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ResearchArticle

ANALYSIS OF CYTOLOGICAL FALSE NEGATIVES OF CARCINOMA OF CERVIX

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A B S T R A C T

Introduction: Cervical carcinoma is one of the leading malignancies affecting the women of India. Since the Papanicolaou (Pap) test can very effectively detect the prolonged phase of carcinoma in situ, current policy suggests that all women should have this test done at regular intervals. Monitoring cytohistological discrepancies is a useful quality assurance tool in cytology laboratory. Cervical cancer has been successfully reduced by routine screening and medical intervention.

Aims and objectives: This present study was done to study the prevalence of cytohistological discrepancy in histologically proven cases of carcinoma cervix and to identify the causes for false negativity in the cytological examination in histologically proven cases of carcinoma cervix.

Materials and methods: This study was conducted in the Department of Pathology, Mahatma Gandhi Institute Of Medical Sciences, Sevagram, Maharashtra. This study was done on 209 biopsy proven cases of carcinoma cervix over a period of two years. The original cytological diagnosis of smears available in these cases were co- related with the biopsy results.

Results: In the pre-review, in 80.3% cases the diagnosis of carcinoma was rendered on initial screening in cytology. Cytohistological discrepancies were observed in 19.6% accounting for a false negative rate of 19.6% and a false negative fraction 0.196 %. After post review, the false negative rate reduced to 11.62% and the frequency of the different types of errors were calculated.

Conclusion: The level of agreement between cytology and the histology diagnosis may be used as a measure of laboratory quality. To the best of our knowledge, data on factors associated with cytohistologic discrepancy in Pap smear are limited. Therefore, we conducted this study to evaluate the factors associated with cytohistologic discrepancy in Pap smears and to determine the rate of cytohistologic discrepancy and ways to reduce the false negatives.

INTRODUCTION

After breast cancer, cancer of the cervix, is the second most common cancer in females and one of the major cause of cancer amongst women in developing countries. It accounts for 17% of all cancer deaths amongst women aged 30-69 years. It may occur in approximately 1 in 53 Indian women during their lifetime as compared to1 in 100 women in developed regions. [1]

World wide 266000 women die of cervical carcinoma each year. It is the leading cause of cancer deaths in eastern and Central Africa. 528000 new cases of cervical cancer were diagnosed world wide in the year 2012; about 85% of these occurred in the developed regions. The majority of the deaths due to these cancers can be prevented through universal access to comprehensive cervical cancer prevention and control programmes. Cervical cancer still remains the only human malignancy that has been successfully reduced by routine screening and medical intervention. [2]

122,844 women are diagnosed with cervical cancer in India per year and 67,477 die from the disease. [3] It is the second most common cancer in the age group of 15- 44 years. In South Asia, India has the highest age standardized incidence at 22.[3] Though the main cause of increasing cervical cancer is not known presumably exposure to human papilloma virus (HPV), active sexual life, multiparity, hormonal contraception, genetic factors and smoking are factors that may initiate the process of cervical cancer.[4] Cervicalcancer screening programmes play animportant role in the reduction of cervical cancer in developed countries, though in many developing

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countries they still have not been implemented. The 10 -20 year lag between pre cancer and cancer offers ample opportunity to screen , detect and treat the pre cancerous lesions and contain its progression to frank cancer.[2]

Since the test can effectively detect the prolonged phase of carcinoma in situ, current policy suggests that all women should have this test done at the beginning of their sexual activity and thereafter every six months. Hence, the ideal screening strategy ought to identify those cervical cancer that are likely to progress to invasive cancers, thus maximizing the benefits obtained from cervical screening.[4]

Cytological screening leads to the determination of precursors and their mimics. The practical value of these precursor lesions is their presence in cervicovaginal smears and their early detection by cytological screening. On the assumption that the treatment of these pre-cancerous lesions would prevent invasive cancer of the cervix, the test has been hailed as the ultimate tool in cancer detection and prevention. Cancer of the cervix grows slowly over a period of time from precancerous dysplasia / Cervical Intraepithelial Neoplasia (CIN) to preinvasive to invasive cancer. However it is important to know that most CIN do not develop into cancer. [5] High grade squamous intraepithelial lesion (HGSIL) Pap smear carries a high risk for significant cervical pathology. Around 1-4% of women with HGSIL Pap smear had invasive cervical cancer. [6] Cervical cancer screening programmes play an important role in the reduction of cervical cancer in developed countries. On the assumption that the treatment of these precancerous lesions would prevent invasive cancer of the cervix, the test hailed as the ultimate tool in cancer detection and prevention. Since the name, Papanicolau, was too long, the term Pap test was coined for this procedure which now has come into colloquial use and this test has now entered the mainstream of laboratory testing.

