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BRIEF ARTICLE

Smoking and genital human papilloma virus infection in women attending cervical cancer screening in Greece

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Abstract

AIM: To investigate whether smoking is associated with human papilloma virus (HPV) infection.

METHODS: HPV infection is considered to be a necessary condition for cervical cancer development. The study population included 1291 women, aged 25-55 years, attending cervical cancer screening. All women had a Papanicolaou (Pap) test, with liquid-based cytology (Thinprep®), an HPV-DNA test and an evaluation of smoking habits. The COBAS® 4800 system was used for HPV-DNA testing, enabling identification of the following high-risk HPV (hrHPV)-types: each of HPVs 16 and 18 separately, and HPVs 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 as a cocktail. The evaluation of smoking habits was assessed using the smoking intensity index (SII), a variable formed as the product of cigarettes consumed per day by the days (years × 365) that a woman was a smoker, divided by 1000.

RESULTS: There were 136 smokers among 238 women tested positive for hrHPV-types (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and/or 68), and 463 smokers among 1053 hrHPV-negative women (OR = 1.7, $P < 0.001$). This association was attributed to the youngest age group of women, aged 25-34 years (OR = 2.3, $P < 0.001$), while there was no association in other age groups. The intensity of smoking (increasing SII) showed no statistically significant association with hrHPV infection. Cervical infection with HPV 16 and/or HPV 18 was also not associated with age or smoking habits. Finally, no association was found between Pap test status and smoking habits or smoking intensity.

CONCLUSION: Smoking appears to be associated with hrHPV infection of the uterine cervix, particularly in younger women. Further studies should investigate whether this association is based on causality and evaluate the role of other possible co-factors.

Key words: Human papilloma virus; High-risk human papilloma virus; Human papilloma virus-DNA test; Smoking; Cervical cancer; Screening

Core tip: Human papilloma virus (HPV) infection is a prerequisite for cervical cancer development. We investigated whether smoking can influence the course of HPV infection, in 1291 women attending cervical cancer screening. Smoking appeared to be associated with high-risk HPV (hrHPV) infection of the uterine cervix, particularly in younger women, aged 25-34 years. In addition, women in this younger age group with a negative Pap test were more likely to have hrHPV infection if they were smokers than if they did not smoke. Further studies should investigate whether this association is based on causality and evaluate the role of other possible co-factors.

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INTRODUCTION

Genital infection by human papilloma virus (HPV) is considered to be a *sine qua non* condition for development of cervical cancer[1]. In general, HPVs are divided into two groups, according to their oncogenic potential: The first group consists of the so-called low-risk and the second of the high-risk HPV (hrHPV)-types. Specific types of hrHPV can cause cervical cancer, and more than 95% of cervical cancer biopsies contain DNA from hrHPV genomes[2].

There are, however, certain other factors that may influence the probability of hrHPV infection or accelerate the carcinogenic processes leading to cervical cancer[3]. Transmission of HPV between sexual partners is influenced by the age of a woman and her partner, the number of sexual partners, the age at first sexual intercourse, use of barrier contraceptive methods, co-infections, male sexual behaviour, and male circumcision[4]. In some studies however, age at first sexual intercourse was not identified as an independent risk factor in multivariate analysis[5-7]. Other factors that have similar impact on disease progression include high parity, long-term oral contraceptive use, and smoking[8-10].

Many studies have shown that cigarette and tobacco smoking in general have a moderate and statistically significant association with cervical cancer and cervical intraepithelial neoplasia (CIN)[10-19], although the latter is not supported by other studies[20]. In detail, Syrjänen *et al*[20], by using multivariate analysis, found that cigarette smoking was not an independent risk factor for CIN2 or more advanced lesions; however, there is evidence that suggests a potential role of passive smoking on invasive cervical cancer[21,22]. There is also evidence suggesting an association between smoking and an increased risk of high grade vaginal intraepithelial neoplasia (high grade VAIN)[23].

It is not quite clear though, if tobacco smoking, apart from increasing the risk for invasive cervical cancer and its precursors, may also influence HPV infection, particularly persistent HPV infection. Recent research focusing on the possible association between tobacco smoking and HPV infection has shown a positive association in women[18,24,25], as well as in men[26]. In the present study, this possible association has been investigated in an urban population of women participating in cervical cancer screening in Greece.

