## ORIGINAL ARTICLE

# DEMOGRAPHIC CHARACTERISTIC OF HPV INFECTION IN WOMEN - A HOSPITAL BASED STUDY FROM GUWAHATI, INDIA

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## ABSTRACT

**Background:** Human papilloma virus is a dominant factor for cervical carcinogenesis. About 70% of cervical cancer cases are related to infection with high risk (H-R) HPV types mostly HPV16 &HPV18. First coitus at a younger (≤ 15) age, high parity, and early age at marriage behave as major risk factors for HPV infection.

**Objective**: On this background, the present study has been carried out to detect genital HPV infection in women between 15-65 years and its association with age factor, marriage and parity.

**Materials & methodology**: The Nested PCR using primers for L1 consensus gene with My9/My11 & GP6+/GP5+ followed by multiplex PCR is carried out in cervical scrapping to detect HPV 16 & HPV18 by respective primers. The HPV positive cases were compared with their corresponding socio demographic data available in the proforma filled up during the sample collection.

**Result**: The study showed significant association of HPV infection in women who had early marriage ($p<0.01$). Though age factor and parity status did not show significant association, they behave as an independent factor for HPV infection.

**Conclusion:** on account of the central role of HPV infection in cervical carcinogenesis, any risk factor other than HPV like early marriage, high parity and age itself may play a role by either increasing the risk of acquisition or duration of the infection, or by increasing the risk of progression from HPV infection to cervical cancer.

**Key Words:** H-R HPV, nested PCR, Cervical smear, cervical cancer

### Abbreviation

| | |
|---|---|
| DNA | De oxy ribo nucleic acid |
| PCR | Polymerase Chain Reaction |
| SIL | Squamous intraepithelial lesion |
| RMRC NE | Regional medical research center for north east (India) |
| ECA | Epithelial cell abnormality |
| NILM | No intra epithelial lesion/malignancy |

### INTRODUCTION

Human papilloma virus is a dominant factor for cervical carcinogenesis and has been established as the major cause of cervical cancer in women[1]. About 70% of cervical cancer cases are related to infection with high risk (H-R) HPV types mostly HPV16 &HPV18. 15% are related to H-R HPV types 31, 33, 35, 52, and 58 collectively and 15% are related to other less common types, with some geographical variation. India has a population of 366.58 million women ages 15 years and older who are at risk of developing cervical cancer[1]. About 7.9% of women in the general population are estimated to harbour cervical HPV infection at a given time, and 82.5% of invasive cervical cancers are attributed to HPVs 16 or 18[1]. In India, HPV type 16 alone in cervical cancer is 70-90% while occurrence of HPV type 18 varies from 3 to 20%[1]. HPV is a necessary cause of cervical cancer, but it is not a sufficient cause. Other cofactors are necessary for progression from cervical HPV infection to cancer. Long-term use of hormonal contraceptives, high parity, early initiation of sexual activity, multiple sex partners, tobacco smoking and co-infection with HIV have been identified as established cofactors; co-infection with *Chlamydia trachomatis* and herpes simplex virus type-2, immunosuppression, low socioeconomic status, poor hygiene and diet low in antioxidants are other probable cofactors [2]. First coitus at a younger (≤ 15) age, increased number of pregnancies, and early age at

marriage were recognized as the significant risk factors for HPV infection[3].

## OBJECTIVES

To detect cervical HPV infection in women who attended a tertiary hospital in Guwahati, India and its association with some of the demographic factors like age factor, marriage and parity.

## METHODS & MATERIALS:

Total 226 no. of cervical scrapping were collected from married non pregnant women aged 15-55 years during the period January '2011 till June'2012. The study was approved by Institutional Ethical Committee vide letter No.MC/190/2007/Pt-1/71 dated 15/12/10. Prior to the collection of the specimen, the written consent from the patients attending the GOPD has been taken.