The efforts of the physicians and national health system has been aimed at the early recognition of these precancerous cells in cervical smears and hence allow treatment at an earlier stage. Accordingly a few women will develop cervical cancer despite adherence to accepted screening protocols. In addition, problems inherent with sampling, interpretation, and effective clinical follow up preclude total prevention of cervical cancer.[7]

Monitoring cytohistologic discrepancies is a useful quality assurance tool in cytology laboratory. As a part of continuous quality improvement program, cytohistologic correlation may help laboratories to refine diagnostic criteria and improve diagnostic accuracy and reproducibility.[8]

Cytohistologic correlation entails the concomitant review of cytological and histological specimen that were obtained in a narrow time frame from the same site in a given patient. The level of agreement between cytologic and the histologc diagnosis may be used as a measure of laboratory quality. Few objective studies of errors in pathology have been performed apart from interobserver variability studies and studies concerning false positive and false negative diagnosis. Using the metric of cytohistologic discrepancy, several avenues of investigations were carried out to better elucidate the nature of errors in cytology and histology, as well as to examine the effects on patient outcome.[9] Necrosis, inflammation and bleeding can obscure, dilute or alter the diagnostic cells. This explains another Pap smear paradox, namely, invasive cancer have a higher false negative rate than pre-cancerous lesions.[7] Using the metric of cytohistologic discrepancy, the following study was carried out to better elucidate the nature of errors in cytology and histology, as well as to examine the effects on patient outcome.

Aims and objectives

1. To study the prevalence of cytohistological discrepancy in histologically proven cases of carcinoma cervix.
2. To identify the causes for false negativity on cytological examination in histologically proven cases of carcinoma cervix.
3. To evaluate the causes and suggest means of decreasing the false negativity rates in cervical cytology.

MATERIAL AND METHODS

The present study was entitled "Analysis of cytological false negatives of carcinoma of cervix'' was conducted in the Department of Pathology in Mahatma Gandhi Institute Of Medical Sciences, Sewagram, Wardha, Maharashtra. A total of 209 biopsy proven cases of carcinoma cervix over a study period of two years were included in the present study. The original cytological diagnosis of the smears available in these cases was correlated with the biopsy. All the cases in the present study were histologically proven to be squamous cell carcinoma (SCC). The exclusion criteria in the study were women who had prior hysterectomy, no available histological data, patients on radiotherapy for carcinoma cervix, and known cases of carcinoma cervix. The available cytologic and the histologic slides were reviewed.

The cervical cytology specimen was obtained by cervical scrape with disposable Ayre's spatula. The smears were made by scraping the cervix from the squamocolumnar junction in a clockwise direction (360 degrees rotation) and fixed immediately in 95% alcohol.After the receipt of the specimen in the cytology section in the Department of Pathology; labeling and Pap staining was performed on the smears according to the method proposed by Milner *et al*.[10] Cervical cytology reporting were done according to the Bethesda system 2001 for cervical cytology reporting.[11]

In the histopathological division of the Department of Pathology, the biopsy specimens were received in formalin as a fixative. The specimens were then further processed in automatic tissue processor and paraffin sections were cut into 3 μm thickness diameter and stained by routine haematoxylin and eosin method. Correlation between the original cytological diagnosis and the biopsy was done in all biopsy proven cases of carcinoma cervix.

The following statistical evaluation were done

1. ***The false negative rate:*** the percentage of cases in which cytolological diagnosis missed the diagnosis of malignancy
2. ***The false negative fraction:*** false negative / true positive + false negative. As a total of true positives and false negatives were the total cases in the present study and all were biopsy proven malignancies, hence false negative rates and false negative fraction was the same.

The false negative cytological smears were reviewed again to differentiate between screening errors, diagnostic errors and sampling errors.

1. Screening errors were defined as those in which the abnormal cells were present in the cytology smear but the screener failed to detect them.
2. Diagnostic errors or interpretation errors were defined as the failure to properly categorize the cells once they have been found.
3. Sampling errors were defined as those in which the smears failed to show abnormal cells on re examination.

RESULTS

1. Maximum number of cases of carcinoma cervix were in the age group 41-50 years (30.14%) followed by 31-40 years (27.2%) . (Table 1)

Table 1 Age distribution of patients

Age group (years)	Number of cases	%
31-40	57	27.2
41-50	63	30.14
51-60	54	25.83
61-70	27	12.91
>70	8	3.82
Total	209	100

2. Out of the total 209 biopsy proven cases of carcinoma cervix with co relating conventional Pap smears ; pre review ; 168 cases showed the presence of malignancy , i.ecytohistological correlation was 80.38 %. (Table 2)

Table 2 Pre review cytological diagnoses in biopsy proven cases of carcinoma cervix

	Cytology diagnosis	Number of cases	%
1.	NILM	02	0.95
2.	ASCUS	15	7.17
3.	LGSIL	12	5.74
4.	HGSIL	09	4.30
5.	Malignancies	168	80.38
6.	AGUS	3	1.43
7.	Unsatisfactory	-	
	Total	209	100

3. Pre review, 41 cases did not show features of malignancy on cytology accounting for a false negative rate of 19.6%.
4. False negative rate was higher in the pre menopausal category [28/41 (68.29 %)] followed by post menopausal category [13 /41 (31. 70%)]. (Table 3)

Table 3 Pre review distribution of discrepant cases in pre and post menopausal women

Pap smear Diagnosis	Pre Menopausal	%	Post Menopausal	%
ASCUS	12	42.85%	3	23.07
LGSIL	9	32.4	3	23.07
HGSIL	5	17.85	4	30.76
AGUS	1	3.57	2	15.38
NILM	1	3.57	1	7.69
Total	28 (68.29 %)		13 (31.70 %)	41

