

## ORIGINAL ARTICLE

# Comparative Study of Pap Smear Quality by using Ayre's Spatula versus Ayre's Spatula and Cytobrush Combination

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## Abstract

**Background:** This study tried to evaluate the quality of Pap smear samples obtained by two methods Ayre's Spatula and Ayre's spatula and cytobrush combination. **Methods:** The study was conducted in 200 patients, at Yashoda hospital, Hyderabad. For each patient first, ectocervical sampling was done with Ayre's spatula. Cytobrush (Cytobrush<sup>R</sup>Plus) GT, Medscand Medical AB, Sweden) was then introduced into the endocervical canal and smear was obtained. Ayre's spatula smeared on two slides (A & B) followed by cytobrush on B slide. After fixing the slides, they were sent to the Pathology Department, where they were stained with Pap stain and a single cytologist read all the slides. The slides were reported following The Bethesda system (2001) (TBS) of reporting. **Results:** There was a significant difference between the two groups while comparing smear quality 184 (92%) of 200 patients had a satisfactory smear when cytobrush was used along with spatula as compared to 106 patients (53%) when spatula was used. Inadequate smears in the form of absent endocervical cells occurred in 10 (5%) patients when smear was taken using cytobrush-spatula as compared to 94 (47%) when spatula was used alone. Thus there is better sampling of the transformation zone with the cytobrush than the spatula alone. Positive pathology was seen in 11 (5.67%) with use of cytobrush-spatula, 5 (2.6%) when the spatula was used. This was statistically significant, which might be due to better sampling of the transformation zone. **Conclusion:** Cytobrush is an easy instrument to use and is well tolerated by patients, it appears to be more effective than the spatula alone in obtaining evaluable specimens, adequate smears and is far superior to the Ayre's spatula in its ability to obtain endocervical cells.

**Keywords:** Pap smear, Ayre's Spatula, Cytobrush

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## Introduction

Yearly cervical cancer affects 3,70,000 women worldwide and kills 1,90,000 with almost 1,25,000 new cases of cervical cancer in India alone (2001 estimate).<sup>[1]</sup> The incidence of disease in India ranges from 21.8 to 41.2 per 1,00,000 people compared to 7.4 to 11.9 in developed countries (USA, UK, Canada).<sup>[2]</sup> This disparity is the result of effective screening programs adopted in developed countries to bring down the prevalence of invasive disease. To reduce the incidence of cervical cancer in our country we must spread awareness and educate the population about the importance of

cervical screening. Although the Pap smear is considered as the gold standard in cervical cancer screening. The only limitation of the conventional Pap smear is the potential for sampling and preparation errors, which can occur when the cells on the slide are not representative of the condition of the cervix. Only a fraction of the epithelial cells collected on the sampling device are actually deposited on the slide. Theoretically, endocervical element should be excellent marker of accurate sampling of the squamo-columnar junction, resulting in increased sensitivity in screening for cervical carcinoma. Attempts to improve the yield of endocervical cells by various collection

techniques have not eliminated the occurrence of smears without endocervical cells but significant improvement has been reported. Use of both an endocervical brush and a spatula has shown to collect a better sample of cells than either spatula alone, or a spatula used in combination with a cotton-tipped swab. According to the National Cancer Control Program, which was launched in 1984, and FOGSI Recommendations, developing countries like ours should achieve highest possible coverage rate (80%) and target high-risk women between 30-60 years where 90% of cancer occurs.<sup>[1,3]</sup> The American cancer society (ACS) updated recommendations 2002 states that screening with conventional Pap testing every year. If liquid based cytology is being used, screening can be extended to every two years, screening should begin at the age of 21 or within 3 years of onset of sexual activity, and screening can stop at the age of 70 if there has been no abnormal Pap test result in the past 10 years. The ACS also states that screening after of hysterectomy for benign diseases is not necessary<sup>[4]</sup> The "WHO progressive model"<sup>[6]</sup> states that Screen every woman once at the age of 45 years. Once resource permits, expand to screen every woman at 35, 45 and 55 years. Presently we should aim for one Pap smear per woman in her lifetime starting at age 45, we should do it with the best available screening method at hand. Therefore The present study was conducted to test whether the use of cytobrush and Ayre's spatula combination improves sample quality with better pick up rate of atypias than Ayres spatula alone, as it is the routine practice in our institution.

## Materials and Methods

This is a prospective analytical study where the cases were taken from the patients attending the Gynecological Out Patient Department attached to Yashoda Super Specialty Hospital, Malakpet, Hyderabad during the period from September 2010 to September 2012. The patients were selected randomly; all women who were sexually active either of reproductive age group or menopausal were included in the study group. A total of 200 cases were studied.