MATERIALS AND METHODS

Participants and specimen collection

Women attending cervical cancer screening in two outpatient clinics of the Hippokrateio Hospital in Thessaloniki, Greece, were recruited for the present study, as well as for an ongoing multi-center study dealing with screening for cervical cancer based on hrHPV-DNA detection as primary test, using the Cobas® 4800 HPV Test. In total, 1291 women were recruited for the present study between August 2011 and January 2013. Participants were 25-55 years old, living in the urban area of Thessaloniki. The following exclusion criteria were used: pregnancy, treatment for CIN during the previous 5 years, history of hysterectomy, and use of conventional cytology.

Every woman was informed about all aspects and the rationale of the study, and signed a consent-form in order to participate. After recruitment, women answered certain questions about their smoking habits; if they smoked, and if yes, how many cigarettes per day and for how many years. According to this information, the number of cigarettes per day, multiplied by years of smoking [n (cig/d) × 365 × n (years) divided by 1000] was calculated, creating a new variable, the smoking intensity index (SII), by which the sample was stratified. Following completion of the questionnaire, all women were examined by a specially trained healthcare professional (either a gynecologist or a midwife), who took cell samples from both the ecto- and the endocervix, using the Cervex brush® (Rovers® Medical Devices, B.V. Oss, The Netherlands). Brushes were inserted in Thinprep® vials containing PreservCyt® Solution (Hologic, Inc, Marlborough, MA, United States), and were discarded after handling according to the instructions of the manufacturer.

Cytology

Papanicolaou (Pap) smears were prepared using the Thinprep® liquid-based methodology, as previously described[27] and examined by a specially trained cytologist. The cytological assessment was performed according to the Bethesda 2001 classification[28]. The remaining liquid sample was then sent to the Peripheral Laboratory of Public Health of the Hellenic Center for Disease Control and Prevention and Laboratory of Hygiene and Environmental Protection/Laboratory of Microbiology of the Democritus University of Thrace in Alexandroupoli, Greece, where it was tested for HPV-DNA, using the

Cobas® 4800 system (Roche® Molecular Diagnostics, CA, United States).

HPV-DNA detection

The Cobas® 4800 HPV Test is based on two main procedures[29]: (1) automated isolation of viral and human nucleic acids from clinical samples; and (2) enhancement and detection with real-time-polymerase chain reaction of 22 target-DNA sequences. Specifically, the Cobas® 4800 HPV Test employs primers for the determination of a sequence of approximately 200 nucleotides within the L1 polymorphic region of the HPV genome. The HPV primer concentration that exists in the Main Compound has been designed for the DNA enhancement of 14 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The oligonucleotide fluorescent tracers are bound to the polymorphic regions within the sequence determined by those primers. An additional primer couple and an additional tracer target the β-globulin human genome -clone (amplicon) 330 bp- in order to supply a control serum. Simultaneous infusion, enhancement and detection of human β-globulin genome and viral sequences using Cobas® 4800 HPV Test gives the user the added advantage of control during all stages of the examination. The HPV primer concentration in the main compound reagent for the Cobas® 4800 HPV Test has been designed for the DNA enhancement of 14 hrHPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. The enhanced signals from HPV 16 and 18 are each detected with a separate special fluorescent stain, while the other twelve hrHPV types are detected using the same fluorescent stain[29].

Statistical analysis

The association between HPV infection and smoking was evaluated using the χ^2 test and Fisher's exact test. The influence of smoking and smoking intensity on hrHPV infection as well as on cytology status was evaluated with the use of cross-tabulation matrices associating age. Frequencies and relative frequencies, as well as their corresponding *P*-values of the χ^2 test and the ORs are calculated. *P*-values < 0.05 were considered statistically significant. Analyses were performed using the SPSS 20.0 statistical software (IBM Co., New York, United States).

RESULTS

Recruited women (n = 1291) were divided into two groups: (1) hrHPV-positive women, *i.e.*, women positive for any of the 14 hrHPV types (n = 238); and (2) hrHPV-negative women, *i.e.*, women negative for all 14 hrHPV types (n = 1053).

