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MORPHOMETRIC EVIDENCE OF A RELATION BETWEEN HUMAN PAPILLOMA VIRUS INFECTION AND CERVICAL INTRAEPITHELIAL NEOPLASM

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ABSTRACT

We report a quantitative method applied to histological sections to ascertain the disturbances of cellular and nuclear maturation in cervical squamous epithelium. Using this method, we could postulate which changes can be attributed to carcinogenesis, and which to infection with Human Papilloma Virus. In the context of the problem distinguishing these two entities in a diagnostic setting, and because there are indications that Human Papilloma Virus is involved in cervical carcinogenesis, our observations might be of importance for both pathologists and epidemiologists. Our findings indicate that there is a morphological continuum ranging from purely viral induced changes to morphological features of neoplasia, and that during these events the viral related changes are suppressed.

Keywords: Carcinogenesis, histomorphometry, Human Papilloma Virus, koilocytosis.

INTRODUCTION

The papilloma viruses are unique among the viruses in that they can produce tumors in their natural host. The majority of lesions are benign but the viruses supposedly play a role in the development of cancers (Coleman et al., 1985). The relation between infection of the cervical epithelium with human papilloma virus, causing condylomatous (koilocytotic) lesions, and cervical intraepithelial neoplasia (CIN) is a matter of contention. In cervical pathology, the following findings are relevant: papilloma virus antigens were found with variable frequency in cervical intraepithelial neoplasia, and viral DNA was found in the majority of biopsies with CIN (McCance et al., 1983). In the latter study, in only half of the cases in which the viral DNA was demonstrated in the affected nuclei, the immunostaining for viral antigens was also positive. This indicates that cells having incorporated the viral DNA nevertheless can lack expression of antigens related to viral proteins.

Kurman et al. (1982) demonstrated that there is a relationship between the grade of CIN and positivity of the immunostaining for viral antigens, and Ferenczy showed in 1977 that the more koilocytotic the lesion, the higher the chance for a positive immunostaining result.

In histological sections it was found that in the beginning of the koilocytotic CIN spectrum there are multipolar and dispersed polyploid mitotic figures, and at the end aneuploidy with the occurence of abnormal mitotic figures with clumped and lagging chromosomes (Winkler et al., 1984).

From the quoted studies the hypothesis can be derived illustrated in Fig. 1.

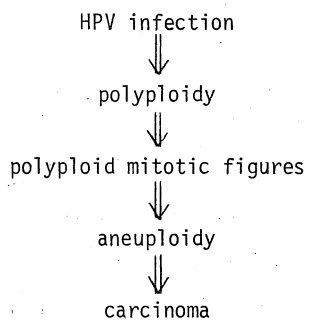

Fig. 1. HPV infection and cervical carcinoma.

The morphological expression of polyploidy is nuclear enlargement without changes in chromatin pattern, and that of aneuploidy is polymorphia and changes in chromatin pattern.

It seemed attractive to us to study the presence of human papilloma virus antigens, the degree of koilocytosis, and morphometric features of the koilocytotic lesions in order to try to find evidence of a relation between human papilloma virus infection and cervical intraepithelial neoplasia. For the morphometrical approach, we devised a quantitative method to express (disturbances of) maturation in cervical epithelium. Maturation of stratified squamous epithelium cells causes a decrease in nuclear size and an increase in cytoplasmic areas in tissue sections of cervical epithelium. Maturation of stratified squamous epithelium cells causes a decrease in nuclear size and an increase in cytoplasmic size, so it can be quantified by measuring the nuclear and cytoplasmic areas in tissue sections of cervical epithelial cells in the deep layer, the middle layer and the superficial layer, respectively. (See Fig. 2). Maturation from the deep layer to the middle layer can be expressed by comparing the properties of the cells in these layers. This can be achieved by calculating ratios. The three ratios used in our study are tabulated in Table 1.

For our study we used 59 cervical lesions with koilocytotic and nuclear changes. In the histological sections the immunoperoxidase technique was performed to localize human papilloma virus antigens, and the degree of koilocytosis was established according to Ferenczy et al. (1981). In addition, we used a control series of sections of immature metaplasia and mature cervical squamous epithelium. The histological features of CIN are: nuclear atypia (nuclear enlargement and chromatin changes) and, with increasing CIN grade, increasing immaturity of the epithelium. Immature metaplastic epithelium does *not* display atypia, and the immaturity of the epithelium is visible in the small amount of cytoplasm of the superficial layer. Mature squamous epithelium does *not* display nuclear atypia and the superficial cells are flattened and have small, often pyknotic nuclei.

