Why is high grade squamous intraepithelial neoplasia under-diagnosed on cytology in a quarter of cases? Analysis of smear characteristics in discrepant cases

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Abstract

BACKGROUND: The accuracy of cervical cytology has been questioned due to high false negative rate. In order to improve the sensitivity of cytology it is prudent to analyze the factors which hamper with the diagnosis of high grade lesions. AIMS: To study the cyto-histologic agreement in High grade squamous intraepithelial lesions (HSIL) of uterine cervix and to analyze the smear characteristics in discrepant cases. SETTINGS AND DESIGN: Cervical smears of 100 histology proven cases of Cervical intraepithelial neoplasia III (CIN III) were retrieved and reviewed to study cyto-histologic agreement in the diagnosis of high grade lesions. The discrepant smears, undercalled on cytology, were further analyzed to determine the reasons for misinterpretations. Statistical analysis was performed to find out any significant factors for discrepancies. RESULTS: Cytology was able to correctly identify 74 HSILs while in 26 cases a diagnosis of Low grade squamous intraepithelial lesions (LSIL) or below was given. On review, 16 of these non correlating cases could be reclassified as HSIL on cytology while in 10 the diagnosis of LSIL or less persisted. 12/16 (75%) discrepant cases, reclassified as HSIL represented interpretive errors. Sampling errors (7/10) and air drying (5/10) were more frequent in under diagnosed cases. The statistical analysis did not yield any significant differences in the two review groups. CONCLUSION: 26% of HSIL cases were underdiagnosed on cervical smears. The major confounding factors responsible for under interpretation on cytology included air drying artifacts and metaplastic maturation of abnormal cells.

Key Words: High grade squamous intraepithelial lesion (HSIL), Low grade squamous intraepithelial lesion (LSIL), Cervical Intraepithelial Neoplasia (CIN III), cervical smear, colposcopy, cyto-histologic correlation

Introduction

Despite the undoubted utility of cervical cytology screening in prevention of cervical cancer, it is well accepted that some significant lesions would be missed or underdiagnosed on Papanicolaou test due to inherent limitations of cytology in terms of sensitivity, specificity and predictive value. However, in resource poor settings where facilities of colposcopy are limited or non-existent, cytology is the preferred method of screening. It is therefore essential to maintain strict quality control to improve the sensitivity of cytology and reduce the false negative results. Cyto-histologic correlation is one of the accepted methods of internal quality assurance which allows the pathologists to analyze the various factors leading to discrepant diagnosis.

We undertook a retrospective study with the aim to assess the reliability of cytology in correctly identifying
HSILs in biopsy proven cases of Cervical Intraepithelial Neoplasia III (CIN III). We also attempted to analyze the smear characteristics and other factors in discrepant cases which were diagnosed as CIN III on histology but were undercalled on cytology.

**Materials and Methods**

Cervical smears of 100 consecutive histology proven cases of CIN III, diagnosed over a four and a half year period (January 1999 to July 2003), under a hospital based cervical cancer screening programme, were retrieved from the records and reviewed retrospectively. All these women had undergone colposcopic evaluation before taking the biopsy. The study was approved by the institutional review board and ethical committee. An informed consent had been taken from each subject before collecting the smears. The cervical smears were conventional and consisted of one smear in each case containing both ectocervical scraping and endocervical brush sample. The surgical pathology samples consisted of cervical punch biopsies, cones and hysterectomies. All the smears had been diagnosed according to the Bethesda 1991 system of reporting cervico-vaginal smears and the biopsies interpreted according to the CIN classification. The cervical smears signed out originally as LSIL or less were reviewed by one cytopathologist (SG) to analyze the likely reasons for cyto-histo discrepancies. The smear characteristics assessed included

(a) relative number of abnormal cells present on the slide:
   - few : <10 cell groups or single cells
   - some : >10 cell groups but not many groups
   - many : abundant abnormal cells easily recognizable on smear

(b) adequacy of smear as per the Bethesda system

(c) presence of associated LSIL cells (mild dysplasia/koilocytes)

(d) overall assessment of confounding factors for the discrepancies noted.

**Statistical analysis**

Fisher’s exact test was applied to derive the significance of different parameters studied in the two review groups (HSIL versus LSIL or below)

**Results**

Mean age of the patients was 39.4 years (range 27-67 years) and mean interval between the cervical smear and biopsy was 4.6 months

Out of 100 histology proven cases of CIN III, 74 had a cytology diagnosis of HSIL and were thus the concordant cases. The remaining 26 cases which were diagnosed as LSIL or less on cytology formed the discrepant cases which were reviewed and further analyzed for possible reasons of underdiagnosis. The original cytological interpretation of these non correlating cases was LSIL (encompassing mild dysplasia) in 11, LSIL (encompassing Human Papilloma Virus [HPV]) in 5, ASCUS – SIL (squamous intraepithelial lesion) in 6 and ASCUS – NOS (not otherwise specified) in 4 cases. On review, out of 26 non correlating cases, 16 cases could be reclassified as HSIL. These included 10 originally diagnosed LSILs (7 mild dysplasia and 3 HPV), 4 ASCUS -SIL and 2 ASCUS -NOS. The remaining 10 cases were still called as LSIL (6), ASCUS –SIL(2) and ASCUS –NOS (2) even after review. On tracing the previous records of these 10 cases, 4 were found to have an earlier Pap smear test, but in none of these, a diagnosis of HSIL had been made previously also.

