PAP SMEAR WITH ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE

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Objective: This study was conducted to obtain histological results in cases with atypical squamous cells of undetermined significance (ASCUS) found on Papanicolaou (Pap) smears.

Methods: We reviewed cases with ASCUS found in cervical cytology from March 1999 through February 2002 of patients who attended Imam Khomeini or Mehr General Hospitals (n = 104). Except for one patient in whom cervical biopsy was done without colposcopy, the remaining 103 had biopsy under direct colposcopy.

Results: Histological examination revealed 28.8% squamous intraepithelial lesion (SIL) (14 low-grade SIL [LSIL] and 16 high-grade SIL [HSIL]), 1 invasive carcinoma, and 1 endometrial carcinoma. A Pap smear was repeated in 60 women with ASCUS before colposcopy and was normal in 7 (11.7%) cases, revealed ASCUS in 45 (75%) cases, and SIL in 8 (13.3%) cases including 6 LSIL and 2 HSIL. Among the 7 normal cases after the repeated Pap smear, histological examinations showed 2 cases of LSIL. In colposcopic examinations of 103 cases, 22 (21.4%) were diagnosed as normal, while one case had a histological report of LSIL.

Conclusion: Since the cytopathologists’ reports do not differentiate between ASCUS and atypical squamous cells seen in HSIL, to detect any underlying SIL, it seems that immediate colposcopy and direct biopsy are more appropriate methods for managing cases with ASCUS.

Keywords: Atypical squamous cells of undetermined significance • cervical biopsy • colposcopy • Papanicolaou (Pap) smear • squamous intraepithelial lesion

Introduction

The term atypical squamous cells of undetermined significance (ASCUS) was first introduced in the Bethesda system in 1988. ASCUS was defined as abnormal cellular changes that though were not negative, did not meet the criteria of squamous intraepithelial lesion (SIL).

Although this is the recommended label for those smears in which it is not possible to clearly distinguish between a benign lesion and a neoplastic process, it may contain a significant number of intraepithelial lesions.

Based on many reports describing the chance of intraepithelial neoplasia of the cervix (CIN) in those with ASCUS in their Papanicolaou (Pap) smears, the method of management of such cases is a matter of debate.

In 2001, the Bethesda system recommended the term “atypical squamous cells” (ASC) instead of ASCUS and categorized it into two subclasses: ASC-US, which stands for ASC-unknown significance, and atypical squamous cells “can not exclude HSIL” (ASC-H), which suggests a high-grade SIL.

The cytological diagnosis of ASCUS has a 10% – 20% chance of having CIN I while the chance of CIN II and CIN III on histological examination is about 3% – 5%.

While the chance of CIN II or III in ASC cases is about 5% – 17%, in ASC-H this increases to
24% – 94% and the risk of cervical cancer will be about 0.1% – 0.2%. These risks indicate that a Pap smear with ASCUS (or using the new term ASC) is in need of follow-up.

Since the cases of CIN I are almost always accompanied by benign human papillomavirus (HPV) infection, which regresses spontaneously in 60% of patients, the main goal is to detect the more progressive lesions of CIN II and III in those with ASCUS. Repeating the Pap smear at specified intervals, performing immediate colposcopy, testing for HPV-DNA for high-risk types, or combining repeated Pap smear with other adjunctive methods, are all widely used for managing patients with ASC. Each of these approaches has advantages and disadvantages.

Although repeated Pap smear is widely used for managing women with ASC, the sensitivity of a single repeat of Pap smear for detecting CIN II or III is relatively low (67% – 85%).

Ergeneli et al showed that immediate colposcopy and colposcopy-directed biopsy are the best methods for following patient with ASCUS in their Pap smears. On the other hand, Gerber et al suggested colposcopy be performed after repeat of Pap smear.

In the present study, we discuss the histological findings in patient with ASCUS in regard to repeat of Pap smear and colposcopy in Imam Khomeini and Mehr General Hospitals.

Patients and Methods

All cases with a cervical cytology with ASCUS between March 1999 and March 2002 referred to Imam Khomeini Hospital who had undergone colposcopy and biopsy were included in this study (n = 104).

In 44 patients, colposcopy and biopsy were performed immediately. The remaining 60 patients underwent a repeated Pap smear after which colposcopy and biopsy were done. A written informed consent was obtained for each patient prior to treatment.

Colposcopy, as well as biopsies during colposcopy, were performed by specialized gynecologists. Colposcopy and biopsy were performed for all patients with ASCUS. The results of this study were used to determine the sensitivity and specificity of a repeated Pap smear and colposcopy for the detection of SIL and malignancy.

