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Biopsy vs Brushing: Comparison of two sampling methods for the detection of HPV-DNA in low grade and high grade squamous intraepithelial lesion cases in colposcopy screening

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Abstract
Cervical cancer is the most common cause of cancer mortality in women worldwide. Human papillomavirus (HPV) identified as the necessary cause of cervical cancer. HPV testing is widely incorporated into cervical cancer screening strategies in western countries. Current screening requires pelvic examination for cervical sampling, which may compromise target population participation due to the fear of the invasive procedures. The acceptance could be raised by introducing non invasive procedures during screening as much as possible. We explored the interchangeability of cervical brush samples and colposcopy directed biopsies for HPV DNA testing, by means of a prospective study conducted in screening populations who tested positive for abnormal cellular change in Pap smear earlier and called for colposcopy for further clinical detection of malignancy and recorded as low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesion (HSIL) cases.

Keywords: cervical cancer; human papillomavirus; colposcopy; squamous intraepithelial lesion

1. Introduction
Cervical cancer is ranked as the second most frequent cancer in Indian women. Approximately 122,844 new cases of cervical cancer and 67,477 deaths are reported in India annually [1, 2]. Human papillomavirus (HPV) infection is a common sexually transmitted infection. A strong association correlates between persistent HPV infection and risk of cervical lesions, especially for HPV types 16 and 18 [3]. Intriguingly, prospective studies consistently show that only a small fraction of HPV infected women do eventually develop cervical cancer [4]. Cervical cancer begins with the pre-cancerous, benign lesions in the cervix. According to WHO classification, the first stage of development is mild dysplasia, which can then progress to become moderate dysplasia, severe dysplasia and then carcinoma in situ (CIS) or invasive cervical cancer. Mild dysplasia usually regresses on its own without treatment, and doesn't progress to moderate dysplasia. A small fraction of women with mild or moderate dysplasia progresses to cervical cancer, which can take as long as 10years. According to the Cervical Intraepithelial Neoplasia (CIN) system, mild to moderate dysplasia are classified as CIN 1, intermediate dysplasia as CIN 2, and severe dysplasia and carcinoma in situ are together classified as CIN 3. The Bethesda system simplifies it further, by classifying CIN 1 as Low Grade Squamous Intraepithelial Lesion (LSIL) and both CIN 2 and CIN 3 as High Grade Intraepithelial Lesion (HSIL). Based on this knowledge, we aimed in our study to compare the two effective sampling procedures for the detection of HPV DNA in low grade and high grade squamous intraepithelial lesion cases in colposcopy screening**.**

2. Material and Methods
2.1 Sample collection
A prospective cross-sectional study was conducted on 31 women (graded as CIN 1, CIN 2 and CIN 3) during the study period of 2013 to 2014, who had abnormal pap test results earlier on their routine screening and called for colposcopsy clinic of Obstetrics and Gynecology Department, JIPMER for further visual aided screening. The study was approved by the Institute Ethics Committee. All the participants were explained about the purpose of the study prior to their participation and prior approval was taken in a written informed consent. Women with suspicious lesions suggestive of neoplasia and reported as abnormal cervical cytology on Pap smear test results were subjected to colposcopy screening. From the aceto-white lesions, cervical cell samples were collected using cytobrush (BD surePathTM) in the HiViral Transport medium and followed by colposcopy directed biopsy samples were collected in 0.1M Phosphate Buffered Saline (PBS).

2.2 DNA extraction
Cervical cells and cervical biopsies were subjected to DNA extraction using QIAamp DNA Mini Kit according to manufacturer's protocol. Aliquots of 200µl of cervical cell samples were digested with 20 µl of proteinase K and 200 µl of AL buffer at 56°C. DNA precipitation was done by adding 200µl of AE buffer and the extracted DNA was stored at -20°C further use for the study. DNA quantity and quality was

checked by electrophoresing on 1% agarose gel stained with ethidium bromide.

2.3 β-globin PCR (Internal control)

As an internal control of the PCR reaction, DNA samples were subjected to β-globin gene amplification, using HMBB01, PCO4 and GH20 [5]. PCR amplification conditions were as follows: Initial denaturation at 94°C for 10 mins followed by 35 cycles of 94°C (30 sec), 55°C (30 sec) and 72°C (1 min) and final extension at 72°C for 7 min. PCR products were run on 1.5% agarose gel stained with ethidium bromide.

