Cellular and Molecular Biology [TM] **55**, OL1175-OL1185
DOI 10.1170/134

ISSN 1165-158X
2009 Cell. Mol. Biol.[TM]

RISK FACTORS OF INVASIVE CERVICAL CANCER IN MOROCCO

M. MEFTAH EL KHAIR[1, 2], R. AIT MHAND[1], M. EL MZIBRI[2], M. M. ENNAJI[1]

1 Laboratory of Virology and Hygiene & Microbiology, Department of Biology, Faculty of Science and Technology. University Hassan II-Mohammedia. Mohammedia, Morocco.
2 Molecular biology laboratory, National Center for Energy, Nuclear Science and Technology (CNESTEN). Rabat, Morocco.
M.M. Ennaji, Professor, Laboratory of Virology and Hygiene & Microbiology, Department of Biology, Faculty of Science and Technology, University Hassan II-Mohammedia. Mohammedia, Morocco 20650.
E-mail: m.ennaji@univh2m.ac.ma; m.ennaji@yahoo.fr
Tel: +212 23 31 53 52/212 23 314705/08 ext. 301, Fax: +212 23 31 53 53

Received December 29th, 2008; Accepted October 1st, 2009; Published November 15th, 2009

Abstract – It is now well established that infection with oncogenic human papillomavirus (HPV) types is the necessary cause of cervical cancer (CC) and its immediate precursor cervical intraepithelial neoplasia 3. However, HPV infection alonemay not be sufficient to cause CC, and other exogenous and endogenous factors may exist that, in conjunction with HPV, influence the risk of progression from cervical HPV infection to CC. In this chapter, we review the evidence for the role of parity, oral contraceptive (OC) use, and tobacco smoking in CC. In this study, molecular detection of HPV by PCR using consensus primers MY09/MY11 able to amplify the L1 gene present in all types of HPV from fresh frozen biopsies showed that the virus is present in 88% (99/113) of cases. The viral typing, carried out on HPV positive cases through hybridization using specifics probes MY14, WD74, WD126, MY16, MY70 and MY115 which correspond respectively to HPV 16, 18, 31, 33, 45 and 59 has revealed the prevalence of HPV 16 and 18 respectively with 65% (64/99) and 44.4% (44/99). Several combinations of double and multiple infections were also observed. Another part of this work has been devoted to the study of risk factors associated with the development of cancerous cervical lesions. The study is based on a questionnaire during the sampling and also on data collected from clinical records and records of hospital patients.

Key words: Cancer of the cervix; Human Papillomavirus, HPV oncogenes; risk factors.

INTRODUCTION

Cervical cancer is still one of the leading cancers worldwide, although there is adiscrepancy between developed and developing countries. In developed countries, there is a continuous decline in incidence and mortality, whereas in developing countries, there is a more stable or even increasing pattern. The latter is more likely to be due to the lack of screening and infectious cofactors than to ethnic differences (31).

Cervical cancer is considered as a sexually transmitted disease. The clinical and epidemiological studies have identified human papillomavirus (HPV) as the central risk factor and the major infectious etiologic agents of genital precancerous lesions and cancers. HPV are strictly epitheliotropic viruses infecting cutaneous or mucosal surfaces and display a very high selectivity for the specific epithelium infected (35).

Approximately 200 types of the human papillomavirus (HPV) have been identified, of which 85 have been genotypically characterised, and the number is rapidly increasing. About 40 different types of HPV specifically infect the genital area. According to their malignant potential, these viruses can be classified as high- or low-risk. The low-risk types of HPV are usually associated with condyloma acuminata and low-grade squamous intraepithelial lesions (LSIL), while the high-risk viruses, predominantly HPV 16 and HPV18, are related to high-grade squamous intraepithelial lesions (HSIL) and invasive cervical carcinoma (3).

In Morocco, as it is the case in the other North African countries, uterine cervix cancer is second in frequency after breast cancer with predominance of HPV types 16 and 18 as

deduced from a previous Moroccan case-control study of invasive cervical cancer (1,8).

Persistent viral infection with the high-risk types of HPV is established as a necessary cause for the development of squamous cell carcinoma (SCC) and the majority of cases of adenocarcinoma of the uterine cervix. HPV 18 is a risk factor for the development of adenocarcinoma whereas HPV 16 is associated with both histologic types. The natural history studies of HPV infection have shown that, different from those that progress to cancer, most infections are transient and not associated with detectable cytological abnormalities. The reasons for this variable natural history are poorly understood but it has been generally assumed that other causes or co-factors must be important for the development of neoplasia in HPV-infected women. Although several co-factors have been associated with the risk of SCC, their impact on the progression of HPV-infected cervical cells to adenocarcinoma remains unclear (34).

