سلام به من آمد
Squamous intraepithelial lesion (SIL)

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A cytological diagnosis is a prediction of what is going to be seen on a histological resection specimen or biopsy.
Squamous Intraepithelial Lesions (SIL)

- Low-grade squamous intraepithelial lesion (LSIL)
- High-grade squamous intraepithelial lesion (HSIL)
  - With features suspicious for invasion (if invasion is suspected)
• Even with only two categories of SIL, there is an overall 10–15 % inter-pathologist discrepancy rate between LSIL and HSIL interpretations on cervical cytology slides.

• 15–30% of women with LSIL on cervical cytology will have CIN II/III on a subsequent cervical biopsy.
<table>
<thead>
<tr>
<th>Benign criteria</th>
<th>Neoplastic criteria</th>
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<tbody>
<tr>
<td><strong>Chromatin</strong> is fine and evenly distributed</td>
<td>Chromatin is course, clumped and unevenly distributed</td>
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<td><strong>Nuclear size and shape</strong> are within normal physiological limits for the level of maturation of the epithelium</td>
<td>Nuclei showing size and shape variation within the same cell population</td>
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<td><strong>Nuclear membranes</strong> are even</td>
<td>Nuclear membranes are irregular in outline and show indentation and/or thickening due to chromatin margination and clumping</td>
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<tr>
<td><strong>Nuclei</strong> are normochromatic and staining intensity maintained through all cells</td>
<td>Nuclei are usually hyperchromatic and staining varies from one group to the next.</td>
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<td><strong>Multinucleation</strong> can be seen but rarely (repair, reactive endocervicals)</td>
<td>Multinucleation is common, reflecting rapid cell turnover</td>
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<tr>
<td><strong>Nucleoli</strong> are small, even in number and even in size, but may be enlarged but regular in repair/regeneration</td>
<td>If present, nucleoli are large, irregular and vary from nucleus to nucleus; nucleoli are more prominent in cancer than dysplasia</td>
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<tr>
<td><strong>Cohesiveness</strong>, cells are cohesive; architecture is maintained</td>
<td>Loss of cohesiveness. Nuclei tend to crowd each other in sheets/clusters.</td>
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• Apart from the distinction between benign(reactive) and SIL, the most critical distinction is between LSIL and HSIL because this affects decisions for patient management and the necessity for treatment.
• Most LSIL, especially in young women, **will regress** as a result of self-limited HPV infections.

• Transient infections generally regress over the **course of 1–2 years**

• HPV DNA testing is not useful in triaging women with LSIL because 83% were positive for HPV.
• The **majority of LSILs are caused by HPV** whether or not koilocytosis is present.

• **Koilocytes** are most often seen with LSIL but may also be seen in HSIL.

• It is impossible to be certain from histology, cytology or HPV testing whether a **productive infection will persist or progress rather than regress**.
HPV interacts with squamous epithelium in two ways:

1. Active virion production: morphologically low grade lesion, transient, most regress

2. Integration of HPV DNA to host cells DNA: viral persistence; clonal expansion create high grade lesions
Four important features of SIL

- Increasing nuclear/cytoplasmic (N/C) ratio
- Discrepancy between nuclear and cytoplasmic maturation
- Nuclear membrane irregularity
- Irregular chromatin distribution and increased granularity
The distinction between HSIL and LSIL depends on the degree of nuclear and cytoplasmic maturation as demonstrated by nuclear/cytoplasmic (N/C) ratio.

Slater et al. (2005a and b) proposed, as a guide based on mean diameter of the nucleus and cytoplasm, a NC ratio for LSIL less than 50%, 50% or more for HSIL and 25% for a normal intermediate cell.
Distinction between HSIL and LSIL

• **Nuclei** tend to be **larger in LSIL than HSIL**
• **N/C** is increased in **HSIL largely** because of **reduced cytoplasmic size**
• Cytoplasmic maturation may be seen in some cells in HSIL favor CIN2
• If in **doubt report as SIL of uncertain grade** rather than ASC-H
Low-grade squamous intraepithelial lesion (LSIL)
Low-grade squamous intraepithelial lesion (LSIL)

• **Presentation:**
  - mature cells that are **polygonal** in shape (normal intermediate or superficial cells)
  - singly and in sheets but mainly as single cells.