5. The commonest discrepant cytological diagnosis, pre review , in the pre menopausal category was Atypical squamous cell of undetermined significance[ASCUS] (42.85%), followed by low grade squamous intraepithelial lesions [LGSIL] (32.14 %). (Table 3)
6. The commonest discrepant cytological diagnosis, pre review, in the post menopausal category was High grade squamous intraepithelial lesion [HGSIL] (30.76%), followed by LGSIL and ASCUS (23.07 %). (Table 3)
7. On rescreening of the cervical smears by applying the criteria of The Bethesda System 2001, eleven cases were unsatisfactory for evaluation where as seven additional cases of invasive cell carcinomawere detected. (Table 4)

Table 4 Post – review cytological diagnoses in biopsy proven cases of carcinoma cervix

	Cytology diagnosis	Number of cases	%
1.	NILM	02	1.01
2.	ASCUS	03	1.51
3.	LGSIL	11	5.55
4.	HGSIL	6	3.03
5.	Squamous cell carcinoma	175	88.38
6.	AGUS	1	0.5
	Total	198	100

8. Hence, the cytohistological correlation seen increased to 88.38% and the false negative rate reduced to 11.62% after rescreening. (Table 4)
9. Post review of the cervical smears, false negative rate was higher in the pre menopausal category [17/23 (73.91 %)] followed by post menopausal category [06 /23 (26.08 %)]. (Table 5)

Table 5 Post review distribution of discrepant cases in pre and post menopausal woman

Pap smear Diagnosis	Pre Menopausal	%	Post Menopausal	%
ASCUS	3	17.64		
LGSIL	9	52.94	2	33.33
HGSIL	3	17.64	3	50
AGUS	1	5.88		
NILM	1	5.88	1	16.66
Total	17 (73.91%)		6 (26.08%)	23

10. Post review, commonest discrepant cytological diagnosis in the pre menopausal category was LGSIL (52.94%) followed by ASCUS and HGSIL (17.64 %). (Table 5)
11. Post review, commonest discrepant cytological diagnosis in the post menopausal category was HGSIL (50%) followed by LGSIL (33.33%). (Table 5)
12. The comparison of the false negative rate and the false negative fraction was done pre and post review. (Table 6)

Table 6 Comparison of false negative rates pre and post review

	Pre review	Post review
Cytohistological Correlation	80.38%	88.38%
False negative rates	19.61%	11.61%
False negative fraction	0.196	0.116

13. Comparison of distribution of the discrepant cases in the pre and post menopausal women (pre and post review) was done. (Table 7)
14. In the pre menopausal group, the most common cytological error was sampling error seen in 14 cases and three cases also showed screening error. (Table 8)

Table 7 Comparison of distribution of discrepant cases in pre and post menopausal women (Pre and post review)

Pap smear Diagnosis	Pre review		Post review	
	Pre Menopausal	Post Menopausal	Pre Menopausal	Post Menopausal
ASCUS	42 %	23.07%	17.64 %	
LGSIL	32.4%	23.07%	52.94 %	33.33 %
HGSIL	17.85 %	30.76%	17.64 %	50%

Table 8 Types of errors

Category	Sampling Error	%	Screening Error	%	Total
Pre Menopausal	14	82.35	3	17.65	17
Post Menopausal	4	66.66	2	33.33	6

15. In the post menopausal group, the most common cytological error was sampling error seen in four of the cases and two cases showed screening error. (Table 8)
16. In the pre menopausal group, of the three cases that showed screening error, the most common diagnosis was LGSIL (two cases) followed by HGSIL (one case). All these five cases were found to be cases of carcinoma cervix after re screening. (Table 9)

Table 9 Analysis of screening errors in pre menopausal group

Initial diagnosis	Review diagnosis Squamous cell carcinoma
ASCUS	
LGSIL	2
HGSIL	1
AGUS	-
NILM	-
Total	3

17. In the post menopausal group, of the two cases that showed screening error, the most common diagnosis was HGSIL (two cases). (Table 10)

Table 10 Analysis of screening errors in post menopausal group

Initial diagnosis	Review diagnosis Squamous cell carcinoma
ASCUS	-
LGSIL	-
HGSIL	2
AGUS	-
NILM	-
Total	2

18. Out of the 11 unsatisfactory cases, pre menopausal women had more number of unsatisfactory smears were than post menopausal women. Obscuring inflammation was the most common cause in the pre menopausalwomen, where as obscuring inflammation and haemorrhage both were present in the post menopausal age group. (Table 11)
19. Comparison of concordance rates for squamous cell carcinoma on cytopathology and biopsy in various studies. (Table 12)

Table 11 Evaluation of unsatisfactory smears in pre and post menopausal group

S.no	Causes	Pre menopausal	Post menopausal
1.	Obscuring Inflammation	4	2
2.	Obscuring Haemorrhage	2	2
3.	Low celularity	1	-
	Total	7	4

Table 12 Comparison of concordance rates for squamous cell carcinoma in various studies

	Study	Rate in percentage
1.	Present study	88.38
2.	Wei *et al*	88
3.	Yoshida *et al*	73.3
4.	Chaithanya *et al*	86.65
5.	Jain *et al*	83.6
6.	Yeoh *et al*	54.5
7.	Nawaz *et al*	97.3