A hospital based case control study investigating the role of HPV and other risk factors in the origin of cervical cancer has been completed at the oncologic center, located in the Casablanca area.

MATERIALS AND METHODS

Cases and Specimens

One hundred and thirteen fresh frozen biopsies of cervical specimens were collected from women visiting the oncologic center in Casablanca (Ibn Rochd Hospital, Morocco) and the National Institute of Oncology Rabat (INO) for cervical cancer symptoms. The tissue samples were frozen immediately after collection and stored at -80˚C until use. Three of the samples were adenocarcinoma, nine undertemined and the rest were squamous cell carcinoma type. One hundred cervical scrapes were used as controls. Controls were recruited from several gynaecologic centres. For this study, recruitment was made from women who were presenting for their annual routine Pap smear test and had no previous cervical abnormalities based on their medical chart information.

DNA extraction

DNA was collected from scrapes or from 10 µm thick sections of tissue (2,16) and the sections were treated for 3 h with 200 µg/ml proteinease K in 500 µl digestion buffer (50 mM KCl, 10 mM Tris-HCl (pH 8), 0.5% Tween) at 56°C then at 37°C overnight, the enzyme was subsequently inactivated by heating for 10min at 95°C. The DNA was purified from digested samples using standard phenol-chloroform extraction and ethanol precipitation and then resuspended in 50 µl of TE (pH 8). DNA was immediately used for PCR amplification or stored at -20°C until use.

HPV detection and typing

The presence of HPV in the cervical lesions was detected by performing the polymerase chain reaction (PCR) using primers (MY09 and MY11) which amplify a highly conserved 450-bp segment in the L1 HPV gene (Ting Y and Manos MM, 1990). Typing of the amplified products was done by hybridisation with specific probes. The probes used in this study were MY14 (C A T A C A C C T C C A G C A C C T A A), WD74 (G G A T G C T G C A C C G G C T G A), WD126 (C A A A A G C C C A A G G A A G A T C), MY16 (C A C A C A A G T A A C T A G T G A C A G), MY70 (T A G T G G A C A C T A C C C G C A G) and MY115 (C T G C T G T G T C T T C T A G T G A C A G) that are specific to HPV 16, 18, 31, 33, 35 and 45 respectively (14).

Study of risk factors associated with cervical cancer

The study is based on the questionnaire establishes on data sets collected from records of clinical oncology Center-CHU Ibn Rushd in Casablanca and the National Institute of Oncology Rabat (INO). A combination of factors has been studied to better understand the socio-economic study population. Other factors such as reproductive factors (age of marriage, age of first pregnancy, number of sexual partners, and taking oral contraceptives), smoking and genital infections were selected to study their associations with the development of cervical cancer.

Statistical analysis was done by software Epi-Info 6, which allows you to collect and analyze quantitative data in public health. It allows:

To quickly create forms for entering data controlled; Carry out simple statistical analysis;

To prepare analytical reports, activity reports;

To export the data to statistical software (SAS, SPPSS, Stata, etc.) or office (Excel).

In this study, the software Epi-Info 6 was used to estimate the association between the various factors studied and the risk of developing cervical cancer. A variable was considered significant if $p < 0.05$.

RESULTS

Molecular typing of HPV DNA positive samples

Molecular detection of HPV DNA, using PCR amplification and molecular hybridization, revealed the presence of viral DNA in 88% of cases (99/113). The HPV DNA distribution is shown in (Table I). HPV 16 and HPV 18 were the more frequent genotypes found in this study with respectively, 65% (64/99) and 44.4% (44/99). Several combinations of double and multiple infections were also observed. In the control group, no HPV DNA sequences were detected by using consensus primers MY09/MY11 **(Figure 1 and Table I).**

Study of risk factors associated with cervical cancer

Demographic and socio-economic populations

The study of the distribution of cancer cases and normal according to demographic and socioeconomic characteristics showed that:

