



Original Research Article

Comparison and Correlation of PAP Smear, Colposcopy and Histopathology in Symptomatic Women and Suspicious Looking Cervix in a Tertiary Hospital Care Centre

Ashmita.D¹, Shakuntala.P.N^{1*}, Shubha.R.Rao¹, S.K.Sharma¹, Geethanjali.S²

¹ Department of Obstetrics and Gynaecology, ² Department of Pathology, St.Martha's Hospital, Nrupathunga Road, Bengaluru, Karnataka, India.

*Correspondence Email: shakuntala_pn@yahoo.com

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ABSTRACT

Aims and objectives:

- To study the socio demographic parameters of women with symptomatic and suspicious looking cervix.
- To compare and correlate the findings of papsmear, colposcopy and histopathology in detecting premalignant lesions of cervix.

Material and Methods: This was a prospective observational study conducted from 01/02/07 to 28/02/09 in the Department of Obstetrics and Gynaecology. Pap smear was performed by the conventional method and Colposcopy was done for all 52 sexually active women who came with complaints of discharge per vagina, inter menstrual or post coital bleeding and women with an abnormal pap smear. Final correlation of pap smear and colposcopy were based on histopathology.

Results: 80.8% of women were in the age group of 30-50 years. 94.2% of women were literate and multiparous. 26.9% of women were willing to undergo the tests due to awareness and fear of cancer. Commonest complaint was white discharge per vaginam in 26.9% and polygamy in 21.1% of their husbands. Per speculum examination revealed abnormalities in 86.5% of women. There was no history of use of oral contraceptives (OC), intrauterine contraceptive device (IUCD) in 53.9% of women. Only 32.7% had used IUCD. 1.9% of women were treated for sexually transmitted disease in the past. Use of tobacco in the form of smoking or chewing tobacco products were encountered in 7.7% and 11% of women respectively. However, 32.7% were exposed to passive smoking. Child marriage and early marriage were prevalent in 13.5% of women (<15 years) and 68.75% of women (<18 years) respectively. 82.7% of women had a normal pap smear, high grade squamous intraepithelial lesion in 3.8%. Colposcopy findings suggestive of low grade infection and high grade or suspicious of malignancy in 55.8% and 25.2% of women respectively. Final histopathology correlated with a diagnosis of CINII/CIN III in 3.8% of women. 63.5% had a report of chronic cervicitis with metaplasia.

Overall pap smear had a poor sensitivity compared to colposcopy, 19.5% vs 90.24% (p<0.001) respectively. Pap had a better specificity though not significantly compared to colposcopy, 83.33% vs 72.73% (p<0.3) respectively. Descriptive statistical analysis has been used in the present study.

Conclusion: Significant risk factors associated with premalignant lesions of cervix were younger age at initiation of intercourse (coitarchae) and high parity. No significant correlation conclusions could be drawn from the use of OC/IUCD and use of tobacco contributing to premalignant lesions of cervix. Pap smear had a poorer sensitivity especially in the low grade squamous intraepithelial lesions. Colposcopy on the other hand had better sensitivity. Hence it may be better to utilise both tests as they complement each other in screening of premalignant lesions of cervix.

Key words: PAP smear, Colposcopy, Premalignant Lesion of Cervix, Suspicious looking Cervix.

INTRODUCTION

Cervical cancer is a largely preventable disease, yet it tragically remains the third most common cancer worldwide with over 5,30,232 (8.8%) new cases and mortality of 2,750,087(8.2%) annually.⁽¹⁾ Cancer in India accounted for 3.3% of the disease burden and about 9% of all deaths in 2004.⁽²⁾

It has been observed since screening was introduced in 1955 in the developed countries, the incidence of invasive cervical cancer has decreased more than 74%.⁽³⁾ Further, the changing socio economic profile and initiation of different screening strategies have depicted a decline in the cervical cancer incidence and mortality worldwide.⁽⁴⁾ The conventional Pap technique nonetheless is far from perfect and carries a 10-70% false negative rate due to collection and processing errors necessitating that the test be performed annually for optimal performance. Also, in a developing country like India, cytology based screening programmes are difficult to organize because of absence of trained manpower, infrastructure, logistics, quality assurance, frequency of screening and costs involved.⁽³⁾ Cancer of cervix is now thought to be a sexually transmitted disease. Its association with early sexual activity and sexual promiscuity in particular, has been well established. It has been suggested that cervical cancer might be caused by sexual transmission of an infectious agent and HPV has been identified as the leading candidate for the sexually transmitted etiological factor in cervical cancer.⁽⁵⁾