Smoking and HPV status

In the younger group of women, aged 25-34 years, 95 out of 158 hrHPV-positive women were smokers, as compared with 125 out of 316 hrHPV-negative women (60.1% *vs* 39.6%, $P < 0.001$). Hence, in this age group, smokers were 2.3 times more likely to have a hrHPV infection than non-smokers. There was no statistically significant difference between hrHPV-positive and hrHPV-negative women in the 35-44 years old (46.7% *vs* 45.4%) and the 45-55 years old age groups (57.1% *vs* 46.3%). Regardless of age, a significantly higher proportion of hrHPV-positive women were smokers, as compared with hrHPV-negative women (57.1% *vs* 43.8%, $P < 0.001$). The OR for smokers to be hrHPV-positive in all age groups, *i.e.,* for ages between 25 and 55, was 1.7 ($P < 0.001$). An overview of these results is presented in Table 1.

Table 1 Association between smoking and high-risk human papilloma virus status, stratified according to age *n* (%)

Age (yr)	Smoking	HPV (+)	HPV (-)	Total	*P*	OR
25-34	Yes	95 (43.2)	125 (56.8)	220	< 0.001	2.3 (1.6-3.4)
	No	63 (24.8)	191 (75.2)	254		
	Total	158	316	474		
35-44	Yes	21 (12.1)	152 (87.9)	173	NS	1.1 (0.6-2.0)
	No	24 (11.6)	183 (88.4)	207		
	Total	45	335	380		
45-55	Yes	20 (9.7)	186 (90.3)	206	NS	1.5 (0.8-3.1)
	No	15 (6.5)	216 (93.5)	231		
	Total	35	402	437		
Total	Yes	136 (22.7)	463 (77.3)	599	< 0.001	1.7 (1.3-2.3)
	No	102 (14.7)	590 (85.3)	692		
	Total	238	1053	1291		

P-value of χ^2 test and corresponding OR (95%CI). NS: Not significant; HPV: Human papilloma virus.

Table 2 Association between smoking intensity and high-risk human papilloma virus status stratified according to age *n* (%)

Age (yr)	Smoking	HPV (+)	HPV (-)	Total	*P*
25-34	< 50	58 (41.4)	82 (58.6)	140	NS
	50-100	23 (44.2)	29 (55.8)	52	
	100-150	10 (50.0)	10 (50.0)	20	
	150-200	1 (33.3)	2 (66.7)	3	
	> 200	3 (60.0)	2 (40.0)	5	
	Total	95	125	220	
35-44	< 50	5 (9.6)	47 (90.4)	52	NS
	50-100	5 (11.6)	38 (88.4)	43	
	100-150	6 (11.1)	48 (88.9)	54	
	150-200	0 (0.0)	5 (100.0)	5	
	> 200	5 (26.3)	14 (73.7)	19	
	Total	21	152	173	
45-55	< 50	2 (4.3)	44 (95.7)	46	NS
	50-100	2 (6.5)	29 (93.5)	31	
	100-150	5 (9.4)	48 (90.6)	53	
	150-200	3 (11.5)	23 (88.5)	26	
	> 200	8 (16.0)	42 (84.0)	50	
	Total	20	186	206	
Total	< 50	65 (27.3)	173 (72.7)	238	NS
	50-100	30 (23.8)	96 (76.2)	126	
	100-150	21 (16.5)	106 (83.5)	127	
	150-200	4 (11.8)	30 (88.2)	34	
	> 200	16 (21.6)	58 (78.4)	74	
	Total	136	463	599	

P-value of χ^2 test. NS: Not significant; HPV: Human papilloma virus.

