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A Study of Cytological Findings in Cervical (PAP) Smears in Correlation With Cervical Biopsies.

Alok Shetty K, Jyoti R Kini*, and Hema Kini.

Department of Pathology, Kasturba Medical College, Manipal University, Mangalore, Karnataka, India.

ABSTRACT

Uterine cervix carcinoma is one of the main causes of cancer death in women worldwide. The use of the cervical (PAP) smear as a screening tool has significantly reduced the incidence of cervical cancer. Cytohistopathologic correlation of Pap smears is a widely accepted method of internal quality assurance of laboratories and to assess its utility as a screening test. The aim of our study was to compare the cytological findings in cervical smears with the histopathological features of the corresponding biopsies and calculate the sensitivity, specificity and accuracy rates of cervical cytological examination and to analyse the errors that occurred on reporting of cervical smears. Cervical smears with corresponding histopathological biopsies were reviewed. Accuracy rates of cytological examination, before and after blind review, were calculated and compared. Slides with cyto-histopathologic discrepancies were then reviewed unblinded to analyze the type of errors. Out of 2911 cases sent for cervical cytological evaluation, 210 were available for cytohistopathological correlation. Positive correlation was found in 198 cases and discrepancies in 12 cases. This gave an overall accuracy rate of 94.3%, sensitivity of 68% and specificity of 99.5% .On blind review, cytological diagnosis changed in seven cases. The 12 discrepant cases were analysed again, revealed that on initial reporting there were four screening errors, three interpretive errors, and five sampling errors. Cytohistopathological correlation is essential to assess the quality of cervical cytological examination in the laboratory. Cautious reporting is essential when smears are associated with artifacts due to radiotherapy, inflammation and in smears with low cellularity.

Keywords: Cytohistopathological correlation, Pap smear, screening, quality control

Corresponding author

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INTRODUCTION

Uterine cervix carcinoma is one of the main causes of death from cancer in women worldwide, and the leading cause of death from cancer in several developing countries including India [1,2]. There is a decrease in the Age Adjusted Incidence Rates (AARs) of cervical cancer in the urban population based cancer registries in India. However, since over 70 per cent of the Indian population resides in the rural areas, cancer cervix still constitutes the number one cancer in our country [1].

The clinical and histological importance of gynaecologic cytology as a cancer screening tool cannot be overemphasized as it has played a pivotal role in significantly reducing the incidence of cervical cancer. However due to inherent limitations of cytology in terms of sensitivity, specificity and predictive value, some significant lesions could be missed or under diagnosed on Papanicolaou test [3]. An incorrect cytologic diagnosis is pardonable if it is due to the ability of the lesion to evade accurate diagnosis, however misdiagnoses due to inefficient screening or overestimation of cytologic appearances are not at all acceptable.

Laboratory confirmation of any positive screening test result by histopathology is critical for optimal patient care [4]. Cytohistopathological correlation is a widely accepted method of internal quality assurance and allows the pathologists to analyze the various factors leading to discrepant diagnosis [5-13]. Thus, it is part of a laboratory's patient safety armamentarium and a strongly advocated component of medical practice. This study underlines the need for every cytology laboratory to review itself via the technique of cyto-histopathologic correlation in order to deliver optimum services and offer suitable advice to the clinicians.

MATERIALS AND METHODS

The study was conducted in the Department of Pathology, of our institution which receives cervical cytology smears and histopathology specimens from a Government Obstetric & Gynaecological Care Hospital, two private hospitals affiliated to the institution & other private hospitals and consultants, in and around the city. It was a retrospective study and samples submitted for cervical cytological examination over a one year period were included in the study. The Institutional Ethics Committee clearance was also obtained for the study. Cervical smears with corresponding histopathological biopsies were collected.

The smears were conventional Pap smears,stained by Papanicolaou and were reported according to the Bethesda system. The biopsies were Hematoxylin and Eosin (H& E) stained sections prepared from paraffin blocks reported using the Cervical Intraepithelial Neoplasia (CIN) classification. The initial cytological and histopathological diagnosis was recorded along with other relevant data such as the nature of histopathology specimen, time elapsed between biopsy and cytology. Then, the cytological smears and histopathological slides with discrepancy were reviewed blindly and a diagnosis assigned. The accuracy rates of cytological examination, before and after review, were calculated and compared. The slides with cyto-histopathologic discrepancies were then reviewed again unblinded to analyze the type of errors and understand the possible pitfalls while reporting.

