Research Article

COMPARATIVE STUDY OF LIQUID BASED CYTOLOGY (LBC) AND COLPOSCOPY FOR DETECTION OF PREMALIGNANT LESIONS OF CERVIX

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ABSTRACT

Background: To compare and analyze Liquid Based Cytology (LBC) with colposcopy in detection of premalignant lesions of cervix.

Material and methods: A randomized, observational, prospective cohort study was conducted in the Department of Obstetrics and Gynecology, Government Medical College, Patiala. 324 females were screened, 18 withdrew their consent and 6 dropped out as they fail to turn up during the later stages of study. Finally, N=300 patients were enrolled who fulfilled both inclusion and exclusion criteria. All patients were aged more than 18 years and complained of discharge per vaginum, post-coital bleeding, post-menopausal bleeding, intermenstrual or abnormal bleeding per vaginum. These patients were subjected to LBC and Colposcopy screening. However, on detection of any premalignant lesions of cervix, cervical biopsy was taken for confirmation by histopathology.

Results: Out of 73 cases detected positive by LBC, 29 cases were true positive (TP) while 44 cases were false positive (FP). 54 cases were true negative (TN) and 18 cases were false negative (FN) with accuracy of 57.24%. In colposcopy, 38 cases were TP, 3 cases were FP, 95 cases were TN and 9 cases were FN making the colposcopy 91.72 % accurate. The relationship of sensitivity results between LBC and colposcopy was p value of 0.000 whereas between LBC and Histopathological examination (HPE) was p<0.01. The relationship was comparable at p value of 0.103 between colposcopy and HPE, which was non-significant.

Conclusions: The study concluded that colposcopy to be better modality than LBC for early detection of premalignant lesions of cervix. The sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy of colposcopywas 80.85%, 96.93%, 92.68%, 91.34% and 91.72% as compared to LBC with 61.70%, 55.10%, 39.72%, 75% and 57.24% respectively.

INTRODUCTION

Cervical cancer has an uneven geographical distribution. Globally, it is the second most common cause of cancer among women after breast cancer.[1] Nearly 5 lakhs new cases of invasive cervical cancer are diagnosed annually worldwide and 80% of them occur in developing countries.[2] In India, cervical cancer is the commonest cancer among women and a largest burden to the world. Out of every 5 women, one patient suffers from cervical cancer, which belongs to Indian subcontinent. World Health Organization (W.H.O) study showed that amongst 1.3 lakhs women in India with a diagnosed cervical cancer, approximately 74,000 die every year.[3] The majority of cervical cancers are caused by infections with a specific subtypes of human papilloma virus (HPV), which is a sexually transmitted virus that infects cervical cells and may result in pre-cancerous lesions and invasive cancers.[4] More than 80% of cases are diagnosed at an advanced clinical stage of cervical cancer.[5] Cervical cancer is proceeded by preinvasive and early invasive stage for 10-15 years. Therefore, if preinvasive or early invasive lesions are detected at an earlier stage by screening method then they can be treated adequately. In developed countries, the initiation and sustenance of any cervical cytology programme involves screening of sexually active women once in 3-5 years. The incidence of cervical cancer can be reduced by 80% if the quality, coverage and follow-up of screening is high.[6] There are various screening methods for cervical cancer e.g., PAP smear, LBC and Colposcopy. The present study was an attempt to compare the results of LBC and colposcopy for an early detection of precancerous lesions of carcinoma cervix.

MATERIAL AND METHODS

The present study screened 324 patients, aged above 18 years who reported to Out Patient Department (OPD) of Obstetrics

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And Gynecology, Government Medical College, Patiala from January 2013 to August 2014.18 patients withdrew their consent and 6 patients were lost for follow-up. Finally, N= 300 patients were enrolled for the study. The study had an approval of Ethical Review Committee. Written informed valid consent was taken from all participants. Patient's confidentiality was maintained throughout the study and data was analyzed statistically.

The socio-demographic profiles of the patient were recorded in the proforma during the initial workup. The procedure was fully explained to the patient, questions asked by the patients, were answered and then written consent were taken. All patients were screened for carcinoma of cervix/ cervical intraepithelial neoplasia (CIN) by LBC and Colposcopy.

Inclusion criteria

Women aged more than 18 years with complaints of discharge per vaginum, post-coital bleeding, irregular bleeding per vaginum, post-menopausal bleeding, chronic backache, naked eye abnormality of suspicious cervix and patient with high risk factors like early age of marriage, high parity and multiple partners.