Sexually active women of reproductive and menopausal age group coming to OPD for Gynecological check up included from the study.

Women with menstruation/active vaginal bleeding, Vaginal infection, Patients with obvious and frank lesions on the cervix, Pregnant women, Use of vaginal cream/pessary/sexual intercourse/douche/tampon within last 24 hours, Women who have undergone total hysterectomy were excluded from the study.

### *Materials used for the procurement of specimens in this study*

1. Ayre's wooden spatula
2. Disposable plastic cytobrush
3. Clean glass slides labeled A and B prior to collection for Ayre's spatula and cytobrush respectively
4. Wide mouthed bottles containing the fixative solution i.e: 95% alcohol
5. Printed formats where clinical information is recorded

### *Pap smear devices used*

#### **Ayre's Spatula**



#### **Cytobrush**



Each prospective patient was asked for the consent in writing and explained regarding the study to be conducted on them. All the procedures conducted were in accordance with Ethical Conduction of Studies on Human subjects. Institutional Ethical Committee permission was obtained for the study. Gynecological and obstetrical history in detail was taken. Information was also recorded regarding marital status, age at first coitus, methods of contraceptive used (if any), History of multiple sexual partners in the patient or the spouse and prior cytological examination if any. After a through general physical examination, patient was put on the examination table in dorsal position and speculum was introduced into the vagina, exposing the cervix. Two samples were taken first using the Ayres spatula and then the cytobrush.

**Technique of Smear Preparation**

|   | <b>Sampling technique</b>   | <b>Smear preparation</b>  |
|---|---|---|
| Ayres spatula   | Rotate 360° on cervix (pointed end in the cervical os)  | Spread the material from both sides of spatula on 2 slides A&B. Fixed by 95% alcohol. |
| Cytobrush (Cytobrush® plus GT Medscand Medical AB Sweden) | Introduce brush into the cervical canal till few bristles are seen outside the OS. Rotate the brush through 180°. | Unroll the brush material on glass slide B. Fixed by 95% alcohol                      |

The slides were reported following The Bethesda system (2001) (TBS) of reporting.<sup>[6]</sup> The rate of adequate smears, presence of endocervical cells, associated benign changes, inflammatory elements and positive pathology were noted in spatula slide and combined slides (spatula + cytobrush), two groups was compared using the Z test for proportions. If it was found necessary then a diagnostic cervical biopsy was followed.

**Results**

As shown in the table 1, there was a significant difference in Pap smear quality between the two collection groups. 184 i.e. (92%) of 200 patients had a satisfactory smear when cytobrush was used with spatula as compared to 106 (53%) patients, when spatula was used alone. This was found to be statistically significant (P<0.005). Inadequate smears in the form of absent endocervical cells occurred in 10 (5%) combined smears, as compared to 94 (47%) of spatula smears, which was also statistically significant (P<0.005). Inadequacy due to hemorrhage was seen in 2 (1%) combined smears while no spatula smears had absent squamous cells or hemorrhage. There was no statistical difference between both the collection groups in number of slides that could not be read and were advised to repeat smear.

After fixing, the slides were sent to the Pathology Department, where they were stained with Pap stain. All slides were read by a single cytopathologist. The spatula slides were read first and presence of endocervical cells, along with pre-invasive or occult lesions noted. Then spatula-cytobrush slides were read for adequacy of cells for endocervical cells, benign changes, and for the presence of other positive pathology.

**TABLE 1: Smear Quality**

|                               | <b>Spatula</b> |          | <b>Combined (brush+spatula)</b> |          |        |
|-------------------------------|----------------|----------|---------------------------------|----------|--------|
|                               | <b>N</b>       | <b>%</b> | <b>N</b>                        | <b>%</b> |        |
| Satisfactory                  | 106            | 53%      | 184                             | 92%      | <0.001 |
| Absent Endocervical cells     | 94             | 47%      | 10                              | 5.0%     | <0.001 |
| Absent Squamous cells         | 0              | 0        | 0                               | 0        |        |
| Repeat smear / unsatisfactory | 8              | 4%       | 4                               | 2%       | > 0.05 |
| Inadequate due to hemorrhage  | 0              | 0        | 2                               | 1%       |        |

