

Impact of education level and life-style related factors on high-risk human papillomaviruses infections

Received for publication, March 05, 2013
Accepted, March 27, 2014

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Abstract

OBJECTIVES: To examine the impact of educational level and of the related life-style and behavioral factors on the acquisition of HPV infections.

METHODS: A retrospective analysis of the socio-demographic variables in a cohort of 360 married women (aged 25-40 years), with a positive HPV-DNA result, who underwent cytological screening and HPV genotyping.

RESULTS: A lower education level was positively correlated with an early beginning of sexual life, a lower age at first pregnancy and an increased number of lifetime sexual partners, abortions and multiparity. Use of oral contraception was higher in women with a high level of education (68.3% versus 19.3%, $p < 0.0001$). High risk HPV genotypes (16, 31, 51, 18) were present in 38.8% of the cases, with a significantly higher incidence in women with lower level of education (57% vs. 30.5%, $p < 0.0001$); nevertheless, the prevalence of severe cytological lesions was similar in both groups.

CONCLUSION: Life-style and reproductive factors associated with a lower level of education determine an increased risk of acquisition of high risk HPV infections, but do not influence the progression to carcinogenesis.

Keywords: HPV carcinogenesis, life-style and reproductive cofactors for HPV carcinogenesis; education level; high risk HPV genotypes in Romania

Introduction

Romania has the highest cervical cancer incidence rate of the European Union (age-standardized rate 23.9 per 100000 women-years versus 14.5 in Eastern Europe and 15.3 worldwide) and one of the highest cancer death rates: 11.8 versus 6.3 in Eastern Europe and 7.8/100 000 worldwide (J FERLAY & al. [1]). In 2008, in Romania, 3402 women were diagnosed with cervical cancer and 2005 women died from the disease; the age-specific cervical cancer incidence among women aged 15-44 years was 24.4/100.000 (WHO/ICO [2]). Deficiencies in the population's accessibility to health services can partially explain this harmful situation: in 2005, there was only one general practitioner per 578 women aged 25-65 years; one gynecologist per 3012 women; one oncologist per 21,195 women and one histopathologist per 13,258 women (I APOSTOL & al. [3]). Overall, there are 19 physicians per 10,000 persons, the expenditure on health is 5.6 % of the gross domestic product of 250 USD per capita (WHO [4]). Other important factors are related to the lack of knowledge and the untruthful attitude towards both cytological screening (F BADULESCU & al. [5]), as a tool for early detection of cervical cancer, and prophylactic interventions, such as human

papillomaviruses (HPV) vaccination. A national school-based program to vaccinate females aged 11 years (aimed at targeting 110 000 subjects) was launched in 2008, but was dropped-out due to the low acceptance (vaccine uptake was limited to 2615 doses).

Persistent infections with high-risk (HR) HPV genotypes, found in virtually all cases of cervical cancer, play a necessary role in the initiation of cervical cancer (JM WALBOOMERS & al. [6], FX BOSCH & al. [7]), but are not sufficient for the carcinogenic process (PE GRAVITT [8], CB WOODMAN & al. [9]). Conversion of a malignant lesion from a dormant to a progressing state is governed by a series of cofactors, whose influence needs better understanding (J STEBBING & al. [10]). Therefore, there is a continuous need for the identification of viral and host features that modulate the risk of disease progression. An increasing number of reports focus on life style-related and reproductive risk factors that can act as potential cofactors in the HPV induced - cervical carcinogenesis (JS SMITH & al. [11], DM PARKIN & al. [12]). A higher level of education have been consistently associated with better health and reduced disease risk (WHO [13]), yet there is little evidence on whether this is true in the context of HPV infection and subsequent carcinogenesis.

This study examines the impact of educational level and of related life-style non-occupational factors on the acquisition of HPV infection.

Materials and methods

Patients. The study cohort consists of 360 married women, aged 25-40 years, resident within the Bucharest City area, Romania, who had repeated cytological examinations of the cervical smears and positive HPV-DNA testing results. None of the patients underwent hysterectomy, nor was ever treated for invasive cancer of the cervix, or other genital cancers. All patients filled-in a socio-demographic questionnaire (adapted after www.yourdiseaserisk.wustl.edu) that included educational, behavioral, reproductive and life style information, as potential risk factors for HPV infection and cervical cancer. The present analysis is based on the following recorded epidemiological variables: age, marital status, years of education, age at first sexual intercourse, number of pregnancies, live births and abortions, lifetime number of sexual partners, lengths and type of contraception methods used, previous history of STIs, smoking habits. All patients gave informed consent for the study, which was approved by the ethic committee of the Institute of Virology.

