Morphological diagnosis of HPV lesions and cervical intraepithelial neoplasia (CIN) is highly reproducible

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Summary

Purpose: to assess the value of individual histological criteria in the diagnosis of cervical HPV lesions.

Methods: 138 women referred for colposcopic evaluation (due to abnormal PAP smears) were subjected to cervical punch biopsy. The biopsies were classified as no HPV lesion, CIN 1, or CIN 2-3 by two observers independently. Kappa tests were used for inter-observer agreement of the diagnosis. The presence of binucleation, multinucleation, abnormal mitosis, koilocytosis, spindle koilocytosis and dyskeratosis was similarly assessed.

Results: the Kappa statistic was 0.638 (CI 95% 0.533 - 0.743), showing substantial inter-observer agreement. Abnormal mitosis and multi-nucleation were the two most powerful discriminators between CIN 2-3 and CIN 1. Koilocytosis proved to be the single most powerful discriminator between CIN 1 lesions and non-HPV lesions.

Conclusion: the results advocate the use of histology as the gold standard in diagnosing cervical precancerous lesions. The classical criteria can be also used to differentiate low-grade lesions, which has practical implications by avoiding the unnecessary treatment of minor abnormalities.

Key words: Papillomavirus; CIN; Histology; Diagnosis.

Introduction

Papillomaviruses are obligatory intranuclear organisms infecting mitotically active cells at the squamo-columnar junction within the immature transformation zone (TZ). In productive HPV infection, the number of viral particles increases in parallel with terminal differentiation towards the epithelial surface, where keratinocyte nuclei are packed with complete virions [12]. This viral replication in the epithelial cells is accompanied by a number of morphological alterations that parallel the molecular changes in the proliferating epithelial cells. The virus appears to interfere with the mitotic spindle and with cytokinesis, resulting in multinucleated cells and significant cytological atypia attributable to polyploid. Individual epithelial cells also develop perinuclear halos under the influence of HPV-encoded proteins, resulting in cytologically and histologically diagnostic koilocytes [5, 12, 15], which is the cytopathic effect of HPV. Hyperchromatic, unusually enlarged nuclei and/or abnormal mitosis are found significantly more often in lesions infected with HPV 16, 32, and 35 than in those infected with HPV 6, 11, 18, 31, and 45, and may represent a useful histological marker of HPV lesions with significant oncogenic potential [5, 6].

Productive HPV infection in squamous epithelium is characterized by cellular changes identified in lesions with different stages of evolution [1, 9]. Classical histopathological features of HPV infection were described long ago [7] but their significance was not recognized until 1976 when Meisels *et al.* [11] reported their association with low grade CIN. These changes were characterized by dyskeratosis and koilocytosis. According to this orginal study, the most useful parameters suggesting the presence of HPV were: "meganuclei", dyskeratosis, koilocytosis, multicleation and hyperchromatic nuclei [11]. Subsequently, binucleation and/or multinucleation have been categorized as secondary characteristics or non-classical signs of HPV, with somewhat low reproducibility [13]. Similarly, the reproducibility of the histological biopsy, used as the gold standard in grading CIN [3, 6, 9], has been demonstrated to show some inter-observer variability as well [2, 4, 10]. In this study, we tested the reproducibility of the suggested histopathological characteristics of HPV infection and their appearance in a series of 138 colposcopic biopsies with different grades of CIN and non-CIN lesions.

Methods

The material for this prospective study consisted of 138 consecutive women referred for colposcopy at Leonor Mendes de Barros Hospital, São Paulo and Centro de Atenção à Saúde da Mulher, Campinas (UNICAMP). All women underwent careful gynecological examination, including colposcopy and directed punch biospy from the atypical transformation zone. The bio-

Revised manuscript accepted for publication August 30, 2000

psies were submitted to the reference laboratory (Institute Adolfo Lutz) to be processed for HE-stained sections, according to standard procedures. The biopsies were examined using the WHO classification [13] and categorized as either: 1) no HPV lesion (including normal histology and inflammation), 2) CIN 1 (including condyloma acuminatum and CIN 1), or 3) CIN 2-3, grouped together. In addition, the presence of binucleation, multinucleation, classical koilocytosis, spindle koilocytes, aberrant mitosis and dyskeratosis was recorded in all lesions. All samples were examined by two observers, independent of each other, to test the reproducibility of the suggested morphological characteristics of HPV, and their association with different grades of cervical intraepithelial neoplasia (CIN).

