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article

Association of oncogenic human papillomavirus DNA with high grade cervical intraepithelial neoplasia: the role of cigarette smoking

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Introduction

Human papillomavirus (HPV) is currently considered as the main aetiological factor of cervical cancer and its precursor cervical intraepithelial neoplasia (CIN).¹ The integration of HPV DNA in the human genome is crucial for persistence of the virus and its carcinogenic effects. Molecular mechanisms and cellular events associated with viral persistence are still poorly understood, although it seems likely that a permissive viral replication increases the viral load in infected cells. Correlation between the viral load and persistence of the infection is indirect; in women with HPV induced cytopathic changes, a high load of oncogenic HPV types is strongly associated with CIN 2 and CIN 3.² The role of smoking as a risk factor for CIN and cervical cancer has been repeatedly emphasised starting from the late 1980s, although the results still remain controversial.³⁻⁸ This study was designed to evaluate the independent role of current smoking and viral load of the oncogenic (high risk) HPV types as predisposing factors for CIN 2 and CIN 3.

Material and methods

The subjects of this study were selected among 163 women referred to the colposcopy clinics of Leonor Mendes de Barros and CAISM/UNICAMP hospitals, between September 1995 and August 1997 because of HPV suggestive abnormalities in their Papanicolaou smears. The protocol was approved by the institutional review board, and all women gave informed consent. The patients filled in a detailed questionnaire requesting their social, sexual, and reproductive histories.

A total of 148 women aged between 18 and 36 years (mean 27 years), who presented with an abnormal pattern on colposcopy warranting a directed punch biopsy, were included. Histological changes were evaluated according to the WHO classification⁹ into the following categories: absence of HPV lesion, CIN 1, CIN 2, or CIN 3. On light microscopy, 42 women showed no HPV lesion on the cervix, 69 had CIN 1, 24 showed a CIN 2, and 13 showed CIN 3.

For the HPV DNA analysis, the first generation hybrid capture (HC) HPV DNA assay, including high risk HPV probes for types 16, 18, 31, 33, 35, 45, 51, 52, and 56 was used according to the instructions of the

manufacturer (Digene, USA). A cut off level of 1 relative light unit (RLU) was used, signifying that the samples with light emission higher than 1 RLU are considered HPV positive. Based in the data of Wright *et al*¹⁰ the viral load was classified as low when ranging from 1 to 5 RLU, intermediate when ranging from 5.1 to 20 RLU, and high when above 20 RLU. HC, colposcopy, and directed biopsy were collected with a median interval of 13 weeks (minimal of 4 and maximal 21 weeks) after the Pap smears.

The distribution of the risk factors was compared among women with no HPV lesion and CIN by univariate logistic regression, and the odds ratio was calculated with a confidence interval of 95%. After this, a polytomous logistic regression analysis model was used to check the independent associations between the studied variables and CIN. Because CIN 2 and 3 are considered high grade lesions they were analysed together. By choosing the women with no HPV lesions as the base category for the dependent variable, the model estimated two risk equations simultaneously: (a) the odds for having CIN 1 *v* no HPV lesion and (b) the odds for having CIN 2 and 3 *v* no HPV lesion. In all data analysis, EPI-INFO 6.02 and SAS (Statistical Analysis System) were used.

Results

The overall detection rate of HPV DNA was 48% (71/148 women) and the detection rate increased significantly with the grade of CIN. The probability of CIN 2 and CIN 3 was also directly related to the viral load. Accordingly, odds ratio (OR) for CIN 2 or 3 was 14.57 in women with low viral load, reaching the value of 20.64 in those with a high viral load, compared with the women with a negative HC test (table 1).

An early onset of sexual activity, cigarette smoking, and the presence of HPV DNA were factors associated with CIN 2 or 3 in the polytomous logistic regression analysis (table 2). Women who smoke presented with an adjusted OR of 4.37 for having developed CIN 2 or 3 compared with the non-smokers. Women with positive HC test for the oncogenic HPV DNA had an adjusted OR 14.40 of presenting CIN 2 or 3 compared with those who tested as HPV negative.

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Table 1 Lifestyle variables and HPV status of the cervical lesions in univariate regression analysis

	Histological diagnosis			Without HPV lesion v CIN 1		Without HPV lesion v CIN 2 and 3	
	Without HPV lesion No (%)	CIN 1 No (%)	CIN 2 or 3 No (%)	Crude OR	(95% CI)	Crude OR	(95% CI)
Race							
White	30 (71)	53 (77)	28 (76)	1.0		1.0	
Non-white	12 (29)	16 (23)	9 (24)	0.76	(0.32; 1.81)	0.86	(0.29; 2.20)
Schooling							
>4 years	9 (21)	20 (29)	15 (41)	1.0		1.0	
<4 years	33 (71)	49 (71)	22 (59)	0.67	(0.27; 1.65)	0.40	(0.15; 1.07)
Lifetime number of sexual partners							
One	23 (55)	23 (33)	17 (46)	1.0		1.0	
More than one	19 (45)	46 (67)	20 (54)	2.42	(1.10; 5.32)	1.18	(0.49; 2.80)
Age at first sexual intercourse							
>18 years	23 (55)	33 (48)	8 (22)	1.0		1.0	
<18 years	19 (45)	36(52)	29 (78)	1.32	(0.61; 2.85)	4.39	(1.63; 11.82)
Parity							
Nulliparous	9 (21)	15 (30)	3 (8)	1.0		1.0	
One childbirth or more	33 (79)	35 (70)	34 (92)	0.64	(0.25; 1.65)	3.09	(0.77; 12.43)
Current smoker							
No	30 (71)	44 (64)	11 (30)	1.0		1.0	
Yes	12 (29)	25 (36)	26 (70)	1.42	(0.62; 3.26)	5.91	(2.24; 15.62)
High risk HPV DNA							
Absent	34 (81)	36 (49)	7 (19)	1.0		1.0	
Present	8 (19)	37 (51)	30 (81)	4.37	(1.78; 0.71)	18.21	(5.90; 56.12)
Viral load in HPV DNA positive women							
HPV negative	34 (80)	36 (52)	7 (19)	1.0		1.0	
Low (1–5 RLU)	2 (5)	9 (13)	6 (16)	4.25	(0.86; 21.10)	14.57	(2.42; 87.32)
Median (5.1–20 RLU)	2 (5)	8 (12)	7 (19)	3.78	(0.75; 19.07)	17.00	(2.90; 99.76)
High (>20 RLU)	4 (10)	16 (23)	17 (46)	3.78	(1.15; 12.44)	20.64	(5.30; 80.39)