DISCUSSION

The aim of using the cervical smear test (Papanicolaoutest) is to enable the early detection and treatment of pre cancerous lesions and reduce the mortality rate in females due to carcinoma cervix. Cytological screening leads to the detection of precursor lesions of carcinoma cervix and their mimics. Both squamous cell carcinoma and adenocarcinoma develop through distinctive precursor lesions that are liable to detection by the Pap smear test. The practical importance of the precursor lesions is that they are present in the cervicovaginalsmears, and hence are liable for early detection by cytological screening. [12]

It is well accepted now that the Pap smear test has been the most effective cancer screening test ever introduced. There has been a reduction in the death rate of more than 70 % for this prevalent cancer in recent times. [13]

Although research suggests Pap smear screening is relatively common, there are subgroups which remain resistant to screening efforts. Cervical cancer screening guidelines were developed to screen the general population. Special high risk populations are not adhered to in these guidelines. These include (1) women with history of carcinoma cervix (2) women who were exposed in utero to di-ethyl stilbesterol and (3) women who are immune compromised.[14]

Both liquid based cytology (LBC) and conventional method, as a part of cervical cytology screening are accepted. Conventional papanicolaou smears, though most commonly used, are accompanied by some drawbacks. Smearing problems, drying artefacts, inadequate fixation, presence of background obscuring material and thick smears are problems at times encountered with conventional smears.

Liquid based cytology smears (LBC) like Thin Prep processor, Auto cyte and Sure Path or such other systems have minimal drying artefacts and minimum background material leading to optimal cellularity of smears and reduction of background obscuring material.[15]

Though human papilloma virus (HPV) , is an important factor for the development of squamous cervical neoplasia , still most HPV infected women do not develop significant cervical abnormalities. Factors that determine which HPV infection

will develop into squamous intraepithelial lesions (SIL) have been poorly determined. Young females with an effective immune response clear the infection or reduce the viral load to undetectable levels in an average of 8.24 months. The HPV infection found in older females reflects the persistent past infection and correlates with increased rates of high grade squamous intraepithelial lesions (HGSIL) with increasing age. [16]

Low or intermediate type HPV (6, 11) are mostly associated with LGSIL and are usually polyclonal; whereas HSIL harbors clearly oncogenic high risk HPV DNA such as 16,18,31,33 and 35 that are usually monoclonal with a tendency to progression. In LGSIL, there is typically no accumulation of abnormal DNA. Koilocytic atypia is related to the expression of viral E4 protein and is classified as LGSIL in TBS [Figure 1]. Contrary to this in HGSIL, the disrupted cell cycle due to the high risk HPV DNA leads to the accumulation of aneuploid cells that are able to replicate and survive. This phenomenon is mainly induced by the viral proteins E6 and E7 of the high risk oncogenic HPV types. From a bilological point of view, the Bethesda approach is very realistic because LGSIL and HGSIL reveal different pathogenesis.[12] In the WHO classification cervical intraepithelial neoplasia CIN 1 relates to LGSIL; whereas CIN 2 and CIN 3 relates to HGSIL. [12]

This terminology and the process that created The Bethesda System (TBS) have had a profound impact on the practice of cervical cytology for laboratorians and clinicians equally. The Bethesda conferences and their ensuing output have also set the stage for standardization of terminologyacross multiple organ systems, including both cytology and histology.[12]

Quality control in cervical cytology is carried out with the objective to improve the performance of the test to eliminate the false negative results. Cytohistological correlation (CHC) is used most frequently by cytopathology personnels for evaluation of failures in cytological screening. This is a process by which cytologic and histologic interpretations are compared, generally from the same anatomic site, to determine whether they are concordant or discordant.[17]

Monitoring cytohistological discrepancies is an effective tool in this direction. This involves processing of the samples for cytological screening and comparing it with the gold standard of histopathology.[8]

In the present study, the biopsy proven cases of carcinoma cervix were reviewed and compared with their cytology counterpart. The cytohistlogical correlation in the present study was 80.4% that increased to 88.38% post review of the cytological smears that were negative for malignancy in the initial reporting. This was similar to the rate of 88% of cytohistlogical correlation found in the study by Wei *et al*.[18] Similarly studies by many authors like Yoshida *et al*[19] , Jain *et al*[20],Chaithanya*et al*[13] and Yeoh*et al*[21] , found rate of correlation of 73.3%, 83.6% , 83.6% and 54.5% in their study that was lesser than the concordance rate of our study. However, Nawaz *et al*[22] had a rate of 97.3% that was much higher than ours (Table12).