### A) Specimen Collection for HPV DNA by PCR and Preparation and Reporting:

Cervical scraping from the posterior vaginal pool was smeared and dried on to Whatman 3MM filter paper, and stored individually at room temperature after labeling for HPV DNA testing by PCR. The dry paper smears were carried to RMRC, NE for detection of HPV DNA. The test is carried out according to the method described by Kailash *et al*,.(2002)[4] and Sotlar K *et al*,.(2004)[5] .

### B) Methodology for HPV DNA Testing by PCR

**DNA Preparation:**

A small piece (2-3 mm) of the dried paper specimens was punched out with the help of a sterile puncher ,transferred into 1.5 ml micro centrifuge tube (eppendorf, Hambarg, German,) containing 100 μl of distilled water. This way all the samples were made ready for DNA extraction. The centrifuge tubes containing the dry smear punch were boiled for 15 minutes, immediately placed on ice for 5 minutes followed by centrifuge at 10,000 rpm for 3 minutes at 4 $^{0}$ C. 70 ul volume of supernatant kept into a fresh 1.5 ml centrifuge tube and stored at -20 $^{0}$ C for PCR. PCR mix with Taq DNA Polymerase (Promega, USA) and the primers were added to the tube. Amplification of DNA by conventional PCR was performed in a 20μl reaction mix in a DNA Thermal cycler (Applied biosystem, thermal cycler,) using primer My09/My11 which is a primer for consensus L1 gene of HPV. The primer sequence used in the study is:

| HPV genotype | Amplicon (bp) | Sequence (5`-3`) |
|---|---|---|
| MY09/11 | 450 | FP- GCM CAG GGW CAT AAY AAT GG<br>RP- CAA CTT CAT CCA CGT TAC ACC |
| GP 5+/6+ | 150 | FP- AAT GCC TGT GTT CAT TGC TG<br>RP- TTC AAG GTC AGC CCC TAC AC |
| 16 | 457 | CAC AGT TAT GCA CAG AGC TGC<br>CAT ATA TTC ATG CAA TGT AGG TGT A |
| 18 | 322 | CAC TTC ACT GCA AGA CAT AGA<br>GTT GTG AAA TCG TCG TTT TTC A |

| HPV genotype | GP-E6-3F | GP-E6-5B | GP-E6-6B |
|---|---|---|---|
| Consensus | GGG WGK KAC TGA AAT CGG T | CTG AGC TGT CAR NTA ATT GCT CA | TCC TCT GAG TYG YCT AAT TGC TC |
| Common | ––– C–– A––– ––– ––T ––– – | | |
| 16 | | ––– ––– ––– ––T T––– ––– ––– –– | |
| 18 | | | –– ––– ––– ––– –T– ––– ––– –– |

**Single-letter code: W, A/T; K, G/T; R, A/G; Y, C/T; N, A/C/G/T; X, unknown nucleotides. –, identity with consensus sequence.**

**PCR amplification and amplicon detection:**

PCR for HPV detection was carried out using MY09/11 (Baay et al.1996)[6] and GP5+/6+ (Evans et al. 2005)[7], HPV 16 & HPV 18(Sotlar K *et al*,.(2004)[5] . Amplifications were performed with the following cycling profile: AmpliTaq Gold activation was performed by incubation at 95$^{0}$ C for 5 min followed by 40 cycles of 1-min denaturation at 94°C, 1-min annealing at 55°C, and 1-min elongation at 72°C. The last cycle was followed by a final extension step of 7 minutes at 72 $^{0}$ C. After PCR, 10 μl of the amplified product were run on an ethidium bromide-stained 2% agarose gel ((BioRad, USA) and visualized with a UV Transilluminator .The gel picture is photographed in a gel documentation system (GELLOGIC 2200 Image System). Every PCR reaction included positive and negative controls. Strict laboratory precautions and control measures were followed to avoid cross-contamination and carry-over in the PCR assay.