Exclusion criteria

1. Patients with frank growth of cervix.
2. Pregnancy and immediate postpartum period.
3. Acute cervical infection
4. Unmarried women

Techniques of sample collection

LBC: After inserting Cusco's speculum in vagina, area was cleaned with normal saline. Rover's cervix brush was then inserted into endocervical canal and rotated for 5 turns in a clockwise direction gently pushing towards the cervix until shorter bristles begin to bend and extend over the ectocervix. Detachable head of device was then dropped into B.D. sure-path-vial containing preservative (24% ethanol) that serves as a transport, preservative and antibacterial medium for gynecological specimen and the sample then sent to pathology department for examination. The prepared smear were analyzed as per Bathesda system, 2001 for reporting of cervical/vaginal cytological diagnosis.[7]

Colposcopy was performed on day 8-12 of menstrual cycle in pre-menopausal and in post-menopausal women, it was performed on 10-12 days after estrogen therapy. In patients, where any other procedure was done on the cervix, it was performed after 6 weeks. Technique: Patient was laid down in lithotomy position. After introducing self-retaining Cusco's speculum in the vagina, the mucus was swabbed-off with cotton swabs moistened with normal saline. Green filter was used to evaluate the vascular details to detect any findings suggestive of precancerous stage like punctuation, mosaic pattern, irregular branching vessels etc. The cervix was swabbed with a cotton wool soaked in freshly prepared 3% diluted acetic-acid solution for 3 minutes. Epithelium with high nucleo-cytoplasmic ratio turned to white after acetic-acid application. Next, iodine test was performed. Results were interpreted for normal squamous epithelial cells as mahogany brown. The normal columnar epithelium, immature squamous metaplasia, regenerating and inflamed epithelium cells contains very little glycogen and thus, either do not or partially stain with iodine. Findings were noted in Odell's

diagram in which colposcopic lesions were represented in a circular diagram in relation to external os. The results of colposcopy were analyzed as per scoring system proposed by Reid and Scalzi and IARC (Table A and B).

Table A Modified Reid's colposcopic Index- Adapted from Reid R, Scalzi and International Agency for Research in Cancer (IARC) manual [8,9]

Colposcopy Sign	Score 0	Score 1	Score 2
Margin	Condylomatous or micropapillary contour. Floccculated or feathered, jagged, angular, satellite lesion, AWA beyond original squamo-columnar junction.	Regular lesion with smooth indistinct borders.	Rolled, peeling edges, sharp margins.
Colour	Shiny, snow white, areas of faint (semi transparent) whitening.	intermediate shade(Shiny but grey white)	Dull, oyster grey
Vessels	Uniform, fine caliber non dilated capillary loops fine punctuation or mosaic	Absence of surface vessels	Definite,coarse punctuation or mosaic.
Iodine staining	Any lesion staining Mahagonybrown;mustard yellow staining by a minor lesion(by first three criteria).	Partial iodine uptake(mottled pattern)	Mustard Yellow staining of a significant lesion(an acetowhite area scoring 3 or more points by the first three criteria)

Table B Reid's Colposcopic Index /Score [RCI][8]

Score	Colposcopic findings
0-2	Normal colposcopy
3-5	Low grade disease(HPV infection of CINI)
6-8	High grade
>8	Invasive Lesions

Punch/wedge or cone biopsies along with endocervical curettage were taken in LBC and or colposcopy screen positive patients from the areas with worst features. All the specimens were sent in 10% formalin solution to Department of Pathology, Government Medical College, Patiala for histopathological reporting.

Statistical analysis: Data were entered in Microsoft Excel (Microsoft office excel standard edition 2003 Microsoft Corporation) and analyzed using SPSS (Statistical Package for Social Sciences) version 16.0 statistical software. Descriptive analysis was computed in terms of mean and standard deviation for continuous variables and frequency with percentage for ordinal and nominal variables. Prevalence of outcome variables along with 95% confidence interval was calculated. Pearson's x^2-test used to observe and quantify an association between the categorical outcome and different study variables. Student's t-test for independent samples was used to compare the mean values. A P value of less than 0.05 was considered to be statistically significant. LBC and colposcopy were analyzed for comparison, correlation and test of association if any by taking histopathology as gold standard. Sensitivity, specificity, PPV and NPV of both modalities were calculated in reference to histopathology.