Table 3 Association between smoking and human papilloma virus-16, human papilloma virus-18 status stratified according to age *n* (%)

Age (yr)	Smoking	HPV-16					HPV-18				
		(+)	(-)	Total	*P*	OR	(+)	(-)	Total	*P*	OR
25-34	Yes	24 (10.9)	196 (89.1)	220	NS	1.5 (0.8-2.8)	8 (3.6)	212 (96.4)	220	NS	1.3 (0.5-3.7)
	No	19 (7.5)	235 (92.5)	254			7 (2.8)	247 (97.2)	254		
	Total	43	431	474			15	459	474		
35-44	Yes	4 (2.3)	169 (97.7)	173	NS	0.8 (0.2-2.9)	1 (0.6)	172 (99.4)	173	NS	0.4 (0.1-3.8)
	No	6 (2.9)	201 (97.1)	207			3 (1.4)	204 (98.6)	207		
	Total	10	370	380			4	376	380		
45-55	Yes	-	206 (100)	206		-	-	206 (100.0)	206		
	No	-	231 (100)	231			-	231 (100.0)	231		
	Total		437	437			-	437	437		
Total	Yes	28 (4.7)	571 (95.3)	599	NS	1.3 (0.8-2.3)	9 (1.5)	590 (98.5)	599	NS	1.0 (0.4-2.6)
	No	25 (3.6)	667 (96.4)	692			10 (1.4)	682 (98.6)	692		
	Total	53	1238	1291			19 (1.5)	1272 (98.5)	1291		

P-value of χ^2 test and corresponding OR (95%CI). NS: Not significant; HPV: Human papilloma virus.

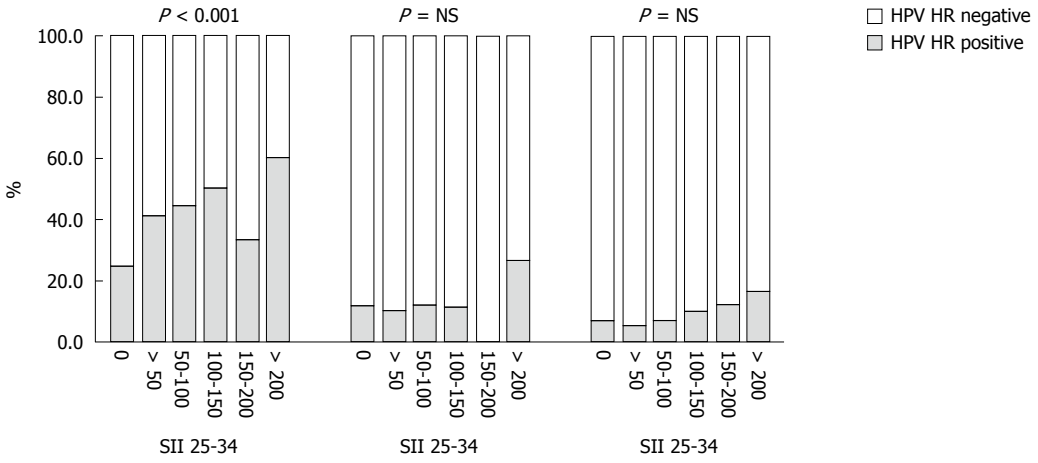

Figure 1 Stratification of human papilloma virus-positive and human papilloma virus-negative women according to age and smoking intensity. There was no statistically significant association between smoking intensity and (h) human papilloma virus (HPV) status. SII: Smoking intensity index. HR: High risk; NS: Not significant.

No association was found between smoking intensity and hrHPV status. These results are presented in detail in Table 2. Figure 1 shows an overview of hrHPV-positive women stratified according to age and smoking intensity. The latter is defined according to the calculated smoking intensity index (SII = cigarettes/d × 365 × years of smoking divided by 1000), and a woman is classified as non-smoker if she had never smoked or if she had stopped smoking at least 1 year earlier. Smokers were classified into one of the following categories according to the previously described variable: SII < 50, if she had smoked less than 50000 cigarettes during her life as a smoker, SII = 50-100, if she had smoked between 50000 and 100000 cigarettes, SII = 100-150 if she had smoked between 100000 and 150000 cigarettes, SII = 150-200 if she had smoked between 150000 and 200000 cigarettes, and SII > 200 if she had smoked more than 200000 cigarettes.

There was no statistically significant association between smoking habits (*i.e.,* smokers *vs* non-smokers) or smoking intensity (increasing SII) and infection from HPV types 16 or 18 in any age group. Table 3 show an overview of the association between smoking and HPV-16 and HPV-18 status, respectively, stratified according to age.