### C) Interpretation of PCR testing:

The size of amplified PCR products was observed against a 100bp DNA ladder. The band that correlated with the expected product size (450bp), was considered positive.( cite Figure 2). The all negative samples for MY09/MY11 that was observed in First round of nested PCR were further processed for 2$^{nd}$ round PCR using primers GP6+ and GP5+ using same PCR

protocol and PCR profile and run 2% agarose gel for confirmation of results. 1 µl of the first round nested PCR amplicon was used as template for the 2nd round nested PCR.

**The statistical Analysis:**

The association/contingency between demographic factors and HPV infection was statistically calculated by using the Chi-Square (**χ2**) test of significance. The HPV prevalence and the sensitivities, together with the 95% confidence intervals (CI), of the present study were estimated for subjects with age factor, age at marriage and parity status. The calculations were performed by adopting the statistical software Graph Pad InStat.

## RESULTS & OBSERVATION:

HPV DNA is detected in 22 samples. HPV prevalence of the study is 9.73%. High risk HPV16/18 prevalence is 81.8%. HPV16/18prevalence is more common in SIL and cervical cancer. (Cite table 1).

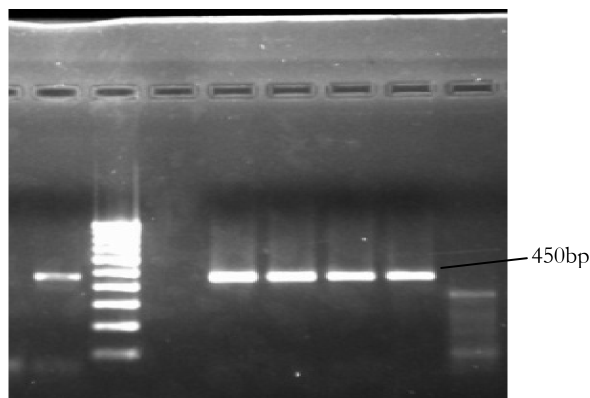


**Fig 1: L1 PCR Figure showing presence of HPV in Lane 4,5,6,7. M=100bp marker, lane1=+ve control, lane3= -ve control**

**Table 1: Results of HPV Status of the Patient with cervical abnormality**

| HPV Status | NLIM | LSIL | HSIL | SCC | Total (22) |
|---|---|---|---|---|---|
| HPV 16 | 1 | 1 | 4 | 6 | 12 (54.5%) |
| HPV 18 | 1 | 1 | 4 | 0 | 6(27.3%) |
| HPV Other than 16/18 | 2 | 2 | 0 | 0 | 4(18.2%) |

**Table2: Association of Age factor with HPV infection**

| Age in Years | Women | HPV 16 | HPV 18 | HPV Others | Total HPV +ve (%) |
|---|---|---|---|---|---|
| <30 | 48 | 2 | 1 | 1 | 4 (8.3) |
| 30-39 | 81 | 3 | 2 | 1 | 6 (7.4) |
| 40-49 | 69 | 2 | 2 | 2 | 6 (8.7) |
| ≥50 | 28 | 5 | 1 | 0 | 6 (21.4) |
| Total | 226 | 12 | 6 | 4 | 22 (9.73) |

χ2 value=3.819, df=6, P=12.592 Not significant

**Table 3: Age at marriage & HPV**

| Age at marriage | Women | HPV 16 | HPV 18 | HPV Others | Total HPV +ve (%) |
|---|---|---|---|---|---|
| <16 | 20 | 3 | 0 | 0 | 3 (15.0) |
| <26 | 140 | 8 | 5 | 4 | 17 (12.1) |
| <36 | 66 | 1 | 1 | 0 | 2 (3.03) |
| Total | 126 | 12 | 6 | 4 | 22 (17.5) |