RESULTS

The socio-demographic profiles of the patient showed that majority of women (71.33%) were in the age group of 21-40

years. Mean age was 37.28 years with standard deviation±9.59 years. Maximum numbers of cases having CIN (8.33%) were of same age group. 46.34% of study population was Para 2 with mean parity of 2.30 ± standard deviation 1.07. Among the cases with biopsy proven CIN, the majority (6%) belonged to same parity (P2).

The population distribution amongst rural and urban showed that 40.33% belonged to urban area whereas 59.63% belonged to rural area. The incidence of CIN was more in rural population than urban population, being 8.66% and 7% respectively. The education status of the women revealed that 24.67% were illiterate, 65.33% had primary/high school education and 10% had higher education.

Among contraceptive measures, results revealed that 37% were permanently sterilized, 11.66% practiced coitus interruptus, 1.33% used oral contraceptives and 5.67% had intrauterine copper devices insertion. However, 13% of patient did not practice any form of contraception. Furthermore, maximum cases of CIN (8%) were reported in women who were tubectomized which may be attributed to larger number of rural population in study group, who consider permanent sterilization as a convenient method of contraception.

The commonest complaint reported by patients for attending to OPD was discharge per vaginum and pain abdomen i.e., 94.66% and 92% respectively, whereas 10% of women came with complaint of post-coital bleeding and 17.67% had irregular bleeding per vaginum. Maximum numbers of CIN (10.33%) were detected in patients with discharge per vaginum.

Per speculum findings revealed 39.33% subjects with normal looking cervix, chronic cervicitis in 30.33%, hypertrophy in 9.67%, ectropion and erosion in 11.66% and 9% of women had unhealthy looking cervix. Incidence of CIN was maximum (6.66%) in women with unhealthy looking cervix, thus substantiating the role of down staging of carcinoma cervix by simple per speculum examination.
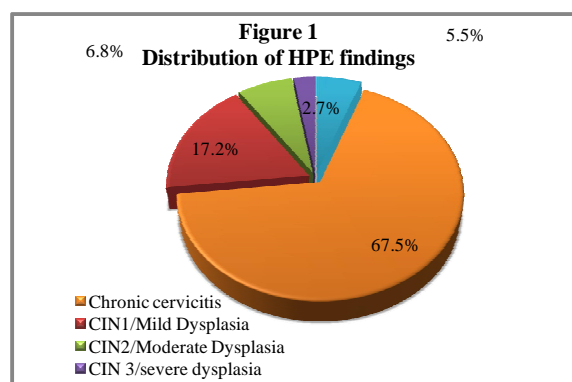
The commonest finding of LBC was inflammatory smear present in 39%, followed by normal smear seen in 36.67% of women. 73 out of 300 smears (24.33%) were abnormal and their distributions are enumerated in table-1. Maximum (4.66%) cases of CIN were detected in smears with ASCUS or ASC-H as LBC result.

Table 1 Distribution of LBC Findings

LBC	No. of Cases (n=300)	%age	CIN cases on biopsy	%age
ANS	110	36.67	6	2.00
Inflammatory	117	**39.00**	12	4.00
ASCUS , ASC-H	39	13.00	14	**4.66**
LSIL, Mild dysplasia	22	7.33	06	2.00
HSIL , Severe dysplasia	11	3.67	09	3.00
Squamous cell carcinoma	01	0.33	00	0
Total	300	100%	47	

ANS: Adequate normal smear; ASCUS: Atypical squamous cells of unspecified significance; ASC-H: Atypical squamous cells, cannot exclude HSIL; LSIL: Low grade squamous intraepithelial lesion; HSIL: High grade squamous intraepithelial lesion

Cervical biopsies were taken in 145 women who had abnormal results of either LBC or colposcopy or both. Pie chart distribution of various histopathological findings is shown in figure 1.

Figure 1 Distribution of HPE findings

Agreement between LBC and biopsy results is depicted in table 2. Number of cases of TP, FP, TN and FN were calculated and results are shown in table 3. Sensitivity, specificity, PPV, NPV and accuracy of LBC in detecting premalignant lesions was then calculated accordingly taking biopsy as reference standard.

