ORIGINAL ARTICLE

RANDOMISED COMPARISON OF THREE PAP SMEAR COLLECTION METHODS

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ABSTRACT: INTRODUCTION: Worldwide, According to statistics every year 8,000,000 new cases of cancer are diagnosed and about 5,000,000 people die from the disease. The early diagnosis will play a great role in reducing the mortality and morbidity. India being a developing country, an early and low cost screening procedure with good detection rate will play great a role in reducing the mortality and morbidity. Cytology is the time tested procedure in this screening modality. **AIM:** The purpose of the study is to compare smear quality and endo-cervical cells recovery of three Papanicolau smear collecting devices and to determine which method produces the highest quality smear. **MATERIALS AND METHODS:** It is a prospective study for a period of 18 months conducted at Vanivilas hospital and Bowring and lady Curzon hospitals attached to Bangalore Medical College. 300 women were chosen among perimenopausal, pregnant, and postmenopausal both symptomatic and asymptomatic women attending the OPD, were randomized to be tested with one of the 3 methods of PAP smear examination each group with 100 patients. Smears were reported by Bethesda method, compared for adequacy, inflammatory pattern, infection, presence of endocervical cells etc. Analysis was done with chi square test. **RESULTS**: 55% were in the age group of 20-25 years. 43% presented with leucorrhoea. 36% cases had healthy cervix and 27% showed cervical erosion. Adequacy was best with cytobrush and spatula. Comparison of adequacy showed a significant p value with cytobrush and spatula. 13% smears showed trichomoniasis. Ayre's spatula alone was not an ideal method as endocervical cell presence is least with this. **CONCLUSION**: Use of cytobrush + Ayre spatula in routine pap smear screening is effective in obtaining adequate smears when compared to smears taken with cotton swab + Ayre spatula or spatula alone. Keeping in mind that ours is a developing country with financial problems, cotton swab along wuthAyre spatula may be recommended for improving percentage of adequate smears. However to reduce the percentage of false negative smears cytobrush along with Ayre spatula should be used.

KEYWORDS: Cancer screening, PAP smear, Ayre's spatula, Endocervical cells.

INTRODUCTION: Worldwide, According to statistics every year 8,000,000 new cases of cancer are diagnosed and about 5,000,000 people die from the disease. Out of all cervical cancers seen in the world 14% occur in the developed countries and 86% occur in developing countries. The early diagnosis will play a great role in reducing the mortality and morbidity associated. India being a developing country, an early and low cost screening procedure with good detection rate will play great a role in reducing the mortality and morbidity. One such method is cytologic examination.

The cytologic examination has been regarded for many years as a highly accurate method to able to detect cervical epithelial atypias. However, several authors have reported high rates of

false negative examinations. The rate of false negative examination could vary between 12 to 69%.

In reports concerning the standard of adequacy of the cytologic examination two main sources have been considered. One related to adequacy of cell collection by gynaecologists and other connected with screening by the cytopathologist.

False negative rates are increased when an exocervical scrape or endocervical swab is used alone. Paired simultaneous sampling decreases the false negative rate. Many studies have demonstrated that the endocervical brush obtains samples that contain endocervical cells more effectively than spatulas or cotton tipped swabs.

MATERIALS AND METHODS:

Source of data: the patients attending OPD, Department of OBG, VaniVilas Hospital and Bowring and Lady Curzon hospital, attached to Bangalore Medical college, Bangalore.

INCLUSION CRITERIA:

1. Patients in the reproductive age group, perimenopausal and menopausal group both symptomatic and asymptomatic patients.
2. Even pregnant patients are included.

EXCLUSION CRITERIA: Acute cervical infections, patients after total hysterectomy, women with invasive cancer cervix. Patients who have been previously treated for cervical cancer.

After considering the inclusion and exclusion criteria, a total of 300 patients were randomised to be tested with one of three cervical cytologic sampling devices. All cases were subjected to history taking, physical examination was done and later pap smear was taken.