χ2 value=17.15, df=6, P<0.01 Significant

**Table 4: Association of Parity with HPV infection**

| Parity | Women | HPV 16 | HPV 18 | HPV others | Total HPV +ve (%) |
|---|---|---|---|---|---|
| 1-2 | 127 | 3 | 1 | 2 | 6 (4.7) |
| 3-4 | 76 | 5 | 4 | 2 | 11 (14.3) |
| ≥5 | 23 | 4 | 1 | 0 | 5 (21.7) |
| Total | 226 | 12 | 6 | 4 | 22 (17.5) |

χ2 value=3.128, df=4, P=9.488, Not Significant

The study showed higher HPV prevalence below 30 year age group (8.3 %,); <40 years group 7.4%, <50 years group 8.7% and >50 years group the prevalence increases up to 21.4% (Cite Table2). Women married before 16 years of age showed increased HPV prevalence (15.0%) (Cite table 3). Women with 5 children or more show higher HPV prevalence with 21.7% where as those who had 1-2 children the prevalence is 4.7% (Cite table 4). The association/contingency between HPV infection and different demographic characters like age, early marriage and parity was statistically calculated by using the Chi-Square (**χ2**) test of significance. The study showed significant association of HPV infection with early marriage. (cite Table 3)

## DISCUSSION

In India, the association of the infection of high-risk HPVs with the age of marriage below 18 years has been found to increase the risk of cervical cancer by 22 fold[2]. The study carried out by Dutta S et al [2011] showed that the risk of HPV infection was higher in women aged 25 to 34 years (odds ratio, 1.11), in married women below 20 years of age (odds ratio, 1.80), and in women with parity ≥4 (odds ratio, 1.04). High-grade squamous intraepithelial lesion cytology was more frequent in women infected with HPV 16 than in those infected with HPV 18 and other types[8]. A meta-analysis done by Silvia de Sanjosé *et al.*[2007] confirms that HPV infection is most common in women younger than 25 years of age .However; a second peak of HPV prevalence is seen in women aged 45 years or older in all regions[9]. In the Osmanabad district study, the prevalence of HR-HPV types in the 30–39, 40– 49 and 50–59 age groups were 9.8%, 10.4% and 12.2%, respectively. In the multicentre cross-sectional study in India, these were 7.0%, 6.8% and 7.5%, respectively (*J* Giftson Senapathy et al[2011])[10]. The present study also establishes similar result with higher HPV prevalence in < 30 year age group. The prevalence increases up to 21.4%. in the >50 years group which may be due to

persistent HPV infection. HPV prevalence in cervical smears compared with study done by Laikangbam P *et al*,. (2007) showed HPV16/18 prevalence at age < or =30 years in West Bengal (8.8%) is comparable to that our study with 8.3%[11]. Hildesheim *et al.* (2001) observed risk of HSIL/SCC cases increased with increasing number of live births in HPV positive cases[12] . Though the present study reveals no significant association of HPV with age and parity status of woman, it showed high H-R HPV prevalence in women who had 5 or more children. HPV infections are at least two-times more frequent in pregnant women than in non-pregnant women showing a gradual but statistically significant ($P < 0.001$) increase in HPV infection with the increasing number of pregnancies[2]. Perhaps this is why in India early marriages, normally resulting in higher number of pregnancies, is a high-risk factor for cervical cancer due to immature cervix[2]. Long duration of sexual activity in women married at young age below16 years is more prone for HPV infection. Hence the present study showed early marriage in a woman is significantly associated with HPV infection. The possibility that young age and high parity may not show significant association with HPV infection, but may suggest that these factors may enhance the probability of malignant transformation of HPV infection and behave as an independent factor for HPV infection.

### Limitation of the study

It is a cross-sectional study. Hence it only provides information on HPV prevalence. Primers for HPV 16/18 are used in the study, so some of the other HPV types could not be interpreted.

## CONCLUSION

On account of the central role of HPV infection in cervical carcinogenesis, any risk factor other than HPV like early marriage, high parity and age itself may play a role by either increasing the risk of acquisition or duration of the infection, or by increasing the risk of progression from HPV infection to cervical cancer.

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