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To Study Human Papilloma Virus Serotype Prevalence In Selected Married Sexually Active Women

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ABSTRACT

Objective - To study prevalence of various serotypes of Human Papilloma Virus in selected married sexually active women. **Methods** - Study period from June 2009 to Dec 2010. 500 PAP smears were done. Women with abnormal PAP smears, normal PAP with unhealthy cervix & abnormal colposcopy underwent HPV DNA PCR testing. **Result** - Six cases were positive with subtype 31, 66, 18 & three cases were with 16 number serotype out of 60 cases tested. **Conclusion** - Prevalence of HPV in selected women was about 10%. Prevalence of serotype 16 was 50%. Prevalence of serotypes 31, 66, 18 was 16.66% each.

Key word : HPV, Cancer cervix, Serotype

MATERIAL & METHODS

- 500 PAP smears were performed during study period from June 2009 to Dec 2010.
- Present study had a sanction from college ethical committee.
- After written consent and detailed information women with abnormal PAP, normal PAP with suspicious unhealthy cervix were taken for colposcopic exam with VIA/VILI positive areas underwent HPV DNA PCR testing with subtyping.
- Endocervical scrapings on cytobrush with sterile saline obtained for testing.
- Detection for HPV DNA in samples is done by using gel electrophoresis technique.
- HPV types are identified by direct nucleotide sequencing of PCR products, using automated gene analyser AMI 3100.
- The PCR based assay along with nucleotide sequencing analysis of HPV typing has specificity of 98-100%.

Criteria for exclusion

- Individuals concurrently enrolled in clinical studies of investigational agents or studies involving collection of cervical / genital specimens.
- History of known prior vaccination with HPV vaccine.
- Subjects who had undergone hysterectomy or treatment for cervical cancer in past.
- Pregnancy
- Active bleeding or infective discharge or UTI (screening can be postponed till completion of treatment)
- Any condition which in the opinion of investigator might interfere with the evaluation of study objectives.
- Inability to give consent.

Criteria for inclusion - All included except as given above.

Study conduct

Ethical committee sanction was taken for the study.Written informed consent was taken from women. Women underwent PAP smear test. Abnormal PAP smear or women with unhealthy cervix underwent HPV DNA PCR testing.

Laboratory assessment

Endocervical scraping on cytobrush with sterile saline obtained and transported at room Temperature. Sample processed and DNA subjected to amplification and reamplification By PCR. Detection for HPV DNA in samples was done by using gel electrophoresis technique. Genotype identification of HPV was done by DNA PCR using MY09 and MY11 primers. HPV

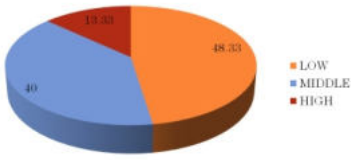
serotypes were identified by direct nucleotide sequencing which is gold standard for identification of HPV subtypes. The PCR based assay along with nucleotide sequencing for HPV typing has specificity of 98-100%. The assay has high sensitivity with lower limit of detection being 1250 viral particles per mg of tissue.

Findings and results

Analysis of HPV positive cases -

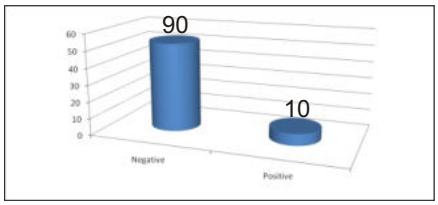
- 2 cases with HPV 16 had a Ca Cervix I b and they underwent Wertheims hysterectomy.
- 1 case with HPV 16 had Ca Cervix IIb, which underwent radiotherapy
- 1 case with HPV 18 had Ca Cervix Ib, which underwent Wertheims hysterectomy.
- 1 case with HPV 31 had Ca Cervix Ib, which underwent Wertheims hysterectomy.
- 1 case with HPV 66 was advised biopsy, reporting chronic cervicitis.

Figure 1 - Socioeconomic status of women



This figure shows that most women with cervical disease are from Lower socio-economic strata.