Cytological screening. The same cervical sample was used for the Pap test and for the HPV DNA assay. Cervical cells, collected with a cervical brush were fixed for cytological screening, and transported in Cobas PCR Cell-Collection Media for viral examination, respectively. Separated blind investigators reported the results for the two tests. Cervical cytology was performed with conventional Pap smears and the results were reported using the 2001 Bethesda System (D SOLOMON & al. [14]) as negative for intraepithelial lesion, ASCUS (atypical squamous cells of undetermined significance); ASC-H (atypical squamous cells cannot exclude a high grade squamous intraepithelial lesion); LSIL (low-grade squamous intraepithelial lesion); HSIL (high-grade squamous intraepithelial lesion) and squamous cell carcinoma, endocervical adenocarcinoma *in situ* and adenocarcinoma.

After DNA isolation with High Pure PCR Template kit (Roche), HPV detection and genotyping was performed using Linear Array HPV Genotyping Test (Roche) according to the manufacturer's instructions. The biotinylated HPV primers target an approximately 450 base pairs sequence within the polymorphic L1 region of the HPV genome. Additional primers, which target the human β -globin gene, provide a control for DNA extraction and amplification. The kit allows the detection of 37 high- and low-risk HPV genotypes, including those strongly associated with cervical cancer.

Statistical analysis was performed with GraphPad InStat3 (GraphPad Software Inc. San Diego, CA, USA). The **Student t** test was used to compare differences between groups for quantitative variables. Double-sided *P* values of ≤ 0.05 were considered significant. For contingency table analysis, Fisher's exact test was used and odds ratios were calculated. An odds ratio of 1 means that the event is equally likely to occur in both investigated groups.

Results and discussions

Education level and differences in the reproductive factors. The number of women with higher education (with diploma awarded by a college) exceeded two times the number of women with elementary studies (less than 8 years of school education). The average age in both lots was similar (range: 21-39 years), young subjects (<30 years) prevailed in both groups.

Table 1. Constitutive variables as potential co-determinants in the acquisition of HPV infection

Variable	Level of education		<i>p</i> -value	Odd Ratio
	High N=246	Elementary N=114		
Mean age-years \pm SD	27.2 \pm 5.2	29.1 \pm 4.2	NS	
Mean age at menarche-years \pm SD	13.04 \pm 0.9	12.08 \pm 0.6	NS	
Length of menstruation-days	4.8	3.5	NS	
Women who had sexual contact before age 16 -number (%)	55 (22.4)	47 (41.2)	<0.0001	
Mean age at first pregnancy; years \pm SD	24 \pm 4.4	21.3 \pm 2.4	NS	
Average number of abortions	1.37	3.31	0.0013	
Average number of live births	0.82	1.2	NS	
Multiparity-number (%)	9 (3.6)	19 (16.6)	<0.0001	5.267
Cesarean deliveries -number (%)	44 (17.9)	8 (7)	0.0059	0.3465

* NS- statistically non-significant ($p > 0.05$)

No consistent differences have been established in respect with age at menarche or length of menstruation. As shown in table 1, a lower level of education was positively correlated with several reproductive factors that augment the risk of HPV acquisition: lower age at first pregnancy, increased number of abortions and multiparity. Until 1995, the total fertility rate (TFR) in Romania was rather high (1.3 births per women) and the mean age of women at childbearing was relatively low (with a peak in the age group of 20-24 years). In a population-based, multi-stage nationwide reproductive health survey (MINISTRY OF HEALTH OF ROMANIA & al. [15]) women with incomplete primary and secondary education had higher TFR compared to those with post-secondary education (1.8 vs. 0.9 births per woman). According to this 2004 survey, at the population level, sexual experience among women under 15 years of age was very uncommon (2.8%), higher percentages being registered in rural areas (4.2% versus 1.8% in urban areas). In our study, however, although the mean age at first sexual contact was similar (19.8 \pm 2.8 vs. 18.1 \pm 2.4 years) for both study groups, a higher percentage of women with elementary studies (41.2 vs. 22.4%, $p < 0.0001$) started sexual life before the age of 16 years, and this factor was directly correlated with the acquisition of HPV infection.

Correlation between the level of education and lifestyle related factors. A significant difference in the prevalence of modifiable life-style related factors according to the education level was found (table 2).

Women with lower level of education reported more frequently an increased number of life-time multiple sexual partners (49.1% vs. 26.8%, $p < 0.0001$). Accordingly, the incidence of previously diagnosed STD infections (HSV1, Chlamydia and Mycoplasma) was three times higher in this group (45.6% vs. 14.6%, $p < 0.0001$).

