Cytohistological Correlation of Cervical PAP Smears - A Two-Year Retrospective Study from Ongole, Andhra Pradesh

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ABSTRACT

BACKGROUND

Cervical cancer is one of the most common forms of cancer. In India it is the second most common cancer after breast carcinoma. PAP smear is the best screening method to reveal spectrum of lesions in cervix ranging from inflammatory to the neoplastic process. Because of easy accessibility of cervix, cervical screening is helpful for ready diagnosis of 90 % to 96 % cytological abnormalities even in the pre-invasive stage. We wanted to evaluate the importance of PAP smear examination as a preliminary method accommodating histopathology and assess the spectrum of lesions.

METHODS

The study was done to identify the target age groups for the early detection of cancerous cervix by PAP smear examination. The study was done on patients who attended government general hospital (GGH), Ongole, from January 2018 to December 2019. Lesions were categorized based on 2014 Bethesda system as negative intraepithelial lesion for malignancy (NILM) / Inflammatory, benign, premalignant, and malignant. The clinicocytological and histopathological examination (HPE) of cervical biopsy of corresponding smears were compared and analysed in the present study.

RESULTS

Of the 584 cases studied by PAP smear examination, maximum number of patients were between 21 and 60 years of age. Maximum number of patients came with the complaint of white discharge. Highest number of cases reported were NILM / inflammatory (54.18 %), followed by atypical squamous cells of undetermined significance (ASCUS) (20.68 %), high-grade squamous intraepithelial lesion (HSIL) (13.84 %), squamous cell carcinoma (SCC) (6.84 %), low-grade squamous intraepithelial lesion (LSIL) (1.65 %), metaplasia (1.19 %), atypical glandular cells of undetermined significance (AGUS) (0.51 %), adenocarcinoma (0.17 %).

CONCLUSIONS

Cervical PAP smear screening is simple, cost effective and reliable method for early detection of cervical cancers. Classification of lesions based on Bethesda terminology is most informative and useful. Correlation of PAP smear cervical cytology with gold standard HPE provides greater efficacy in diagnosis.

KEYWORDS

PAP smears, NILM, ASCUS, AGUS, LSIL, HSIL

BACKGROUND

Dr George Papanicolaou is the pioneer for the introduction of Papanicolaou screening to identify various cervical lesions ranging from inflammatory to cancerous lesions. It is one of the most reliable cancer screening technique for cervical cancers.[1] PAP screening is most widely and commonly used current cancer screening test throughout the world.[2] Cancer cervix is the major health problem in many women of developing countries constituting nearly 7.5 % of all female cancer deaths. India accounts for about 20 % of all cervical cancers throughout the world. It is the second most common cancer next to carcinoma breast. As the carcinoma cervix has long pre-invasive stage of nearly 10 to 20 years from mild epithelial abnormality to established carcinoma, PAP screening became a very important test for detection and prevention of cancerous process.[3] It is a simple, safe, non - invasive, outdoor, and effective method for detection of various cervical lesions.

As early as 1976, Dr Harald Zur Hasen and his colleagues postulated the role Human Papilloma Virus (HPV) in cervical oncogenesis, for which he was awarded Nobel prize in Medicine in 2008.[4] Boshart M et al. demonstrated that the integration of HPV 18 viral DNA into host cell genome was responsible for the development of cervical carcinoma.[5] Currently evolved diagnostic ways in western countries are HPV genetic testing and computer assisted interpretation.[6] Even though invasive technique, cervical biopsy is the gold standard for diagnosing cervical lesions.

METHODS

The present study was a retrospective study done in the Department of Pathology, Government Medical College, Ongole over a period of two years from January 2018 to December 2019. A total number of 641 PAP smears were studied. Out of 641 cases 584 cases were taken into study because 32 cases were inadequate and 25 nil pathological cases. All conventional PAP smears were reported based on Bethesda classification system 2014. Out of 584 cases 105 cases were turned out for histopathological examination.
The main objective of the study was:

1. To correlate the PAP smear diagnosis with that of histopathology.
2. To know the common age group.
3. To know the most common presenting symptom.
   Before taking PAP smears patients were informed about the procedure.