Various smear characteristics like the number of abnormal cells, adequacy of sample, presence of associated LSIL cells and presence of metaplastic

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### Table 1: Analysis of smear characteristics in discrepant cases

<table>
<thead>
<tr>
<th></th>
<th>HSIL (n=16)</th>
<th>LSIL or below (n=10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Original diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSIL (mild dysplasia)</td>
<td>7</td>
<td>4</td>
<td>0.59</td>
</tr>
<tr>
<td>LSIL (HPV)</td>
<td>3</td>
<td>2</td>
<td>0.66</td>
</tr>
<tr>
<td>ASCUS (SIL)</td>
<td>4</td>
<td>2</td>
<td>0.58</td>
</tr>
<tr>
<td>ASCUS (NOS)</td>
<td>2</td>
<td>2</td>
<td>0.50</td>
</tr>
<tr>
<td>2. Adequacy of sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Satisf</td>
<td>11</td>
<td>4</td>
<td>0.15</td>
</tr>
<tr>
<td>Satisf but limited</td>
<td>5</td>
<td>6</td>
<td>0.15</td>
</tr>
<tr>
<td>Endocervical cells absent</td>
<td>2</td>
<td>1</td>
<td>0.68</td>
</tr>
<tr>
<td>Air drying/poor fixation</td>
<td>3</td>
<td>5</td>
<td>0.11</td>
</tr>
<tr>
<td>3. Number of abnormal cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Many</td>
<td>5</td>
<td>1</td>
<td>0.22</td>
</tr>
<tr>
<td>Some</td>
<td>6</td>
<td>5</td>
<td>0.41</td>
</tr>
<tr>
<td>Few</td>
<td>5</td>
<td>4</td>
<td>0.48</td>
</tr>
<tr>
<td>4. Associated LSIL/HPV changes</td>
<td>7</td>
<td>6</td>
<td>0.34</td>
</tr>
<tr>
<td>5. Metaplastic maturation of cells</td>
<td>11</td>
<td>4</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Satisf - satisfactory
ASCUS: Atypical squamous cells of undetermined significance
LSIL - Low grade squamous intraepithelial lesion
HSIL - High grade squamous intraepithelial lesion
HPV - Human papilloma virus
NOS - Not otherwise specified
maturation in abnormal cells were examined in the discrepant cases to find possible causes of underdiagnosis on cytology. (Table 1)

**Number of abnormal cells**
Out of a total of 26 discrepant cases, 9 had a few abnormal cells, 11 had some, while 6 had many readily discernible abnormal cells on the smears. Thus 20/26 cases had only scanty number of abnormal cells which were missed on original screening. This could have been possibly due to under representation of the lesion in these smears. On careful review of the smears, abnormal cells, representative of HSIL could be picked up in 16 cases [few in 5 (Figure 1), some in 6 and many in 5]. In the remaining 10 cases, cells indicative of HSIL were not identified even after meticulous review by the cytopathologist and this could probably be attributed to sampling errors.

**Adequacy of sample**
15/26 (57.6 %) smears were satisfactory for evaluation while 11/26 (42.3 %) were labeled as satisfactory but limited due to various factors according to the Bethesda system. Among the discrepant cases, 11/26 (42.3 %) cases which were reclassified as HSIL were optimal for interpretation, whereas 4/10 (40%) cases which were LSIL or below were optimal. Air drying artifact was the most common reason for the suboptimal smears, being present in 8/11 (72.7 %) cases (Figure 2). Obscuring blood / inflammation was present in 2/11 (18.1 %) of satisfactory but limited smears among discrepant cases. Endocervical component was absent in 3/11(27.2 %) of the limited smears. However, there were no significant differences between the reclassified HSIL and LSIL and below categories.

**Presence of atypical cells with metaplastic differentiation** : In 15/26 (57.6 %) cases abnormal cells revealed metaplastic maturation with rounder, denser cytoplasm and high nuclear/cytoplasmic ratio. Such cells were observed in 11 cases reclassified as HSIL (Figure 3) and 4 cases reclassified as LSIL and below.

**Presence of associated LSIL cells:** 13/26 (50%) non correlating cases had prominent LSIL cells (including koilocytes) and these included 7/16 (62.5 %) cases reclassified as HSIL (Figure 4)

**Overall confounding factors for under interpretation:** On final analysis, it was observed that 12/16 (75%) cases reclassified as HSIL represented interpretive errors; 8 had both sampling and interpretive

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**Figure 1:** Few high grade abnormal cells in a smear classified as HSIL on review. (originally called ASCUS-NOS)

**Figure 2:** A smear limited by air drying artifact showing high grade lesion on review. (originally called LSIL)

**Figure 3:** Metaplastic differentiation of abnormal cells in a smear classified as HSIL on review. (originally interpreted as ASCUS-SIL)
errors while 4 (25%) had sampling errors. On the other hand, sampling errors, (7/10) and air drying (5/10) were more frequent in cases that were still called as LSIL or below, after review.