Smoking and cervical cytology results

Regarding cervical cytology results, women were divided into two groups: (1) Women with a negative (normal) Pap-smear (n = 1246); and (2) women with abnormal Pap smear [atypical squamous cells of uncertain significance (ASCUS) or worse] (n = 45). Comparisons according to smoking habits (smokers *vs* non smokers) or smoking intensity between women with normal and women with abnormal cytology results, using the χ^2 test, did not show any statistically significant differences between different age groups (data not shown).

Smoking, HPV status and cervical cytology results

Comparisons according to smoking habits between the two groups of women regarding cervical cytology and the two groups regarding hrHPV status showed that women with a negative Pap test were more likely to have hrHPV infection if they were smokers than if they did

Table 4 Association between smoking habits and high-risk human papilloma virus status according to Papanicolaou test results *n* (%)

Cytology	Smoking	HPV (+)	HPV (-)	Total	*P*	OR
Pap (+)	Yes	18 (69.2)	8 (30.8)	26	NS	1.6 (0.5-5.6)
	No	11 (57.9)	9 (42.1)	19		
	Total	29	16	45		
Pap (-)	Yes	118 (20.6)	455 (79.4)	573	0.001	1.7 (1.2-2.2)
	No	91 (13.5)	582 (86.5)	673		
	Total	209	1037	1246		
Total	Yes	136 (22.7)	463 (77.3)	599	< 0.001	1.7 (1.3-2.3)
	No	102 (14.7)	590 (85.3)	692		
	Total	238	1053	1291		

P-value of χ^2 test and corresponding OR (95%CI). Papanicolaou (Pap) test (+) = atypical squamous cells of uncertain significance or worse; Pap test (-) = Normal. NS: Not significant; HPV: Human papilloma virus.

Table 5 Association between smoking intensity and high-risk human papilloma virus status according to Papanicolaou test results *n* (%)

Cytology	Smoking	HPV (+)	HPV (-)	Total	*P*
Pap (+)	< 50	8 (66.7)	4 (33.3)	12	0.045
	50-100	4 (80.0)	1 (20.0)	5	
	100-150	5 (100.0)	0 (0.0)	5	
	150-200	0 (0.0)	3 (100.0)	3	
	> 200	1 (100.0)	0 (0.0)	1	
	Total	18	8	26	
Pap (-)	< 50	57 (25.2)	169 (74.8)	226	NS
	50-100	26 (21.5)	95 (78.5)	121	
	100-150	16 (13.1)	106 (86.9)	122	
	150-200	4 (12.9)	27 (87.1)	31	
	> 200	15 (20.5)	58 (79.5)	73	
	Total	118	455	573	
Total	< 50	65 (27.3)	173 (72.7)	238	NS
	50-100	30 (23.8)	96 (76.2)	126	
	100-150	21 (16.5)	106 (83.5)	127	
	150-200	4 (11.8)	30 (88.2)	34	
	> 200	16 (21.6)	58 (78.4)	74	
	Total	136	463	599	

P-value of χ^2 test and corresponding OR (95%CI). Papanicolaou (Pap) test (+) = atypical squamous cells of uncertain significance or worse; Pap test (-) = Normal. NS: Not significant; HPV: Human papilloma virus.

not smoke (OR = 1.7, $P = 0.001$). This was not the case if cervical cytology showed ASCUS or worse; however the number of these women was rather low, given that the study was conducted among women attending screening. These findings are presented in Table 4.

On the other hand, similar comparisons for smoking intensity between the two groups of women regarding cervical cytology and the two groups regarding hrHPV status showed, based on very low numbers, that smoking intensity (increasing SII) was associated with a higher probability of a positive ($P = 0.045$), but not a negative Pap test (Table 5).

Finally, stratification of all these associations between smoking habits, Pap smear results and hrHPV status according to age showed that only younger women (25-34 years old) with a negative Pap test were more likely to have hrHPV infection if they were smokers than if they did not smoke (OR = 2.3, $P = 0.001$). These findings are presented in Table 6.

DISCUSSION

In the present study, smokers were more likely to be tested positive for hrHPV types among women 25-55 years of age in an urban area of Greece. Similar results were found in a population of young women in Brazil: certain HPV types were significantly more frequent in current smokers than non-smokers[30]. Likewise, in a study conducted in Portugal, the CLEOPATRE study group showed that smoking was associated with an increased risk of HPV infection[25]. The association between HPV infection and smoking has also been a consistent finding in other studies, conducted in various countries, including Russia, Belarus and Latvia[20], Germany[31], Costa Rica[7], and Canada[32].

