Original Article

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Correlate Abnormal Pap Smears with Colposcopic Findings, HPV Testing, FHACT and Cervical Biopsy Findings

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Abstract

Introduction: Almost one-fourth of the global cervical cancer burden is in India. There is an enormous opportunity for screening with the prospect of detection of cancer at the preclinical stage and hence, cervical cancer control programs become important.

*Aims and Objectives***:** To correlate abnormal Pap smears with colposcopic findings, HPV testing, FHACT and cervical biopsy findings.

Materials and Methods: The present study was a prospective observational study carried out at Kamineni Hospital, LB Nagar, Hyderabad, over a period of two years. The study group consisted of 150 women with abnormal cytology report (ASCUS and above) in whom colposcopy guided biopsy, HPV test and FHACT test were done and results were correlated.

Observations and Results: The prevalence of abnormal Pap smears was 2.91%. In the 150 patients with abnormal pap smears, 63.3% cases, showed normal per speculum findings.

Pap smear test had a very low sensitivity of 34.29% and high specificity of 95.65%, PPV of 92.31% and NPV of 48.89%. Colposcopy had a sensitivity of 60% and specificity of 73.91 %, PPV of 77.78%, NPV of 54.84%. HPV test was positive in 40% cases.

Conclusions: It is recommended that colposcopy when available, should be used as the next step in evaluation of women with ASCUS/ASCH/LSIL instead of following them up with repeat smears. The FISH-based HPV-associated cancer test can be used as a secondary screening assay, along with HPV subtyping to assist in patient management decisions.

Keywords: Pap Smears; Colposcopic Guided Biopsy; FISH; HPV Subtyping; FHACT.

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Introduction

Cervical cancer is the fifth most common cancer in humans, the second most common cancer in women worldwide and the most common cause of cancer death in the developing countries [1]. Almost one-fourth of the global cervical cancer burden is in India [2]. Hence, cervical cancercontrol programs are importantas there is a potential for effective prevention via screening and treatment of the precursor lesions.

Cancer screening can be effectively done by Pap smears, colposcopy and cervical biopsies. Traditionally the goldstandard for assessing the performance of Pap smear has been histopathology [3]. Hence, there is a need to study preneoplastic and neoplastic cervical lesions by Pap smears and correlate with histopathological findings.

Recently introduced FISH-Based HPV-Associated Cancer Test (FHACT) assesses genomic alterations in the HPV infected cervical cells. Our aim is to correlate abnormal Pap smears with colposcopic findings, HPV testing, FHACT an dcervical biopsy.

Materials and Methods

The present study was a prospective observational study carried out in the department of Obstetrics and Gynaecology, at Kamineni Hospital, LB Nagar, Hyderabad, over a period of two years from May 2014 to May 2016. The study group consisted of 150 women with abnormal cytology report (ASCUS/LSIL/HSIL) in whom colposcopy guided biopsy was done from the most suspicious areas; HPV test and FHACT test were done.

Inclusion Criteria

All women who attended the gynaecology OPD with abnormal Pap smear report (ASCUS and above) with or without symptoms were included.

Exclusion Criteria

Women who were not willing to participate in the study, women with frank cancer, pregnantwomen and post hysterectomy patients were excluded.

Pap smear test was done as part of routine screening during the study period.Those with abnormal Pap smear (ASCUS and above)were taken for colposcopyguided biopsy which was done from the most suspicious areas; HPV test and FHACT.

Histological prediction of colposcopic findings was done according to the Reid's modified colposcopic index (RCI)[14]. When RCI is 0-2, the histology is likely to be CIN 1, with RCI of 3-4, the histology is likely to be of overlapping lesion CIN 1 or CIN 2 and with RCI 5-8, histology is likely to be CIN 2-3.

The results of the colposcopy findings,cervical biopsy, HPV subtyping and FHACT were analysed and correlated.

Data was entered in Microsoft excel and analysis was done using SPSS version 20. Descriptive statisticalanalysis was done. Results on categorical measurements are presented as percentages. Significance was assessed at 5 % level of significance. Chi square testwas used to find out the significance of study parameters on a categorical scale between two groups. Diagnostic ability of Pap smear and Colposcopy was assessed for Sensitivity, Specificity, Positive predictive value (PPV), Negative predictive value (NPV) and Accuracy.

Results

A total of 5138 women were screened with Pap smear during the study period, out of which 150 women had abnormal pap smears (ASCUS and above). Among these 150 women, 73 underwent colposcopicbiopsy. Among these 73 women,30 women consented for HPV subtyping and 12 women consented for FHACT. The prevalence of abnormal Pap smears in the screened population was (150/ 5138) 2.91%.