Table 2 Agreement between LBC Smear and Biopsy

L B C	HPE					
	Chronic cervicitis	CIN 1 /Mild dysplasia	CIN2/ Moderate dysplasia	CIN3/ Severe dysplasia	Squamous cell carcinoma	Total
Normal, inflammatory (n=72)	54	15	03	-	-	72
ASCUS, ASC-H (n=39)	25	07	03	02	02	39
LSIL(n=22)	16	01	02	01	02	22
HSIL(n=11)	02	02	02	01	04	11
Squamous cell carcinoma(n=1)	01	-	-	-	-	1
Total	98	25	10	4	8	145

Table 3 Evaluation of LBC for CIN

LBC	Biopsy Positive	Biopsy Negative	Total
Positive	29(TP)	44(FP)	73
Negative	18(FN)	54(TN)	72
Total	47	98	145
Chi square value	3.58		
P value	0.001		

Thus our study revealed a low sensitivity and specificity of 61.70% and 55.10% respectively for LBC, missed 18 cases out of 47 biopsy positive cases making this modality 57.24% accurate. PPV was 39.72% and NPV was 75% for this screening method.

A highly significant p value of 0.001 was obtained on chi square test between LBC and HPE, which indicated that the results of both modalities differ significantly.

Results of colposcopic assessment of 300 patients are shown in table 4 with maximum number of CIN cases (9.66%) seen in patients with Reid's scores between 3-5. Agreement between colposcopy and biopsy results done in 145 patients is depicted in table 5.

Table 4 Colposcopy Results

Reid's Score	No. of Cases(n=300)	%age	CIN cases on biopsy	%age
0-2	257	85.66	9	3
3-5	32	10.66	29	9.66
6-8	09	3.00	9	3
Unsatisfactory	02	0.67	0	0
Total	300	100%	47	

Table 5 Agreement between Colposcopy and Biopsy

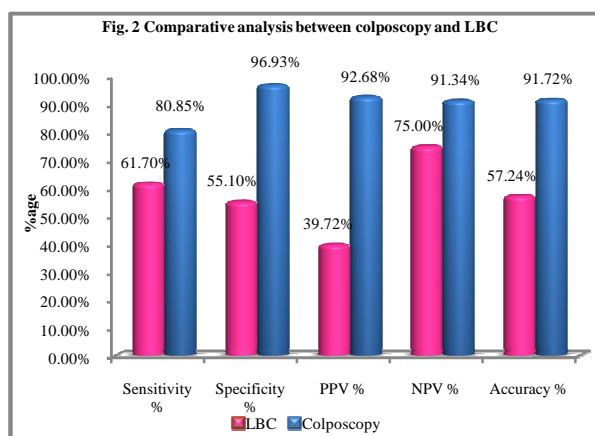| Reid's Score	Chronic cervicitis	CIN1/ Mild dysplasia	CIN2/ Moderate dysplasia	CIN3/ Severe dysplasia	Squamous cell carcinoma	Total
0-2 (n=104)	95	07	01	01	00	104
3-5 (n=32)	03	12	09	03	05	32
6-8 (n=9)	00	06	00	-	03	9
Total	98	25	10	04	08	145

Number of cases of TP, FP, TN and FN were calculated and results are shown in table 6. Sensitivity, specificity, PPV, NPV and accuracy of colposcopy in detecting premalignant lesions was then calculated accordingly taking biopsy as reference standard. A non significant p value of 0.103 was obtained on chi square test, which revealed that results of colposcopy were comparable with that of histopathology

Table 6 Evaluation of Colposcopy for CIN

Reid score	HPE		Total
	Positive	Negative	
Positive	38(TP)	3(FP)	41
Negative	9(FN)	95(TN)	104
Total	47	98	145
Chi square value	94.78		
P value	0.103		

The sensitivity of colposcopy in detecting CIN was high being 80.85%. Specificity, accuracy, PPV, NPV and accuracy were 96.93%, 92.68%, 91.34% and 91.72% respectively.

Comparative Analysis between Colposcopy and LBC

Fig. 2 Comparative analysis between colposcopy and LBC

p value obtained on chi square test was 0.000 which was highly significant and hence findings of LBC were not comparable with colposcopy. Comparison of results of both modalities is shown in figure 2.Thus, Colposcopy showed better results in detecting precancerous lesions in carcinoma cervix as compared to LBC with sensitivity, specificity of 80.85% and 96.93% versus 61.70% and 55.10% respectively. The incidence of preinvasive lesions –LSIL(mild dysplasia) and HSIL(moderate to severe dysplasia) was 17.2% and 9.5% respectively while the incidence of invasive lesion 5.5% was in our study.