• **Nuclei:**
  - show **variable size** (anisonucleosis) & **staining**
  - Nuclear enlargement/ increased chromatin granularity/
    Bi/multinucleation
Low-grade squamous intraepithelial lesion (LSIL)

- Cytoplasm
  - The cell and has a similar density to that of a normal intermediate squamous cell.
  - HPV-associated cytoplasmic changes are not a prerequisite for LSIL
1 – enlarged nuclei (approximately 5 x intermediate cell nucleus);
2 – multinucleated cells;
3 – irregular nuclear margins;
4 – presence of rough chromatin deposits;
5 – presence of koilocytes.
Cytological features of koilocytes

**Cytoplasmic criteria:**

- There is a circumscribed area of clearing around the nucleus known as a koilocyte (koilos means ‘hollow’ in Greek’). This area of clearing is, when examined ultrastructurally, **filled with viable virus particles.**

- A **thick cytoplasmic rim** is present around the vacuole representing **margination of the cytoplasmic contents.**

- halo is not uniform
The cells have well-defined zones of perinuclear clearing & dense eosinophilic or cyanophilic peripheral cytoplasm.
Mimics of LSIL

• Pseudokoilocytosis
• Radiation
• Hyperkeratosis
• Tight halos in TV
• Herpes Cytopathic Effect
Mimics of LSIL

- **Pseudokoilocytosis:** Cytoplasmic vacuolization due to glycogen often takes on a yellow refractile, “cracked” appearance
Mimics of LSIL

- **Radiation Changes:** The cytoplasm of these cells is usually quite distinctive with a two-toned, *vacuolated appearance* that lacks the perinuclear clearing and *peripheral condensation* present in a typical koilocyte.
Mimics of LSIL

• Tight haloes of reactive changes:
  - Small tight halo usually due to organisms
  - No peripheral condensation of cytoplasm
  - Equal distance between edge of nucleus and halo rim
  - Lack of nuclear features of LSIL
High-grade squamous intraepithelial lesion (HSIL)
Cytological features of HSIL

• Presentation
- single cells, sheets or crowded groups of cells
- there are usually separated cells at the margins or between the cell groups in which the features of SIL can more clearly be seen.
- loss of polarity and some crowding
- dyskaryotic cells will be immature indicating incomplete epithelial maturation.
Cytological features of HSIL

• Nucleus:
  - chromatin is more granular and irregularly distributed than in LSIL.
  - true nuclear membrane irregularities with irregular borders and focal thickening, nuclear notches and folds.
  - most often hyperchromatic nuclei but may be normochromatic or hypochromatic.
  - nuclei in HSIL are not necessarily enlarged and may be little larger than that of a normal intermediate cell (Smith & Turnbull 1997; Denton et al. 2008; Wilbur et al. 2015).
Cytological features of HSIL

• Cytoplasm:
  - The cytoplasm will vary in size between rather smaller than a normal intermediate cell and almost complete absence.
Cytological features of HSIL

Nuclear/cytoplasmic ratio

- As the cells are less mature than in LSIL there is a higher NC ratio: the nucleus in HSIL occupies at least 50% of the diameter of the cell. The increased NC ratio is more due to reduced cytoplasmic size than nuclear enlargement (Nayer et al. 2004; Slater et al. 2005a, 2005b).
Major presentations of HSIL

- Keratinizing
- Non-keratinizing large cell
- HSIL in immature squamous metaplasia
- Small cell severe dyskaryosis
- HSIL in atrophic samples
Keratinising HSIL

• Keratinizing cells with nuclear atypia and high N/C ratios such as these represent HSIL.
• Keratinizing HSIL may display more abundant cytoplasm.
• The cytoplasm is generally eosinophilic or orangeophilic but may sometimes be cyanophilic.
Keratinising HSIL

• High-grade squamous intraepithelial lesion: keratinizing dysplasia, aka pleomorphic dysplasia (elongate, spindle, caudate & tadpole cells) - a good descriptive term for higher grade keratinizing lesions.

• No prominent nucleoli, no tumor diathesis
Non-keratinizing large cell HSIL

- In view of the poorly defined cytoplasmic margins and delicate cytoplasm, N/C ratio may be difficult to assess. The diagnosis is made on the abnormal chromatin pattern and irregular nuclear membranes (dyskaryosis) and the disorderly arrangement of cells.
Non-keratinizing large cell HSIL

• Nucleus:
  - The nuclei will show variation in size and shape but the shape is generally round to oval.
  - Irregular nuclear borders can be appreciated in the dissociated single cells.
  - Although they should never be used to make a definitive diagnosis, bare abnormal nuclei are a feature.
  - The chromatin, in this case, is variable as there are variations in presentation.
    • Nuclei may be hypochromatic with bland chromatin
    • Nuclei may be normochromatic with a finely granular but irregular chromatin
    • Nuclei may be hyperchromatic with granular, coarse, irregular chromatin

HSIL- stripped nucleus pattern
• HSIL typically involves the TZ of the cervix, which is derived from immature metaplasia.
• The cytoplasm is thick with well-defined cell borders.
• The NC ratio must be considered in comparison with that of normal immature metaplastic cells but is likely to be more than 50% in HSIL.
• The diagnosis of HSIL depends on the abnormal chromatin pattern and nuclear membranes.
HSIL in immature squamous metaplasia

• A very important feature of these cells is that on conventional cytology they can be seen trapped in streaks of mucus, which at times can be helpful in searching for more abnormal cells.