When the highest grade diagnosis of the biopsy specimens is the same as that of the Pap smear result (ie, no evidence of squamous intraepithelial lesion or malignancy [NILM] versus negative for dysplasia, LGSIL versus CIN1, HGSIL versus CIN2 ; it is considered that biopsy correlates with the Pap smear results. Further discrepancies can be considered as minor (one step discrepancy) or major (two and three step discrepancy). One step discrepancy is between Pap smear and biopsy results are (NILM versus CIN 1 or LGSIL versus CIN 2). Two step discrepant diagnosis is (NILM versus CIN 2) and three step discrepant diagnosis is (LGSIL verses carcinoma cervix). Many institutions elect to evaluate only a two or three step discrepant diagnosis as one step discordant diagnoses often resulted in greater number of discordant pairs .[23]

To determine whether a diagnosis is discrepant or not, the cytological and the histological diagnoses must be carried out within a short time frame, and should be compared using different scales of measurement. Many cytology laboratories use semiquantitative scales in which the standard diagnosis are associated with a graded probability of the disease, whereas some cytology laboratories prefer using more descriptive interpretation. [24]

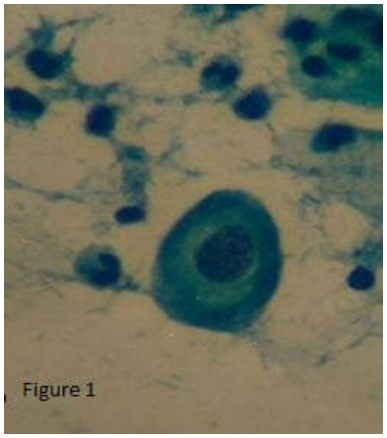

Figure 1 Smear showing a Koilocyte with thickening of the cytoplamic borders and perinuclear halo in case of LGSIL (Pap 400 x)

The 2001 Bethesda system classification of Pap smear diagnoses is a typical example of a semiquantitativescale. This is so because interpretations in the 2001 TBS do not have exact co relates in the CIN system. [24] The Bethesda system classification along with being a uniform system of reporting, also provides effective communication portals amongst cytopathologists and the referring clinician. It also is a very important means of cytohistopathological correlation.[20]

In our study, both one and two step discordant diagnoses were evaluated. In the pre menopausal age group two step discrepant diagnoses was 52.94 % [LGSIL vs SCC], and one step discordant diagnoses was 17.64 % [HGSIL vs SCC] (Table 5). Similarly, in the post menopausal category, two step discrepant diagnoses was 33.33 % [LGSIL vs SCC], and one step discordant diagnoses was 50 % [HGSIL vs SCC] (Table 5). It was seen that in both the age groups, HGSIL was the most common cause of one step discordant diagnosis, henceforth the need for proper identification of HGSIL on Pap smears [Figure 2]. Hence many studies have been been done to better elucidate the causes of cytohistologic discrepancies of HGSIL on cervical smears.[23, 24]

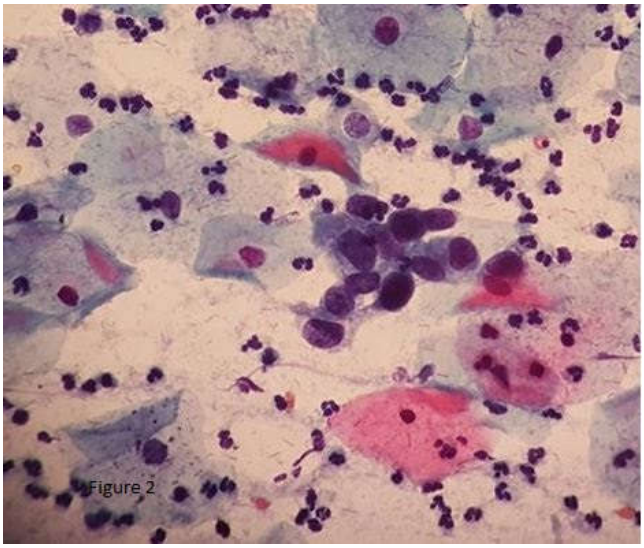

Figure 2 Smear shows the presence of cluster of cells with high nuclear cytoplasmic ratio, hyerchromatic nucleus, irregularly dispersed chromatin and immature cytoplasm consistent with HGSIL (Pap 400 x)

Atypical squamous cell of undetermined significance (ASCUS) interpretation entertains a lot of inter observer variability and does not have a clear representation in its biopsy counterpart. Hence its recommend that when reporting ASCUS, to connote it as favoring reactive or favoring neoplasia [11] [Figure 3].

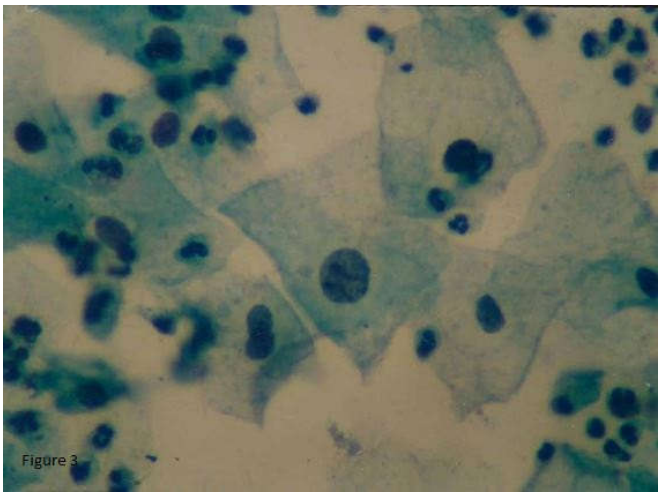

Figure 3 Smear shows marked nuclear enlargement and hyperchromasia in a case of ASCUS(Pap 400 x)