In statistical analysis, frequency tables were analyzed using the Chi-square test. Risk estimates expressed as Odds Ratio (OR) and 95% CI were used to assess the significance of the correlation between the individual variables in univariate analysis. The degree of agreement between observers was characterized by kappa statistics. Kappa is an index of inter-observer agreement that as been corrected for chance and therefore indicates the degree of inter-observer agreement over and above what would be expected by chance alone. For most purposes, kappa values greater than 0.60 and 0.80 may be taken to represent, respectively, a substantial and almost perfect agreement beyond chance, values between 0.00 and 0.60, slight to moderate agreement beyond chance and values less than 0.00, very poor agreement beyond chance [8].

Results

The inter-observer agreement between the two observers in classifying the 138 cervical lesions is shown in **Table 1**. There was complete inter-observer agreement in 98 cases (71%), while in 34 cases (25%) a variation of only one degree was detected. In the final examination, 25 cases were considered to be under-scored (CIN 2) by observer 1 and no lesion by observer 2). Only one lesion was over-scored as CIN 3 by observer 1 and as no lesion by observer 2. The final consensus diagnoses of the 138 cases were: no HPV lesion - 32 cases; CIN 1 - 62 cases; CIN 2 or 3 - 44 cases. The kappa statistic for all grades of cervical lesions taken together was 0.638 (CI 95% 0.533 - 0.743). This is substantial inter-observer agreement in diagnosing the condition.

Table 1. — *Agreement between two observers in categorizing 138 cervical biopsies*

Observer 2	Observer 1				Total
	No lesion	CIN 1	CIN 2	CIN 3	
No lesion	19	10	2	1	32
CIN 1	6	54	2	0	62
CIN 2	2	9	15	1	27
CIN 3	0	1	6	10	17
TOTAL	27	74	25	12	138

Kappa coefficient = 0.638 CI 95% (0.533-0.743)

Table 2 summarizes the detection of the different HPV-suggestive morphological characteristics in different lesions. All these changes were rare in lesions classified as non-HPV lesions. All except multinucleation were good discriminators between non-HPV and CIN 1 category, with OR ranging from 2.7 to 140.0. Among CIN biopsies, abnormal mitosis and multinucleation were the two most powerful (OR 116.7 and 66.8) discriminators between CIN 2-3 and CIN 1 lesions. However, seven CIN 2-3 cases (16%) did not show either abnormal mitosis or multinucleation. The single most powerful discriminator between lesion grade proved to be koilocytosis in distinguishing between CIN 1 lesions and non-HPV lesions (OR 140; CI 26.6-736.8).

Discussion

Conventional morphological examination of the histological biopsy remains to be the gold standard in the diagnosis of cervical cancer precursors. The reproducibility problems of different histological classifications are well known [16, 17], particularly the difficulty in making a distinction between severe dysplasia and carcinoma in situ (CIS), as well as low-grade CIN from non-CIN lesions. This variable inter-observer agreement does not, however, invalidate the use of biopsy as the cornerstone in cervical cancer detection, as recently argued [2, 4]. This was also convincingly demonstrated in the present study, where two observers graded a series of 138 cervical biopsies to test the inter-observer agreement of their diagnosis. Indeed, there was complete inter-observer agreement in 98 cases (71%) and in 34 cases (25%); variation was only one degree. In the consensus assessment, 25 cases were found to be underscored (as CIN 2 by observer 1 and as no lesion by observer 2). Only one lesion had been overscored. The kappa coefficient calculated according to Landis *et al.* (1989) [8], was 0.638 (CI 0.533-0.743) which indicates