Results

The mean ± SD age of the patients was 45.1 ± 12 years (median: 46; range: 25 – 85 years). In 60 patients, the repeated Pap smear was performed 4 – 6 months after the initial Pap smear, that was normal in 7 (11.7%), ASCUS in 45 (75%), LSIL in 6 (10%), and HSIL in 2 (3.3%) cases. All these cases, but one, had subsequent colposcopy and directed biopsy. The results of histological examinations were: 39 (65%) benign or normal, 19 (31.6%) SIL (10 LSIL and 9 HSIL), 1 (1.7%) invasive carcinoma, and 1 (1.7%) endometrial carcinoma (Table 1).

The histological findings in the 44 cases in which colposcopy and biopsy were done immediately after the ASCUS was diagnosed in their Pap smear were 32 (72.7%) benign or normal, 11 (25%) SIL (4 LSIL and 7 HSIL), and 1 (2.3%) invasive carcinoma (Table 1).

The differences in histological findings between the two groups—those who underwent colposcopy-directed biopsy immediately and those who had a repeated Pap smear before colposcopy-directed biopsy—were not statistically significant.

If we consider the ASCUS threshold for referring to colposcopy, then the repeated Pap smears as a test for identifying intraepithelial lesions or malignancies would have a sensitivity of 91%, a specificity of 13%, a positive predictive value of 36%, and a negative predictive value of 71% while referral cases will be 88% (53 cases) (Table 2).

When we consider the LSIL threshold for

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>Repeat Pap smear (n = 60)</th>
<th>Immediate colposcopy (n = 44)</th>
<th>Total (n = 104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-benign</td>
<td>39 (65)</td>
<td>32 (72.7)</td>
<td>71 (68.3)</td>
</tr>
<tr>
<td>LSIL *</td>
<td>10 (16.6)</td>
<td>4 (9.1)</td>
<td>14 (13.5)</td>
</tr>
<tr>
<td>HSIL **</td>
<td>9 (15)</td>
<td>7 (15.9)</td>
<td>16 (15.4)</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>1 (1.7)</td>
<td>1 (2.3)</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>1 (1.7)</td>
<td>0</td>
<td>1 (0.9)</td>
</tr>
</tbody>
</table>

*LSIL = low-grade squamous intraepithelial lesion; **HSIL = high-grade squamous intraepithelial lesion.
referring to colposcopy, the sensitivity of repeated Pap smears would decrease to 23.8% (Table 2).

The results of colposcopy in 103 cases revealed normal in 22 (21.4%) cases, abnormal in 55 (53.4%), LSIL in 12 (11.6%), and HSIL in 14 (13.6%) patients. Colposcopy as a diagnostic test for detection of intraepithelial squamous and malignant lesions had a sensitivity of 97%, a specificity of 30%, a positive predictive value of 38%, and a negative predictive value of 96% (Table 2).

Of the seven cases with normal repeated Pap smear, two and of 22 cases with normal colposcopy, one found positive for LSIL.

We had 45 cases of ASCUS on repeated Pap smear in whom there were 12 patients with SIL, one with invasive carcinoma, and another with endometrial carcinoma (Table 3).

**Discussion**

In this study, histological findings of cervical biopsy in 104 cases with ASCUS, found in their Pap smear, were evaluated and methods of follow-up were assessed. Colposcopy-directed biopsy was performed immediately in 44 cases and after repeating another Pap smear in the remaining 60 cases.

The repeated Pap smear showed normal results in only 7 (11.7%) cases. If we consider the ASCUS threshold for case referral, 53 (88%) of the cases would need colposcopy. The sensitivity of repeated Pap smear for detection of SIL or malignancy was well enough high (91%) but its specificity was rather low (13%).

Other studies have reported that the specificity of repeated Pap smear for the detection of CIN II and III is between 67% and 85%.

If we use LSIL on repeated Pap smear as a threshold criteria for case referral to colposcopy, the sensitivity of the test decreases significantly and, as demonstrated in this study, 12 cases of SIL (7 LSIL and 5 HSIL) and one patient with invasive carcinoma would have been missed.

According to the guidelines of the National Cancer Institute (NCI), published in JAMA 2002, whenever the result of a repeated Pap smear is ASC or higher, it should be referred for colposcopy. These guidelines also warn that referring based on an LSIL or HSIL result could cause many cases of CIN II or III to be missed.

