

## RESEARCH COMMUNICATION

# The Prevalence of Human Papillomavirus in Cervical Cancer in Iran

SH Mortazavi<sup>1</sup>, MR Zali<sup>2</sup>, M Raoufi<sup>3</sup>, M Nadji, P Kowsarian<sup>3</sup>, A Nowroozi<sup>3</sup>

## Abstract

**Background:** The human papilloma virus (HPV), which is sexually transmitted, and most commonly causes genital warts, has been linked to cervical intraepithelial neoplasia and invasive carcinoma. Of ninety plus types of HPV, HPV-16 is the most prevalent in cervical cancer, followed by HPV-18, and HPV-33. As HPV's implication has not been assessed in the Middle East the main focus of this retrospective study was to determine the prevalence of HPV -16,18, and 33 in cases of cervical cancer from Iran.

**Material and Methods:** This retrospective study covered 100 patients with uterine cervical carcinomas who were referred to two referral centers for cancer in Tehran-Iran. Pathological blocks were collected for these cases and initial review of the blocks showed poor specimens in 18 cases, which left 82 cases for the study. These samples were histologically examined to verify the presence and the type of carcinoma. The next step was in situ hybridization for the detection of HPV common DNA. In Situ hybridization was preformed on all samples. Finally, Polymerase Chain Reaction (PCR) was preformed for the HPV types 16, 18, and 33. PCR amplification of exon 5 of the p53 gene was used as an internal control for the integrity of DNA. Takara PCR Human papilloma Detection method was used which includes primer for HPV 16, 18, and 33. Three primers were used alone, or in combination, in order to increase the sensitivity of the detection.

**Results:** The majority of tumors were squamous cell carcinomas (87%). The rest were adenosquamous carcinoma and adenocarcinomas. None of the 82 different cervical carcinoma tissue samples were found to be positive by in situ hybridization. In the PCR samples, amplification of DNA was observed for 69 tumor specimens. In the remaining 13 cases, the DNA in fixed tissue was degraded, as verified by the absence of an internal control band (p53). Out of the total 69 tumors (85.5%) with adequate DNA contained HPV band on PCR. The majority (73.9%) of HPV positive tumors contained HPV-16; the rest (11.6%) demonstrated type 18 and 33. There was no correlation between the histology of carcinoma and presence of types of HPV.

**Conclusion:** The prevalence of HPV in carcinomas of uterine cervix in Iran is similar to those reported in other regions of the world. Similarly, it appears that HPV-16 is the most common type associated with cervical cancer in Iran. Further studies on larger samples of patients, particularly in those with pre-invasive forms of the disease, are needed to elucidate the carcinogenic role of HPV types in cervical cancer in Iranian women.

**Key Words:** HPV - cancer - cervix - Iran

*Asian Pacific J Cancer Prev*, 3, 69-72

## Background

Human papilloma virus (HPV), with double stranded DNA is the cause of sexually transmitted disease and also has been linked strongly with cervical cancer. HPV DNA

has been detected in over 90% of cervical biopsies, which had signs of dysplasia or cancer. HPV can also cause warts on the vulva, perineum, or smooth chondilomas in cervix or vagina. More than 90 types of HPV (pathogenic and non-pathogenic) have been identified. The most common ones

<sup>1</sup>Department of Radiation Oncology and Nuclear Medicine, Jorjiani Hospital, Shahid Beheshti University of Medical Sciences. Tehran,

<sup>2</sup>The Director for Research, The Academy of Medical Sciences. Tehran Iran, <sup>3</sup>The Academy of Medical Sciences, Tehran Iran. + Pathology Department, University of Miami, USA.

All Correspondence: Dr. S H Mortazavi, P.O.Box 11365-3175, Tehran, Iran. E-mail: mortazav@ams.ac.ir Fax: 98-21-7561875

associated with cervical cancer are types 16, and 18, which have been reported in 70% of the cases. The implication of the importance of pathological human Papillomavirus has not been studied in the Middle East where the incidence of cancer of cervix is high. As cancer of cervix is the second commonest cancer in Iranian females (Mortazavi et al., 2000) this retrospective study has been preformed to determine the prevalence of HPV and its type in cervical cancer in Iran.