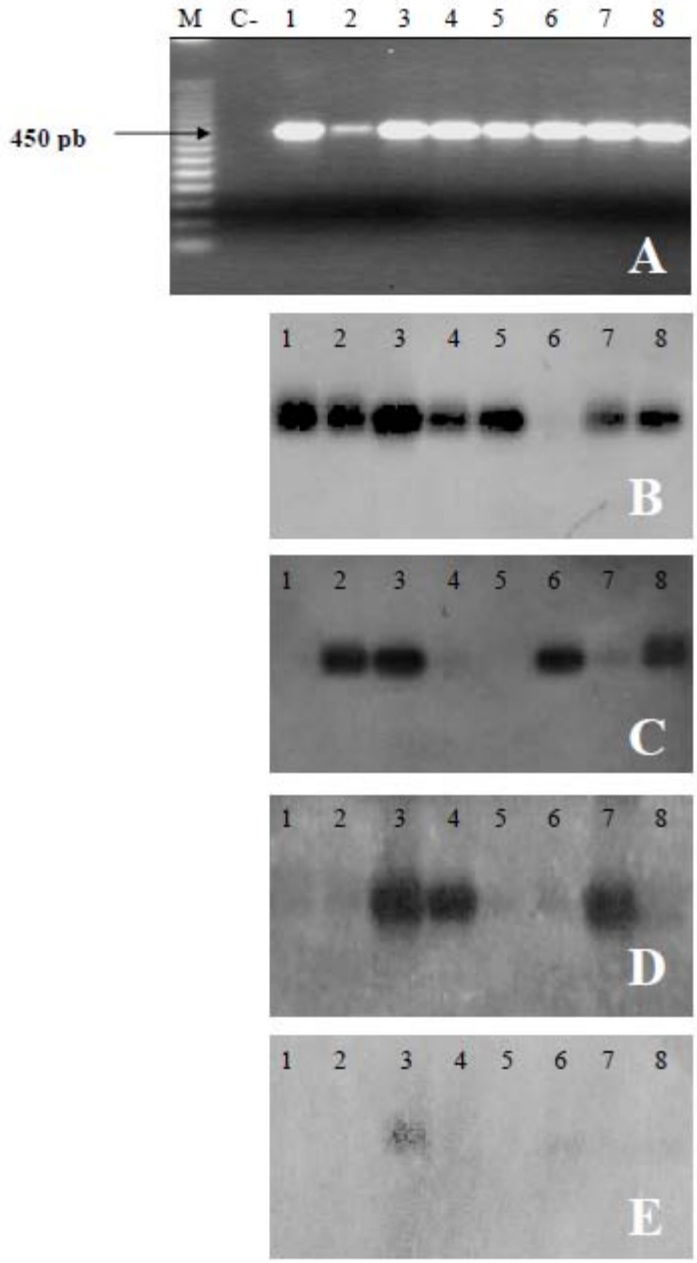

Figure 1: Representative illustration of the HPV detection and typing.
Electrophoresis gel photo (A). Lanes 1 to 8 correspond to HPV positive cervical specimens; C-: negative control (sterile distilled water); M: 50 bp ladder molecular weight marker. For HPV typing, PCR products were hybridized with MY14 (B), WD74(C), MY70 (D) and MY115 (E) that specific respectively to HPV16, 18, 45 and 59.Specimen 1and 5 are infected only by HPV16; specimens 2 and 8 are infected by HPV16 and 18; specimen 3 is infected by HPV 16, 18, 45 and 59; specimens 4 and 7 are infected by HPV 16 and 45; specimen 6 is infected only by HPV18.

Table I: Distribution of HPV types by histology

	Uterine cervical lesions		
	Squamous Cell Carcinoma	***Adenocarcinoma***	***Undetermined***
Negative	12	1	1
Positive	89	2	8
Single infection			
16	29	1	2
18	15		2
45	2		
Double infection			
16/18	15	1	1
18/45	2		
31/33	1		
16/45	7		
Triple infection			
16/18/45	3		
16/18/31	2		
Multiple types			
16/18/33/45	1		
16/18/45/59	2		
Untyped	10		3

The population of our study had an average age of 61 with extreme age of 34 and 88. When compared to the general population, women with cancerous lesions of the cervix were significantly older.

Marital status for the study population consists mostly of married women (58% cancer for women and 88% of healthy women) and widows (35% of women cancer). The marital status of patients is similar to that of the entire population of the study. This observation is related to the overall structure of the population in which almost every woman has lived as husband and wife to a period of his life.

Education and level of education have also been studied in cancer cases and controls 97% of women with cancerous lesions were illiterate.

In our series 62% of patients in a rural area and were unemployed. In addition, since all women with cervical cancer presented a certificate of need for joint exercise their professions with a very low income, we can say the image of the present economic situation that these patients are a low socioeconomic level.

Table II below shows the demographic and socio-economic consequences of the general population of the study (patients and controls).

The analysis of various risk factors among women with cervix cancer compared to controls is represented in **Table III**.

Study of risk factors

Age at first marriage

Of 156 women with cancer married at an age above 15 years, only 41% had developed cancer. This proportion is higher among women married at an early age. Indeed, 73% of women married to the age of 15 (49/67) had cancer. Statistical analysis showed a significant relationship between age of first marriage and the development of cervical cancer ($p = 0.0011$).