**TABLE 2: Epithelial Changes**

|       | <b>Spatula (n=192)</b> |          | <b>Combined (cytobrush+spatula) (n = 194)</b> |          |
|-------|------------------------|----------|---|----------|
|       | <b>N</b>               | <b>%</b> | <b>N</b>                                      | <b>%</b> |
| ASCUS | 0                      | 0        | 0   | 0        |
| AGCUS | 0                      | 0        | 1   | 0.51     |
| LSIL  | 4                      | 2.08     | 8   | 4.12     |
| HSIL  | 1                      | 0.52     | 2   | 1.03     |
| SCC   | 0                      | 0        | 0   | 0        |

As shown in table 2, there was one (0.5%) smear, which had atypical glandular cells of undetermined significance in the cytobrush-spatula smears. Low-grade squamous intraepithelial lesions were seen in 4.12% of smears obtained by cytobrush-spatula compared to 2.08% smears by the Ayre's spatula. High-grade intraepithelial lesions occurred in 1.03%

of the cytobrush-spatula smears compared to 0.52% in the spatula smears.

Positive pathology was seen in 11 of 194(5.67%) patients in the cytobrush-spatula smears as compared to 5 (2.60%) patients in the spatula smears. The difference was found to be statistically significant (P<0.005).

**TABLE 3: Correlation with Biopsy in Combined Smears**

|                                  |   | Normal | Chronic cervicitis | CIN I | CIN II | CIN III | SCC |
|----------------------------------|---|--------|--------------------|-------|--------|---------|-----|
| Normal                           | 0 | 0      | 0                  | 0     | 0      | 0       | 0   |
| Benign changes with inflammation | 0 | 0      | 0                  | 0     | 0      | 0       | 0   |
| LSIL                             | 8 | 0      | 2                  | 3     | 2      | 0       | 1   |
| HSIL                             | 2 | 0      | 0                  | 0     | 0      | 1       | 1   |
| AGCUS                            | 1 | 0      | 0                  | 1     | 0      | 0       | 0   |

**TABLE 4: Correlation with Biopsy in Spatula Smears**

|                                  |  | Normal | Chronic cervicitis | CIN I | CIN II | CIN III | SCC |
|----------------------------------|--|--------|--------------------|-------|--------|---------|-----|
| Normal                           |  | 0      | 0                  | 0     | 0      | 0       | 0   |
| Benign changes into inflammation |  | 0      | 0                  | 0     | 0      | 0       | 0   |
| LSIL                             |  | 4      | 1                  | 1     | 0      | 0       | 1   |
| HSIL                             |  | 1      | 0                  | 0     | 0      | 1       | 0   |
| SCC                              |  | 0      | 0                  | 0     | 0      | 0       | 0   |

Low-grade Squamous Intraepithelial Lesion (LSIL), High-grade Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC), atypical squamous cells of undetermined significance (ASCUS).

A total of 11 biopsies were taken from the patients, who showed dysplastic changes in spatula or cytobrush smears. The above table 5 shows the correlation of biopsy report with that of the combined smears. Among the 8 cases diagnosed to be low grade intraepithelial lesion by cytology, 2 case had benign changes, 3 were CIN I and one case was diagnosed to be squamous cell carcinoma on histology. In the 2 cases found to be of high grade squamous intraepithelial lesion, 1 were CIN III, while one was a case of SCC on histology.

Low-grade Squamous Intraepithelial Lesion (LSIL), High-grade Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC)

Table 4 shows the correlation of biopsy, with that of the spatula smears, in the same 5 cases, where biopsy was taken. Among the 4 cases

diagnosed to be low grade intraepithelial lesions, 1 was CIN I, 1 was squamous cell carcinoma, and 2 were benign. Out of the 1 case that were diagnosed to be of high grade intraepithelial neoplasia, was found to be CIN III. As shown biopsy correlated with cytology finding of positive pathology in 27% of spatula smears as compared to 81% in the combined smears, thus it showed a significant difference for both the collection groups.

## Discussion

The present study was conducted to see if cytobrush and Ayre's spatula is better than Ayre's spatula alone, for cervical cytological screening by assessing the adequacy of smears and the likelihood of detecting abnormal cells. 200 patients reporting to the gynecology OPD with various complaints were included in the study. According to Bethesda Classification 2001 [6] satisfactory smear is presence of approximately 8000 to 12000 well-preserved, well-visualized squamous cells and presence or absence of endocervical cells/ transformation zone component to be noted. In the present

study significantly more number of satisfactory smears were obtained with combined (cytobrush + spatula) (92%) compared to the Ayre's spatula (53%).