• These cells usually present as single cells as opposed to immature metaplastic cells, which are usually in small sheets.
HSIL in post-menopausal samples

• Atrophic samples are innately difficult as the atrophic pattern is composed of hyperchromatic parabasal sheets of cells with a high NC ratio in a background of inflammation and autolysis. All these features are seen in HSIL.

• Attention should be paid to samples with a separate population of cells, which should stand out from the uniform atrophic pattern.
HSIL in post-menopausal samples

- HSIL will show the features of dyskaryosis: irregular chromatin, anisocytosis and irregular nuclear membranes.

- In the presence of atrophic vaginitis, when the normal cells may be enlarged and somewhat atypical, a short course of topical estrogen should be recommended.

- HPV triage may be useful in this situation.
Postmenopausal atypia- NILM

• Mild nuclear enlargement without significant hyperchromasia or nuclear irregularity may be seen in atrophic smears and termed “postmenopausal atypia”.

• These changes are not generally associated with HPV-related disease. In the absence of any other definitive abnormalities, such cases should be interpreted as NILM.
HSIL - Small cell severe dysplasia

- The abnormal cells will be either in hyperchromatic crowded groups or single cells.
- The nuclei may be little larger than lymphocytes, polymorphs or normal intermediate squamous cell nuclei.
- The groups may be crowded with no definition to the cytoplasmic cell borders.
- The nuclei will show a loss of polarity. This type of HSIL is seen in CIN3 rather than CIN2 and is hardly ever combined with LSIL or CIN2.
HSIL - Small cell severe dysplasia

- Due to lack of maturation of the NC ratio may approach 100% and will be higher than immature metaplastic or parabasal cells.
- The cytoplasm may be inconspicuous; when visible, it will be basophilic.
- Nuclear membrane irregularities and indentations are frequently seen.
Syncytial Aggregates/Hyperchromatic Crowded Groups

The DD for syncytial groups includes a variety of benign entities such as:

1. immature squamous metaplasia
2. atrophy
3. benign endocervical or endometrial cells.
4. follicular cervicitis
5. IUD changes
6. Tubal metaplasia
7. AIS
Syncytial Aggregates/Hyperchromatic Crowded Groups

• If the cells are abnormal squamous cells, but not diagnostic of HSIL, the appropriate interpretation would be ASC-H.

• If the cells are abnormal but with glandular features, the differential considerations would include endocervical adenocarcinoma in situ or endocervical or endometrial adenocarcinoma.
Syncytial Aggregates/Hyperchromatic Crowded Groups

- Flattening at the edges of the cell cluster, whorling of cells in the center, and lack of glandular architectural features (feathering, rosettes, and pseudostratified strips) favor HSIL over a glandular abnormality.
Syncytial Aggregates/Hyperchromatic Crowded Groups

Separated cells at the margins or between the cell groups in which the features of SIL can more clearly be seen.
Syncytial Aggregates/Hyperchromatic Crowded Groups

Whorling and flattening at edge of the cluster suggest HSIL.

Follow-up:
HSIL (CIN 3) with endocervical glandular involvement
SIL with Endocervical Gland Involvement

- Centrally located cells showing spindling or whorling with flattening of the nuclei at the periphery of the cluster, giving a smooth, rounded border.
SIL with Endocervical Gland Involvement

Normal columnar cells with residual mucin
LSIL with Some Features Suggestive of the Presence of a Concurrent HSIL

• A judgment must be made after examining the whole slide based on the degree of nuclear abnormality as well as the predominant NC ratio.

• The most severe abnormality should be reported.
Inflammatory Cells Mimicking CIN

• Lymphocytes are seen in follicular cervicitis and may mimic small cell HSIL. The key to recognizing them is the presence of tingible-body macrophages in a mixed population of large and small lymphocytes.

• Histocytes have coffee bean-shaped nuclei, finely textured chromatin, longitudinal groove and loose cytoplasm.

Vacuolated cytoplasm, reniform nucleus, and lack of hyperchromasia
Causes of false negative cytology

- Most false negatives are well-recognised types of HSIL, which will not be missed if cytologists are familiar with them.

- Small, sparse or pale dyskaryotic cells
- Hyperchromatic crowded groups of cells
- Dyskaryosis mistaken for or masked by inflammation
How to avoid false negative results?

• Careful examination of the whole slide
• Familiarity with the main causes of false negatives
• Attention to nuclear changes of dyskaryosis
• Correlation of cytological findings with histology
Causes of false positive cytology

- Reactive inflammatory changes (e.g. atrophic vaginitis, follicular cervicitis, endocervicitis, repair)
- Metaplasia (e.g. immature squamous metaplasia, tubal metaplasia, tubo-endometrioid metaplasia)
- Endometrial cells – especially from the lower uterine segment
- Inflammatory cells (e.g. lymphocytes and histiocytes)
The key to avoiding false positives is familiarity with the full range of reactive and metaplastic processes that involve the cervix in the absence of neoplasia.
Happiness depends on your attitude, not on what you have.