Technique of Collection of PAP Smears[7]

Sample collection was avoided in Patients with active menses and who were on vaginal medication for at least 48 - 72 hours before taking the smears.

Keeping the patient in dorsal position, with good illumination, Ayre's spatula is inserted with long end going into the cervical canal till the small end rests on the ectocervix. Then rotate the spatula 360^0 keeping contact with ectocervix throughout without giving much force to prevent haemorrhagic sample. By using endocervical brush, sample is collected by rotating brush through 180^0 in endocervical canal. The sample should be evenly spread on grease free slides and fixed in 95 % isopropyl alcohol or ethanol and air dried for May Grunwald-Giemsa (MGG) and Leishman stains as per the requirement and then proceeded for staining. The cervical biopsies / hysterectomised specimens were fixed in 10 % neutral buffered formalin, processed routinely and finally sections were stained with Haematoxylin and Eosin. After microscopic observation of sections, PAP smear findings were correlated with histopathological findings.

RESULTS

A total number of 584 conventional PAP smear cases were studied and various lesions were diagnosed basing on Bethesda classification 2014 for a period of 2 years. Highest number of cases were seen in between 21 to 60 years. Maximum no. of patients came with complaint of white discharge (52 %) followed by abdominal pain (22 %), abnormal uterine bleeding (18 %), postmenopausal bleeding (06 %), post coital bleeding (02 %).
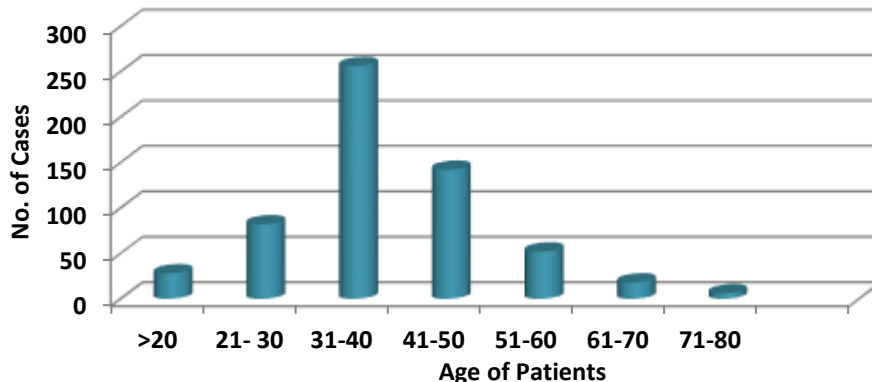
Graph 1. Age Wise Distribution of Cases

| Compliant	Cases (Percentage)
White discharge	304 (52 %)
Abdominal pain	128 (22 %)
AUB & Others	106 (18 %)
Post-menopausal bleeding	35 (6 %)
Post coital bleeding	11 (2 %)
Table 1. Compliant Wise Distribution of Cases	

Out of 584 PAP smear cases, NILM / Inflammatory constituted 311 (53.18 %) cases, Metaplasia in 07 (1.19 %) cases, ASCUS in 121 (20.68 %) cases, AGUS in 03 (0.51 %) cases, LSIL in 10 (1.654 %), HSIL in 81 (13.84 %) cases, Squamous cell carcinoma in 40 (6.849 %) cases, Adenocarcinoma in 01 (0.17 %) case, Radiation changes in 02 (0.34 %) cases and Atrophic changes in 08 (1.36 %) cases. Atrophic changes were seen in 50 to 70 years age range and Radiation changes were seen in 2 cases with history of previous radiotherapy for Endometrial Adenocarcinoma.

