A cyto-histopathological correlation study of lesions of uterine cervix

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Abstract

Background: Carcinoma uterine cervix is one of the leading causes of cancer death among women worldwide. To detect this widely prevalent cancer at an early stage, the simplest test has been a pap smear. Reporting of pap smears is done by using The Bethesda System 2001. However, various benign processes can also be recognized morphologically, and diagnosis of these entities can make an important contribution to patient care.

Methods: Cyto-histopathological correlation of lesions of uterine cervix in 150 cases.

Results: Total numbers of cases correlated in this study were 150. Age group of women, ranged from 23 to 75 years. Majority of the patients belonged to the group of 21 to 30 years.

Conclusion: 75% of the cases showed cyto-histopathological correlation. One rare case of adenosquamous carcinoma was detected cytologically and was later confirmed by histopathology.

Key Words: Uterine-cervix, cytology, histopathology, correlation, PAP smear.

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Introduction

Cytological examination by Papanicolaou smears is not only confined to the diagnosis of premalignant and malignant lesions of the uterine cervix but also for identifying the reactive and infective conditions. Cervical smears may include cells exfoliated from body of the uterus and even at times from fallopian tubes and ovaries.

The potential threat of cancer is the main reason for Pap smear screening programs and if abnormalities are detected, they have to be confirmed subsequently by histopathologic examination.[1]

Pathologists are frequently faced with the problem of reconciling the cytology report with the histopathological findings. That is the reason why we have taken up the present study.

Aim of the study

To study the cytopathology of various lesions of the cervix and to correlate the cytological features with the histopathological findings.

Materials and Methods

This is a prospective study, undertaken in the department of Pathology KBNIMS Gulbarga, during the period of January 2013 to January 2014. Patients were from in and around Gulbarga.

During this period cervical smears of 150 women were studied in whom histopathological study was possible.

Cervical smears were obtained by Gynecologists, using Ayres spatula. Smears were covered with Biofix spray, and sent to Pathology department. These smears were immersed in Papanicolaou fixative (equal volumes of 95% ethanol and ether) for minimum of 30 minutes and then stained with Papanicolaou stain.

Cytological features in these smears were examined in detail. Histopathological study was possible in all these cases, which included 80 cervical punch biopsies; 60 total hysterectomies and 10 pan-hysterectomies.

Histopathological specimens were grossed, processed and blocks were made. Then sections of 3-5 microns were prepared, stained with H&E stain and microscopically examined.

Adequacy of the cytological material: Satisfactory smears which consisted of well preserved and well visualized squamous cells, that are spread over more than 10% of the slide surface, and with adequate endocervical/transformation zone component (minimum of two clusters of well-preserved endocervical and /or squamous metaplastic squamous cells), were only included in the study.

Biopsy and hysterectomy specimens were fixed in 10% formalin, were processed by routine procedure to obtain paraffin sections, and were stained with Haematoxylin and Eosin.

Histopathology reporting was done separately without the knowledge of cytology report. Later cytological features were correlated with histopathological findings.

To substantiate the correlation, results of the present study were calculated by using the methodology of Galen and Gambino.

Observations and Results

This study was undertaken to correlate the cytological features with the histopathological findings in various lesions of the cervix .During this period Pap smears of 150 women were examined and were subsequently correlated with histopathological findings.

The actual findings in our study are as follows: Age: Age group of women, ranged from 23 to 75 years. Majority of the patients belonged to the group of 21 to 30 years.

Overall cytologic diagnoses in this study are shown in Fig. 1.

Smears within normal limits: Among four cases of cytologically normal smears, histopathology showed features of chronic cervicitis in two cases and chronic papillary endocervicitis in two cases.

Benign cellular changes shown in Table 1.