Biopsy was performed in all patients with ASCUS. In keeping with the previous reports, about 29% SIL (14% LSIL and 15% HSIL) and

### Table 2. Validity of repeated Pap smear and colposcopy in detecting SIL and malignancy in patients with ASCUS in Imam Khomeini and Mehr General Hospitals.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Repeated Pap smear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with ASCUS threshold</td>
<td>91 (78 – 100)</td>
<td>13 (2 – 23)</td>
<td>36 (23 – 49)</td>
</tr>
<tr>
<td>with LSIL threshold</td>
<td>24 (6 – 42)</td>
<td>92 (84 – 100)</td>
<td>63 (29 – 96)</td>
</tr>
<tr>
<td>Colposcopy (n = 103)</td>
<td>97 (91 – 100)</td>
<td>30 (19 – 40)</td>
<td>38 (28 – 49)</td>
</tr>
</tbody>
</table>

**Figures in parentheses are 95% confidence interval; The gold-standard test was biopsy in all cases.**

### Table 3. Distribution of histological findings according to the results of repeated Pap smear and colposcopy in patients with ASCUS in Imam Khomeini and Mehr General Hospitals, 1999 – 2001.

<table>
<thead>
<tr>
<th>Repeat Pap smear</th>
<th>Normal</th>
<th>Chronic cervicitis</th>
<th>Endocervical polyp</th>
<th>LSIL</th>
<th>HSIL</th>
<th>Invasive carcinoma</th>
<th>Endometrial carcinoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>ASCUS*</td>
<td>10</td>
<td>20</td>
<td>1</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>LSIL**</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>HSIL***</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>26</td>
<td>1</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Colposcopy</th>
<th>Normal</th>
<th>Abnormal changes</th>
<th>LSIL</th>
<th>HSIL</th>
<th>Invasive carcinoma</th>
<th>Endometrial carcinoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>17</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Abnormal changes</td>
<td>0</td>
<td>45</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>LSIL</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HSIL</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>52</td>
<td>1</td>
<td>14</td>
<td>16</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*ASCUS = atypical squamous cells of undetermined significance; **LSIL = low-grade squamous intraepithelial lesions; ***HSIL = high-grade squamous intraepithelial lesions.
3% malignancy were detected on histological examination.\textsuperscript{4, 5, 7}

In this study, 75% of patients with ASCUS had the same result as on repeated Pap smear, however, 13% of patients progressed to SIL, i.e. 88% of patients truly needed colposcopy. In another study, it was shown that 50% of cases with ASCUS needed colposcopy due to abnormal cytology on repeated Pap smear.\textsuperscript{17}

It seems that repeated Pap smear cannot decrease the frequency of referrals for colposcopy. Repeated Pap smear also requires frequent visits, which may be unacceptable to some patients, causing delays in detecting important lesions (CIN II and III) or cervical carcinoma. It is, therefore, recommended to consider performing immediate colposcopy. Ergenoli et al reported colposcopy-biopsy in 64 patients with ASCUS and detected 14% L SIL and 11% H SIL. They concluded that ASCUS is a marker for the detection of SIL and that immediate colposcopy-directed biopsy is an appropriate method of follow-up for such patients.\textsuperscript{18}

Gerber et al followed patients with ASCUS primarily with repeated Pap smears, but also with colposcopy in those with persistent ASCUS.\textsuperscript{5} The persistence of ASCUS on repeated Pap smear examinations has been about 16%, of which 49% had a normal view on colposcopy so that biopsy was not performed. That is why Gerber et al believe that colposcopic evaluation of patients with persistent ASCUS in repeated Pap smear tests is an appropriate method for follow-up of such patients.\textsuperscript{5}

In our study, the sensitivity and specificity of colposcopy were 97% and 30%, respectively. These figures can be compared to the results of a metaanalysis in which sensitivity and specificity were reported as 96% and 48%, respectively.\textsuperscript{19}

It must be noted that the procedure of colposcopy is expensive, uncomfortable, and that referral for colposcopy causes stress and anxiety. For these reasons, the use of colposcopy may among other things potentially lead to over-diagnosis and over-treatment.

The reflex HPV-DNA test is another method that can be used to follow up patients with ASCUS. This test helps spare 40% – 60% of women from undergoing colposcopy and whenever the test is negative, the patient can be assured that she has no lesion of concern.\textsuperscript{17}

The availability of the appropriate laboratory facilities for performing the HPV-DNA test is still limited in Iran and the test is expensive.

Since it is currently not routine to cytopathologically distinguish between ASC-US and ASC-H in Iran, we recommend immediate colposcopy-directed biopsy as the method of choice for follow-up of patients with ASCUS in their Pap smears.

Acknowledgment

We would like to thank Dr. Mahshid Hormazdi and Dr. Nasser Rakhshani, who helped us with the cytopathologic reports for some of patients with ASCUS in their Pap smears.

References

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