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Original Research Article

Comparative study of the cytologic diagnosis, specimen adequacy, sensitivity, and cost effectiveness of liquid-based cytology with that of conventional PAP tests

Manickadevi M. S.[1]*, S. V. Hemalatha[2], Thangamani M. [1]

[1]Department of Obstetrics and Gynecology, Stanley Medical College, Chennai, Tamil Nadu, India
[2]Department of Obstetrics and Gynaecology, Chengelpet Medical College, Tamil Nadu, India

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***Correspondence:**
Dr. Manickadevi M. S.,
E-mail: harishma75@gmail.com

ABSTRACT

Background: Cervical cancer is the most common cancer among women in many developing countries constituting 20-30% of female cancers. In the developed western countries, it accounts for only 4-6% of female cancers. This difference largely reflects the impact of mass screening using cervical cytologic methods.
Methods: Prospective observational study.300 patients were selected from the gynaecology OPD of Government RSRM lying In Hospital. A detailed history was taken and they were informed about the screening procedures and counselling given regarding the necessity for their participation in screening program. Both conventional pap and liquid-based cytology were performed in the same patient and results were analyzed.
Results: Out of 300 patients screened for cancer cervix with conventional Pap and liquid-based cytology in the same patient, LBC detection rate of cervical abnormalities and HSIL was higher than that of CP. False -ve rate of LBC was only 2%. Sensitivity is 97% and false positive rate is 44%. The sensitivity is equal to that of the gold standard method. Infection detection rate increased with LBC. Specimen adequacy improved with LBC substantially. The collection sample device brush was found superior to the wooden Ayre's spatula in both not injuring tissues and specimen collection. The adverse impact on a woman's quality of life caused by unnecessary repeat smears and possible investigations would reduce the cost with LBC. The economic evidence suggests that LBC screening every 3 years or longer may be cost-effective. LBC reduced the pressure on a skilled workforce.
Conclusions: As stated in WHO press release, dated 11 October 2001 it can be concluded through the current study that we should aim to divert our resources to screening and treating the high-risk groups of women. The effort once or twice in their lifetime will reduce the incidence of cervical cancer by 50%.

Keywords: PAP smear, Liquid based cytology, Cervical cancer

INTRODUCTION

Cervical cancer is the fourth most common cancer among women globally, with an estimated 604 000 new cases and 342 000 deaths in 2020. About 90% of the new cases and deaths worldwide in 2020 occurred in low- and middle-income countries.[1] In the developed western countries, it accounts for only 4-6% of female cancers. This difference largely reflects the impact of mass screening using cervical cytologic methods.

The purpose of cytologic screening is to identify those women who have intraepithelial lesion (CIN) and not those who have cancer. If disease can be detected & treated at this preinvasive stage, outcome should significantly improve.

Aim and objectives

Aim and objectives of current study were (1) to compare the cytologic diagnosis, specimen adequacy, sensitivity, and cost effectiveness of liquid-based cytology with that of conventional pap tests, (2) to correlate diagnostic colposcopy with histopathology in patients with abnormal cytology reports and (3) to study the accuracy of combined screening procedures.

METHODS

Study design, location and duration

Current study was a prospective observational study conducted at Government RSRM hospital from October 2020 to October 2021.

Selection of cases

A random selection of 300 patients attending as outpatient in gynaecology department of Government RSRM Hospital, Chennai during the year 2020-2021.

Inclusion and exclusion criteria

Women who are sexually active, including post-menopausal women and with no previous screening test taken were included in the study. Antenatal and postnatal mothers, previous screening test taken, proven preinvasive lesions and on treatment, menstruating women were excluded from the study.

Procedure

A detailed history of the patient taken, and the patient informed about the screening procedures and counselling given regarding the necessity for their participation in screening program. Both conventional PAP and liquid-based cytology were performed in the same patient.

PAP smear

Materials: Cusco's bivalved self-retaining speculum nulliparous; 28 mm, post menopause; 28mm, multiparous -36mm, light source, Ayre's spatula, glass slide, marker pencil, sterile glove and Cytofix spray. Method: patient was put in lithotomy position and a suitable size Cusco's speculum was introduced without lubricant. Cervical smear taken with Ayre's spatula rotating it through 360° over the squamocolumnar junction. The smear was evenly spread over the glass slide, marked with the pap smear number for that patient and immediately spray fixed.