Table 1: Benign cellular changes

Sl. No.	Benign cellular changes	No. of cases
1	Chronic cervicitis	83
2	Chronic cervicitis with squamous metaplasia	8
3	Chronic cervicitis with keratinisation	3
4	Chronic papillary endocervicitis	22
5	Chronic papillary endocervicitis with squamous metaplasia	1
6	Chronic cervicitis with Trichomonas vaginalis infestation	2
7	Atrophic cervicitis	1
	Total	120

In this study, 83 cases showed features of chronic cervicitis, in which the smears showed admixture of superficial and intermediate squamous epithelial cells, marked cytolysis, and inflammatory exudate consisting of neutrophils, lymphocytes, and macrophages. Lactobacilli were seen in all cases and "clue cells" were seen in two cases.

In two smears there were Trichomonas vaginalis organisms which had pear-shaped appearance and pale blue to green in color, with an eccentric, slightly basophilic nucleus.

Histopathological diagnoses in cytologically diagnosed cases of chronic cervicitis shown in Table 2.

Table 2: Histopathological diagnoses in cytologically diagnosed cases of chronic cervicitis

Sl. No.	Histo-Pathological Diagnosis	No. of cases
1	chronic cervicitis	46
2	chronic papillary endocervicitis	26
3	chronic papillary endocervicitis ,with chronic follicular cervicitis	01
4	chronic papillary endocervicitis, follicular cervicitis with squamous metaplasia	01
5	chronic cervicitis with mature squamous metaplasia	06
6	chronic cervicitis with mild dysplasia	01
7	chronic cervicitis with moderate dysplasia	01
8	infiltrating ,large cell, non-keratinising squamous cell carcinoma	01
	Total	83

In cytologically diagnosed cases of chronic cervicitis with squamous metaplasia, the additional features were presence of varying numbers of immature, maturing and mature metaplastic squamous cells. Immature metaplastic cells were seen in sheets and had dense cyanophilic cytoplasm with occasional cytoplasmic vacuolations. Maturing metaplastic squamous cells were recognized by the cytoplasmic elongations, indicating spider cells. Mature metaplastic squamous cells doubly thick cell borders. Histopathological diagnosis in cytologically diagnosed cases of chronic cervicitis with squamous metaplasia showed in Table 3.

Table 3: Histopathological diagnosis in cytologically diagnosed cases of chronic cervicitis with squamous metaplasia

Sl. No.	Histopathological Diagnosis	No. of cases
1	Chronic cervicitis.	2
2	Chronic cervicitis with squamous metaplasia	2
3	Chronic papillary endocervicitis with squamous metaplasia	1
4	Chronic cervicitis with epidermidization	2
5	Chronic cervicitis with moderate dysplasia	1
	Total	8

Smears showing polypoidal and papillary processes lined by columnar endocervical cells, accompanied by inflammatory infiltrate, diagnosis of chronic polypoidal (papillary) endocervicitis were made.

In smears of chronic cervicitis with epidermidization, there were parakeratotic cells and anucleate squames.
In our study epithelial cell abnormalities on cytology were seen in 26 cases showed in Table 4.

Table 4: Epithelial cell abnormalities in cytological study

Sl. no.	Epithelial cell abnormalities in cytological study	No. of cases	percentage
1	Chronic cervicitis with atypical squamous metaplasia	2	7.69%
2	Chronic cervicitis with LSIL	3	11.55%
3	Chronic cervicitis with HSIL	4	15.38%
4	Invasive ,keratinising, squamous cell carcinoma	6	23.08%
5	Invasive ,large cell, non-keratinising ,squamous cell carcinoma	2	7.69%
6	Chronic cervicitis with endocervical glandular cell dysplasia	4	15.38%
7	Chronic cervicitis with endometrial hyperplasia	4	15.38%
8	Adenosquamous carcinoma	1	3.85%
	Total	26	100%

Two case of chronic cervicitis with atypical squamous metaplasia showed a few clusters of endocervical cells, accompanied by immature and mature metaplastic squamous epithelial cells. There were also accompanying atypical metaplastic squamous cells having large, densely staining nuclei with thick cytoplasm and sharply defined cell borders.