Discussion

Due to various well recognized inherent limitations of cervical cytology, a percentage of high grade lesions is missed/undercalled on Pap test.\(^1\,\,^5\) It is argued that in the absence of colposcopy and histology facilities in resource limited areas, these cases are likely to progress to invasive cancer. Thus in order to reduce the under reporting of HSILs on cytology, it is imperative that we analyze the smear characteristics and other confounding factors which might lead to underdiagnosis of HSIL. The present study was designed with this objective in mind.

Out of 100 histology proven cases of CIN III, cytology was able to correctly identified 74 cases, while in 26 cases a diagnosis of LSIL or below was given on cytology. Other workers have reported similar results. In a study by Sodhani et al,\(^4\) cytohistological correlation was obtained in 77.5% of high grade lesions. Zuna et al\(^8\) in a retrospective review found accuracy of cytology in correctly predicting high grade lesions to be 62.7%. The non correlating cases in our study were retrospectively reviewed carefully by one of the cytopathologists (SG) and an attempt made to analyze various clinical and laboratory factors responsible for discrepant results. After review, 16 /26 discordant cases were reclassified as HSIL while in 10, the diagnosis of LSIL or less still persisted. The preparatory factors which resulted in underdiagnosis of lesions included inadequate sampling and suboptimal smear preparation, especially air drying of the smears. The analytical factors included the screening and interpretive errors on the part of cytotechnologists and cytopathologists. Metaplastic differentiation of abnormal cells was responsible for many cases of HSIL being undercalled originally. Also in some cases coexistent LSIL cells with prominent koilocytic change probably distracted the pathologist from recognition of HSIL cells. Previous studies have also reported similar difficulties in interpretation of HSIL smears. In a study by Zuna et al,\(^8\) on review of cytology smears of 39 discrepant cases which were histologically proven CIN III but were called LSIL or below on cytology, 28 could be reclassified as HSIL whereas 11 cases did not have cells representative of high grade lesion and were categorized as sampling errors. Lyall and Duncan\(^9\) reported that their cases of CIN III were preceded by smears diagnosed as mild dyskaryosis in 13.8-29.3% cases per year over a five year period. Klinkhamer et al\(^10\) attributed the cyto-histologic discrepancy in under interpretation of HSIL cases to intraobserver variation. In a College of American Pathologists (CAP) Q – Probes study of 22,349 cervical biopsy-cytology correlations, 9.2% of HSIL biopsies were preceded by ASCUS interpretations whereas 26.5% had LSIL cytologies.\(^1\)

Various explanations have been offered for under diagnosis of HSIL in some cases on cytology. One of the reasons may be presence of only a few abnormal cells representative of high grade lesion on the smear, and these may be missed on screening.\(^11\) 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. Misinterpretation of cohesive tissue fragments of HSIL as immature metaplastic cells may also lead to under reporting on cytology.\(^13\,\,^14\) In our study, the major confounding factors which led to discrepant diagnoses included the suboptimal smears due to air drying artifacts and difficulty in interpretation of metaplastic differentiation of abnormal cells. Air drying was observed in 8/11 (72.7%) of limited smears and these comprised (30.7%) of non correlating smears. Though the differences between the two review groups are not statistically significant, we feel that the sample size in this study is probably too small to draw definite conclusions. Our findings are in agreement with those of Zuna et al\(^8\) who have performed a detailed analysis of cervical smear characteristics in non correlating cases of high grade lesions undercalled on cytology. They

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**Figure 4**: Prominent associated LSIL cells (koilocytes) in a smear reclassified as HSIL. (originally undercalled as LSIL encompassing HPV)
have concluded that the main confounding factors to be specimen inadequacy and lack of consistency among pathologists for interpretation of metaplastic patterns.

In the present system of patient follow up in our set up, these discrepant cases undercalled on cytology, may finally be detected on colposcopy as all persistent ASCUS and above cases are referred for colposcopic evaluation. However, this entails a lot of burden of the health care system and tremendous referral load. In resource limited settings, if we wish to reduce the referral load by referring only high grade lesions for colposcopy and following up the low grade lesions by cytology alone, we must ensure efficient quality control in the cytology laboratory so that majority of high grade lesions are picked up on cytology. It is therefore imperative that the cytopathologists be aware of the difficult smear patterns and other factors which may hamper with the diagnosis of high grade lesions. Improved specimen handling to avoid drying artifacts, careful screening by the cytotecnologists and diligent reporting by the cytopathologists might help reduce to some extent the discrepancies in cytological interpretation of high grade lesions.

References

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