Liquid based cytology

Materials: Cusco's bivalved self-retaining speculum, light source, cervical brush, vial with 6 ml of methanol based preservative solution and sterile glove. After conventional PAP is taken, the cervical brush is inserted into the cervical canal. Some bristles should still be visible. If inserted too far, there may be inadvertent sampling of the lower uterine segment (LUS), which causes diagnostic difficulties because its epithelium resembles HSIL and adenocarcinoma in situ (AIS). A brush is used to take both ecto and endo-cervical sampling. The brush is rotated in the canal 180 degrees to limit bleeding and to increase specimen adequacy. A full rotation is unnecessary because the circumferential bristles are in contact with the entire surface the moment the brush is inserted. The brush is immediately rinsed in to 6 ml of vial containing preservative fluid and capped. The slide and the vial are labelled and sent to the laboratory. The conventional smear-containing slide is stained directly by the Papanicolaou staining method.

Lab processing

Cytospin is a cytocentrifuge that concentrates only cells in the liquid sample onto the slide ·The supernatant fluid containing no cells and obscuring material like blood and mucus is absorbed by the filter card. The cytospin is operated and the cell spin rotor is subjected to 3,000 rpm for 10 minutes. The slide with the resulting cell sample is now taken from the cell clip and stained with the routine Papanicolaou stain. Patients who were found to have abnormal cytology reports were subjected to colposcopy-directed biopsy.

Cell staining

Consistency and reliability in staining are the cornerstones of cytological interpretation. The universal stain for cytological preparation is the Papanicolaou stain. Harris haematoxylin is the optimum nuclear stain and the combination of 0G6 and EA gives the subtle range of green, blue, and pink hues to the cell cytoplasm.

Microscopic appearance

Nucleus- blue colour, cytoplasm of superficial cells - pink, intermediate cell bluish green, non-keratinizing squamous cells - blue/green and keratinizing cells - pink/orange

Colposcopy

Materials: colposcope, bivalved cusco speculum, cotton tipped swabs, sterile glove, normal saline, 3% acetic acid, Lugol's iodine, examination table with height adjustment, the magnification is 13.5 times. With the patients in lithotomy position, the colposcope was positioned and the objective lens adjusted to bring the cervix into focus. The cervix and vagina were cleaned with a cotton swab with normal saline. The cervix was inspected for lesions such as leucoplakia, carcinoma, and viral condylomata. Then a solution of 3% acetic acid was liberally applied over the cervix. The solution is mucolytic and changes the colour and vascular pattern after an internal of 10-30 seconds.

The cervix was inspected for colour, surface, columnar epithelium, transforming zone, SCJ, and vascular pattern. The vascular pattern was again studied using a green filter. Schiller's iodine test was done for patients with suspicious lesions. The special symbols for the different colposcopical patterns are used by the colposcopist to document the clsposcopic findings that imitate as closely as possible the picture observed in the colposcope. The two recording systems in vogue are: Odell diagram colposcopic lesions may be represented in a circular diagram in relation to the OS and modified Hammond's graph of the cervix. It consists of 3 concentric circles with 12 radial lesions in a clockwise fashion. The innermost represent endocervix, the intermediate one is the transformation zone, and the outermost is the ectocervix.

Biopsy cervix

Materials: sterile glove, vulsellum, light source, tischler biopsy forceps and container with 10% formalin**.** The biopsy material was fixed with 10% formalin immediately. The fixed material was then imbedded in wax for section cutting. The sections were then subjected to haemotoxylin and eosin staining to read the changes in the squamous lining in respect of inflammation, ulceration, dysplasia, CIS, micro invasive carcinoma, and invasive carcinoma. The management of abnormal lesions is finally dependent upon the histopathological diagnosis. Biopsies were taken from iodine-negative areas or areas of atypical colposcopic findings. All biopsies were done under colposcopic guidance.

Statistical analysis

Statistical analysis was done utilizing Microsoft Excel and SPSS statistical software.