RESULTS

A total of 2911 cervicovaginal smears and 828 histopathology specimens related to the uterine cervix were received over a period of one year in the pathology department. The cervical smears were conventional Pap smears and the histopathology slides prepared from H&E stained sections. On assesment of the histopathology and cervical cytology reports, 272 cases had Pap smears followed by a biopsy or hysterectomy. Out of these 210 cases were selected, as 33 cases had polyp as the histopathology report, 19 had inadequate cervical smears and 10 cases had minimal tissue or only fibrinohemorrhagic material on biopsy. These 210 cases were used for all further analysis.

The histopathology specimens were received within 6 months of cervical smear examination. The types of biopsy specimen available for these 210 cases were punch biopsies, curettings and hysterectomy specimens. Histologic diagnosis was considered as the final diagnosis. In cases where more than one histopathology specimen was available and there was variability in the histopathology reports the highest grade of dysplasia was considered as the diagnosis. Based on these criteria the selected cases were reviewed for discrepancies in the cytology and histopathology reports. (Table1)

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Table 1: Cytohistological correlation (NILM: Negative for Intraepithelial Lesion/ Malignancy, LSIL: Low Grade Intraepithelial Lesion, HSIL: High Grade Intraepithelial Lesion, SCC: Squamous Cell Carcinoma, AGC: Atypical Glandular Cells, CIN: Cervical Intraepithelial Neoplasia)

		HISTOPATHOLOGY								
		NILM	CIN I	CIN II, III	SCC	Adeno carcinoma	Small cell carcinoma	Endo-metrial carcinoma	Radiation induced changes	**Total**
CYTOLOGY	NILM	**184**	2	3	1	1	-	-	-	191
	LSIL	1	**1**	-	-	-	-	-	-	2
	HSIL	-	-	**2**	1	-	-	-	-	3
	SCC	-	-	1	**6**	1	-	-	-	8
	AGC	-	-	-	-	-	-	**1**	-	1
	Adeno carcinoma	-	-	-	-	**2**	-	-	-	2
	Small cell carcinoma	-	-	-	-	-	**1**	-	-	1
	Endometrial carcinoma	-	-	-	-	-	-	**1**	-	1
	Radiation induced changes	-	-	-	1	-	-	-	-	1
	Total	185	3	6	9	4	1	2	-	**210**

Out of the 191 cases reported as negative for intraepithelial lesion or malignancy (NILM) on cytology, 184 were negative on histopathology also. Of the two cases of low grade intraepithelial lesion (LSIL) on cytology, one was negative on histopathology. Of the three cases of high grade intraepithelial lesion (HSIL) on cytology one was reported as LSIL. Out of the eight cases of squamous cell carcinoma (SCC) on cytology, six cases concurred with histopathology, one was reported as cervical intraepithelial neoplasia (CIN) III and one was reported as moderately differentiated adenocarcinoma. A case reported as having atypical glandular cells on cytology was discovered to be a case of atypical endometrial hyperplasia due to an ovarian thecoma on post-hysterectomy histopathological examination. This was not considered a discrepancy. One case reported as radiation induced changes on cytology, was found to be a case of post radiotherapy recurrence of squamous cell carcinoma on biopsy.Thus, out of these 210 cases, a positive correlation was obtained in 198 cases and only 12 cases had discrepancy between the cytology and histopathology reports. Eight were false negatives, only one was a false positive, two had discrepancy in the grading and in one case a moderately differentiated adenocarcinoma was reported as squamous cell carcinoma. Based on this the overall accuracy rate, sensitivity, specificity and positive-predictability rates and other parameters regarding the cervical cytological examination done was calculated. The overall accuracy rate was 94.3 %(198 out of 210 cases), underestimation on cytology was 4.3% cases (nine out of 210) and overestimation on cytology was 0.9% cases (two out of 210). The percentage of false negatives was 3.8% (eight out of 210) and that of false positives was 0.5 % (one out of 210).The case where a moderately differentiated carcinoma was reported as an SCC, was considered as pure morphology interpretive error and not as an over or underestimation on cytology. Hence, the sensitivity was 68 %, specificity 99.5%, and the positive predictive rat**e** was 94.44%.