Discussion

Among the 148 young Brazilian women analysed in the present study, the association of current smoking with the severity of CIN was evident and remained as an independent risk factor, significantly associated with CIN 2 and 3 in the multivariate analysis as well. The results are in alignment with other recent studies. Thus, Olsen *et al* established an intimate interrelation between smoking, high risk HPV type 16, and CIN 2/3: the OR for CIN 2 and 3 in HPV type 16 positive smokers and non-smokers was 65.9 and 15.7, respectively.⁸ The same was true with the detection of high risk HPV DNA in these women, which is in alignment with the data of Kanetsky *et al* who also demonstrated an association between cigarette smoking and cervical dysplasia in non-Hispanic black women, after adjustment for HPV infection (HC assay).⁶

Recently, the viral load has been associated with CIN in cross sectional studies. In our series, the adjusted OR of presenting with CIN 2 or CIN 3 was 14.40 times higher in women with positive HC test (for oncogenic types), and the viral load was directly related to the grade of CIN. However, to more accurately

predict the prognostic value of the viral load, it would be important to specifically assess the role of individual HPV types, and not merely examine the load of the virus pool included in the HC test.

Of the other risk factors established in the present series, the onset of sexual activity before the age of 18 contracted an adjusted OR of 3.22 for CIN 2/3. An early age at the first intercourse predisposes the woman to any sexually transmitted agents (such as HPV). The same effect is exerted by the number of lifetime sexual partners, established as a risk factor for CIN 2/3 in a number of epidemiological studies.^{9–11}

Although subjected to the potential temporal biases of a cross sectional study, the present series clearly demonstrates that the severity of the cervical precancer lesions is closely associated with (1) the presence of oncogenic HPV DNA, (2) cigarette smoking, and (3) early onset of sexual activity. The increase of the viral load was directly related to CIN 2/3. Taken together with the current data on the causal role of HPV in cervical carcinogenesis, the present results imply that smoking and HPV might act synergistically in this process.^{3–8} To further elucidate the mechanisms of these suspected synergistic actions between HPV and cigarette smoke, however, additional experimental studies are necessary.

Table 2 Factors significantly associated with CIN 2 and 3 in the polytomous logistic regression analysis

	CIN 1 v without HPV lesion			CIN 2 and 3 v without HPV lesion		
	Adjusted OR	(95% CI)	p Value	Adjusted OR	(95% CI)	p Value
Current smoker						
No	1.0			1.0		
Yes	1.25	(0.52; 2.96)	0.62	4.37	(1.48; 12.92)	<0.01
Age at first sexual intercourse						
≥18 years	1.0			1.0		
<18 years	1.17	(0.52; 2.6)	0.71	3.22	(1.05; 9.83)	0.04
High risk HPV DNA						
Absent	1.0			1.0		
Present	3.76	(1.51; 9.34)	<0.01	14.40	(4.47; 46.40)	<0.01

The likelihood ratio statistic has a value of 11.70 with eight degrees of freedom, which is indicative of a good fit ($p=0.17$), adjusted by race, schooling, lifetime number of sexual partners, and parity.

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Contributors: SFM Derchain had the initial idea for this project focused on analysing the interrelations of smoking and HPV in the development of CIN; CM Roteli-Martins, examined all patients and collected the data. Together, these two authors also wrote the project plan and the first version of this manuscript. KJ Syrjänen has given constructive advice concerning the project design, as well as the composition of the manuscript, including the discussion of the results in proper context, statistical tests, and revising the English language of the paper as well as making suggestions to pertinent references. HJ de Abreu and EZ Martinez participated in the design of the study and they also made the statistical analysis. VAF Alves suggested the use of hybrid capture, and also read and classified all the cervical biopsies.

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Development of further national guidelines

Following the publication of national management guidelines on 22 conditions as a supplement to the August edition of the journal *Sexually Transmitted Infections*, the Clinical Effectiveness Group of the MSSVD and AGUM is seeking the opinion of the wider specialty as to whether there is a need to develop guidelines on additional topics.

If you have any suggestions for future guideline development, please contact: Dr Keith Radcliffe, Chairman CEG, Consultant in GU Medicine, Whittall Street Clinic, Whittall Street, Birmingham B4 6DH; fax: 0121 237 5729; email: k.w.radcliffe@bham.ac.uk