With respect to smoking intensity, the International Agency for Research on Cancer (IARC), in a pooled analysis demonstrated that smoking intensity played a significant role in HPV infection risk[24]. Furthermore, similar results to the IARC study were found in a cohort of Tuscan women[33]. In contrast, Collins *et al*[12] found no evidence linking the risk of acquiring an HPV infection with the intensity of smoking. Likewise, in the present study, there was no association between smoking intensity (expressed by a Smoking Intensity Index) and hrHPV infection of the uterine cervix. These contradictory findings may be due to geographic variations in the prevalence of HPV-types[34], as well as due to differences in study design and methods used.

Despite the fact that smoking is a well established risk factor for cervical cancer, there are still contradicting reports regarding the association between tobacco use and hrHPV infection. In a study conducted in Denmark, current smokers were found to have similar HPV prevalence as compared with women who had never smoked, whereas past smokers had a decreased prevalence of HPV[35]. A significant association between HPV prevalence and smoking was found for HIV positive but not for HIV negative women, in a study comparing these two groups[36]. In a nested case control study, smoking was associated with CIN3, but not with HPV infection[37]. In, a study conducted in the United States and Venezuela, risk factors for cervical cancer development appeared to vary

Table 6 Association between smoking habits and high-risk human papilloma virus status according to Papanicolaou test result and stratified according to age *n* (%)

Age (yr)	HPV (cytology)	Smoking	HPV (+)	HPV (-)	Total	*P*	OR
25-34	Pap (+)	Yes	13 (76.5)	4 (23.5)	17	NS	
		No	9 (64.3)	5 (35.7)	14		
		Total	22	9	31		
	Pap (-)	Yes	82 (40.4)	121 (59.6)	203	< 0.001	2.3 (1.5-3.5)
		No	54 (22.5)	186 (77.5)	240		
		Total	136	307	443		
	Total	Yes	95 (43.2)	125 (56.8)	220	< 0.001	2.3 (1.6-3.4)
		No	63 (24.8)	191 (75.2)	254		
		Total	158 (33.3)	316 (66.7	474		
35-44	Pap (+)	Yes	3 (75.0)	1 (25.0)	4	NS	
		No	1 (100.0)	0 (0.0)	1		
		Total	4	1	5		
	Pap (-)	Yes	18 (10.7)	151 (89.3)	169	NS	
		No	23 (11.2)	183 (88.8)	206		
		Total	41	334	375		
	Total	Yes	21 (12.1)	152 (87.9)	173	NS	
		No	24 (11.6)	183 (88.4)	207		
		Total	45 (11.8)	335 (88.2)	380		
45-55	Pap (+)	Yes	2 (40.0)	3 (60.0)	5	NS	
		No	1 (25.0)	3 (75.0)	4		
		Total	3	6	9		
	Pap (-)	Yes	18 (9.0)	183 (91.0)	201	NS	
		No	14 (6.2)	213 (93.8)	227		
		Total	32	396	428		
	Total	Yes	20 (9.7)	186 (90.3)	206	NS	
		No	15 (6.5)	216 (93.5)	231		
		Total	35	402	437		
All ages	Pap (+)	Yes	18 (69.2)	8 (30.8)	26	NS	
		No	11 (57.9)	8 (42.1)	19		
		Total	29	16	45		
	Pap (-)	Yes	118 (20.6)	455 (79.4)	573	0.001	1.7 (1.3-2.3)
		No	91 (13.5)	582 (86.5)	673		
		Total	209	1037	1246		
	Total	Yes	136 (22.7)	463 (77.3)	599	NS	
		No	102 (14.7)	590 (85.3)	692		
		Total	238	1053	1291		

P-value of χ^2 test and corresponding OR (95%CI). Papanicolaou (Pap) test (+) = atypical squamous cells of uncertain significance or worse; Pap test (-) = Normal. NS: Not significant; HPV: Human papilloma virus.

between the two countries[38], and this might be possible for HPV infection as well. Therefore, studies evaluating risk factors for HPV infection should be conducted in various places around the world. An interesting finding in the present study was that younger women (25-34 years old) with a negative Pap test were more likely to have hrHPV infection if they were smokers as compared with non-smokers. This finding may suggest that smoking might enhance HPV-infection in its early stages, before development of pre-invasive lesions. On the other hand, it might be argued that this is a casual rather than a causal association, since younger women are more likely to smoke, as well as to be HPV-positive.