## Material and Methods

This retrospective study consisted of 100 patients who were referred to Jorjiani and Madaen Hospitals in Tehran from other medical centers in Tehran and provinces over a three-year period. These patients had been referred for treatment of cancer of cervix and had received treatment for this disease. Pathological blocks were collected for these cases and initial review of the blocks showed poor specimens in 18 cases, which left 82 cases for the study. In order to perform in situ hybridization and PCR, the collected blocks were sent to Department of Pathology where the tests were carried out by one of the authors in Miami University, USA. The samples were first assessed histologically for the presence of cancer, and its type. The next step was to identify the common DNA in HPV. In Situ hybridization was preformed on all samples. Enzo Pathogen Human Papiloma Virus in situ Screening Assay for tissue Section was done in accordance with the producer's instruction. The last stage, Polymerase Chain Reaction (PCR), was done for HPV types 16, 18, and 33. In order to control the DNA integrity, PCR amplification was preformed in exon 5 of p53 gene. In this part Takara PCR Human Papillomavirus was used, which includes HPV types 16, 18, and 33 primers. These primers were used alone or in combination in order to increase the detection sensitivity.

## Results

The types of different cancer of cervix and their numbers are shown in Table 1. The majority of the tumors were squamous cell carcinomas. The remainders were

**Table 1. Patients Distribution According to Histology of Cancers**

Histology	Number	Percent
Squamous cell ca.		
Grade	8	9.6
Grade II	14	17.0
Grade III	29	35.4
Adenosquamous Ca	17	20.7
Adenocarcinoma, others	4	4.9
Total	72	87.6

adenosquamous and adenocarcinoma. None of 82 cervical tissues were positive for HPV by the in situ hybridization. This was almost certainly due to age of blocks, tissue fixation, and possibly lack of sensitivity of the method in detecting incorporated viral genome. For the PCR, there was amplification of 69 tumor samples. In the remaining 13 samples the DNA in fixed tissue was degraded as verified by the absence of internal control band (p53). Fifty nine tumors with adequate DNA revealed HPV band in PCR. The majority of them (85.5%) were HPV-positive tumors of HPV16, 18, and 33 types as shown in Table 2. No relationship was found between histological types and differentiation and various types of HPV in the tumor specimens.

## Discussion

Cancer of cervix is one of the commonest female cancers in developing nations, and its association with the most common sexually transmitted virus, which is HPV, has long been established. The aim of this retrospective study was to show the prevalence of this virus in the pathological specimen of Iranian women suffering from cancer of cervix for the first time. Although the prevalence of cervicle cancer is gradually decreasing in the world, it is however, the second most common cancer in women through out the world, and of about 500,000 new cases each year; about 80% are from the developing countries (Eduardo, 1996). In Iran the most prevalent female cancers was cancer of cervix, first reported in 1966, (Armin, 1966) and a few years later by Habibi et.al in 1973 in a study of 44,000 cancer cases.(Habibi, 1974) This high rate fell over the years from 19.4% to 11.4% (Mortazavi et al., 2000) mostly due to early detection and treatment of dysplasia's and carcinoma in situ. Our reliance has been on Pappaniclous Staining to pick up early changes in cervical tissue and to treat it. However, this we find now is not sufficient and early detection of virulent HPV strains in cervicle tissues and its treatment is indicated, as HPV plays a pivotal role in the causation of this cancer.