On review of the slides of the 12 cases with discrepancy, the following results were obtained. (Table 2). Thus there was a change in the cytological diagnosis in 7 out of the 12 cases. Based on this, the above mentioned parameters were recalculated and overall accuracy rate was 96.67% (203 out of 210 cases), underestimation on cytology was 2.86% cases (6 out of 210) overestimation on cytology was 0.5%cases (1 out of 210). The percentage of false negative was 1.4% (3 out of 210) and that of false positives was 0% (0 out of 210). The sensitivity was 87.5% and specificity was 100% with a positive predictability rate of 100%

As evident from Table 2 on unbiased blind review there was change in diagnosis in 7 cases. The cases were then reviewed a second time unblindedly to analyze the type of errors that had occurred initially and even on blind review. Initially there were 4 screening errors, 3 interpretive errors and 5 sampling errors. Based on this, the screening sensitivity rate was calculated. Screening sensitivity rate (Sensitivity calculation including only the false negatives identified as resulting from screening and/or interpretive errors) was 70.83%. On

review discrepancies there were no screening errors, and interpretive errors but only sampling errors in five cases and grading difference in two cases.

Table 2: Revised cytological diagnosis on blind review of discrepant cases

CASE No.	INITIAL CYTOLOGICAL DIAGNOSIS	DIAGNOSIS AFTER BLIND REVIEW	HISTOPATHOLOGICAL DIAGNOSIS
1.	Radiation induced changes	Squamous cell carcinoma	Squamous cell carcinoma
2.	Squamous cell carcinoma	Poorly differentiated carcinoma	Moderately differentiated adenocarcinoma
3.	HSIL	HSIL	Squamous cell carcinoma
4.	LSIL	NILM	Chronic cervicitis
5.	NILM	NILM	CIN-1
6.	NILM	LSIL	CIN-III
7.	NILM	NILM	CIN-III
8.	Squamous cell carcinoma	Squamous cell carcinoma	CIN-III
9.	NILM	ASCUS	CIN-I
10	Inflammatory smear	ASC-H	CIN-II
11.	NILM	HSIL	Squamous cell carcinoma
12.	NILM	NILM	Adenocarcinoma

Table 3: Comparison of screening parameters of 2 studies

	Chhabra et al.	Present Study (Prior To Blind Review)	Present Study (After Blind Review)
Sensitivity	81%	68%	87.5%
Specificity	95%	99.5%	100%
Positive Predictive Value	92.8%	94.44%	100%
Percentage Of False Negatives	18.7%	3.8%	1.4%
Percentage Of False Positives	4.8%	0.5%	0%
Overall Accuracy	88%	94.3%	96.67%

DISCUSSION

The aim of the present study was to evaluate the ability of the simple Pap test to accurately diagnose precancerous and cancerous lesions of the cervix, so that suitable clinical management of the patient is possible. Simultaneously, the exercise acts a quality control study of the gynaecologic cytology laboratory. Out of the 210 cases used for analysis, 198 cases had a positive correlation giving an overall accuracy rate of 94.3%. Even other parameters such as specificity(99.5%) and positive predictability rate (94.4%) were appreciable, except sensitivity which was 68%. The present study is compared to a study by Chhabra et al[14] in which 320 satisfactory smears with corresponding histopathology were reviewed. The two studies are comparable except the categories LSIL and SCC which were very high in the study by Chhabra et al [14]. This could be because the study by Chhabra et al was conducted in an apex institution with high risk cases presenting late in the course of the disease whereas the present study was an opportunistic screening program, and also may be due to the shorter timeframe for sample selection in the present study. On comparison of the two studies with respect to screening parameters, the findings were comparable (Table 3).

In a study by Salvetto and Sandiford(2004) [10], sensitivity and specificity levels of cervical cytological examination in Nicargua (sensitivity 88.94%, specificity 90.71%), Peru (sensitivity 95.18%, specificity 88.55%), and UK (sensitivity 99%, specificity 96%), were compared. In our study the screening sensitivity and other parameters are comparable to these countries.

Cytohistopathologic discrepancies can occur due to two reasons. Firstly, due to over or under estimation on cytological examination, which in turn can be due to inadequate screening or due to inherent properties of the lesion itself. Secondly, due to inadequate sectioning of histopathology specimens [4]. As seemingly adequate sections are taken in our lab for histopathological examination of specimens related to the uterine cervix, it was assumed that the histopathological diagnosis was the final accurate diagnosis. And this histopathological diagnosis was used as a yardstick to measure the accuracy of the cervical smear examination as has been done routinely for many years [5].