The exact biological mechanisms by which tobacco use is associated with HPV infection, are not clearly understood yet. Persistence of an HPV infection might have been enhanced, leading to an increased risk of progression to cancer, coupled by the carcinogenic effect of polycyclic aromatic hydrocarbons contained in tobacco smoke[21]. These known carcinogens exert a transformation effect on the epithelium of the cervix uteri[21,39,40]. Smoking may also act by increasing cell-turnover in the transformation zone of the cervix[41]. Finally, another possible mechanism might be aberrant, HPV-induced DNA methylation[42].

HPV infections are usually transient. On the other hand, persistence of HPV infection and progression to high grade lesion are probably facilitated by smoking, due to a local immunosuppression that it causes[21,38,43]. This local immune dysfunction results in prolonged duration of oncogenic HPV infections, as well as a decreased probability of clearing the oncogenic infection[44,45]. Furthermore, smoking appears to decrease the capability of the immune system to develop HPV-16/18 antibodies or maintain HPV-16/18 antibody positivity over time, after a natural HPV infection[46]. In addition, smoking has been also associated with a higher baseline HPV-16 and HPV-18 DNA load[47]. In our study, however, smoking did not seem to be associated with cervical HPV-16 and/or HPV-18 infection. Finally, another possible factor that appears to play a role in the persistence of HPV in-

fection, as well as in its progression to high-grade lesions and invasive cervical cancer, is the interaction between smoking and the genetic background of an individual, which determines her susceptibility to infection and disease progression[43].

A limitation of the present study, as well as of most relevant publications, is that HPV-DNA-testing was done only once for each woman. Sequential HPV genotyping of all participants, at certain intervals, could possibly show if smoking might also influence the course of HPV infection. Another limitation of the present study and most relevant publications is that possible co-factors including age at first intercourse, number of sexual partners and oral contraceptive use have not been considered, and thus the possibility of bias cannot be ruled out.

In conclusion, smoking has a well-documented synergistic role with HPV infection, leading to cervical cancer development[48,49]. Furthermore, smoking seems to be associated with an increased prevalence of HPV, a finding confirmed by the present study. Further studies should investigate whether this association is based on causality and evaluate the role of other possible co-factors. In any case, current smokers with either HPV infection or CIN lesion should be managed cautiously, and they should be advised to quit smoking.

COMMENTS

Background

Human papilloma virus (HPV) infection is considered to be a necessary condition for cervical cancer development. High-risk HPV (hrHPV) types can cause cervical cancer, and more than 95% of tumour samples contain DNA from hrHPV genomes. Many studies have shown that smoking is associated with cervical cancer and its precursors.

Research frontiers

Several studies in various countries, including Brazil, Portugal, Russia, Belarus, Latvia, Germany, Costa Rica, and Canada, have shown that smoking is associated with an increased risk of HPV infection. Regarding smoking intensity, results have been contradictory.

Innovations and breakthroughs

In the present study, smokers were more likely to be tested positive for hrHPV types, among women 25-55 years of age, in an urban area of Greece. In respect to smoking intensity, no association with hrHPV infection of the uterine cervix was found in the present study.

Applications

The association between smoking and hrHPV infection is useful for planning cervical cancer prevention strategies. Furthermore, this association may help to clarify or identify new mechanisms of carcinogenesis.

Terminology

HPVs are divided into two groups, according to their oncogenic potential: low-risk HPVs, usually leading to genital warts or low-grade intraepithelial lesions, and hrHPV types, with high oncogenic potential.

Peer review

The paper is interesting and well written. The reviewers encourage authors to continue to explore this issue, improving their analysis with information on general habits of investigated young women and to discover the further correlations with HPV infection/co-infections and other factors, a part smoking.

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