Figure 2 - HPV testing results



This table shows that 10% of women showed positive HPV infection with abnormal PAP smear

Table 1 - PAP Reporting

PAP Smear Report	No	Percentage
HSIL	12	20%
LSIL	15	25%
NILM	20	33.33%
NILM (Inf)	10	16.66%
Unsatisfactory	3	5%
Total	60	

This table shows, that we had 20% of women having HSIL which is very high and again only 16.66% women who had inflammatory smear.

Table 2 - Age wise Population.

Age Group	No.	Percentage
Below 20	0	0
21 – 25	5	8.33%
26 – 30	15	25%
31 – 35	12	20%
36 – 40	14	23.33%
41 - 45	14	23.33%
Total	60	

Abnormal PAP smear was found rampant not only in age group above 36 yrs, but also between 26 - 30 yrs.

Table 3 - Family Size Data.

Age of Marraige	No.	Percentage
12 - 18	40	66.66%
19 - 20	8	13.33%
20 - 25	8	13.33%
26 - 30	3	5%
>30	1	1.66%

Women with 2 or more children showed more PAP abnormalities.

Table 4 - Age Of Marriage

Age of Marraige	No.	Percentage
12 - 18	40	66.66%
19 - 20	8	13.33%
20 - 25	8	13.33%
26 - 30	3	5%
>30	1	1.66%

This table shows the tragic fact that 66.66% women got married between the ages of 12 - 18 yrs, which is a major risk factor in cervical disease.

DISCUSSION

In USA, PAP smear screening is done in all women who are sexually active. Incidence of finding LSIL is 1.5% to 2% and HSIL is about 0.5%. (Rock & Jones, III, 2003) In India, we do not have the luxury of universal screening and therefore patients presenting in Gynaecology OPD from June 2009 to December 2010 underwent PAP smear examination. Out of these 60 women had abnormal PAP smears. This is same as found by Khanna et al in 2009. As women already had some kind of cervical disease, we had high incidence of HSIL which is about 12%. Out of these none were HPV positive.

Our women have lot of non HPV related or come & go HPV insults causing cervical diseases where colposcopy & HPV serotype testing helps us in knowing if that lesion can undergo malignant change.

Sankaranarayanan and colleagues in 2009 found that HPV testing was more objective and prevented more advanced cervical cancers and cervical deaths. Bigras in 2005 found the probability of PAP test to be abnormal is directly proportional to HPV viral load. Thus whenever HPV is negative in HSIL & LSIL lesions it is reassuring.

Total 6 cases (10%) were positive for HPV DNA subtype. All these were cancer cervix cases. Of which 3 were of subtype 16, one of 18, 31 and 66. The invasive disease does not develop unless there is persistence of HPV DNA. It has been proposed as first ever identified cause of human cancer (Rock & Jones, III, 2003). Out of 80 known HPV genotype thirty are known to infect the genital tract. Out of these 20 have been identified as carcinogenic with types 16 & 18 found most commonly in malignant lesion.

The common types are classified according to their oncogenic potential as follows

1. Low risk - 6, 11, 41, 44
2. Intermediate risk - 31, 33, 35
3. High risk - 16, 18, 45, 56

There are many proposals to include HPV subtyping in management protocols for abnormal cervical cytology. (Bigras 2005) Evaluation of HPV based cytological screening pilot studies in United Kingdom have concluded that caution be exercised in implementing HPV testing in women with minor cytological abnormalities(Rock & Jones, III, 2003).

In our study we found that one case of cancer cervix out of 6 had HPV subtype 31, which is having intermediate risk oncogenic potential and not available in bivalent or quadrivalent vaccines available in the market.

CONCLUSION

Our study shows that it is very important to do HPV DNA PCR testing for HPV typing in high risk as well as cancer cervix patients to know subtype of HPV in a given area so as to know the prevalence of the same. This test is expensive, though very specific, cannot be done frequently. It is very important to continue this study to have a large number of women tested for same, to provide HPV subtype related cervical neoplasia.

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