Colposcopy is an integral part of the management of women presenting with abnormal cervical cytology and those with lesions in the lower genital tract indicative of intraepithelial neoplastic disease. Colposcopy as a subjective modality has a sensitivity for the detection of intraepithelial disease in the range of 60-75%. When employed with exfoliative cytology, this sensitivity can be increased to >90%. The sensitivity of a combined Pap and Human Papilloma Virus(HPV) testing strategy is further enhanced by referring all women with Low grade squamous intraepithelial lesion(LSIL) or High grade squamous intraepithelial lesion(HSIL) cytology to colposcopy regardless of HPV result.⁽⁶⁾

Dindigul Ambillikai Cancer registry (DACR) shows clearly a decline in cervical cancer incidence in Dindigul district, where the crude rate of 23.1 per 100,000 observed during 2003-2006 was lesser compared to 47.7 during 1996-1998. A trend which could be observed due to implementation of cervical cancer screening programme.⁽⁷⁾ The cervical cancer is the leading cause of cancer in Indian women and the burden in India alone is estimated as 1,34,420 new cases(25.9% of all female cancers) and mortality accounts to 72,825(23.3%).⁽¹⁾

The worldwide HPV prevalence in cervical carcinomas is 99.7%.⁽⁸⁾ The presence of HPV in virtually all cervical cancers implies the highest worldwide attributable fraction so far reported for a specific cause of any major human cancer. The cost for HPV testing in the Indian scenario is prohibitive presently and hence

other screening modalities have to be employed. ⁽⁹⁾ Cytology and colposcopy are being widely used for this purpose and we are comparing and correlating the findings of papsmear and colposcopy with the gold standard histopathology in a hospital based setting in symptomatic women and suspicious looking cervix.

MATERIAL AND METHODS

The present study is a Prospective Observational study conducted on 52 women after obtaining informed consent. It was conducted in the Department of Obstetrics and Gynaecology from 01/02/07 to 28/02/09. The study was approved by the Institutional ethical committee.

Pertinent history relating to age, age of marriage (proxy for coitarche), parity, contraceptive use, socio-economic status, educational status, polygamy, use of tobacco or its products predominant symptoms and sexual history were recorded on a computer generated proforma for each woman.

Inclusion criteria:

Sexually active women who came with complaints of discharge per vagina, inter menstrual or post coital bleeding were subjected to papsmear and colposcopy. The diagnosis of pre malignant lesion of cervix would be based on final histopathology obtained following colposcopic directed biopsies.

Exclusion Criteria:

- Prepubertal and postmenopausal women presenting with gynaecological complaints.
- Invasive carcinoma of cervix and other genital malignancies.
- Clinical evidence of acute infection
- Pregnant and puerperial women.
- Women with bleeding at the time of examination.

Procedure: following the description of the procedure and informed consent the observations from the per speculum examination was recorded and a repeat PAP smear would be obtained if there was gross discrepancy in the previous pap report or the visual findings and These findings were recorded under the heading of unaided visual inspection of cervix on the proforma.

In symptomatic women, the exfoliated cells from the cervix was sampled using the Ayer's spatula which fit into the contour of the ectocervix (slide no.1) and endocervix was sampled using a Endocervical Sampling Brush, Boen Healthcare Co., Ltd. (slide no.2). The material obtained was smeared evenly on a glass slide and inserted into a bottle containing 10% formaldehyde. The smears were sent to the cytopathology laboratory at St. Martha's Hospital for reporting. The cervical smear were stained with Papanicolaou technique and then screened and reported according to the Bethesda System. ⁽¹⁰⁾

Then Colposcopy was performed using Dr. Camscope video colposcope manufactured by Sometech, Inc. With an Optical zoom 4x, 6x upto 20x was used.