2.4 HPV DNA detection by MY09/11 PCR

MY09/MY11oligonucleotide primers [6] were used in this study. MY09/11 PCR amplification protocol was followed as described earlier with minor modification in the amplification reaction time [5]. The reaction was carried out in a volume of 25µl. The reaction mixture contains 125ng of each sense and antisense primers, 0.2mM dNTPs, PCR buffer, 2.5µl of sample DNA (100ng) and the final volume was adjusted with sterile MilliQ water. Positive and negative controls were also included in the amplification run. The thermal cycling conditions as per the following protocol:

Table 1: PCR parameters

Step	Temperature	Time	No. of cycles
Initial Denaturation	94°C	10min	1
Denaturation	94°C	1 min	40
Annealing	55°C	30 sec	
Extension	72°C	45 sec	
Final Extension	72°C	7 min	1
Hold	4°C	Indefinite	

3. Results

The mean age of the pre-invasive carcinoma women group is 42 years, the majority of the women falls in the range between 27 to 45 years. The integrity of the genomic DNA was assessed by amplifying 248 bp β-globin gene (Fig 1). In this study, the HPV L1 gene (450bp) was amplified using MY09/11 PCR assay. The HPV positivity rate is 83.37% (26/31) (Fig 2).

The overall HPV DNA positivity was found to be 83.37% (26/31). However, 3 out of the 31 brush samples gave negative results for HPV DNA, which was given positive results in biopsy sample. Therefore, HPV DNA was detected in 83.37% (26/31) of brushings and 74.19% (23/31) of biopsies ($p>0.05$). The agreement between Biopsy vs. Brushing was found to be 98.57%, and K value was 0.9702. The statistical analysis was carried out using MEDCALC online statistical software

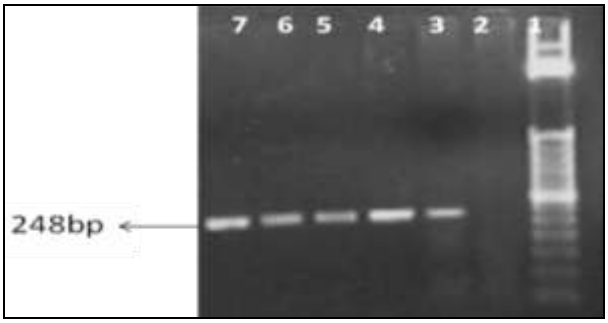

Fig 1: 1.5% gel electrophoresis depicts the amplification of 248bp of β-globin gene. L1: 50 bp ladder and L2: 248bp of β-globin gene.

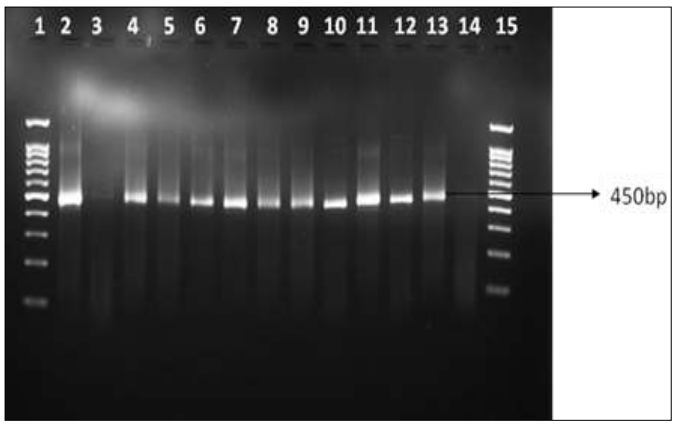

Fig 2: 1.5% gel electrophoresis depicts the amplification of 450bp of HPVL1 gene. L1: 100bp ladder and L2: 450bp of HPVL1 gene

4. Discussion and Conclusion

The success of cervical cancer screening depends highly on the participation of the target population, and screening programs requiring pelvic examination, and colposcopy directed biopsies may pose a barrier. Indeed, women who are not or infrequently screened are known to be at highest risk of developing cervical cancer [7]. Incorporation of non-invasive sampling methods, e.g., cervical cell sampler specimens, might increase the acceptance of testing and optimize screening coverage. However, the clinical validity of cervical cell sampler specimens in pre-invasive carcinoma cases has not yet been well established. The presented work explores the

interchangeability of cervical cell sampler specimens and colposcopy directed biopsies for HPV DNA testing and showed an excellent agreement k value between the both sampling methods.

Colposcopy-biopsy is mostly accepted as diagnostic procedure in cytologic screening abnormalities. The potential strength of cervical cell sampler specimen as a screening sampling method in low grade and high grade squamous intraepithelial lesion cases were explored in our present study. Therefore this sampling modality might be considered in the future cervical cancer screening programs in precancerous lesion cases.

5. Conflict of interest

Authors declare no conflict of interest

6. Acknowledgment

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