Tewari et al in their study found 57 % satisfactory smears with cytobrush compared to 22% with the Ayre's spatula.<sup>[7]</sup> They also found absent endocervical cells in 36% smears in cytobrush group and 75% in spatula group. They concluded that of all the devices studied endocervical brush is better for sampling the endocervix and gives a better quality of smears. A similar result has been obtained from the present study. In the present study we found that 95% of cytobrush smears were adequate and only 47% smears from the spatula were found to be satisfactory. In a similar study by Buntinx et al;<sup>[8]</sup> satisfactory smear were found to be 95.3% in the cytobrush samples and 83.8% in the spatula smears, shows a gain of 12%. They also had absent endocervical cells in 2.70% of cytobrush smears and 16.76 % of the spatula smears. In our study we found inadequate smears in the form of absent endocervical cells in 10 (5%) of cytobrush smears compared to 94 (47%) smears taken by the spatula.

In a study by Rea Rammou-Kinia et al; compared spatula and non spatula methods for cervical sampling satisfactory smears were found to be 99.1% with cytobrush compared to 16.5% with the spatula. They also found absent endocervical cells in 0.9% of cytobrush smears and 59.6% of spatula smears.<sup>[9]</sup> In a study by Boon M et al 0.20% smears in the cytobrush group had no endocervical cells compared to 7.86% of spatula smears.<sup>[10]</sup> Although there can be several factors that could have been affecting the smear that includes the technique and skill of the clinician and Technical support staff. However most studies including this show that the use of cytobrush has some positive impact on improving adequacy rates for pap smears.

In the present study, positive pathology was seen in 11 cases (5.67%) among the smears taken by cytobrush and spatula compared to 5 cases (2.6%) in the spatula smears, and this was statistically significant. Rea Rammou-Kinia et al;<sup>[9]</sup> concluded that the cytobrush was able to sample immature lesions located higher in the endocervical canal and were able to detect abnormalities of columnar epithelium. In their study positive pathology was found to be 4% in

the cytobrush group and 3.8 % in the spatula group. In our study the rate of low grade intraepithelial changes was 4.12% (8 of 194) in the cytobrush-spatula smears as compared to 2.08 % (4 of 192) in the spatula smears. The rate of high grade intraepithelial changes was 1.03 % (2 of 194) in cytobrush-spatula smears and 0.52 % (1 of 192) in spatula smears. This increase, in the pickup rate by the cytobrush, could have been due to better sampling of the endocervical canal and the transformation zone.

Boon et al<sup>[10]</sup> reported a randomized prospective trial in which the rate of mild dysplasia or worse was 0.7 % (29 of 4142) for the cytobrush and spatula group compared to 0.57% (52 of 9140) for the spatula group. Though not statistically significant there was a gain in the pickup rate for cytobrush group. In the same study, the rate of severe dysplasia was 0.22% (9 of 4142) for smears obtained by spatula plus cytobrush and 0.13% (12 of 9140) for smears obtained by Ayre's spatula. Presence of no correlation was noted in both Low Grade Squamous Intraepithelial Lesions (LSIL) and High grade Squamous Intraepithelial Lesions (HSIL) groups. The biopsies from the LSIL smears, showed a wide range of histological results, irrespective of the collection device. The disparity between the smears with HSIL and the corresponding histological result was less in HSIL group.

It has been demonstrated that the combined use of the brush and spatula yields better results than use of spatula alone. The better yield and preservation of endocervical cells seen with the cytobrush can be attributed to the fact that the instrument is better suited for the endocervical canal, and assures a better sampling of the transformation zone. The best way to carry a cervical sampling is to begin with the ectocervical smear using the spatula and end with endocervical sampling with the cytobrush. In the present study two smears were obtained from the same patient. This allowed us to exactly assess the contribution of the cytobrush in the detection of preinvasive lesions of the cervix, which was found to be significant. The cytobrush is an easy instrument to use and is well tolerated by patients. It appears to be more effective than spatula alone in obtaining cytology specimens. Women with stenotic cervical os especially in post menopausal, the

ability of cytobrush to collapse to Imm helps to pick up endocervical elements.

### **Conclusion**

To conclude, the cytobrush is an easy instrument to use and is well tolerated by patients, it appears to be more effective than the spatula alone in obtaining valuable specimens, adequate smears and it is far superior to the Ayre's spatula in its ability to obtain endocervical cells. Added advantage of cytobrush is in postmenopausal woman and those having stenoticos where cytobrush could be easily used. The only concern is cost-effectiveness of the cytobrush which has to be worked out before implementing the cytobrush-spatula in routine cancer screening program.

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