Histopathological study in these two cases showed features of chronic papillary endocervicitis.

Cervical smears in LSIL showed varying numbers of parabasal cells, intermediate and superficial squamous epithelial cells. All these cases showed slightly enlarged nuclei in superficial and intermediate squamous epithelial cells. (Picture 1)

Histopathological diagnoses in cytologically diagnosed cases of chronic cervicitis with LSIL shown in Fig. 2. (Picture 2)

Smears of HSIL showed scattered parabasal, intermediate and superficial squamous epithelial cells. In all these cases a few intermediate squamous epithelial cells showed moderately enlarged hyperchromatic nuclei with prominent chromocenters and coarse chromatin. (Picture 3)

Histopathological diagnoses in cytologically diagnosed cases of chronic cervicitis with HSIL shown in Fig. 3. (Picture 4)

In all the six cytologically diagnosed cases of invasive keratinizing carcinoma the smears showed abnormal squamous epithelial cells with marked nuclear pleomorphism. Elongated tumour cells and tadpole cells were seen. Multinucleated tumour giant cells were sees in one case. Dyskeratosis was noted in all the cases. In all these cases background showed tumour diathesis. (Picture 5)

All these cases were confirmed by histopathology. (Picture 6)

Two cases of invasive, large cell, non –keratinizing squamous cell carcinoma showed scattered as well as loose aggregates of abnormal squamous cells having cyanophilic cytoplasm, large vesicular nuclei and occasional irregular nucleoli. Mitoses were sparse. Background showed tumour diathesis.

The diagnosis was confirmed by histopathology.

Smears of chronic cervicitis with endocervical glandular cell dysplasia showed sheets and clusters of endocervical cells with nuclear overlapping; elongated, hyperchromatic nuclei and increase in nuclear-cytoplasmic ratio.

Histopathological diagnoses in cytologically diagnosed cases of chronic cervicitis with endocervical glandular dysplasia shown in Fig. 4.

One case was diagnosed on cytology as Adenosquamous carcinoma. The smears showed clusters of endocervical cells showing nuclear pleomorphism, and there were also pleomorphic squamous epithelial cells. (Picture 7)

The diagnosis was later confirmed by histopathology. (Picture 8)

In four cytologically diagnosed cases of chronic cervicitis with endometrial hyperplasia, the smears showed clusters of endometrial cells, and histopathological examination of cervix showed features of chronic cervicitis.

One case of atrophic cervicitis showed predominantly degenerating parabasal and intermediate squamous epithelial cells, with nuclear pyknosis, margination of chromatin, karyolysis and karyorrhexis. The hysterectomy specimen showed features of atrophic cervicitis.

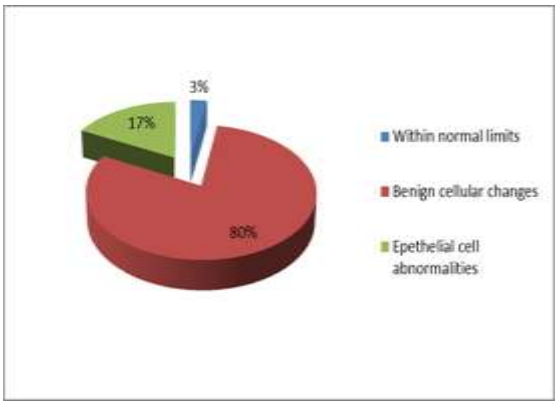

Fig. 1: Over all cytologic diagnoses in this study

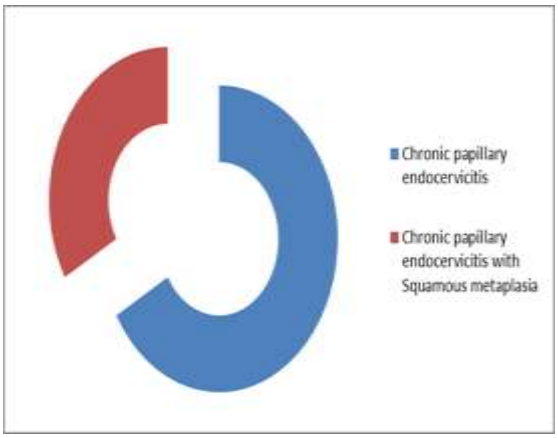

Fig. 2: Histopathological diagnoses in cytologically diagnosed cases of Chronic cervicitis with LSIL