Although cervical cancer is influenced by many environmental and genetic factors, HPV infections of specific pathological strains has been shown to act as initiator. (Bauer et al., 1991) One should also take into account other factors such as age of first intercourse, parity, contraception, health care and nutrition and smoking. (Becker et al., 1994) We preformed hybridization and PCR

**Table 2. Patients Distribution According to HPV Types**

HPV type	Number	Percent
16	51	73.9
18/33	8	11.6
No HPV	10	14.5
Total	69	100

**Table 3. Relationship of Pathology to HPV Types**

Histology	HPV-16	HPV-18/33	No HPV
Squamous cell ca.	48	4	9
Grade I	4	0	1
Grade II	39	3	2
Grade III	5	1	2
Adenosquamous Ca	3	3	1
Adenocarcinoma, others	0	1	0
Total	51	8	10

methods to make our result comparable with other centers. According to our results, 86% of the examined samples were HPV positive, with 73.9% HPV 16, 11.6% HPV 18 and 33, and 14.6% with no HPV. The only other study attempted in Iran were 100 cases of cervical cancer and dysplasia in 1996 that showed only 8 HPV were positive with 2 dysplasia cases and 6 cases with squamous cell carcinoma, using peroxides method. (this was an informal report of the study) (Danesh, 1995). In our study, as shown in Table 1, HPV was present in 87% of squamous cell carcinomas, majority were Grade 2 type. HPV was present in 9% of cases of adenosquamous carcinoma. The result of this study is parallel to many other studies. In one study, some researchers in Britain, France, Spain, and US collected samples from about a thousand patients with invasive cervical carcinoma from 32 hospitals of 22 countries. By using sensitive DNA experiment more than 25 types of different HPV DNA were recognized, and achieved a detection rate of 93%. The most prevalent type was HPV-16, with the exception of Indonesia, where HPV-18 was commonest.

Other types which were uncommon was detected in some specific areas, as HPV-45 in West Africa, and HPV-39, and 59 in Central and South America. (Eduardo, 1996) In Costa Rica, 324 women were examined for HPV using PCR method, they found 73% in cases of low grade squamous intraepithelial were positive with HPV-16, and 88% in squamous cell carcinomas. (Herrero et al., 2000) Similar studies in Paraguay of 113 pathological samples of invasive squamous cell carcinoma revealed 97% presence of HPV-16 using PCR method. (Rolon et al., 2000) In China 71.6%

**Table 4. Patients Distribution According to Age Groups**

Age groups	Number	Percent
<=10	0	0
31-40	8	9.8
41-50	15	18.3
51-60	30	36.6
61-70	17	20.7
>70	4	4.9
Unknown	8	9.7
Total	82	100

of patients with CIN, 44.2% of blood donors and 27.3% of children were HPV positives. (Guzick, 2000) In Sweden, a study on penile brush samples was

Preformed using PCR method, in which 13% of 147 cases were positive for, and 6.4% were HPV type 16. (Wikstrom et al., 2000) Recent study in South Africa shows a high rate of infection with HPV type 16, 18, 31, 33, and 45. (Marais et al., 2000) All the studies show the main types and some geographical variation.

Therefore it would appear that HPV is the initiating factor in causation of dysplasia leading in some cases to carcinoma of cervix. We do know that the transition from dysplasia to invasive carcinoma is over a long period, perhaps even up to ten years.

Perhaps one of the best evidence that shows the prolonged period needed between presence of the virus and invasive carcinoma of cervix was shown by Wallin and his associates, (Wallin et al., 1999) who matched 118 cases with baseline Pap smear that showed HPV against same number of women with no such a change. After an average of 5.6 years 30% of the infected group had cancer of cervix as against 2.5% in the control group. Not surprisingly, 77% of women with cancer had the same strain of HPV in their cancer as they had in their baseline Pap smear.

Therefore early detection of the virus by diagnostic and screening methods would be the first step with our present knowledge to detect and treat this virus as early as possible. (Guzick, 2000) The treatment has to be subsequently checked so that we can be sure that the virus has been totally eradicated. This will also require the treatment of male partner if infected. Perhaps in the future vaccination will result in almost complete eradication of this cancer.