Number of sexual partners

The distribution of cancer cases based on the number of partners shows that 53.51% of patients with cervical cancer were monogamous. The statistical study has shown that there is no significant relationship between the development of cervical cancer and the number of sexual partners.

Number of pregnancies

The study of the distribution of cases depending on other risk factors showed 60.81% of women had cancer more than 6 pregnancies, however,

Table II: The demographic and socio-economic of the general population of the study (patients and controls).

	Cases (N=113)		controls (N=100)	
	Nb	%	Nb	%
Age				
<40	8	7	30	30
40-59	68	60	50	50
60-79	31	28	10	10
>80	6	5	10	10
Marital status				
- Married	65	58	88	88
- Divorced	8	7	6	6
- Widow	40	35	5	5
- Single	0	0	1	1
Education				
- No	110	97	58	58
- Primary	1	1	14	14
- Secondary	2	2	17	17
- Superior	0	0	11	11
Profession				
- With	6	5	47	47
- No	107	95	53	53
Habitual residence				
- Urban / semi urban	43	38	76	76
- Rural	70	62	24	24

Table III: Distribution of patients with cervical cancer according to different risk factors

	General population	Nombre of cancer cases	Percentage of cancer cases	P-value
Age at first marriage				
- > 15	156	64	41.02	
- < 15 years	67	49	73.13	0.0011
Number of sexual partners				
- 1	185	99	53.51	
- 2	28	14	50	No Significatif
Pregnancy				
- 1 - 3	58	28	48,27	
- 4 – 6	71	35	49,29	
- more than 6	74	45	60,81	No Significatif
Age at first pregnancy				
- >20 years	61	26	42,62	
- 18-20	48	24	50	
- 15-17	43	22	51.16	
- <15 years	56	36	64.28	0.02 (significatif between >20 and <15 years)
Parity				
- nulliparous	10	5	50	
- primiparous and pauciparous	61	31	50.81	
- Multiparous (4 - 5 - 6)	75	39	52	
- Large multiparous (7 ors mores)	67	38	56.71	No Significatif
Oral Contraceptive use				
- Yes	109	22	20.18	
- No	104	91	87.5	0.0001
Venereal diseases				
- Yes	44	12	27.27	
- No	92	77	83.69	0.0001
- Unknown	77	24	31.16	
Smoking				
- Yes (passive)	40	30	75	
- No	163	83	50,92	0.01

the statistical study has shown that there is no significant relationship between development of cervical cancer and the number of pregnancies.

Age at first pregnancy

In this study, thirty-six women with cervical cancer is a percentage of 64.28%, had their first pregnancy before the age of 15 years. Statistical analysis is very significant with a p-value = 0.02 indicating that pregnancy at an early age increases the risk of developing cervical cancer.

Parity

According to parity, the patients were grouped into four categories: Never, primiparous and paucipares multiparous (4 - 5 - 6) and large multiparous (7 and over). The average was 7. The largest parity was 13. However, the statistical study has shown that there is no significant relationship between the development of cervical cancer and parity.

Oral Contraception

As illustrated in Table III, 87.5% of women with cervical cancer have never used oral contraceptives this shows that the use of oral contraceptives does not increase the risk of developing cervical cancer in the population studied.

Released-venereal diseases and smoking

Statistical analysis revealed that there was no significant association between the development of cervical cancer and venereal diseases, and for

smoking has no significant relationship was observed.

DISCUSSION

Worldwide, cervical cancer is the second most common cancer among women after breast cancer, with around 493000 newly diagnosed cases and over 273000 deaths estimated to occur annually. It is the leading cause of cancer-related death among women in developing countries, where approximately 80% of cervix cancer cases occur (28).

Invasive cervical cancer can be divided in two major histological types: Squamous Cell Carcinoma and adenocarcinoma with respectively 80–85% and 10% of cases. Adenosquamous carcinoma and other rare tumours represent only 3% of cases (31).

HPV detection and typing

A large number of studies conducted in different parts of the world confirm that HPV infection is a common phenomenon in precancerous and cancerous lesions of the uterine cervix. HPV DNA was detected in 90–95% of cervical cancers (15, 22). In this study molecular detection of HPV by using PCR and hybridization analysis showed that the virus is present in 88% of cases. HPV 16 and HPV 18 were the more frequent genotypes found in this study with respectively, 65% (64/99) and 40.5% (40/99).

It is now well established that infection with oncogenic human papillomavirus (HPV) types is the necessary cause of cervical cancer (CC) and its immediate precursor cervical intraepithelial neoplasia 3 (CIN3).