In all 52 women colposcopy was done according to conventional method and Modified Reids colposcopic index / scoring (RCI) was performed. ^(11, 12)

The cervix was visualized under low power to note any abnormal findings. Capillaries and surface blood vessels of the cervix was visualized under low power to note any abnormal findings. Capillaries and surface blood vessels were examined with a green filter. 3-5% glacial acetic acid was gently applied twice over the cervix for a total period of one minute, to ensure appropriate acetowhite reaction. Transformation zone was defined between the old and new squamo-columnar junctions. Colposcopy was considered unsatisfactory if

the new squamo-columnar junction was not visualized completely and endo-cervical curettage was performed. Examination of each quadrant was done in a clockwise direction. If acetowhite reaction was seen in the transformation zone; then margin, colour, vessels and colposcopy signs were scored using RCI scoring method(table A). Lugols iodine was applied and findings documented. Reids Colposcopic scoring/Index was done as shown in the table B. Colposcopy guided biopsies were

taken with punch biopsy forceps from the site with highest score and transferred to a vial containing 10% formaldehyde and sent for histopathological examination. Unsatisfactory colposcopy was recorded separately. (Endocervical curetings were obtained from all the women for analysis). Pap smear and colposcopy were correlated with histopathology obtained from colposcopic directed biopsies and further management was decided.

Table A-Modified Reid’s colposcopic Index- Adapted from Reid R, Scalzi⁽¹¹⁾ and International Agency for Research in Cancer (IARC) manual⁽¹²⁾

Colposcopy Sign	Score 0	Score 1	Score 2
Margin	Condylomatous or micropapillary contour. Flocculated 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 Mahogany brown;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].^(11,12)

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

Table-C: Evaluation of socioeconomic status we adapted the method followed by Thulaseedharan.JV, et al⁽¹³⁾

Socio-economic Status(SES)	Amount in Indian Rupees
1USDollar=54 Rupees	
Lower class	<1000 rupees
Upper lower class	>1000<2000
Lower middle class	>2000<5000
Upper middle class	>5000

Statistical Methods: Descriptive statistical analysis has been carried out in the present

study. Results on continuous measurements are presented on Mean±SD(Min-Max) and results on categorical measurements are presented in number(%).

Significance is assessed at 5% level of significance.

+ Suggestive significance 0.05<P<0.10.

* moderately significant 0.01< P ≤0.05. *

* Strongly significant P 0.01

Chi- square/Fischer Exact test has been used to find significance of the study parameters on categorical scale between groups. 90% Confidence Interval has been computed to find the significant features. Confidence Interval with lower limit more than 50% is associated with statistical significance.

Statistical software: SPSS 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 were used for

the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc. (14, 15)

RESULTS

80.8% of women were in the age group of 30-50 years (table1) 94.2% of women were literate and multiparous (table2,4). 48.1% of women were belonging to the lower class and 26.9% of women were willing to undergo the tests due to awareness and fear of cancer (table 3). Polygamy in 21.1% of husbands (table 5). Commonest complaint was white discharge per vaginum in 26.9% (table 6). Per speculum examination revealed abnormalities in 86.5% of women (table 7). There was no history of use of oral contraceptives (OC), intrauterine contraceptive device (IUCD) in 53.9% of women. Only 32.7% had used IUCD. 1.9% of women were treated for sexually transmitted disease in the past (table 8). Use of tobacco in the form of smoking or chewing tobacco products were

encountered in 7.7% and 11% of women respectively. However, 32.7% were exposed to passive smoking (table 9). Child marriage and early marriage were prevalent in 13.5% of women (<15 years) and 68.75% of women (<18 years) respectively (table 10).82.7% of women had a normal pap smear, high grade squamous intraepithelial lesion in 3.8%. Colposcopy findings suggest low grade infection and high grade or suspicious of malignancy in 55.8% and 25.2% of women respectively. Final histopathology correlated with a diagnosis of Cervical Intreepithelial Neoplasia (CIN) II/CIN III in 3.8% of women. 63.5% had a report of chronic cervicitis with metaplasia Table 11,12,13,14.