NILM \ Inflammatory	311 (53.188 %)
Metaplasia	07 (1.19 %)
ASCUS	121 (20.68 %)
AGUS	03 (0.51 %)
LSIL	10 (1.654 %)
HSIL	81 (13.84 %)
SCC	40 (6.84 %)
Adenocarcinoma	01 (0.174 %)
Table 2. Cytological (Pap Smear) Diagnosis of 584 Cases	

Radiation changes were seen in 0.342 %, Atrophic changes were seen in 1.346 %. Out of 584 PAP smears studied, 105 cases were coming for histopathological examination. Out of 105 cases, total 39 (37.14 %) cases showed chronic cervicitis changes with or without metaplasia. Of 39 cases only chronic cervicitis changes were seen in 21 (53.84 %) and 18 (46.15 %) showed chronic cervicitis with metaplasia. Cervical intra-epithelial neoplasia:- CIN 1 in 21 (20.00 %) cases, CIN 11 in 22 (20.95 %) cases, CIN111 in 5 (4.76 %) cases, Squamous cell carcinoma (SCC) in 16 (15.23 %) cases and Adenocarcinoma in 2 (1.90 %) cases.

Figure 1 (a). 40X, H & E HSIL

Figure. 1 (b) 10X, H & E SCC

Figure 1 (c) 40X, H & E SCC
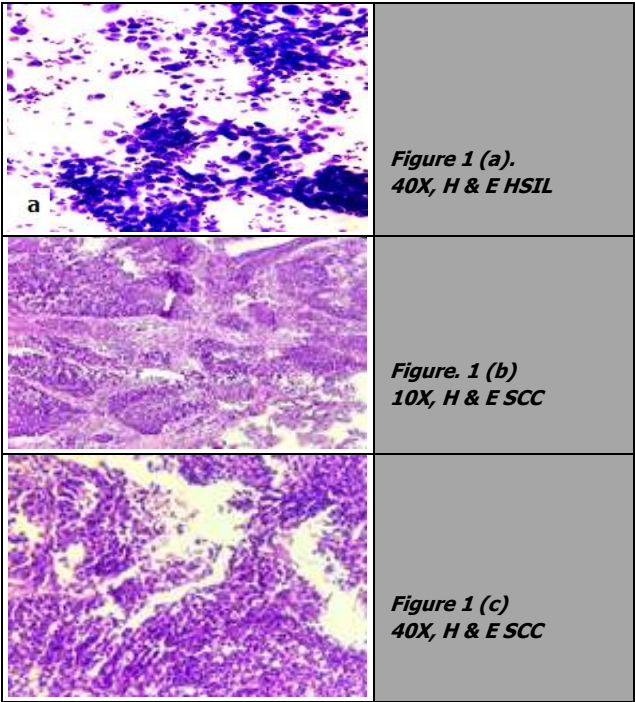
Pap Diagnosis	No. of Cases on PAP Smear Who Turned Out for HPE	HPE Diagnosis						
		Chronic Cervicitis	Chronic Cervicitis with Metaplasia	CIN I	CIN II	CIN III	SCC	Adenocarcinoma
NILM	22	14	8	-	-	-	-	-
ASCUS	26	07	6	11	2	-	-	-
AGUS	02	-	-	-	-	-	-	2
LSIL	05	-	1	4	-	-	-	-
HSIL	34	-	3	6	20	4	1	-
SCC	16	-	-	-	-	1	15	-
Total	**105**	**21**	**18**	**21**	**22**	**5**	**16**	**2**
Table 3. Cytohisto Correlation of Conventional PAP Smears and Cervical Biopsies / Hysterectomies								

DISCUSSION

Age Distribution
In the present study most of the cases were seen in 21 - 60 years and most were in second parity (52 %) compared with the studies of Bhushan M. Warpe et al. (2016) and Rathod S B et al. (2015)[8] followed by para 3 (22.9 %).

(Yrs.)	31 − 40	41 - 50	51 - 60
Present study	36.8 %	26.4 %	08 %
Rathod G B Singla[9]		42.4 %	21.2 %
Bhushan M. Warpe	34.51 %	30 %	10 %
Table 4. Age Wise Distribution of Comparative Studies			

Patient's Complaints
Highest number of patients came with the compliant of leucorrhoea (white discharge) 52 %, similar to the studies of Alakananda et al. (51 %), followed by abdominal pain in 22 %, abnormal uterine bleeding in 18 %, postmenopausal bleeding in 06 %, post coital bleeding in 02 %.