Table 1: Age wise distribution of abnormal Pap smears and Histopathology

Age (years)	Pap Report (n=150)							Histopathology (n=73)			
	ASCUS	ASCH	AGUS	LSIL	HSIL	SCC	Total	Cervicit is	CIN1	CIN2,3	Total
21-30	0	0	0	1	1	0	2 (1.3)	0	0	1	1(1.3)
31-40	15	6	1	0	1	1	24(16.0)	3	3	3	9(12.3)
41-50	29	5	5	3	6	0	48(32.0)	5	10	10	25(34.2)
51-60	31	7	3	5	7	1	54(36.0)	5	7	15	27(36.9)
>/=60	10	8	1	2	1	0	22(14.7)	2	3	6	11(15.0)
Total	85	26	10	11	16	2	150(100)	15	23	35	73(100)

Table 2: Distribution of clinical findings in relation to Pap smears and HPE

Clinical Findings	Pap Smears No. of Cases Percentage	Histopathology Cervicitis	CIN1	>/=CIN2	Total No. of Cases Percentage
Normal	95 (63.3%)	9	19	16	44 (60.2%)
Erosion	26 (17.3%)	1	1	10	12 (16.4%)
Hypertrophy	21(14.0%)	3	2	6	11 (15.0%)
Hypertrophy and erosion	3 (2.0%)	2	1	0	3 (4.1%)
Bleeds on touch	5 (3.3%)	0	0	3	3 (4.1%)
Total	150 (100.0%)	15	23	35	73(100.0%)

Among150 cases, 68%were in the age groups of 41-60 years. Among the women who underwent colposcopic biopsy, 70% were in the age groups of 41-60 years.

Out of 35 women with >/=CIN 2 on histopathology, 15 (42.8%) were in the age group 51-60years, 10 (28.5%).

Distribution of Parity in the Patients with Abnormal Smears (n=150): Of the 150 women with abnormal pap smears,34% cases were 3rd para,26.7% were 2nd para, 18.7% were 4th para, 9.3% were 5th para/more, 8.7% were para 1, and 2.7%were nulligravida.

In the 150 patients with abnormal pap smears, 63.3% cases, showed normal per speculum findings.

Out of total 5138 patients, ASCUS was reported in 1.6% cases. Out of 150 abnormal smears, ASCUS was 56.7%. CIN lesions contributed to 73% cases on biopsy.

Frequency Distribution of Colposcopy Findings by RCI Index: Out of 73 women who underwent colposcopy, 44 cases (60%) had RCI index 0-2(CIN 1), 24 cases (32.9%) had RCI index 3-4 (CIN1/2), 5 cases (6.8%) had RCI index 5-8(CIN 2/3).

Table 3: Distribution of abnormal Pap smears and HPE

Pap Smear			Histopathology	
Report	**No. of all cases (%)**	**No. of abnormal cases (%)**	**Report**	**No. of cases (%)**
Inflammatory/Normal	4988 (97%)	-	-	-
ASCUS	85 (1.6%)	85 (56.7%)	Cervicitis	15 (20.6%)
ASCH	26 (0.5%)	26 (17.3%)	CIN1	23 (31.5%)
AGUS	10 (0.1%)	10 (6.7%)	CIN2	20 (27.4%)
LSIL	11 (0.2%)	11 (7.3%)	CIN3	11 (15.1%)
HSIL	16 (0.3%)	16 (10.7%)	CIS	2 (2.7%)
SCC	2 (0.03%)	2 (1.3%)	SCC	2 (2.7%)
Total	5138 (100.0%)	150 (100.0%)	Total	73 (100.0%)

Table 4: Correlation of abnormal Pap smears with HPE findings:

	HPE Findings		
Pap smear	**CIN1**	**>/=CIN2**	**Total**
ASCUS	16	9	25
ASCH	4	10	14
LSIL	2	5	7
HSIL	1	11	12
Total	23	35	58

χ2- 37.80; P Value– 0.001(significant)

Table 5: Correlation of colposcopy (Reids index) with HPE findings

	HPE findings			
Reids index	**Cervicitis**	**CIN1**	**>/=CIN2**	**Total**
0-2	13	17	14	44
3-4	1	6	17	24
5-8	1	0	4	5
Total	15	23	35	73

χ2- 29.9; P VALUE -0.003 (Significant)

11(91.6%) out of 12 women with HSIL on Pap smear had >/=CIN 2 (high grade) on HPE. 22(47.8%) out of 46 women with low risk on Pap smear(ASCUS/ ASCH/LSIL)were with CIN1 (low grade) on HPE. The results showed good correlation between Pap smear and HPE for high grade lesions.