RESULTS

The numbers of cases screened were more in the age group of 31-40 i.e. 50.3%. Total 61% of the cases screened were multipara ≥3. Total 57.7% of cases screened has their main complaint as white discharge PV. The next common complaint was urinary tract infection and lower abdominal pain. Total 46.7% of cases screened had cervical erosion and 33.3% had normal cervix with speculum examination. About 70.4% cases screened were normal in local examination. 18.6% of cases had discharge. LBC detected LSIL in 10.7% of cases, HSIL in 4.7% of cases and Cancer in 2% of cases. PAP smear detected LSIL 8n 10% of cases, HSIL in 1.7% of cases and cancer in 0.3% of cases 56.7 percent of PAP smear specimen and 96.7 percent of LBC specimens were adequate for examination. Low grade squamous intraepithelial neoplasia (mild dysplasia), out of 32 cases, 20 had cervical changes. LSIL detection rate of LBC in this study was 63%. High grade squamous intraepithelial neoplasia (moderate and severe dysplasia) - out of 14 HSIL cases, 1 was diagnosed as invasive cancer and 1 as LSIL. HSIL detection rate was 85%; total 40% of the study population had normal colposcopic findings and the remaining 60 percent had abnormal findings.

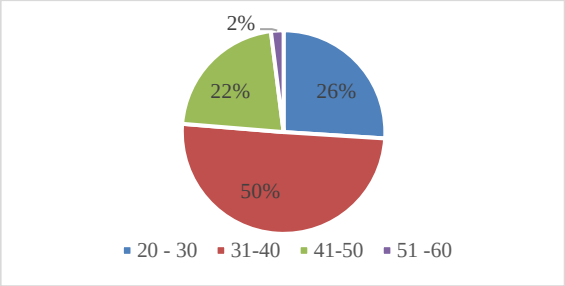

Figure 1: Age distribution of the patients who were screened with cytology.

Table 1: Age distribution of the patients who were screened with cytology.

Age (years)	N	%
20-30	78	26
31-40	151	50.3
41-50	65	21.7
51-60	6	2
Total	300	100
Mean±SD	35.73±7.21	

Table 2: Parity distribution.

Parity	N	%
0-2	117	39
3-5	180	60
>5	3	1
Total	300	100

Table 3: Presenting complaints.

Complaints	N	%
White discharge	173	57.7
UTI	34	11.3
Lower abdominal pain	34	11.3
Low back ache	30	10
Infertility	4	1.3
Mass P/V	11	3.7
Mass P/A	4	1.3
Dysmenorrhea	10	3.4
Total	300	100

DISCUSSION

The present study was conducted to evaluate the efficiency of the liquid-based cytology diagnostic modality in the diagnosis of cervical cancer and to quantify its correlation with the histopathology. In this study, 300 patients were randomly selected, and both conventional pap and liquid-based cytology were applied

to the same women and compared within the subject analysis.

Table 4: Cervical status.

Cervical status	N	%
Normal	100	33.3
Erosion	140	46.7
Hypertrophy	35	11.7
Old cervical tear	25	8.3
Total	300	100

Table 5: Local examination.

Variables	N	%
Normal	211	70.4
Discharge	56	18.6
UV prolapse	9	3
Excoriation	12	4
Wart	7	2.3
Others	5	1.7
Total	300	100

Table 6: Cytologic diagnosis.

Cytology	PAP		LBC	
	N	%	N	%
Normal	64	21.3	232	77.3
Inflammatory smear	200	66.7	16	5.3
Mild dysplasia	30	10	32	10.7
Moderate dysplasia	3	1	3	1
Severe dysplasia	2	0.7	11	3.7
Cancer	1	0.3	6	2
Total	300	100	300	100

The advantage is that many characteristics are equivalent for both tests (including patient attributes, timing in the menstrual cycle, and clinician factors). Within-subject analysis is common in diagnostic accuracy studies for cervical cancer screening because it is relatively simple to collect two samples during a pelvic examination.

Of the total cases examined, 50.3% of them were in the age group of 31-40 years. Total 61% of the cases screened were multiparous women.38% of the cases were illiterate, and 39.3% of the cases had their secondary schooling and above. 45% of the cases screened were from socioeconomic class V, and 97% of the cases were menstruating. 99% of cases in this study followed other than barrier contraception, a main risk factor for cancer cervix in low socioeconomic group.16% of couples had taken treatment for STD. The main complaint among the screened population was white discharge, followed by urinary tract infection and lower abdominal pain.