Table 2. Modifiable lifestyle and reproductive risk factors in the study population

Modifiable factors (n, %)	Level of education		P value	Odd Ratio
	High N=246	Elementary N=114		
Sexual and contraceptive factors				
Use of oral contraception	168 (68.3)	22 (19.3)	<0.0001	0.111
Multiple sexual partners	66 (26.8)	56 (49.1)	<0.0001	2.633
STD infections	36 (14.6)	52 (45.6)	<0.0001	4.892
Life style factors				
Smokers	117 (47.6)	68 (59.6)	NS	1.258
Incidental smokers <5 cigarettes/ day	94 (38.2)	52 (45.6)	NS	
Hard smokers >20 cigarettes/ day	23 (9.3)	16 (14.03)	NS	
Excess body mass	16 (6.3)	21 (18.4)	NS	0.7368
Overweight (BMI>25 kg/m ²)	14 (5.5)	19 (16.7)	NS	
Obesity (BMI>30 kg/m ²)	2 (0.8)	2 (1.7)	NS	

NS- statistically non-significant ($p > 0.05$)

In the general population of Romania, oral contraceptive use is around 14 % (MINISTRY OF HEALTH & al. [15]). Our results showed that women with lower education had a 3.5 times lower rate of oral contraception usage than those with higher levels of education (19.3% vs. 68.3%; $p < 0.0001$). Hormonal contraception is one of the main factors associated with a significant increase in the risk of cervical cancer development: by a factor of 1.07 for each year of use of hormonal contraception or by 1.38 for 5 years of use, (INTERNATIONAL COLLABORATION OF EPIDEMIOLOGICAL STUDIES OF CERVICAL CANCER & al. [16]). On the contrary, in ex-users, the excess risk is approximately halved 2–4 years after cessation, and halved again after 5–9 years, with no significant excess risk reported 10 years after cessation of use (JS SMITH & al. [11], INTERNATIONAL COLLABORATION OF EPIDEMIOLOGICAL STUDIES OF CERVICAL CANCER & al. [16]).

Lifestyle related variables such as smoking or overweight, considered to be predictors for the development of severe dyskaryotic lesions (INTERNATIONAL COLLABORATION OF EPIDEMIOLOGICAL STUDIES OF CERVICAL CANCER & al. [17]), were linked in our study with a low education level, but the differences were not statistically significant. Several large studies demonstrated that current smoking, and especially smoking of 20 or more cigarettes per day is an independent risk factor for both cervical HPV infection, and for high-grade CIN in young women (E ROURA & al. [18], M SCHIFFMAN & al. [19]).

Prevalence of high risk-HPV genotypes infections. Overall, 38.8% the 360 women were infected with high risk (HR) HPV genotypes. HPV 16 was by far the most prevalent genotype; followed, in descending order of frequency, by genotypes 31, 45, 51 and 18. These

results, concordant with other studies from Romania (G ANTON & al. [20]), provide an image of the circulating HPV genotypes in Romania, the country with the highest cervical cancer incidence rate of the European Union, that might prove valuable for future programs on HPV vaccination.

As shown in table 3, the prevalence of HR- HPV genotypes infections was roughly double in the elementary educated women (57 % vs. 30.5% $p < 0.0001$).

Table 3. Prevalence of high risk-HPV genotypes infections

HPV status (n, %)	Education level		<i>p-value</i>	Odd Ratio
	High N=246	Elementary N=114		
HPV 16 single infection	50 (20.3)	48 (42.1)	<0.0001	2.851
HPV 18 single infection	8 (3.25)	4 (3.5)	NS	1.082
All high risk HPV infections	75 (30.5)	65 (57)	<0.0001	3.024
HPV16 coinfection with other genotypes	3 (1.2)	7 (6.1)	NS	2.494
Co-infections with multiple high and low risk HPV genotypes	34 (13.8)	36 (31.6)	0.002	2.878

NS- statistically non-significant ($p > 0.05$)