Co-infection with multiple HPV types is a common finding, of many molecular epidemiologic studies. Some HPV types might interact or act synergistically to induce lesion development or progression (17, 32).

The risk of high-grade lesions and invasive cervical cancer seems to be considerably increased among women with multiple-type infections compared with those harbouring a single HPV type (33). Morrison *et al.* (20) and Herrero *et al*. (12) have shown that the risk of cervical lesions strongly increases when HPV-16 and other types are not present alone. Moreover, another study suggested that some HPV types might cooperate with HPV-16 to produce dysplasia or cancer. In the present study, we found that HPV types 45 and 59 might also interact with HPV 16 and/or HPV 18 and add to the baseline risk observed with single-type infections (32). Our analyses controlling for individual HPV types provide some evidence that risk in the context of the HPV types 16 and 18 may be particularly modulated by co-infections with other types (32).

However, HPV infection alone may not be sufficient to cause CC, and other exogenous or endogenous factors might exist that, in conjunction with HPV, influence the risk of progression from cervical HPV infection to CC.

Study of risk factors associated with cervical cancer

Candidate cofactors may be classified into three groups: 1) environmental or exogenous cofactors, including use of oral contraceptives (OCs), tobacco smoking, diet, cervical trauma, and coinfection with human immunodeficiency virus (HIV) and other sexually transmitted agents; 2) viral cofactors, such as infection by specific types, coinfection with other types, HPV variants, viral load, and viral integration; and 3) host cofactors, including endogenous hormones, genetic factors such as human leukocyte antigen, and other host factors related to the host's immune response (7, 18).

In this work, we are interested, in addition to the viral infection to a number of risk factors related to the socio-economic level, sexual activity, smoking, oral contraception and other sexually pathogens transmitted:

The socio-economic level

A low socio-economic level is considered a risk factor of cervical cancer. The majority of patients in this study with cervical cancer are of a low socio-economic level. Hygienic conditions unfavorable, can cause genital infections and chronic cervicitis (9, 24). Previous studies have shown that women with a higher degree of hygiene (washing the genital area during menstruation for example) have a lower risk of developing cervical cancer (8). In our study, most patients residing in rural areas have a precarious hygiene level, these women are victims of many deliveries and often poorly followed with miscarriages, they often have limited incomes, limited access to health service, Poor food and limited knowledge of health problems and preventive behaviors. This explains the high risk of developing cervical cancer in this population (9, 24).

Micronutrient deficiencies and a low intake of vegetables and fruits may be a risk factor for cancer development. Indeed, a lack of folic acid, vitamins C, A and carotenoids would be an explanation of a higher risk at a multiparity, since folic acid deficiency was found during pregnancy (23).

Sexual Activity

In this study, the distribution of cancer cases in terms of number of partners has shown that 99 patients with cervical cancer were monogamous. Like the various sexually transmitted diseases, studies have shown that the number of sexual partners and sexuality are early risk factors, the most important for the development of cervical cancer. Indeed, the incidence of cancer of the cervix is higher among women who have sex with several partners and especially men with cancer of the penis (25).

Parity

This study indicates that women multiparous do not represent a risk to develop cancerous lesions in the cervix. However, statistical analysis of the association between pregnancy at an early age and the risk of developing cervical cancer is very significant with a p-value = 0.02. Studies have shown that women are more multiparous risk of developing cervical cancer. A study in Morocco, in patients with cancer of the cervix, reported that 72% of patients were multiparous (4 or more pregnancies), 24% were between 1 to 3 children and 4% were nulliparous (10). Similar results were reported in studies conducted in other countries such as Costa Rica (7). This combination has not been reported among women with cervical cancer and HPV positive in Denmark, the United States, Colombia and Spain (Bosch et al. 1992; Castellsague X and N. Munoz, 2003).

The pooled data from eight case-control studies on invasive cancer of the cervix and two cancer studies in situ (CIS) from four continents suggest that, compared to women who never had children, they who had three or four were 2.6 times more risk of contracting cervical cancer and those who had seven or more had 3.8 times more likely (21). Other studies corroborate this positive relationship between the number of children and cervical cancer (7).

The physiological reason for this association is unclear. Indeed, the trauma suffered during childbirth, the change in hormone levels, nutrition and a greater vitality virus during pregnancy may explain the high sensitivity (7, 23).

Age of first sexual intercourse

This study showed a significant association between early age of first marriage and the development of cervical cancer among the Moroccan population with a p-value <0.05. Studies have shown that women who had their first sexual intercourse before age 16 are twice as likely as those whose first report took place after 20 years. This relationship between the early first sex and the risk of cervical cancer could reflect the greater sensitivity of the cervix to the action of various carcinogens during adolescence (5, 13). It seems that early sexual activity is associated with a high risk that during puberty, cervical tissue undergoing various changes that may make this region more vulnerable to injury. The results of several studies argue for sexual transmission and stress the importance of the role of male partner: the male partners of patients with cervical cancer have a lot more sexual partners than women are not affected (25).