17(54.8%) out of 31 women with RCI <3 on colposcopy (low risk) had CIN 1(low grade) on HPE.21(77.7%) out of 27 women with RCI>/ =3had>/= CIN3(high grade) on HPE. Correlation between colposcopic findings and biopsy was good for high grade lesions.

Sensitivity and Specificity of Pap Smear and Solposcopy

Pap smear test had a very low sensitivity of 34.29% and high specificity of 95.65%, PPV of 92.31% and NPV of 48.89%. Colposcopy had a sensitivity of 60% and specificity of 73.91%, PPV of 77.78%, NPV of 54.84%.

HPV subtypes: 30 women in the study group were subjected to HPV subtyping. Of these 18 (60%) were negative and 12 (40%) were positive for HPV. HPV type 16 was detected in 5 cases, both 16 and 18 in one case, 18 in one case and subtype 11, 33, 56, 58, 67 were also detected each in one case.

Table 6: Correlation of HPVsubtype distribution with HPE findings

HPE	HPV									Total
	Negative	11	16,18	16	18	33	56	58	67	
Cervicitis	3	0	0	0	1	0	0	0	0	1
CIN1	5	0	1	0	0	0	0	1	0	2
CIN2	9	1	0	0	0	0	1	0	1	3
CIN3	1	0	0	1	0	1	0	0	0	2
CIS	0	0	0	2	0	0	0	0	0	2
SCC	0	0	0	2	0	0	0	0	0	2
Total	18	1	1	5	1	1	1	1	1	12

There were two cases of SCC, both tested positive for HPV 16.

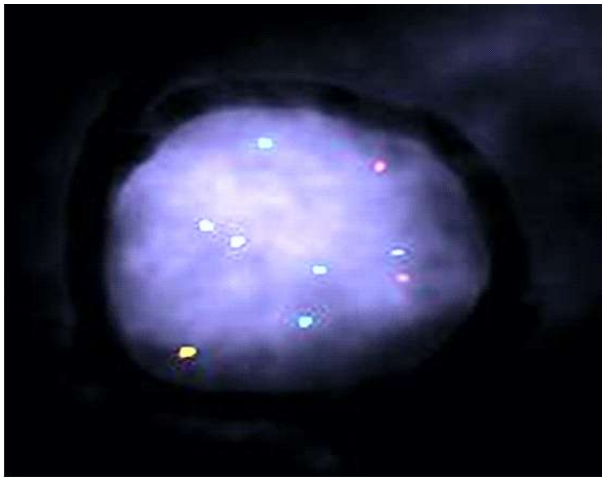

Fig. 1: Abnormal yellow signal indicating amplification of 20q.

Analysis of the FHACT results in the study, (n=12)

Among the 12 women in whom FHACT test was performed, only one case was positive and it showed 20q amplification (yellow signal) in whom Pap smear was reported as HSIL,RCI was 3,HPE was reported as CIN3, and HPV 16 was positive.

Discussion

In the present study clinical profile of the women with abnormal papsmears, the sensitivity, specificity, PPV, NPV of the Pap smear and colposcopy, correlation between Pap smear, colposcopy, HPV, FHACT with histopathology was studied.

The overall rate of abnormal pap smears was 2.91% in our study. In the studies conducted by Sharma et al [4] and Stolnicuet al [5] the abnormal pap smears were detected in 9.54% and 5.9% respectively.

Regarding age distribution, high prevalence of abnormal pap smears was found in higher age group of 51-60years. Similar observations have been made by Kohli et al[6] Sharma et al, [4] Radha et al[7] andChandrakala et al [8]. Majority of the high grade lesions on HPE, CIN 2 and above, were seen in the age group 51-60years. About 71% women with >/= CIN2 on HPE were in age group 41-60years which appeared to be the most significant group for cervical screening.

Regarding parity, in our study, 60% cases were of parity 2 or 3. In the studyby Kohliet al [6] 49%were of parity 3 or 4 and in the study by Sharmaet al [4] 68.3% were of parity 2 or 3 indicating increased prevalence in multipara.

Regarding clinical findings, 63%women with abnormal smears had normal looking cervix and 45.7% women with normal looking cervix had >/ =CIN2 on HPE, indicating that periodic screening is important for all the women,irrespective of the appearance of cervix.

Out of total 5138 smears, 85 (1.6%)were ASCUSand ASCH were 26 (0.5%). This compares well with the observation of Stolnicuet al [5] who reported 2.6% ASCUS and 0.9% of high-grade intraepithelial squamous-type lesions of all cases.In the study by Sharma et al [4] ASCUS was 0.043%, LSIL was 9.28%, HSIL was 0.21%. In present study, about 56% women had ASCUS and 17% had ASCH, which form the major group of women whoneed regular follow up, to rule out premalignant and malignant disease of cervix.