LSIL is rare in post menopausal women as was seen in our study and also by other researchers. [23,25] Lesions with high mitotic index must be upgraded to HGSIL.HGSIL remained the most common discrepant diagnosis in post menopausal women, both pre and post review [Figure 4,5] (Table 3, 5) . This finding was similar to many other studies where HGSIL the most common discrepant diagnosis on cytology and that was later on proved to be carcinoma cervix on histopathology examination. [23, 25] Around 1-4% of women with HGSIL on Pap smear had invasive cervical cancer and 55-66% women have high grade CIN from colposcopic directed biopsies. [6]

In our study the pre review false negative rate was 19.6% that reduced to 11.6% post review (Table 6). Poomtavorn *et al*[23] and Alwahaibi *et al*[25] found 24.2 % and 36.8% of false negative rate , that was slightly higher than our study. Li *et al*[26] and Numnum *et al*[27] reported the prevalence of false negative rates of 7.8% and 16%, respectively, that was lower than our study.

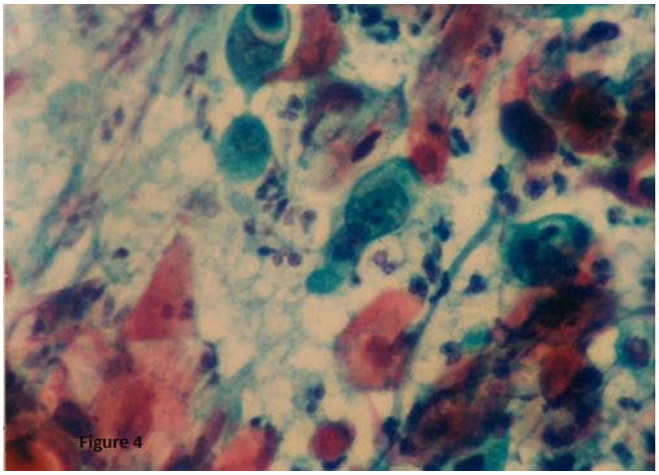

Figure 4 Smear shows the presence of carcinoma cervix cells in a case initially reported as HGSIL(Pap 400 x)

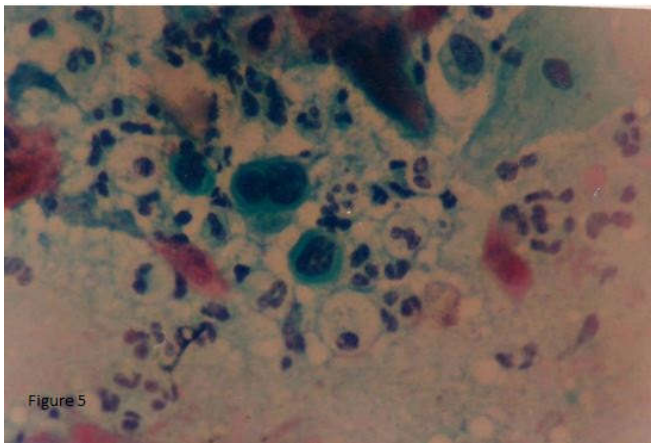

Figure 5 Smear shows the presence of carcinoma cervix cells in a case initially reported as HGSIL (Pap 400 x)

The cytohistologicaldiscrepancy, pre and post review was more prevalent in premenopausal females (73.9%) as compared to post menopausal females (26.08%) (Table 5); where as Poomtavorn *et al* [23] found higher rates in post menopausal women (40 %).

Further, to reduce the discordance rate between cytological diagnosis and follow up histology, a variety of reliable diagnostic tools like cytochemistry have been evaluated .[18] $p16^{INK4a}$, a tumor suppressor protein, is strongly over expressed in almost all HGSIL and invasive cancers of the cervix uteri. It is used as a surrogate marker for the presence of HGSIL or more advanced lesions. [19,28]

IMP3, is an mRNA binding protein, and IMP3 antibody is highly specific marker for malignant lesions on biopsy. P 16 +/ IMP3+, has a higher sensitivity but lower specificity, and is usually positive in cases of SCC and is useful in improvement of cytohistologicaldiscrepancies .[18]

The institute of medicine (IOM) defined a medical error as the failure of a planned action to be completed as intended or the use of a wrong plan to achieve an aim.[29]