1. In the first group of 100 patients pap smear was taken with cytobrush and Ayre's spatula. Per speculum examination with moist sterile Sims speculum was done with good source of light. Two smears were taken- a) from endocerviacl canal with cytobrush: cytobrush gently inserted into the endocervical canal, rotated 360 degrees. The samples applied to the slide with a paint stroke of each side of the bristles.
   b) From transformation zone with Ayres spatula which was rotated circumferentially over the transformation zone. The sample applied to the slide from both sides of spatula.
2. In the second group of 100 patients pap smear done with sterile cotton swab dipped in saline from the endocervical canal. and ectocervix with Ayre's spatula
3. In third group of 100 patients, pap smear done with Ayre's spatula from the ectocervix, single slide.

   Smears were immediately fixed in the fixative, 95% ethyl alcohol. All the smears were evaluated by the same cytopathologist who was blinded to the sampling device.

The reporting was done according to Bethesda classification. The smears which showed adequate number of endocervical cells and not obscured by blood or air drying were reported as adequate smears. The smears showing few endocervical cells, obscured by blood or inflammatory

cells were reported as marginal smears. While those showing no endocervical cells, too thick or obscured by blood or inflammatory calls were reported as inadequate smears.

OBSERVATIONS AND RESULTS: Table 1 shows the age wise smears taken in the study. Majority of patients are between 15-30 years 55%, next group is 31-40 years i.e, 28.7%, followed by 41-50 years i.e., 14% and more than 50 years i.e., 2.3%. Not much of significance could be attached to this information in the study.

Age group(in years)	Cytobrush + Spatula	Spatula	Cotton Swab +spatula	%
<20	5	4	9	55
21-30	51	51	45	
31-40	28	30	28	28.7
41-50	14	14	14	14
>50	2	1	4	2.3

Table 1: Distribution of patients according to age

The symptoms with which these patients presented to the OPD were analysed and the observations have been shown in Table 2. Leucorrhea was the commonest complaint (42.6%) next was pain abdomen with leucorrhea (27.3%) followed by menstrual irregularities with leucorrhea (15%). All the three groups were found to be homogenous with respect to complaints as the p value was not significant.

complaints	Cytobrush + Spatula	Spatula	Cotton Swab +spatula	%
Leucorrhea	36	45	47	42.6
Pain abd +leucorrhea	33	28	21	27.5
Menstrual irregularity +leucorrhea	17	18	10	15
Mass p v	1	1	2	1.3
Mass p a	1	-	2	1
Low back ache	4	4	6	4.8
Secondary amen	-	-	-	-
Secondary infertility	1	2	2	1.6
asymptomatic	7	2	10	6.5

Table 2: distribution according to presenting complaints

When these patients were subjected to physical examination the following observations were made. This is presented in Table 3. Healthy looking cervix in 36.4%, followed by erosion 27.3% and hypertrophy in 24.4%. Not much of a difference was observed in the three groups.

Findings	Cytobrush + Spatula	Spatula	Cotton Swab +spatula	%
Healthy	36	37	36	36.4
Erosion	25	27	30	27.3
Hypertrophy+ erosion	31	22	20	24.4
Bleeding on touch	4	4	3	3.6
Cervicitis	3	8	10	7
prolapse	1	2	1	1.3

Table 3: per speculum findings

The reports were analysed for adequacy it was noted that number of adequate smear was higher in cytobrush + spatula (83%) and cotton swab+spatula (74%) compared to ayre's spatula alone. This is shown in Table 4.

	Cytobrush + Spatula	Spatula	Cotton Swab +spatula
Adequate	83	68	74
Marginal	8	10	14
inadequate	9	22	12

Table 4: comparison of pap smear quality by three collecting methods

When compared pairwise, cytobrush+spatula vs spatula alone p value was 0.027 and was significant. The other group comparisons were not showing any significant difference. The comparison and p value is shown in Table 5.

Groups compared	X^2	df	P
Cytobrush + Spatula versus spatula	7.163	2	0.027
Cytobrush + Spatula versus cottonswab+ spatula	2.58	2	0.27
Spatula versus cottonswab + spatula	3.86	2	0.14

Table 5: Pair wise comparison of three groups with respect to adequacy

Table 6 shows the reproductive tract infections which are sexually transmitted diagnosed by cervical smear examination Trichomonas vaginalis was diagnosed in 13% and candida albicans in 5% cases. A pair wise comparison was made regarding the presence of endocervical cells in the smears by the 3 methods.

groups	Trichomonial infection	Candida albicans infection
Cytobrush + spatula	8(2.6%_)	3(1%)
Spatula	14(4.6%)	6(2%)
Cotton swab+ spatula	17(5.6%)	6(2%)

Table 1: infections diagnosed by cervical smear examinations

Results are shown in Table 7. When cytobrush+spatula (Device 1) was compared with spatula alone (Device 2), the odd's ratio was 2.29 which means the device 1 is 2.29 times more likely to get endocervical cells.