On analysis of the errors in discrepant cases, the number of false negatives was initially eight. On blind review the number fell to three, while two were reported positive but with grading differences. In a study by Joste et al [4], 19% of the false negatives cases were associated with artifacts such as absent or low number of cells. In our study, out of the 3 cases of actual false negatives all 3 were associated with sampling errors such as low cellularity. One of the cases of false negative on initial reporting, reported as radiation induced changes was called squamous cell carcinoma on blind review, which was the correct diagnosis. However, the initial false diagnosis is very obviously due to radiation induced artifacts. This case stresses the need for a careful analysis of the smear in post-radiotherapy patients, as the management would vary greatly.

Out of the 2 cases of CIN III/HSIL which were missed initially, on blind review also there were cyto-histopathologic discrepancies in both cases. One was again assessed as negative and the other as LSIL. Thus in both cases the underdiagnosis has been persistent. Various explanations have been offered for under diagnosis of HSIL in some cases on cytology [3]. One of the reasons may be the presence of only a few abnormal cells representative of high grade lesion on the smear, and these may be missed on screening. This is particularly true of small cell lesions high in the endocervical canal. The confounding effects of excessive inflammation and obscuring blood in undercalled cases have also been cited as important reasons. In our study, the factors which led to discrepant diagnoses in the 2 cases was low cellularity in one case and possibly an interpretive error in another with respect to grading. In 2 cases, squamous cell carcinoma was missed on cytology initially, where one case was reported as HSIL and the other negative. The diagnosis of HSIL persisted on blind review; however a diagnosis of HSIL was made in the other case. Thus, the invasive nature was missed on cytology. This difficulty has been found by other authors also [4]. The most likely reason for the one step downgrading of the lesion in our study seemed to be the absence of tumor diathesis on cytology.

The case which was initially reported as squamous cell carcinoma was confidently called as squamous cell carcinoma on blind review too. However the histopathological diagnosis was that of carcinoma in situ. On unblinded review too it seemed prudent to attach a diagnosis of SCC to the smear. The most probable reason for this discrepancy could be that the histopathological specimen received was a punch biopsy, and it is likely that the invasive tumor may not have been sampled. As stressed in the literature, inadequacy of tissue controls in cervical cytopathology may further compound the problem, especially when biopsy material is scant or when the lesion is small [13,14]. Gilani et al noted in their study that many of the false positive cases on cytology in postmenopausal women had an absent transformation zone (47%) on biopsy, and of those who underwent high-risk HPV testing performed, the majority were positive (83.3%).They therefore suggested a potential sampling error on biopsy, perhaps due to an inability to visualize the involved area in older women because of an upward migration of the transformation zone [13]. The subsequent hysterectomy specimen on thorough sampling showed invasive squamous cell carcinoma.

One of the cases was a false positive on initial diagnosis, however after blind review it was called negative, which concurred with the histopathological diagnosis. On unblinded review it seemed that initially there may have been an interpretative error wherein reactive atypia may have been mistaken for atypical cells suggestive of low grade intraepithelial lesion. Abali et al in their study had high incidence of false positive cases which on follow up showed regenerative changes due to chronic cervicitis or inflammation [15]. The low levels of oestrogen in postmenopausal women results in atrophic smears with predominance of parabasal and intermedio-parabasal cells with high nuclear cytoplasmic ratio. The presence of additional inflammatory/repair related regenerative changes in these women makes distinguishing from neoplastic lesions difficult [15]. Hence there could be a over diagnosis of ASC-US in postmenopausal women. One case where a moderately differentiated adenocarcinoma was initially reported as squamous cell carcinoma was on blind review reported to be poorly differentiated carcinoma which seemed to be a more accurate cytological diagnosis in comparison to the histological diagnosis. This case highlights the possible difficulty of accurately diagnosing poor to moderately differentiated glandular lesions in cytology.

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CONCLUSION

Cytohistopathological correlation is an essential exercise to assess the quality of cervical cytological examination in the laboratory. Analysis of cytohistopathologic discrepancies gives a clear understanding of the possible pitfalls in smear examination and will prevent future erroneous reporting. A methodical blinded review by an unbiased individual during regular reporting will definitely help in improving sensitivity rates. This is proved by the improvement in screening parameters on blind review. Cautious reporting is essential when the smear is associated with artifacts due to radiotherapy, inflammation etc and in smears with low cellularity.

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