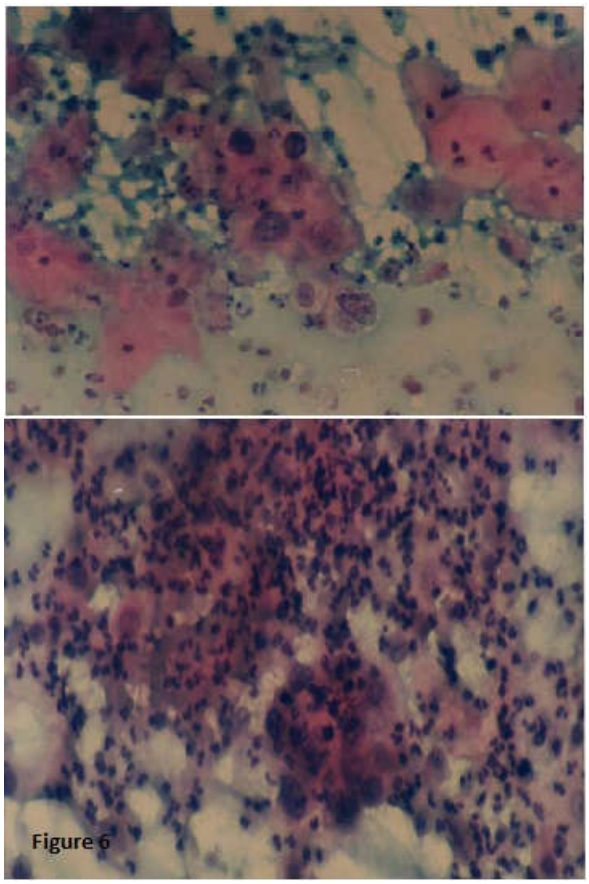

Figure 6 Smear shows the presence of invasive squamous carcinoma cells missed on initial screening obscured by inflammation (Pap 200 x)

All types of error, including those occurring in screening and diagnostic testing, are encompassed in this definition, and it does not link patient outcome to error. Traditionally, two types of errors have been considered by pathology laboratories viz. errors of accuracy and errors of precision. An error detected by cytohistologic correlation is usually an error of accuracy. Disagreement about the cause of correlation error is an example of diagnostic reproducibility, i.e an error of precision.[30]

As cytohistologic correlation generally evaluates cytologic specimens that are generally antecedent or concurrent to the surgical pathology specimens, this process actually focuses more on detecting cytologic, rather than surgical pathology errors.[30]

In some cases, carcinoma cervix goes undetected even after a recent cytology screening test due to errors in either sampling, screening or interpretation. Also necrosis, inflammation and bleeding can obscure, dilute or alter the diagnostic cells in carcinoma cervix explaining another Pap smear paradox that invasive cancers have higher false negative rates pre cancerous lesions.[7] Moss *et al* reported cytologic errors as a major cause of cytohistologic discrepancy. [27] Another study [32] found that other than just sampling, screening or interpretation errors; poor specimen preservation and sub optimal staining are also other causes (preparatory error).

The whole chain of events starting from patient identification till cytology reporting can be divided into pre and post analytic phase.The pre analytic phase deals with patient identification, specimen procurement and transport; where as the analytic phase deals with specimen processing and interpretation. Errors that occur in any of these two phases can lead to leading to cytohistological discrepancies and hence to false negative results. [24]

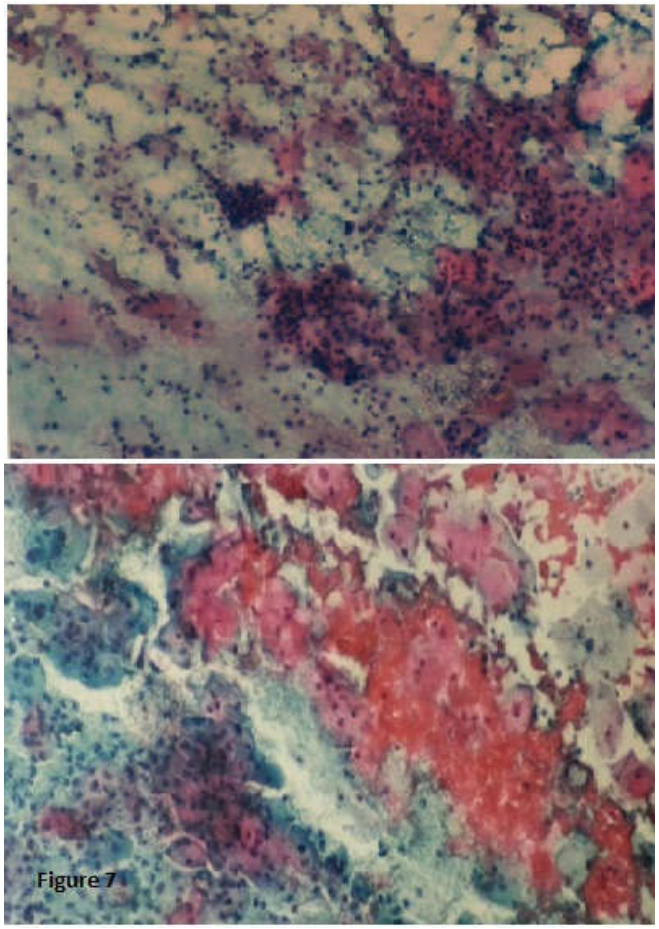

Figure 7 Smear showing obscuring inflammation and haemorrhage in a unsatisfactory smear(Pap 200 x)

False negative findings in cervical smears when are proven on confirmatory histopathology, are a major source of concern for the clinician, cytopathologist and most importantly for the patients.

When the uterine cervix is not adequately represented in case of sampling errors, little can be done then in terms of reducing the false negative rates. Sampling errors occur when the dysplastic cells on the uterine cervix are not adequately transferred on to the slide where they could be seen; though present in the cervix, emphasizing the importance of experienced personnels' participation and the right technique in the sample collection procedure.

In the present study, pre analytic phase error i.esampling errors was the most common error in both pre and post menopausal women (82.35 % and 66.6% respectively). It is the most cause of false negative result in the present study (Table 8) .