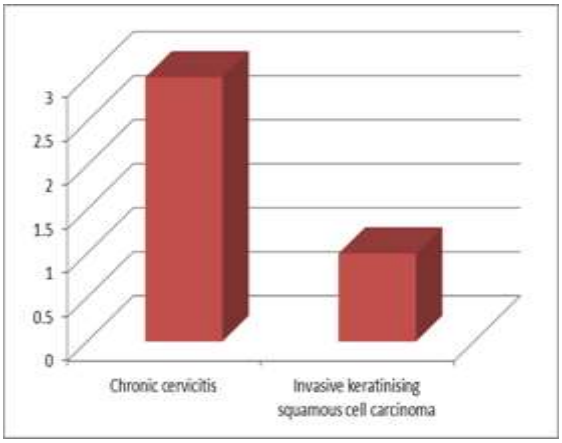

Fig. 3: Histopathological diagnoses in cytologically diagnosed cases of Chronic cervicitis with SIL

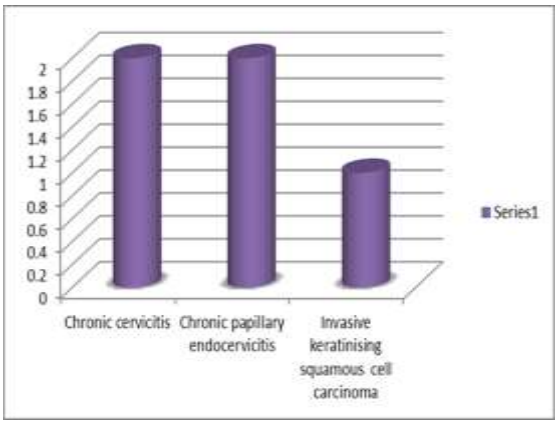

Fig. 4: Histopathological diagnoses in cytologically diagnosed cases of chronic cervicitis with endocervical glandular dysplasia