In the present study inadequacy rate of sample was 4.99 %, comparing with the study of Bhushan M. Warpe et al. (2016) showing 7.4 %. In the present study nil pathological changes were observed in 3.90 %.

NILM
According to the Bethesda 2001 / 2014 PAP smear findings out of total 584 cases, 311 cases (53.188 %) were categorized as Negative for intraepithelial lesion or Malignancy (NILM) compared to the studies of Simridhi Bindroo et al.[10] Saha R et al. (2005)[11] and Selhi PK et al. (2014).[12] NILM cases constitute nonspecific inflammations and specific inflammations due to organisms like Monilia (Candida), Bacterial Vaginosis, *Trichomonas vaginalis*. NILM with bacterial vaginosis seen in 26 cases, with monilial infections in 56 cases and 15 cases showed *Trichomonas Vaginalis*.

22 cases which were diagnosed as NILM on PAP smear examination came for histopathological study. In the present study 100 % of cases of NILM on PAP smear were correlated

with histopathology which was comparable with the study of Simridhi Bindroo et al.

ECA

In the present study after excluding 8 cases of atrophic changes and 2 cases of radiation changes the Epithelial Cell Abnormality (ECA) was observed in 256 cases (41.64 %) which was higher when compared with the studies of Malpani et al.[13] and Sachan et al.[14] but similar to the study of Simridhi Bindroo et al.

In the present study 26 (24.76 %) cases of Atypical squamous cells of undetermined significance (ASCUS) correlated on histopathology, 11 (42.30 %) cases as CIN1, 2 (7.69 %) cases as CIN2 and 13 (50.00 %) cases were correlated as chronic non-specific cervicitis with or without metaplasia which was compared with the study of Yeoh et al.[15] The reason why ASCUS was given on PAP smear was, associated inflammation showed large reactive cells which were misinterpreted as atypical squamous cells.

In the present study 5 (50 %) cases of low grade squamous intra epithelial lesion (LSIL) came for histopathological diagnosis. 80 % of LSIL cases were correlated with histopathological examination which was compared with the study of Atla et al.[16] Out of five cases, 4 cases showed CIN1 change and one case showed chronic cervicitis with metaplastic changes.

In the present study on PAP smear 81 (13.84 %) cases were diagnosed as high grade squamous intra epithelial lesion (HSIL) compared with the study of Chandrakala Joshi PK[17] et al. Out of 81 cases, 34 (32.30 %) cases were turned out for histopathological study. Out of 34 cases 20 (58.83 %) cases showed CIN2 morphology compared to Anuj Poudel et al.[18] 6 (17.64 %) cases showed CIN1, 4 (11.76 %) cases showed CIN3 and one case (2.94 %) showed squamous cell carcinoma. 3 (8.82 %) cases of HSIL showed chronic cervicitis with metaplasia.

In the present study on PAP smear 40 (6.84 %) cases were diagnosed as squamous cell carcinoma. Of 16 (40.0 %) cases which were turned out for histopathology, 15 (93.75 %) cases were correlated and one case (6.25 %) was diagnosed as CIN3 and these findings were similar with the study by Simridhi Bindroo et al.

In the present study on PAP smear two cases (0.342 %) were diagnosed as atypical glandular cells of uncertain significance (AGUS). Out of 2 cases, all two cases were correlated with histopathology as Adenocarcinoma which was comparable with Atla et al.

CONCLUSIONS

PAP smear test is the most important screening modality for detection of premalignant and malignant lesions of cervix. It is most simple, safe, sensitive, rapidly diagnostic, and cost-effective screening tool. As histopathology is the gold standard, it is very much essential to compare and confirm many of the epithelial and non-epithelial lesions of cervix that were diagnosed on PAP smear study. Bethesda system 2014 is used for cytological reporting of cervical PAP smears.

So conventional PAP smear is the most useful method for early detection of premalignant and malignant cervical lesions as well as for appropriate management. PAP smears are also helpful in identifying the aetiology of infectious process and thereby treatment.

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