Among 73 women subjected to colposcopy directed biospy, histopathology was suggestive of CIN lesions in 73% cases and 2% squamous cell carcinomas. Kohli et al [6] have reported CIN lesions in 25% cases and squamous cell carcinoma in 5% cases. Chandrakala et al [8] have reported 43% CIN lesions and 5% squamous cell carcinomas.

Among premalignant and malignant diseases detected in the study group,39.6% were with CIN1 (low grade) on HPE, remaining 60.4 % were with >/ =CIN 2(high grade disease on HPE.

The correlation between Pap smear and histopathology is significant [χ2- 37.80; P Value – 0.001(significant)]. 91.6% women with HSIL on Pap smear had >/=CIN 2 (high grade) on HPE.52.1%

women with low risk on Pap smear (ASCUS/ASCH/LSIL) were with >/=CIN2 (high grade) on HPE. The results were similar to the study conducted by Radha et al [7]. The results showed good correlation between Pap smear and HPE for high grade lesions but poor correlation was seen as far as low grade lesions were concerned indicating that the women with ASCUS/ASCH/LSIL on Papsmears (low risk) also need further evaluation to diagnose premalignant and malignant cervical disease.

The correlation between Reids index and histopathology is significant [X2 29.9; P VALUE - 0.003 (Significant)]. 54.2%women with RCI <3 on colposcopy (low risk) had >/=CIN2 (high grade) on HPE. 77.7% women with RCI>/=3 (high risk) had>/= CIN2(high grade) on HPE. Correlation between colposcopic findings and biopsy was good for high grade lesions and poor for low grade lesions, the results were similar to the study conducted byRadha et al [7]. This might be due to observer variability or due to weaker performance of colposcopy in differentiating normal looking cervix from low grade lesions. None the less, at the same time it provides a good guide for biopsy.

Sensitivity and Specificity of Pap Smears and Colposcopy

Pap smearshad a low sensitivity (34.29%) and high specificity (95.65%). Low sensitivity of Pap test might be due to sampling errors, inadequate samples that are suboptimal for interpretation andobserver variability.The present study showed a higher sensitivity (60%) and a lower specificity (78.95%) of colposcopy when compared to Pap smears. Low specificity might be due to over-diagnosis of theabnormal epithelium which might be due to inflammation, immature metaplasia, and latent HPV infections.

Table 7: Comparison of Pap smears and Colposcopy for sensitivity and specificity

Study	Pap Smears				Colposcopy			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Present study	34.29%	95.65%	92.31%	48.89%	60%	78.95%	72.41%	68.18%
Radha et al[7]	29.4%	87.9%	33.3%	85%	82%	81%	48.2%	96%
Kohli et al[6]	80%	64.29%	48.98%	88.24%	100%	57.14%	50%	100%
Gupta et al[9]	85.85%	87.65%	75.8%	95.3%	-	-	-	-
Chandrakala et al[8]	65%	95%	94%	71%	-	-	-	-
Barut et al[10]	57%	76%	26%	92%	92%	67%	52%	96%

PPV: Positive Predictive Value, NPV: Negative Predictive Value

In the study group high risk HPV subtyping was positive in 40% cases. In present study, the most common subtype of HPV detected was type 16(41.6%) and is mainly associated with >/=CIN2 on HPE.

Among 12 women who underwent FHACT,20q amplification (yellow signal) was seen in one woman with HSIL on Papsmear, CIN 3 on histopathology, HPV 16 positive. More women must be tested forstudying the efficacy of FHACT, which might help in detecting the women at risk for progression to invasive cervicalcancer. Nonrandom host somatic chromosomal alterations are frequently shared across HPV-associated cancers, but with varying frequencies, potentially with functional roles.

At least for 3q26 gain, there is firm preliminary evidence to support that this genomic alteration can serve as a biomarker of disease progression of cervical cancer. Gain of 3q26 (*TERC*) has been detected with increasing frequency in cervical lesions with increasing severity and ultimately is observed in about 75% of cervical cancers [11-14].

The major limitation of genetic tests is high cost and need for experts to interpret the test. It is currently used in developed countries, but is difficult to incorporate it for screening in the developing countries.

Conclusion

It is recommended that colposcopy when available, should be used as the next step in evaluation of women with ASCUS/ASCH/LSIL instead of following them up with repeat smears, for early and accurate diagnosis and to avoid unnecessary anxiety among the patients.

The FISH-based HPV-associated cancer test can be used as a secondary screening assay, along with HPV subtypingto assist in the detection of clinically relevant HPV-associated disease and help guide patient management decisions. This will help in decreasing the discomfort of the patient for repeated follow up.

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