Diagnostic accuracy

In this study, the LBC method of specimen preparation led to the cytological diagnosis of significantly more cervical abnormalities than the conventional method (52 vs. 36). Depending on the prevalence of the disease in a population, there may be more cases of higher-grade abnormalities such as high grade squamous intraepithelial lesion (HSIL) and cancer, which are important outcomes for a test to detect. LBC technique has detected more high-grade lesions than that of pap (20 vs. 6%). In one of the first studies done by Wilbur and associates, a total of 3218 patients had a single cytologic sample that was split into matched pairs. The inferred false -ve rate was 15% for CP and 4% for LBC. In this study, the false negative rate for CP was 61.6% and for LBC it was 2%. In the study by Lee, there was a 14% difference between the CP and LBC. Roberts and associates did a split sample paired CP & LBC in 35,560 patients and found LBC showed these severe abnormalities in 1194 cases compared with CP.[2] In this study, cases with severe abnormalities had LBC of 6.7%, CP of 2%.

Table 7: Comparison of specimen adequacy of LBC with that of concentional PAP

Variables	PAP		LBC	
	N	%	N	%
Satisfactory	170	56.7	290	96.7
Satisfactory for evaluation but limited by				
Air drying artifact	39	13	1	0.3
Thick smear	37	12.3	4	1.3
Endocervical component absent	14	4.7	2	0.7
Scant squamous epithelial component	12	3.3	2	0.7
Obscuring blood	24	8	1	0.3
No clinical history	-	-	-	-
Cytolysis	2	0.7	-	-
Others	2	0.7	-	-
Unsatisfactory for evaluation				
Air drying artifact	-	-	-	-
Thick smear	-	-	-	-
Endocervical component absent	-	-	-	-
Scant squamous epithelial component	-	-	-	-
Obscuring blood	-	-	-	-
No clinical history	-	-	-	-
Cytolysis	-	-	-	-
Others	-	-		

Infection

The detection of infections was very much increased in the LBC technique. Out of 40 Trichomonas infections detected, 26 cases were detected with LBC. Out of 20 cases with Candida, LBC diagnosed 11 cases.

Specimen adequacy

Specimen adequacy was also improved significantly with the LBC method. The reduction in the number of slides

compromised by obscuring amounts of blood, mucus, inflammation, and air-drying artifacts may significantly reduce the need to repeat the screening procedure. An increase in specimen adequacy is due to the fact that the cervical cells that are removed with a brush are rinsed in a "direct to vial" method. All the cells are collected in the preservative solution immediately, which also reduces the likelihood that a patient cell sample will be damaged by air, clumping, etc. The sample is also subjected to a device where it is spun, and the obscuring materials are absorbed by the filter card. In cases of PAP, the whole sample is not smeared on the slide, and if there is a delay in fixation, the sample will be damaged by air drying artifacts, clumping, etc. A 1994 study published in the American journal of clinical pathology found that up to 80% of a sample taken from a patient using the conventional pap smear technique is not smeared on the slide but remains on the collection device.

Table 8: Cervical changes detected by LBC with that of histology.

Histology						
LBC	**Cervicitis**	**MD**	**MOD**	**SD**	**Ca**	**Total**
MD	12	19	1	-	-	32
MOD	-	1	1	1	-	3
SD	-	-	2	8	1	11
Ca	-	-	-	-	6	6
Total	12	20	4	9	7	52

Table 9: Colposcopy results (n=69).

Grading	N	%
Normal	27	40
I	15	22
II	19	28
III	7	10

Table 10: Comparative analysis with various studies.

Authors	Sensitivity %		Specificity %	
	LBC	**PAP**	**LBC**	**PAP**
Sulik et al (2001)	70	79	85	89
Bergeron (2001)[4]	66	61	9.6	11
Park (2001)	45	49	27	23
Bishop (1998)[5]	89	78	44	17
Bolick (1998)[6]	95	85	58	36
Ferris (2000)[7]	52	63	99	99
Brown & Garber (1999)[8]	93	82	96	96
Maxwell (2003)[9]	67	60	97	97
Nice (2003)	67	60	97	97
Present study	97	38	56	93

Collection device

As the pap smear has been taken with a wooden spatula, it is thought to reduce the sensitivity because of the absorptive properties of wood. In the case of LBC, a brush has shown a significant difference in the quality of the sample prepared. There is evidence that the device used to collect cervical cell specimens plays a significant role in determining the sensitivity and specificity of the test (Austin and Ramsey 1998). According to the ASCCP guidelines, the increased sensitivity of the brush makes it the preferred instrument for cervical sampling. As spatula is used for pap, sometimes scarring of the cervix, mucosal tears may happen, and the blood is also collected on the slide, making it difficult to read the slide. The transformation zone as the age advances, goes deeper into the cervical canal, for which a brush will be a better option than a spatula.