Overall pap smear had a poor sensitivity compared to colposcopy, 19.5% vs 90.24% (p<0.001) respectively. Pap had a better specificity though no significantly compared to colposcopy, 83.33% vs 72.73% (p<0.3) respectively (table 11)

Table 1: Distrubution of Age.

Age in years	Number of women(n=52)	%
Up to 29	04	07.70
30-39	21	40.40
40-49	21	40.40
50-59	06	11.50
Total	52	100.00
Mean SD	39.85±7.97	
80.8% of women were in the age group of 30-50 years		

Table 2: Levels of Education in women.

Education levels	Number (n=52)	%
Uneducated	03	05.80
Primary(first standard to seventh)	20	38.50
High school(eight to tenth standard)	10	19.20
Pre University College	09	17.30
University qualification	10	19.20
Total	52	100.00
38.5% received only primary education. However 94.2% had some form of education.		

Table 3: Socio-economic Status(SES)

SES- in Indian rupees-1 US Dollar-54 rupees	Number (n=52)	%
Lower class -<1000 rupees	14	26.9
Upper lower class->1000<2000	11	21.10
Lower middle class-->2000<5000	24	24.00
Upper middle class-->5000	03	03.00
Total	52	100.00
48.1% of women were belonging to the lower class. However 26.9% had awareness of cancer.		

Table 4: Parity Distribution

Parity	Number(n=52)	%
Nullipara	01	01.90
Primipara	02	03.80
Multipara	49	94.20
Total	52	100.00
94.2% were multiparous and average number of children were 3		

Table 5: Polygamy in Husband

Polygamy in husband	Number(n=52)	%
Monogamous	38	73.10
Polygamous	11	21.10
Unknown	03	05.80
Total	52	100.00
21.10% of women had husbands with polygamous relationship. 2 of the wives had died of cervical cancer and they had remarried, the rest of them had unsafe sexual practices.		

Table 6: Presenting complaints

Presenting Complaints	Number (n=52)	%	95% CI
White discharge per vaginum	14	26.90	16.7-40.30
Pain abdomen	11	21.20	12.24-34.00
Menorrhagia	10	19.20	10.80-31.90
Irregular cycles	06	11.50	05.40-22.97
Asymptomatic	06	11.50	05.40-22.97
Urinary tract infection	05	09.60	04.20-20.60
Commonest complaint was discharge per vaginum (26.90%).			

Table 7: Per speculum examination of the Cervix.

Cervical examination	Number (n=52)	%	95% CI
Normal	07	13.50	06.70-25.30
Cervical polyp/ectopy	44	84.60	72.50-91.90
Suspicious looking cervix	01	01.90	0.030-10.10
Total	52	100.00	
86.50% of women had positive clinical (cervical) findings. It could be due to hospital based study.			

Table 8: OCP/IUCD/H/o STD

OCP/IUCD/H/o STD	Number (n=52)	%	95% CI
No family planning methods used	28	53.84	-
OCP use	06	11.50	05.40-22.90
IUCD	17	32.70	21.5-46.20
H/o STD	01	01.90	0.03-10.10
TOTAL	52	100.00	
53.84% of women did not use any type of contraception. 32.7% had used IUCD in the past.			

Table 9: Tobacco use

Tobacco use	Number(n=52)	%
Yes	04	07.70
No	48	92.30
Passive smoking		
Yes	17	32.70
No	35	67.30
Chewing tobacco products was encountered in 7.70% of women. 32.70% were exposed to passive smoking.		

Table 10: Age at Marriage.

Age at marriage(years)	Number(n=52)	%
11-15	07	13.50
16-20*	32	61.50
>20	13	25.00
Total	52	100.00
Mean SD	18.81±3.59	
13.5% of the women were married before 15 years of age(early coitarchae). *22/32(68,75%) women were less than 18 years before marriage.		

Table 11: Correlation of Pap smear, Colposcopy and Histopathology(HPE) findings.

Correlation	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
Pap smear vs HPE	19.51	83.33	80.00	23.26	33.96	0.329
Colposcopy vs HPE	90.24	72.73	92.50	66.67	86.54	<0.001**
Pap vs Colposcopy	22.50	100.00	100.00	27.91	40.38	0.097+
Pap smear was less sensitive -19.51% and had a better specificity-83.33% compared to colposcopy with a sensitivity of 90.24 and specificity of 72.73 respectively.						