HISTOMORPHOMETRY

In Table 2, numerical results computed from the measurement of the 59 koilocytotic cervical lesions are presented. It is clear that nuclear and cellular changes in koilocytotic cervical epithelial lesions have a definite pattern, which is most pronounced in those cases with a low CIN grade, which were also most often positive in the immunostaining. These cases have more excessive nuclear and cytoplasmic enlargement in the middle layer (high values for ratios R_1 and R_2). To our knowledge, this phenomenon has not been

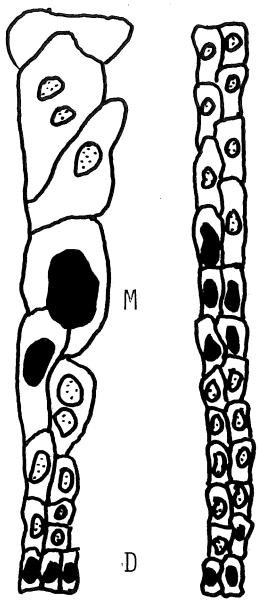

Fig. 2. Schematic representation of cervical epithelium infected with HPV (left) and of CIN III. The measured nuclei in the middle (M) and deep (D) are blackened. Note differences in the morphometric properties.

Table 1. Morphometric features of maturation from the deep to the middle layer in squamous epithelium.

feature	notation	description in normal maturation
R_1	$\frac{\text{nucleus M}}{\text{nucleus D}}$	smaller nuclear area in middle layer compared to nuclear area in deep layer (i.e., $R_1 < 1$)
R_2	$\frac{\text{cytoplasm M}}{\text{cytoplasm D}}$	larger cytoplasmic area in middle layer compared to cytoplasmic area in deep layer (i.e., $R_2 > 1$).
R_3	$\frac{\text{N/C M}}{\text{N/C D}}$	smaller nuclear/cytoplasmic ratio in middle layer compared to nuclear/cytoplasmic ratio in deep layer (i.e., $R_3 < 1$)

Table 2. Morphometric features and CIN I, II, and III, immature metaplastic epithelium and mature squamous epithelium. The three features are dimensionless, sd = standard deviation. The arrows indicate the order of increasing numerical value.

feature		CIN I (n=36)		CIN II (n=18)		CIN III (n=5)		Immature metaplastic epithelium (n=5)		Mature squamous epithelium (n=5)	
no.	notation	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
R_1	nucleus M / nucleus D	← 1.95	0.71	1.81	0.85	1.24	0.37	0.87	0.21	0.80	0.28
R_2	cytoplasm M / cytoplasm D	← 3.44	0.68	2.52	0.51	1.31	0.21	1.45	0.18	2.96	0.59
R_3	N/C M / N/C D	0.58	0.16	0.74	0.21	0.83 →	0.28	0.88	0.36	0.27	0.09

previously reported in the literature. These findings are in accordance with earlier cytologic studies of koilocytotic cells in smears (Boon et al., 1981) and with the hypothesis of a human papilloma virus-induced polyploidy. Immature metaplastic epithelium displays the same ratios as CIN III, however the nuclei are smaller than those of CIN III (data not shown here).

DISCUSSION

The data presented in this paper give us the tools to speculate which morphometrical changes can be attributed to virus infection (viral-related changes), and which are related to cervical intraepithelial neoplasia. These speculations can be made on the basis of the fact with increasing degree of koilocytosis (a viral-related effect) the ranking order of the morphometric features listed in the tables are exactly opposite to that for cervical intra-epithelial neoplasia. This is indicated by the direction of the arrows in Table 2. With increasing grade of CIN, the morphometric expression of human papilloma infection decreases.

In this paper we have shown that the three maturation indices, defined in Table 1, display shifts. However, also other morphometric features including cytoplasmic size in the cells in the superficial layer, N/C ratio in the superficial layer, nuclear size of the middle layer, cytoplasmic size in the middle layer, and N/C ratio in the middle layer change with increasing koilocytosis and with increasing CIN grade (Boon and Kok, 1985). Also here, the ranking order of the values for koilocytosis is opposite to that for carcinogenesis for all features mentioned.

In Table 2 also the values for the standard deviations for each of the subpopulations are given. One may ask whether the observed trends (ranking orders) are statistically significant. They are. To substantiate this contention one may first use a rough method: plot the mean values, and error bars, as a function of CIN grade. For the error bars one can take the standard deviations of the mean. One then is immediately convinced for R_2 and R_3, but possibly not for feature R_1. Therefore one invokes a more refined method to check the statistical significance, discussed in the work of Schaafsma (1966). Ch. 5 of that book discusses the problem of testing homogeneity against trend. In particular Theorem 1 of p. 75 formulates the relevant test for our problem. For the trend observed in R_1 we find a significance level corresponding to 5%, but not to the somewhat more satisfying level of 2% or less, as is found for the other features. Similar conclusions can be drawn for the other considered features for which we have given no numerical data in the present paper.

The advantage of the use of the parameters of Table 1 lies in the fact that because only dimensionless ratios are involved, the precise magnification factor of the studied photographs needs not to be known.

It seems likely that the neoplastic changes are superimposed on the viral changes and that the viral-related changes are suppressed in neoplastic transformation. To distinguish the morphological characteristics of viral-induced carcinogenesis in squamous epithelium from those of chemical cancer induction our method in which the degree and direction of disturbance of maturation is expressed in a quantitative manner seems to be very promising.

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