Similar to our study many authors have also found sampling errors as the most common cause of false negative rate.[21,22,33] Pinho*et al*[34] observed in their study sampling limitations as an important major cause of cytohistologic discrepancy. There is also considerable data that suggests that the sampling error rates in cytology range between 6 -18 % [35, 36, 37]

The cause of higher rates of sampling errors in the post menopausal women is because the squamocolumnnar junction or the transformation zone retreats up the cervical canal and hence at times misses the reach of the spatula or the cytobrush.

Failure of exfoliation of malignant cells is a well documented phenomenon. It is present in some cases of overt carcinoma of the cervix when the necrotic tissue prevents exfoliation of malignant cells and a high proportion of smears are in fact then, sampling errors. Failure of exfoliation is a more common problem in post menopausal women.[38]

Screening errors were the second most common cause of false negative rates in our study, both in the pre menopausal age group 17.64 % and the post menopausal age group 33.33% [Figure 6] (Table 8). It was seen that LGSIL and HGSIL were its most common causes in the pre menopausal and post menopausal categories respectively (Table 9,10).

Husain *et al*[39] found screening error as the most common cause of false negative rate in their study. In screening errors large number of smears contain identifiable neoplastic cells that are missed by the screener. They attributed smears with heavy inflammation, where greater alertness is needed and very thin clean smears as an important causative factor leading to screening errors.

When the diagnostic or abnormal cells, though present in the smear are missed by the screener, it is termed as screening error. Another study reported screening errors as the most common cause of false negative rate in their study .[32] They also reported drying artefacts to be the main reason for 72.7% of discrepant cases they observed. These are more common in the conventional Pap smears as compared to the LBC smears.
Researchers agree that the most rigorous method to avoid screening errors and consequently to monitor the quality control of routine Pap smears in cytology laboratories is to re screen all negative smears, as was done in the present study. This is the most prudent and common approach to detect false negative results. Other methods include review of cases based on clinical risk criteria , 10 % random review of all negative results and most recently rapid pre screening of all smears has been introduced .[40]

Many researchers also suggest that to reduce the screening errors, slides should be reviewed by a second observer from the same laboratory and the repetition of the test should be with knowledge of the clinical data.[33] Apart from avoiding sampling , screening and interpretation errors it is equally important to be aware of all the features that make the smear unsatisfactory or sub optimal. [33]

Interpretation errors were not present in our study. These most commonly occur due to misinterpretation of a reactive atypia, senescent atypia and atypia seen in association with endocervical polyps.[33]

Our study had 5.26% of unsatisfactory smears [Figure 7] (Table 11). In our study, obscuring inflammation was the most common cause of unsatisfactory smears similar to the study by Ransdell*et al*. [42] Jain *et al*[20] had obscuring haemorrhage and low cellularity as the cause of unsatisfactory smears.

False negative rates in our study reduced from 19.6 to 11.6 % after rescreening, stressing the importance of rescreening of all Pap smears that are malignant on histopathological examination. Also as an attempt to reduce the false negative rates, Pap smears should be repeated at regular intervals .Three normal consecutive annual smears make the error rate negligible. [20]

False negative fraction rates in our study was 0.116 . Very few studies calculate the false negative fraction. As a total of true positives and false negatives were the total cases in the present study and all were biopsy proven malignancies, hence false negative rates and false negative fraction was the same. As the prevalence of the disease does not alter the false negative fraction, it is touted as the best current measurement of the accuracy of cervicovaginal smear interpretation. [43, 44] False negative rate in literature ranges from 2 – 72 % , with a recently calculated rate of 16 %. [33,44]

Sensitivity and diagnostic accuracy of any study depend upon the number of true positives and false negatives. A reduction in the false negatives increases the sensitivity of the study. Sensitivity and diagnostic accuracy of the present study was 88.38% that was similar to the study by Wei *et al*.[18] It was lesser that that of the study done by Pinho*et al*[34] which had a sensitivity of 96%. and higher than the study by N.Y. Alwahaibi*et al*[25] and Jain *et al*[20] who had a sensitivity of 63.2% and 84% respectively in their studies.

Another fact that needs mention in this regard is that, tissue interpretations are always easier than cytology preparations. The absence of specialized cytopathologits for the diagnosis of cervical lesions can lead to discrepancies. [25]

However, there is no substitute to proper sampling and preparation of smears and need to avoid of screening and diagnostic errors. Further, as a measure of quality control rescreening of the cytology smears of biopsy proven cases of malignancy are a must to highlight the causes of false negative smears and reduce their occurrence.

CONCLUSION

The level of agreement between cytology and the histology diagnosis may be used as a measure of laboratory quality. To the best of our knowledge, data on factors associated with cytohistologic discrepancy in cases of carcinoma cervix in Pap smears are limited. Most of the studies have been done in relation to determine the cytohistologic discrepancy and sensitivity rates of HGSIL only. Therefore, we conducted this study to evaluate the factors associated with cytohistologic discrepancy in Pap smears of carcinoma cervix and to determine the false negative rates and false negative fraction.
The present study is aims the identification and correction of the false negative rates as a measurement for quality control in cervical cytopathology laboratories.

It also stresses on the identification of the causes of these discrepancies and to asses the false negative rates and fractions so as not to miss any case of carcinoma cervix or its precursor lesions.

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