Devices compared	No of smears		No. of endocervical cells		Odds ratio	95% CI for population odds ratio
	device 1	device 2	device 1	device 2		
Cytobrush + Spatula (device 1) vs Spatula (device 2)	100	100	83	68	2.29	1.82, 2.886,
Cytobrush + Spatula (device 1) vs cotton Swab+spatula (device 2)	100	100	83	74	1.71	1.343, 2.174
Cotton Swab + spatula (device 1) versus Spatula (device 2)	100	100	74	68	1.34	1.106, 1.624

Table 7: presence of endocervical cells

DISCUSSION: as shown by large studies the adequacy of a smear judged by the presence of endocervical cells is very important to achieve higher sensitivity. The number of adequate smear found in the present study is 83% by cytobrush plus spatula as compared to 74% by cotton swab plus Ayer spatula and 68% by Ayre spatula alone. The difference is statistically significant. In a study by Raksha Arora, Surendra Kumar, Habeebullah, JIPMER 63.41% by cotton swab + spatula 64.61% by cytobrush + spatula, 59% by spatula alone. This correlates well with our study. As per their own statement this difference is not statistically significant due to faulty method of spreading the smear on the same slide with the results that many smears were reported as too thick or dry smears.

To avoid the fallacy, in the present study separate slides were taken for ecto and endocervical smears. Hence the number of adequate smears was more.

Though there is significant statistical difference between cytobrush + spatula Vs spatula with regard to adequate smears (83% vs 68%) there is no statistical difference between cytobrush + spatula Vs cotton swab + spatula (83% vs 74%)

As the use of cytobrush is not feasible due to financial constrains for our routine screening of a large no of women, cotton swab along with Ayre spatula may be recommended for the increase in percentage of adequate smears.

In the present study, trichomonas vaginalis was found to be most prevalent i.e, 13%. According to J.S. Mishra et al 3.2%, Sardana et al (1993) 5.1% in Indian population. The infection was associated with premalignant changes in the cervix in 13.5% cases. This should be taken as warning and calls for adequate treatment of the infection to check any risk of malignant transformation of the cells.

So cervical cytology highlights the two fold utility of cytology in picking up the protozoan infection as well as pre-cancerous manifestation in cervical epithelium.

The treatment of vaginal infections seems to be essential before taking pap smear as the no of inflammatory smears is very high 56% with cytobrush + spatula, 52% with spatula, 64% with cotton swab with spatula which could have obscured the visualisation of endocervical cells. J S Mishra et al.study showed a prevalence of trichomoniasis to be 3.2%

Pankaj desai et al study showed the commonest presenting symptom to be painabdomen with leucorrhoea followed by leucorrhoea. The present study found leucorrhoea to be the commonest symptom.

In the diagnosis of cytological abnormalities cytobrush+spatula was found to be the best as shown in the table 8.

Cytology	Cytobrush + Spatula	Spatula	Cotton Swab +spatula
Normal study	12	18	13
INFLAMMTION	56	52	64
ASCUS	6	1	3
LSIL	13	5	7
HSIL	4	2	1

Table 8: cytological examination findings

CONCLUSION:

1. Use of cytobrush + Ayre spatula in routine PAP smear screening is effective in obtaining adequate smears when compared to smears taken with cottonswab + Ayre spatula or spatula alone.
2. According to present study taking PAP smear with Ayre spatula alone, as it used to be done before is not very satisfactory method of collecting endocervical cells. Keeping in mind that ours is a developing country with financial problems, cotton swab along with Ayre's spatula may be recommended for improving percentage of adequate smears. However to reduce the percentage of false negative smears cytobrush along with Ayre spatula should be used.
3. Assessment of endocervical cells (Bethesda system) should be the way to audit the overall quality of a cervical smear screening programme.
4. The presence of vaginal infection increases the number of inadequate smears and there by increases the false negative reports. Hence treatment of vaginal infections is of utmost important before taking PAP smear.

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