Tobacco

In this study none of the patients was an active smoker but for some women with cervical cancer there is a concept of passive smoking. According to some studies, smoking appears to increase the risk of cervical cancer, especially among smokers long (23). Components of tobacco were found in the cervical mucous, the paradoxical effect of inhalation of tobacco components on the cervical epithelium can be explained if it is known that derivatives of these components are distributed in the body fluids, and that nicotine is very concentrated in the cervical mucus. Another constituent of smoke, cotinine, more easily determined by gas chromatography, is also found in the mucus, even among some women non-smokers, victims of passive smoking. However, the biological mechanisms underlying the relationship between smoking and cervical cancer have not been clarified (7, 23, 29).

Contraception

Our study indicates that most patients with cervical cancer have never used oral contraceptives. These data are consistent with the results obtained in other studies in Colombia, Spain, Denmark and America show that there is no association between taking oral contraceptives and pre-malignant lesions among HPV positive women (23). But other studies have shown that

taking a hormonal contraceptive for over 5 years would be an additional risk factor (27). Indeed, contraception would have a role potentiation among women HPV positive. The adjusted relative risk was 2.82 between 5 and 9 years of contraception and 4.03 after 10 years (19).

The choice of contraceptive method also appears to influence the risk of cervical cancer. The type of barrier methods seems to reduce risk, while oral contraceptives seem to increase the contrary. Research has shown that there is a potential long-term prolonged use of oral contraceptives and development of cervical cancer. An analysis of pooled data from 10 case studies - witness patients with invasive cervical cancer or a CIS suggests that the long-term use of oral contraceptives may increase the risk of cervical cancer until Four times in women with HPV infection. The risk associated with oral contraceptives appears to be more pronounced for adenocarcinoma than for malpighiens carcinomas, even after adjustment for various socio-economic and sexual. The evaluation of the effect of oral contraceptives remains delicate because this variable is strongly associated with other factors such as sexual activity (23).

The contraceptive hormones may in part be explained by the existence of elements of response to glucocorticoids and progesterone in the non-coding region of papillomavirus (23).

Other sexually transmitted pathogens

Numerous epidemiological studies on risk factors involved in the development of cervical cancer, reported a large number of sexually transmitted pathogens associated with the development of this malignant disease (23).

In the same context, we evaluated the concept of venereal infection in our study, no association was found between the development of cervical cancer and other agents of sexually transmitted disease.

However, numerous studies have shown that several bacterial infections, viral act on the stroma, the epithelium or both structures at once, causing cervicitis. Among the bacterial infections responsible for cervicitis, citing the gonorrhea, chlamydia trachomatis, the agent of syphilis and Mycobacteria (23). As for cervicitis viral infection herpes simplex virus type-2 (HSV-2) and cytomegalovirus (CMV), are the most recovered (23). Similarly, HIV infection weakens the immune system of women achieved, making the body less able to fight HPV and other cancers, even at their early stage (23).

In conclusion, it is well known now that HPV-16 and persistent infections with other high-risk HPV types are more likely to progress toward cervical neoplasia, but the present study suggests that infections with multiple types might contribute additional prognostic value. This finding has implications in terms of management of cervical lesions and clinical prediction of the outcome of HPV infections. However in Morocco, as in the other developing countries, the lower socioeconomic conditions, voluptuary habits, age at first intercourse, high parity, number of partners, sexual behaviour, familiarity, long-term use of the oral contraceptive and the lack of primary care in the health systems are important risk factors for cervical cancer (1,8).
In Morocco, the national program against cervical cancer rests exclusively on the cytology based screening that offers substantial protection although current coverage is low. Actually joint efforts, between health managers and nongovernmental organizations, are made to introduce HPV detection and typing, in combination with cytological test, in the diagnosis panel.

Combination of Pap smears and HPV testing will improve cervical cancer screening and detect HPV false negative Pap smear results and save women's lives. When available, vaccination against HPV may offer a meaningful alternative to preventing cervical cancer.

Acknowledgements – This work was supported by PARS and PROTARS program of Moroccan National Research Council and also the Ministry of National Education and Scientific Research.
The authors would like to thank all the doctors (Centre d'Oncologie de Casablanca and the Institut National d'Oncologie) who provided us with the biopsy specimens. A special thanks also goes to the biologists and pathologists (Centre d'Anatomopathologie - CHU Ibn Rochd) for their excellent technical assistance.