DISCUSSION

Cervical cancer is potentially preventable cancer. It is preceded by premalignant lesions which take as many as 5-15 years to progress to invasive cancer. If detected and treated timely, preinvasive disease has nearly 100% cure rate.[10] For cancer screening and diagnosis especially in resource limited settings where screening is opportunistic, one needs a test that

has a high specificity and negative predictive value, and that can guarantee a negative screen or diagnosis for cancer of the cervix.

Our goal in the current study was to compare two screening methods of LBC and colposcopy in symptomatic patients in order to introduce the LBC or colposcopy procedures as the preferred and more accurate method to reduce the need for future visit and or patient biopsy.

The incidence of preinvasive lesions in our study was 26.7% which was parallel to the results by Ashmita *et al* (35.2%)

Performance of LBC as a screening test

The result of LBC was not correlated with the biopsy findings in the present study and the sensitivity and specificity of LBC for detecting all grades of CIN was found to be 61.70% and 55.10%, respectively. Comparison with previous studies that have evaluated LBC is shown in table 7. Sensitivity of LBC for detecting premalignant lesions in our study was comparable to the findings of Karimi-Zarchi *et al* (55.3%) and Arbyn *et al* (57.1%) while it was lower than findings of Beerman *et al* (96.24%) and Ilter *et al* (85-95%)

Table 7 Comparision of Different Studies for Evaluation of LBC

Author and year of Study	Age group (In years)	Sensitivity (%)	Specificity (%)
Karimi-Zarchi et al[11] (2013)	21-70	55.3%	77.7%
Macharia et al[12] (2014)	18-60	13%	92%
Ilter et al[13] (2012)	18-72	85-95%	-
Beerman et al[14] (2009)	30-60	96.24%	97.75%
Arbyn et al[15] (2007)	-	57.1%	97.8%
Present study	20-75	61.70	55.10

Performance of colposcopy

The sensitivity of colposcopy in different studies ranged between 50- 88% and specificity between 42 to 91%.Our result of colposcopy was in concordance with previous studies as shown in table 8. Sensitivity of colposcopy in detecting precancerous lesions was comparable to findings of Malur *et al.*, (80%), Zarchi *et al.*, (80%) and Ramesh *et al.*, (83.30%) while it was higher than detected by Macharia *et al.*, (50%).

Table 8 Comparision of Different Studies For Evaluating Colposcopy

Author and year of Study	Age group (Years)	Sensitivity (%)	Specificity (%)
Karimi-Zarchi et al[11] (2013)	21-70	70.90%	44.4%
Zarchi et al[13] (2011)	14-87	80.00%	80.00%
Ramesh et al[16] (2012)	25-65	83.30%	42.42%
Ashmita et al[17] (2013)	30-50	90.24%	72.73%
Macharia et al[12] (2014)	18-60	50.00%	91.00%
Malur et al[18] (2009)	22-65	80.00%	81.54%
Present study	20-75	80.85%	96.93%

Thus, Colposcopy emerged out as a better modality than LBC for detecting early precancerous changes of carcinoma cervix.

CONCLUSIONS

The majority of women with CIN were in the age group of 21-40 years. The most common complaint for seeking gynecological consultation was discharge per vaginum and pain abdomen. The incidence of CIN was more in rural population than urban population and maximum numbers of cases were reported in tubectomized patients with a parity of two children.

In our study, it is evident that colposcopy is definitely more sensitive and accurate than LBC smear. However, it is suggested that the combination of LBC with colposcopy would maximizes the sensitivity and specificity of cervical cancer screening.

Colposcopy in general has a definite role for evaluation of women with abnormal LBC smears, unhealthy cervix and seems to be more accurate in detecting CIN. Hence, primary colposcopy may be incorporated for high risk cases of a genital tract infection as a screening instrument by service providers for women on their first visit. It can be a useful tool for teaching, diagnosis and management of cervical lesions e.g., both neoplastic and non-neoplastic. The early diagnosis of CIN in women of reproductive age group is a desirable goal. It is preventable as it is associated with long preinvasive stage (CIN). There is an urgent need to introduce, encourage and incorporate the practice of screening of clinically suspicious cervix and abnormal PAP or LBC smear results by colposcopy in all the three tiers i.e., primary, secondary and tertiary Health Care System of developing countries.

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