## Conclusion

The prevalence of HPV in cervical cancer in Iran is 85.5% and the commonest type of HPV was type 16, and only 11.6% were types, 18 and 33, which are the similar to most countries. Histological types and differentiation are not found to be significant factors. Further studies should be carried out in larger groups of non-cancer patients to detect and treat pre-invasive cases, and also treatment of male partners appears to be a necessity. Economical serological testing methods and vaccination should be our ultimate goal.

## Acknowledgements

- 1) Our gratitude to The Academy of Medical Sciences of Iran for granting funds for this research project.
- 2) The authors would like to express their thanks to Dr.B.Shahrad, and Dr.K.Mojir Shaibani for allowing us to include their patients in this study.
- 3) We are grateful for the work that Lau Meng and Saum Noori, put into this research at the Pathology Laboratory of Department of Pathology at The Miami University School of Medicine.

## References

- Armin K (1996). Some Statistical Data on Cancer Mortality in Iran. *International Pathology*, **25**, 27-9.
- Bauer HM, Ting Yes, Greer et al (1991). Genital human Papillomavirus infection in female University students as determined by PCR-Based method. *JAMA*, **26**, 472-7.
- Becker TM, Wheeler CM, Mc Gough NS, et al (1994). Sexually transmitted diseases and other risk factors for cervical dysplasia among southwestern Hispanic and non-Hispanic white females. *JAMA*, **271**, 1161-88.
- Danesh Doost B (1995). Infection with HPV and neoplastic lesions of cervical squamous cell carcinoma in Iran. Unpublished.
- Eduardo L Franco (1996). Epidemiology of anogenital warts and cancer, Obstetrics and Gynecology. *Clinics of North America*, **23**, 597-623
- Guzick J (2000). Human Papillomavirus testing for primary cervical cancer screening. *JAMA*, **5**, 108.
- Habibi A (1974). Cancer in Iran. *Iran Journal of Health*, **3**, 113.
- Herrero R, et al (2000). Population based study of Human Papillomavirus Infection and cervical neoplasia in rural Costa Rica. *J Natl Cancer Inst*, **92**, 464-74.
- Marais DJ, Lane C, et al (2000). Sero-response to HPV type 16, 18, 31, 33, and 45 virus like particles in South African women with cervical cancer and cervical intra-epithelial neoplasia. *J Med Virology*, **60**, 403-10.
- Mortazavi SH, Shahidi M, Shahradi B (2000). Changing trend in cancer incidence in Iran. *Cancer Strategy*, **2**, 13-6.
- Rolon PA, Smith JS, Munoz N, et al (2000). Human Papillomavirus infection and Invasive cervical cancer in Paraguay. *Int Journal Cancer*, **85**, 486-91.
- Vernon SD, Unger ER, Reeves WC (?) Human Papillomavirus and cervical cancer. *Curr Probl Obstet Gynecol Fertil*, **198**, 104-24.
- Wallin KL, Wiklund F, Angstrom T, et al (1999). Type Specific persistence of Human Papillomavirus DNA before the development of invasive cervical cancer. *N Engl J Med*, **341**, 1633-8.
- Wikstrom A, Popescu C, Forslund O (2000). Asymptomatic penile HPV infection: a prospective status. *Int J Std AIDS*, **11**, 80-4.



### Personal Profile: Dr. SH Mortazavi

Born in 1938 in Tehran, Iran and I qualified in medicine at Queens University of Belfast (1965), and continued there to specialize in Radiation Oncology, and got DMRT (London). Returned to Iran where I have worked in university since. I am at present Associate Professor, and head of Department of Radiation Oncology and Nuclear Medicine at Jorjiani Hospital, Shahid Beheshti University of Medical Sciences, Tehran. I am fellow of Academy of Medical Sciences of Iran, and various related societies in Iran and UK. I was chairman and examiner in radiation Oncology for about 22 years, and advisor to “WHO” in Radiation 1978-1982. My research interest is in Oncology, and nuclear medicine, changing over the years. It is now more concentrated in molecular epidemiology in cancer, and it's application to patient management.



Left to right Prof MA Zali, P Kowsarian, A Nowroozi, Dr SH Mortazavi