REFERENCES

1. Amrani, M., Lalaoui, K., El Mzibri, M., Lazo, P and Alaoui Belabbas, M.,. Molecular detection of human papillomavirus in 594 uterine cervix samples from Moroccan women (147 biopsies and 447swabs). *Journal of Clinical Virology*.2003, **27**: 286-295.
2. Andersson, S., Rylander, E., Larson, B., Sigurdardottir, S., Backlund, I., Sallstrom, J., et al..Types of human papillomavirus revealed in cervical adenocarcinomas after DNA sequencing. *Oncol Rep.* 2003, **10**: 175-9.
3. Andersson, S., Mints, M., Sällström, J., Wilander, E. The relative distribution of oncogenic types of human papillomavirus in benign, pre-malignant and malignant cervical biopsies.A study with human papillomavirus

deoxyribonucleic acid sequence analysis. *Cancer Detection and Prevention.* 2005, **29**: 37–41.
4. Bauer, H.M., Ting, Y., Greer, C.E., Chambers, J.C., Tashiro, J.C., Chimera, C.L., et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. *JAMA* . 1991, **256**: 472-7.
5. Biswas, L.N., Manna, B., Maiti, P.K. and Sengupta, S. Sexual risk factors for cervical cancer among rural Indian women: a case-control study. *Int J Epidemiol.* 1997, 26(3):491-5.
6. Bosch, F.X., Muñoz, N., de Sanjosé, S., Izarzugaza, I., Gili, M., Viladiu, P., Tormo, M.J., Moreo, P., Ascunce, N., Gonzalez, L.C., *et al*. Risk factors for cervical cancer in Colombia and Spain. *Int J Cancer*. 1992, **52**(5):750-8
7. Castellsague, X. and Munoz, N. Chapter 3: Cofactors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. *J. Natl. Cancer.Inst. Monogr*. 2003, **31**: 20-8.
8. Chaouki, N., Bosch,F.X., Muno, N., Meijer, C.J.L.M., El Gueddari, B., El Ghazi, A., et al.The viral origin of cervical cancer in Rabat, Morocco. *Intenational Journal of Cancer*. 1998, **75**:546-/54.
9. Franceschi, S., Rajkumar, T., Vaccarella, S., Gajalakshmi, V., Sharmila, A., Snijders, P.J., Muñoz, N., Meijer, C.J. and Herrero, R. Human papillomavirus and risk factors for cervical cancer in Chennai, India: A case-control study. *International Journal of Cancer*. 2003, **107**: 127–133.
10. Guerbaoui M. 2000. Le cancer au Maroc. Epidémiologie descriptive.1ère édition: 165-171.
11. Hixon, J.E. and Vernier, D.J. restriction isotyping of humain apolipoprteine E by gene amplification and clivage with Hha I. *J Lipid. Res.* 1990, **31**: 545-548.
12. Herrero R, Castle PE, Schiffman M, Bratti MC, Hildesheim A, Morales J, Alfaro M, Sherman ME, Wacholder S, Chen S, Rodriguez AC and Burk RD. Epidemiologic profile of type specific human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. *J. Infect. Dis.* 2005, **191**:1796 – 807.
13. International Collaboration of Epidemiological Studies of Cervical Cancer. Comparison of risk factors for invasive squamous cell carcinoma and adenocarcinoma of the cervix: collaborative reanalysis of individual data on 8,097 women with squamous cell carcinoma and 1,374 women with adenocarcinoma from 12 epidemiological studies. *Int J Cancer*. 2007, **120**: 885-91.
14. Jeney, C., Tak´acs, T., Sebe, A. and Schaff, Z. Detection and typing of 46 genital human papillomaviruses by the L1F/L1R primer system based multiplex PCR and hybridization. *Journal of Virological Methods.* 2007, 140*:* 32 – 42.
15. Lo, K.W., Cheung, T.H., Chung, T.K., Wang, V.W., Poon, J.S., Li, J.C., et al. Clinical and prognostic significance of human papillomavirus in a Chinese population of cervical cancers. *Gynecol Obstet Invest.* 2001, **51**:202–7.
16. Lungu, O., Wright, T.C. Jr., and Silverstein, S. Typing of human papillomaviruses by polymerase chain reaction amplification with L1 consensus primers and RFLP analysis. *Mol Cell Probes.*1992, **6**:145-52.
17. Meftah el khair, M., Mzibri, M.E., Ait Mhand, R., Benider, A., Benchekroun, N., Fahime, E.M., Benchekroun, M.N., Ennaji, M.M. **.** Molecular detection and genotyping of human papillomavirus in cervical carcinoma biopsies in an area of high incidence of cancer from Moroccan women. *J Med Virol*. 2009, **81:**678-84.2009.