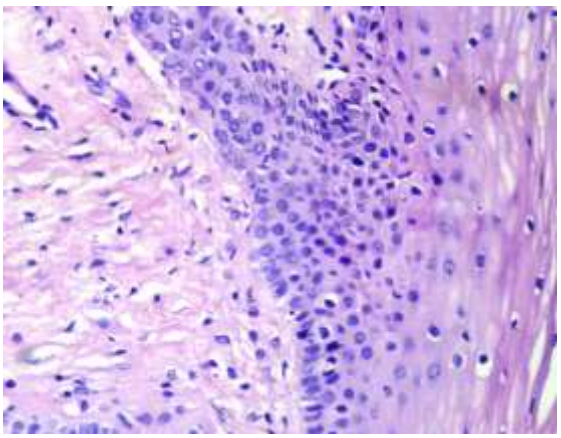

Fig. 2: 40x, Section showing features of mild dysplasia

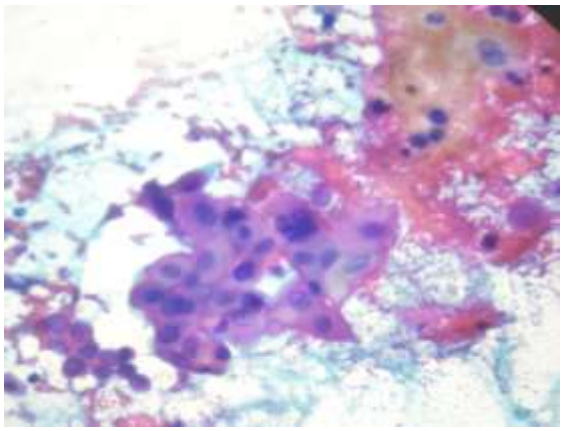

Fig. 3: 40x, Smear showing features of HSIL

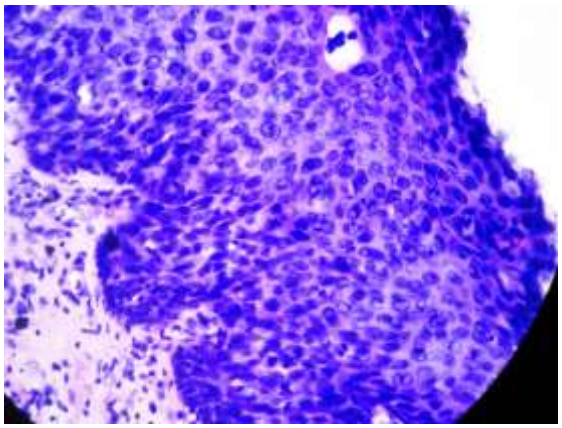

Fig. 4: 40x, Section showing features of severe dysplasia

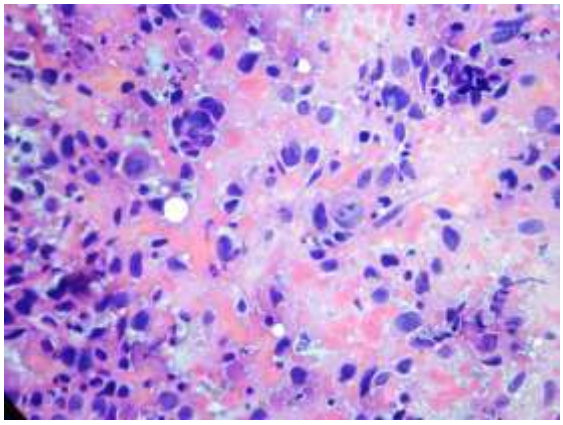

Fig. 5: 40 x, Smear showing features of invasive keratinizing squamous cell carcinoma

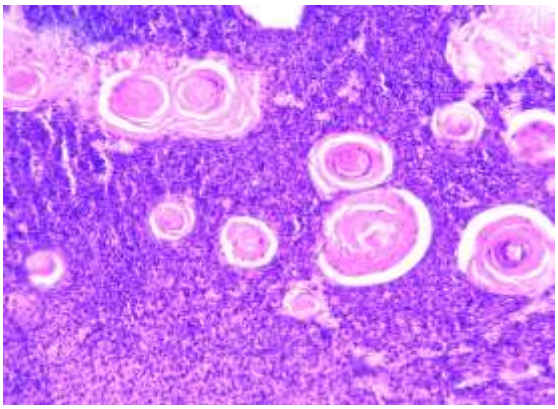

Fig. 6: 10x, Section showing features of invasive keratinizing squamous cell carcinoma