Table 2. — *Presence of morphological criteria in different grades of CIN*

Histological Criteria	No lesion (N=32)		CIN 1 (N=62)		CIN 2-3 (N=44)		CIN 1 vs no lesion	CIN 2-3 vs CIN 1
	N	%	N	%	N	%	OR 1 (95% CI)	OR 2 (95% CI)
Binucleation	4	12%	40	64%	41	93%	12.7 (4.0-41.0)	7.5 (2.1-27.1)
Multinucleation	0	0%	1	2%	23	52%	1.6 (0.1-40.0)	66.8 (8.5-525.6)
Koilocytosis	2	6%	56	90%	43	98%	140.0 (26.6-736.8)	4.6 (0.5-39.7)
Spindle koilocyte	1	3%	37	58%	39	89%	45.9 (5.9-358.2)	5.3 (1.8-15.2)
Dyskeratosis	4	12%	25	40%	40	91%	4.7 (1.5-15.1)	14.8 (4.7-46.6)
Abnormal mitosis	0	0%	2	3%	35	80%	2.7 (0.1-57.6)	116.7 (23.8-570.9)

that inter-observer agreement is substantial. These data indicate that histological assessment is highly reproducible, and a clinically relevant means to classify squamous cell precancerous lesions.

Another aim of the study was to evaluate the accuracy of histological criteria in the diagnosis of cervical HPV lesions. HPV infections induce a variety of proliferative lesions in the squamous epithelia, ranging from benign flat and exophytic condylomas to different grades of CIN. HPV is also the single most important etiological factor of cervical cancer [16]. There is some data suggesting that in CIN 1, the presence of severe nuclear abnormality is indicative of an infection by the oncogenic HPV types [6]. In the present study, all cervical biopsy specimens were evaluated for the presence of individual morphological criteria, suggested to be determinants of HPV infection. Abnormal mitosis and multi-nucleation were frequent findings in the most severe lesions (CIN 2-3). Indeed, these abnormal nuclear features may be predictive of CIN 2-3 even without the presence of significant architectural disarrangement. This is consonant with the view recently adopted by Fox *et al.* (1999), particularly while assessing the lesions in a thin epithelium with few cell layers only [18].

Similarly, the presence of dyskeratosis was more frequent in high-grade lesions (OR 14.8; CI 4.7-46.6), being an important marker in distinguishing CIN 1 lesions. Also classification of these HPV-suggestive criteria showed good inter-observer agreement. Not unexpectedly, the presence of koilocytosis and spindle koilocytes showed a significant association to HPV lesions. Both were also highly powerful markers in differentiating non-HPV lesions from CIN 1 (OR 140.0 and 45.9, respectively). These two features lost much of their distinctive power in determining the difference between CIN 2-3 and CIN 1. This can be explained by the fact that koilocytosis becomes less pronounced in parallel with increasing severity of the lesions, and only infrequently observed in invasive carcinomas [16]. This makes koilocytosis a useful generic marker of HPV, i.e. the cytopathic effect of the virus, particularly prominent in productive HPV infections.

Conclusion

The substantial intra-observer agreement of histological grading of CIN emphasizes the role of histology as the gold standard in the diagnosis of cervical cancer precursors. Of the individual morphological features, abnormal mitosis, multinucleation and dyskeratosis were found significantly more often in high-grade (CIN 2-3) lesions, and as such were significant discriminators between CIN 2-3 and CIN 1. Koilocytosis in turn was the single most powerful criteria between CIN 1 and non-HPV lesions. The present study confirms that some classical criteria can be used to differentiate low-grade lesions, which has practical implications e.g. in the treatment, while avoiding unnecessary treatment of minor abnormalities.

Acknowledgments

This study has been supported by the grant from FAPESP (no 96/1750-3), which is gratefully acknowledged.

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