18. Melnikow, J., Nuovo, J., Willan, A.R., Chan, B.K., and Howell, L.P. Natural history of cervical squamous intraepithelial lesions: a meta-analysis. *Obstet Gynecol.* 1998, **92**(4 Pt 2):727-35.
19. Moreno, V., Bosch, F.X., Munoz, N., Meijer, C.J., Shah, K.V., and Walboomers, J.M. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. *Lancet.* 2002, **359**: 1085-92.
20. Morrison, E.A., Ho, G.Y., Vermund, S.H., Goldberg, G.L., Kadish, A.S., Kelley, K.F. and Burk, R.D. Human papillomavirus infection and other risk factors for cervical neoplasia: a case-control study. *Int J Cancer* .1991, **49**: 6-13.
21. Muñoz, N., Franceschi, S., Bosetti, C., Moreno, V., Herrero, R., Smith, J.S., Shah, K.V., Meijer, C.J., Bosch, F.X. and International Agency for Research on Cancer. Multicentric Cervical Cancer Study Group. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. *Lancet.* 2002, **359**(9312):1093–1101
22. Munoz, N., Bosch, F.X., de Sanjose, S., et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med.* 2003, **348**:518–27.
23. Munoz, N., Castellsague, X., de Gonzalez, A.B., and Gissmann, L. Chapter 1: HPV in the etiology of human cancer. *Vaccine.* 2006, **24:**S3:S1-S10.
24. Murthy, N.S. and Matthew, A. Risk factors for precancerous lesions of the cervix. *European Journal of Cancer Prevention.* 2002, **9**:5–14.
25. Nicolau, S.M., Camargo, C.G.C., Stávale, J.N., CASTELO, A., Dôres, G.B., Lörincz, A., and De lima, G.R. Human papillomavirus DNA detection in male sexual partners of women with genital human papillomavirus infection. *Urology* . 2005, **65**: 251–255,
26. Rombaldi, R.L., Serafini, E.P., Villa, L.L., Vanni, A.C., Baréa, F., Frassini, R., Xavier, M., and Paesi, S. Infection with human papillomaviruses of sexual partners of women having cervical intraepithelial neoplasia. *Brazilian Journel of Medical and Biological Research.* 2006, **39**: 177-187.
27. Sasco, A.J. Epidemiology of uterine cancers. Rev. Prat. 2001, **51**: 1408-12.
28. Stevens, M.P., Tabrizi, S.N., Quinn, M.A., and Garland, S.M. Human papillomavirus genotype prevalence in cervical biopsies from women diagnosed with cervical intraepithelial neoplasia or cervicalcancer in Melbourne, Australia. *Int J Gynecol Cancer*. 2006, **16**:1017–1024.
29. Tay, S.K. and Tay, K.J. Passive cigarette smoking is a risk factor in cervical neoplasia. *Gynecologic Oncology.* 2004, **93**: 116–120
30. Ting, Y., and Manos, M.M. Detection and typing of genital human papillomaviruses. In: Innis MA, Gelfand DH, Sninsky J J and White TJ . editors.1990. PCR protocols: a guide to methods and applications. San diego. Academic press; 356-67.
31. Tjalma, W.A.A., Van Waes, T.R., Van den Eeden, L.E.M., and Bogers, J.J.P.M. Role of human papillomavirus in the carcinogenesis of squamous cell carcinoma and adenocarcinoma of the cervix. *Best Practice & Research Clinical Obstetrics and Gynaecology.* 2005, **19** (4):469–483.
32. Trottier, H., Mahmud, S., Costa, M.C., Sobrinho, J.P., Franco, E.D., Rohan, T.E., Ferenczy, A., Villa, L.L. and Franco, E.L. Human Papillomavirus Infections with Multiple Types and Risk of Cervical Neoplasia. *Cancer Epidemiol Biomarkers Prev.* 2006, **15**: 1274- 1279.
33. Van Der Graaf, Y., Molijn, A., Doornewaard, H., Quint, W., van Doorn, L.J and Van Den Tweel, J. Human

papillomavirus and the long-term risk of cervical neoplasia. *Am J Epidemiol.* 2002, **156**:158 – 64.
34. Zereu, M., Zettler, C.G., Cambruzzi, E., and Zelmanowicz, A. Herpes simplex virus type 2 and Chlamydia trachomatis in adenocarcinoma of the uterine cervix. *Gynecol Oncol.* 2007, **105**(1):172-5
35. Zur Hausen, H. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. *J Natl Cancer Inst.* 2000, **92**:690-698.