Table 12: Correlation of Reid Colposcopy Score with Histopathology(HPE).

Reid's score	Total	No biopsy	Histopathology findings				
			Chr. Cervi. With met.	Cervicitis with mild atypia/dysplasia/ koilocytic changes.	CIN II/III	Cervical endometriosis with sq. metaplasia	Inadequate sample
0-2	25	9(36.0)	16(64.0)	0	0	0	0
3-5	15	0	14(93.3)	1(16.7)	0	0	0
6-8	06	0	2(33.33)	1(16.7)	2(33.33)	1(16.7)	0
US*	06	2(33.33)	1(16.7)	1(16.7)	0	0	2(33.33)
Inference	A Reid colposcopy score above 6 was significantly correlated with abnormal histopathology. P<0.001** (4x6 Fischer Exact test).						
	*US-unsatisfactory colposcopy, chr.-chronic, cervi-cervicitis, met-metaplasia, sq-squamous metaplasia.						

Table 13: Correlation of PAP smear with Histopathology(HPE).

PAP	Histopathology findings						
	Total	Normal	Chr. Cervi. With metaplasia.	Cervicitis with mild atypia/dysplasia/ koilocytic changes/ CIN I.	CIN II/III	Cervical endometriosis with sq. metaplasia	Inadequate sample
NILM*	43	10(23.3%)	28(65.1%)	02(04.7%)	00	01(02.3%)	02(04.7%)
ASCUS**	03	00	02(66.7%)	01(33.3%)	00	00	00
LSIL	03	0	03(100.0%)	00	00	00	00
HSIL	02	0	0	00	02(100.0%)	00	00
US***	01	1(100.0%)	0	00	00	00	00
Inference	52	Abnormal pap smear findings significantly correlated with an abnormal histopathology. P=0.03*(5x6 Fischer Exact test) * NILM-negative for intraepithelial lesion or malignancy, ASC-US**-Atypical Squamous cells – undertermined significance, US***- unsatisfactory smear					

Table 14: Correlation of Pap smear with Colposcopy findings.

PAP smear	TOTAL	COLPOSCOPY FINDINGS		
		Normal -0-2	Abnormal-score >3<6	Suspicious cervix->6
NILM	43	12(27.9%)	22(51.2%)	09(20.9%)
ASCUS	03	00	03(100.0%)	00
LSIL	03	00	02(66.7%)	01(02.3%)
HSIL	02	00	02(66.7%)	00
US*	01	00	00	01(100.0%)
Inference	52	Pap smear and colposcopy findings do not correlate significantly . P=0.455(5x3 Fischer Exact test) *US-unsatisfactory smear.		

DISCUSSION

The prevalence of premalignant lesions of the cervix range from 8.15% to 35.2% (16,17,18,19) similar to the present study with an occurrence of 9.6%.CIN II/III lesions requiring treatment was found in 3.8% of women. Similar observations ranging from 2 to 13.72% has been described in literature. (16, 17)

Pertaining to the socio-demographic parameters, the mean age, mean parity were 39.85±7.97 and 2.52±1.38 respectively. Almost similar observations were also discussed by (13, 16, 17, 18, 19, 20) Mean age of marriage (proxy for coitarche) was 18.81±3.59. Mean age of coitarche in women with a histopathologic diagnosis of CIN I and CIN II/III were 20.33± 4.16 and 14.50±2.12 respectively. 94, 20% were multiparous with an average of 3 children. Many authors have discussed a strong influence of early coitarche and multiparity on the risk of cervical cancer as in the present study. (4, 21, 22) It very thought provoking that inspite of 65 years of

independence and reforms this observation reflects persistence of child marriage/ early marriage which is a social bane and failure of enforcement of law pertaining to age of marriage in our country. During our counselling sessions we have emphasised these matters as it a matter of serious concern.

Present study did not show any correlation between the educational status and preinvasive lesions of cervix. Although, many authors (9,13,23) observed an inverse relationship between the distribution of cervical cancer and the educational status of women.