Single infections with HPV16 were twice more frequent in the group of elementary educated women (42.1 vs. 20.3%, $p < 0.0001$). Lower age at first pregnancy and multiple lifetime sexual partners were factors independently associated with an increased risk of HR-HPV infections in women with lower education level. Interestingly, the diversity of isolated genotypes was also higher in this group, co-infections with multiple HPV genotypes (both high- and low-risk) being near three times more frequent (31.6 vs. 13.8% in those with higher education, $p = 0.002$). In Romania, a population-based national screening program for the early detection of cervical cancer, based on cytological tests, was developed, but its implementation was delayed and the over-time sustainability seems low, due to budgetary constraints and inefficient application. There is no nationwide monitoring of HPV prevalence in women with normal cytology; in Eastern Europe the reported prevalence is 22.3%, compared to 11.8% worldwide (S DE SANJOSE) & al. [21]). A recent study on the HPV prevalence and genotype distribution in women with and without cervical lesions in Northeast Romania (RG URSU & al. [22]), reported a 20.4% rate of HR-HPV infections in women with normal cytology, the most frequent genotypes being HPV 16, 53, 51, 52, 18 and 31. Similar to these results, we noted the low frequency of HPV 18 genotype, the second most important oncogenic HPV type in other countries. The relatively greater propensity for HPV 18 to initiate lesions inside the endocervical canal and in the glandular cells (M SCHIFFMAN & al. [23]) may explain this aspect, since endocervical cells are under-represented in routinely collected PAP smears.

Association between infections with high-risk (HR) HPV genotypes and presence of high grade squamous intraepithelial lesions (HSIL). We analyzed the cytological screening results associated with HR-HPV genotypes infections, taking as a cut-off the difference between HSIL and all other diskaryotic precursor lesions, in order to minimize the variability in interpretation. Out of the total of 140 women infected with high risk HPV genotypes, only 22 (15.7%) had a severe cytological lesion, defined as HSIL (table 4).

Table 4. Age specific prevalence of severe cytological lesions (HSIL) in women infected with HR-HPV genotypes

Age (years)	Level of education for women infected with HR-HPV				
	High		Elementary		
	Number	HSIL (n, %)	Number	HSIL (n, %)	P value
21-30	48	6 (12.5)	26	6 (23.1)	0.0065
31-39	27	4 (14.8)	39	6 (15.4)	NS
Total	75	10 (13.3)	65	12 (18.5)	NS

NS- statistically non-significant ($p > 0.05$)

The fact that detection of an oncogenic HPV genotype was not constantly accompanied by a concurrent severe cytological lesion suggests that other cofactors are necessary for the development and progression of the HPV-induced carcinogenesis. Our results are in accordance with the data from a large study conducted in the New Independent States of the Former Soviet Union and in the Latin American countries (K SYRJÄNEN & al. [24]), in which, although baseline HR-HPV was the single most powerful predictor of incident cervical intraepithelial neoplasia (CIN), different co-factors, indicators of risky sexual behavior, were associated with progression to CIN1-3. Global variations in cancer incidence reported in different socioeconomic groups have been associated with education, behavioral factors, and differences in both access to health care and in health-seeking behavior (F BADULESCU & al. [5], C NAPOLI & al. [25], LA CHOCONTÁ-PIRAQUIVE & al. [26]). In our cohort, severe abnormal smears were slightly more frequent in lower educated women (18.5 % versus 13.3 %), especially in those younger than 30 years (23.1% vs. 12.5%; $p=0.0065$). Even though most of the HPV infections in young women are transitory, several reports have shown that HPV- DNA is a better indicator of a precursor or carcinogenetic lesion than the related cytological abnormalities (M SCHIFFMAN & al. [27]).

For the age group most prone to persistent HPV infection and carcinogenesis (31-39 years), the proportion of HSIL is similar, irrespective of the education level (13.3% vs. 18.5%). HSIL in this age group were associated with HPV-16 infections, supporting its dominant role in the cervical oncogenesis; however the identification of multiple coinfections with other genotypes deserves consideration for the prospect of effective primary prevention and for the possibility of improving the efficiency of cervical screening programs.

A number of limitations of the present study should be addressed. First, because of the limited number of women with elementary education, we might have been unable to distinguish statistical significant differences for some constitutive or modifiable risk factors investigated. Second, this was a retrospective cohort analysis; participants' determination in completing the questionnaires might have introduced a selection bias. Third, we might have missed cytological lesions that occurred transiently; nevertheless, ascertainment bias was minimal, because detection of HPV genotype was done without knowledge of any clinical information.

Conclusions

This study suggests an important impact of the educational level on the reproductive and lifestyle risk factors associated with the acquisition of HPV infections. The effect of the same factors on the establishment of a persistent infection and on the subsequent initiation of carcinogenesis is however small, and potentially incorrectly estimated in conventional analyses. Behavioral strategies directed to modifiable life-style factors might nevertheless

influence the spread of oncogenic HPV genotypes, as well as the burden of other sexually transmitted diseases.

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