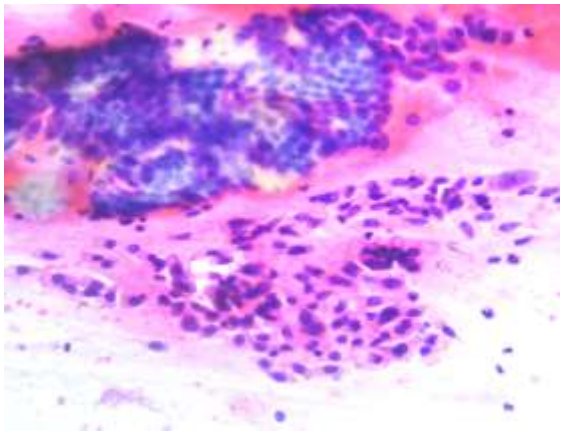

Fig. 7: 40x, Smear showing features of Adenosquamous carcinoma

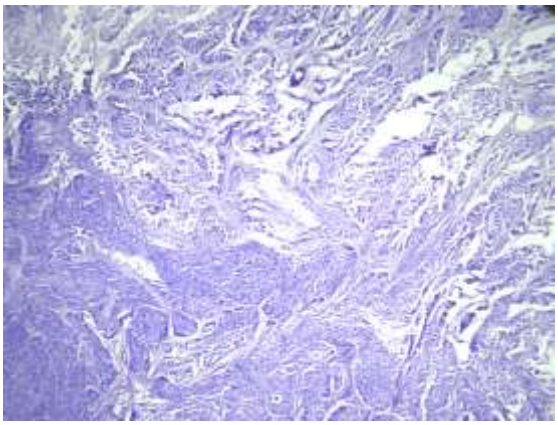

Fig. 8: 4x, Section showing features of Adenosquamous carcinoma

Discussion

The value of cytology diagnosis depends mainly on having good correlation with histopathological features.

P.Sodhani et al, in their cyto-histological correlation study, concluded that, cyto-histological correlation is a measure of quality assurance of a cytology laboratory [2].

In our study cyto-histopathological correlation was done to assess the diagnostic utility of studying the cervical smears.

During this study 150 Pap smears which were satisfactory for evaluation), were examined and subsequently correlated with histopathological features.

Riotton and Cristopherson defined the normal and the abnormal cells of cervical and vaginal smears in the World Health Organization International classification. The abnormal cells were described in terms of the histopathological conditions with which they correlate, i.e., mild moderate and severe dysplasias; carcinoma in -situ, and atypical metaplasia. Grades of dysplasia, carcinoma in-situ and invasive carcinoma have been used by cytologists since many years to describe cervical cytology.[3]

A study was conducted to study the usefulness of cervical cytology by PAP smear and the application of the Bethesda 2001 system in classifying different pre-neoplastic and neoplastic lesions of cervix.

The 2001 Bethesda System for reporting cervical cytologic diagnoses is an incremental change in the uniform terminology introduced in 1988 and revised in 1991. The 2001 Bethesda System includes specific statements about specimen adequacy, general categorization and interpretation of results. The terminology for low- grade squamous intraepithelial lesions (LSILs) and high grade squamous intraepithelial lesions (HSILs) remains unchanged.[4]

In the study of Agnesamma J. et al (1979), among 56 cases of cytological benign lesions, 15 cases were having mild dysplasia on histopathological examination.[5] In the study of Singh V K et al (1984), among 38 cases of cytologically detected chronic cervicitis nine were having mild dysplasia on histology and remaining 29 were chronic cervicicitis.[6] In the study of Talib N.S. et al (1978), among nine cases of chronic cervicitis, histological features suggested chronic cervicitis in seven and two were mild dysplasia.[7]

Comparison of cytological diagnoses with the previous studies shown in Table 5.

Table 5: Comparison of cytological diagnoses with other studies

Sl. no.	Cytological diagnosis	Talib NS et al(1978)	Agnesamma J., et al(1979)	Singh VK. et al (1984)	Present study (2014)
1	Within normal limits	-	-	-	4
2	Benign cellular changes	52	56	98	120
3	Atypical squamous metaplasia	-	-	-	2
4	LSIL	46	12	88	3
5	HSIL	34	42	33	4
6	Carcinoma in- situ	3	7	6	-
7	Invasive squamous cell carcinoma	1	21	2	8
8	Endocervical glandular dysplasia	-	-	-	4
9	Adenosquamous carcinoma	-	-	-	1
10	Endometrial hyperplasia (suspected)	-	-	-	4
	Total	136	145	227	150

Among 120 cases of benign cellular changes observed on cytology there was only one false negative case of malignancy on histopathological correlation.

Among 26 cases of epithelial cell abnormalities, nine cases were true positive and two cases were false negative. There were no false positive malignancy reports.