Cost effectiveness

Even though cost estimation per woman in LBC has increased, the number of inflammatory smears detected in pap has been shown to be normal in LBC (66.7% vs 5.3%). This will reduce the treatment cost as well as the repeat smear cost after treatment for those with inflammatory smears. The economic evidence of trial studies suggests that LBC screening every 3 years or longer after a normal test may be cost-effective compared to pap smear screening every year. As the number of prior normal LBC tests increases, the cost per life year saved will increase substantially. The smaller screening area on the microscope slide and the clarity of the LBC specimen increase the ease of screening.

Results of LBC with that of histology

Detection of invasive or metastatic cancer is considered to be the gold standard. A longitudinal study is best because of the time, expense, and difficulty involved with a longitudinal study, an acceptable surrogate is histology. A clinical diagnosis is based on biopsy confirmation. The evaluation of women with negative test results is considered invasive and costly. As a result, the use of colposcopy and histological follow-up is limited to women with positive diagnoses identified by LBC and PAP tests.

Out of 32 cases in LBC, 19 cases were confirmed by histology as mild dysplasia and 12 cases as chronic cervicitis. 1 case in LBC was diagnosed as moderate dysplasia. Out of 6 cases, 6 were diagnosed as invasive cancer. HSIL out of 14 cases, 1 went as mild dysplasia (LSIL) and 1 as invasive cancer. Duration of marriage had a strong impact on development of cancer cervix. The patients with severe dysplasia and cancer cervix had a longer duration of marriage (>20 years). Those patients with normal cytology had a shorter duration of marriage, less than 10 years.

Treated for STD

Out of 48 couples treated for STD, 13 women had cervical changes in LBC. This shows the impact of STD in the etiology of cancer cervix.

Age distribution

The maximum incidence of cancer cervix is in the age group of 35-64 (ASRW 57.4) (MMTR Chennai, 2002). In this study, patients with HSIL were in the age group of 31-40. This shows the incidence of cancer cervix has shifted to a lower age group. The average age of first coitus was 17 years for cases with cervical changes. This shows that the early age of sexual activity has a strong impact. Of two post-menopausal women with cervical changes, one was diagnosed as severe dysplasia and the other as mild dysplasia. 53% of cases with cervical changes belong to socio economic class V.

Extra/premarital contact (women)

Out of 8 cases, 1 was diagnosed with mild dysplasia, 4 were normal, 2 as severe dysplasia, and 1 as moderate dysplasia by histology.

Result of colposcopy vs. histology

Out of 68 cases of colposcopy done, 27 were normal, of which 3 were diagnosed as LSL and 5 as HSIL. Grade I (insignificant, not suspicious), total 15 cases, 7 cases were LSIL. Grade II (significant, suspicious), total 19 cases, 11 cases were diagnosed as LSIL and 4 cases as HSIL and 4 cases as invasive cancer. Grade III (highly significant, highly suspicious), total 7 cases, 4 cases as HSIL and 3 cases as cancer. The diagnostic accuracy of CIN detected by colposcopy in Present study was 80%. The frequency of cancer detection by colposcopy was 11.3% by Brentran.[3] In the present series the frequency of cancer detection was 10.3%.

Limitations

Cost of LBC was the major limiting factor for the study. Follow up of the patients and the need for further Colposcopy was assessed on a patient-to-patient basis.

CONCLUSION

As stated in WHO press release, dated 11 October 2001 it can be concluded through current study that We should aim to divert our resources to screening and treating the high-risk groups of women. The effort once or twice in their lifetime will reduce the incidence of cervical cancer by 50%. In this scenario, mass screening with LBC, which has a high detection rate for HSIL and a colposcope for abnormal smears, offers a potent method of rapid assessment of any dysplasia possibly present.

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