order to establish the magnitude of anogenital neoplasia and HPV infection in northern Brazil, further and broader investigations should be carried out, possibly involving both urban populations and isolated, Indian communities in the Amazon.

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