Statistical values of detecting malignant lesions shown in Table 6.

Table 6: Statistical values of detecting malignant lesions by cervical cytology using Galen and Gambino method

Sl.no.	Statistical index	Results
1	True positive	9
2	True negative	138
3	False positive	Nil
4	False negative	3
5	False positive error rate	0
6	False negative error rate	2%

7	Sensitivity	75%
8	Specificity	100%
9	Positive predictive value	100%
10	Negative predictive value	97.87%
11	Efficacy	98%

Present study showed 100% specificity and 100% positive predictive value. False positive error rate was zero. Sensitivity was 75%, and efficacy was 98% false negative error rate was 2% and negative predictive value was 97.87%. These statistical values indicate the clinical utility of cervical cytology in detecting the malignant lesions.

Vaishali Jain and A S Vyas studied a total number of 4805 Pap smears over a period of two years (of which 4251 were inflammatory smears, 287 were unsatisfactory and the remaining 276 smears showed epithelial cell lesions) along with their corresponding histopathology findings and they calculated the concordance rate of each variable.[8]

Comparison of sensitivity and specificity with other study shown in Table 7.

Table 7: Comparison of sensitivity and specificity

Values	Vaishali Jain & A S Vyas (2010)	Present study(2014)
Sensitivity	78%	75%
Specificity	26.9%	100%
Positive predictive value	91.1%	100%
Negative predictive value	11.3%	97.87%

Comparison of false negative results shown in Table 8.

Table 8: Comparison of false negative results

Sl. no.	Authors	% of false negative
1	Richart (1964)[5]	2.87
2	Silbar (1966)[5]	18
3	Present study (2014)	25

Among 12 biopsy proved cases of malignancies there were three false negative reports.

As the incidence of malignant lesions in this study is less, there is relatively high percentage of false negative reports.

Discrepencies in cytological and histopathological diagnoses may be attributed to either inadequate sampling, or errors in biopsy techniques.[9]

Sanjay gupta and P. Sodhani in their study, concluded that the major factors responsible for under interpretation on cytology included air drying artefacts and metaplastic maturation of abnormal cells.[10]

Comparative predictive value of invasive cervical malignancies shown in Table 9.

Table 9: Comparative predictive value of invasive cervical malignancies

Authors	Sensitivity of the test	Positive predictive value
GPS Yeoh, KW Chan[11]	91.7%	93.5%
Present study(2014)	75%	100%

In the present study specificity of the cytological screening was found to be high enough to permit surgical intervention after the cytodiagnosis of malignancy. The negative predictive value of 97.87%, when compared with the low prevalence of cervical carcinoma, is relatively low, but the positive predictive value is extraordinarily high.

Most of the cases of cervical cancer are caused by infection with oncogenic, or high risk, types of Human Papilloma Virus. HPV -16 and HPV -18 are the two types that cause most of the cervical cancers.[12]

By 'A Cytology, Histology, and HPV Correlation study From 2 Institutions', Ross A Millller et al, have highlighted the importance of combined Pap and HPV contesting, which may reduce the failure rates especially with regard to glandular lesions.[13]

Conclusion

The effective role of exfoliative cytology in detecting the cellular abnormalities of the cervix is beyond doubt. In our study 75% of the cases showed cyto-histopathological correlation. In our study of 150 cases of cyto-histological correlation, 120 cases showed benign lesions. LSIL were three (2%) and all were benign lesions on histopathology. There were four (2.66%) cases of HSIL, of these three (75%) were benign lesions on histopathology and one (25%) was invasive squamous cell carcinoma. Eight cases (5.33%), of invasive squamous cell carcinoma were diagnosed

on cytology and all were confirmed by histopathological study.

Pap smear screening should still continue according to past guidelines to minimize cancer incidence and all cases of squamous intraepithelial lesions should be subjected to histopathological study to rule out carcinomas.

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