Though our women were of low socioeconomic status (48.1%), they were willing to undergo the proposed tests after counselling, description of the procedures and informed consent. Possibly this may have occurred due to awareness as result of urbanisation. However, many Indian studies have reported that women from lower socioeconomic strata had higher incidence of cervical cancer. (9, 13, 23)

Use of tobacco (passive smoking), chewing tobacco and use of oral contraceptives or intra uterine contraceptive devices did not find any correlation with occurrence of premalignant lesions of cervix, similar observations were reported by other authors. ^(9,17) However, Monsoneg. J et al ⁽⁶⁾ observed that smoking was associated with a 2 fold increased risk of cervical cancer with a significant dose-response relationship. These socio-demographic factors play a vital role in planning screening strategies in a densely populated country like India supporting the contentions by many authors. ⁽¹³⁾

Literature is replete with data pertaining to the sensitivity of pap smear and colposcopy ranging from 27% to 50% vs 44%-89% respectively. Specificity of pap smear and colposcopy ranging from 19.5%-88.71% vs 52%-93.4% respectively. ^(18, 19, 20) In the present study Pap smear had a better specificity (83.33% vs 72.23%) compared to colposcopy and colposcopy had a better sensitivity (90.24% vs 19.5%) compared to Pap smear. Out of the 82.7% of women whose pap smear was reported as negative for intraepithelial lesion or malignancy (NILM,) 72.1% were diagnosed as abnormal or a score more than 3, reflecting a poor pick up rate for low grade lesion by pap smear. Further, on analysing it was observed that Reid colposcopic index correlated significantly with final histopathology with a p value < 0.001. Abnormal pap smear (HSIL) correlated significantly with abnormal histopathology with a p value < 0.03.

Both the women with a histopathology diagnosis of CINII/III had a pap smear report of HSIL. Therefore, pap smear had a better sensitivity for high grade lesions compared to low grade lesions, as evidenced by CINI in final histopathology report as against a normal pap smear. Pap smear reporting is subject to many

disadvantages of specimen collection techniques, fixation, staining process and the experience of the cytopathologist. As in the present study if pap smear was only the modality used, both the cases of preinvasive lesions of cervix would be missed. Similar observations have been discussed by Bhavana et al ⁽¹⁸⁾ and Mallur et al. ⁽¹⁹⁾ this disadvantage of a conventional pap smear may be overcome by using newer techniques like auto pap and pap net.

In the present study, though pap smear and colposcopy correlated with histopathology individually, they failed to correlate with each other when statistically analysed. Since pap smear had a very low sensitivity (19.5%) it would be prudent to add colposcopy as a complementary method to make screening more effective. The obvious advantage by adding both methods is masked by the small number studied.

CONCLUSION

Comparison of Pap smear and colposcopy in a hospital based screening for premalignant lesions of cervix revealed that Pap smear had a very poor sensitivity when compared to colposcopy, especially for low grade squamous intra epithelial lesions. Colposcopy on the other hand errs on the side of over diagnosis and had a higher sensitivity. Hence, both the tests can be used to complement each other in a hospital based screening programme, where facilities for both modalities are available. Early coitarchae and multiparity appeared to be significant risk factors. No significant correlations could be drawn from the use of oral contraceptives, IUCD, tobacco use or polygamy in husbands and premalignant lesions of cervix. However, the possibility of masking may have occurred due to small sample size.

Conflicts of Interest: None.

Authors' contributions: Dr.SPN involved in colposcopic evaluation, manuscript

preparation of the observations, patient care, reference hunting and final editing and photography, Dr. Ashmita was pioneering in reference hunting ,colposcopic evaluation, manuscript preparation editing, keeping record of all the patient data. Dr.Shubha.R.Rao was involved with patient care, editing the manuscript and deciding treatment for the patients. Dr.S.K.Sharma was involved with colposcopic evaluation and final editing of the manuscript and Dr.Geetanjali played a key role in organizing and initiating the Bethesda reporting method, laboratory quality control , cytologic evaluation of pap smear and histopathologic evaluation of all the colposcopic directed biopsies.

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