objectives

- Define diagnostic cytology (clinical cytology).
- Explain the differences between histopathology and cytopathology.
- Recognize the methods for collection of the materials for cytology.
objectives

- Explain FNA.
- Discuss the advantages of cytologic examination
- Identify the criteria for the diagnosis of cellular malignancy.
WHAT IS DIAGNOSTIC CYTOLOGY?
Diagnostic or clinical cytology is the study of the normal and diseased altered cells obtained from various sites of the body, i.e., through the detection of abnormal morphologic characteristics of the examined dissociated human cells.
CYTOLOGY

Is the **science of cell structure**. Compared to histology, the **diagnostic criteria** are few and depend solely on **nuclear** and **cytoplasmic** features of cells.
<table>
<thead>
<tr>
<th>Cytopathology</th>
<th>Histopathology</th>
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<tr>
<td>- Deals with the structural changes within the nucleus and cytoplasm individual cells</td>
<td>- Deals with the form and the structure of the tissue</td>
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<td>- Evaluation requires cells only</td>
<td>- Evaluation usually begins with a tissue biopsy.</td>
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<td>- Inexpensive simple means of diagnosis, allow frequent repetition of cellular sampling (since it causes no tissue injury)</td>
<td>- More invasive traumatic procedure is needed; utilizing surgical instrumentation such as forceps, scissors, etc.</td>
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<td>- Fine needles with 22, 23 or 24 gauge are usually preferred</td>
<td>- Needles if used should have a large gauge (e.g. true-cut needles with a gauge measuring 14, 16).</td>
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<td>Cytopathology</td>
<td>Histopathology</td>
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<td>------------------------------------------------------------------------------</td>
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<tr>
<td>• <strong>Rapid diagnosis</strong> that could be obtained within minutes</td>
<td>• Diagnosis obtained after days</td>
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<tr>
<td>• Basic stain is <em>Pap</em> stain (however <em>H&amp;E</em> could be used as well)</td>
<td>• Basic stain is <em>H&amp;E</em></td>
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<tr>
<td>• Mainly slides are needed</td>
<td>• Paraffin blocks are needed</td>
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<td>• Smears permit better evaluation of the nature of the inflammatory process. fungi and parasites are usually easier to be diagnosed</td>
<td><strong>Difficult to identify specific causative inflammatory pathogen</strong></td>
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Indications (the Advantages) for Cytopathology

Detection of inflammation and certain types of pathogenic agents
Differentiation between benign and malignant
Diagnosis of premalignant diseases lesions
Diagnosis of the type of Malignancy (primary, metastatic or recurrent tumors)
Study of hormonal patterns
Monitoring of response to therapy and
Follow-up of irradiation & chemotherapy
Study of tumour markers
In general **diagnostic cytology** is based upon **three basic sampling techniques**:

1- The collection of **exfoliated cells**.

2- The collection of cells removed by **brushing** or similar abrasive techniques.

3- The aspiration biopsy. **F.N.A. biopsy**.
EXFOLIATIVE CYTOLOGY:

From normal **(physiological)** desquamation products: Is based on a spontaneous shedding of cells derived from a lining of an organ into a cavity, where they can be removed by non-abrasive means. **Examples:**

**Vaginal smears:** cells removed from the posterior fornix of the **vagina** (squamous epithelium, **endocervical** cells, **endometrial** cells).
Bronchial secretion (Sputum): cells derived from buccal cavity, pharynx, larynx, trachea, bronchial tree and pulmonary alveoli.

Urine

Discharge
Nipple
Conjunctival
Ear
ABRASIVE CYTOLOGY:

Obtained through superficial scraping of the lesion *(artificial mechanical desquamation)* examples include:

Cervical scraping so called Pap smear.
Buccal mucosal smear.
Skin scraping.
Direct imprint of a tumor.

**Brushing techniques**: Using rigid endoscopic and fibroptic instruments *(fiber-optic endoscopy)*
Cervico-vaginal PAP smears
Land mark report is by Dr. George N. Papanicolaou on detection of carcinoma of uterine cervix in vaginal smears
Pap smear:
cells are scraped from the cervix and examined under a microscope to check for cancer or other problems.

Cervix viewed through speculum with patient in lithotomy position.

Normal cervix
Cervical dysplasia

Normal cervical cells
Cancerous or pre-cancerous cervical cells
Using a metal cement spatula /tongue blade – scrape the entire surface of the lesion
Fine Needle Biopsy
FNAC/FNAB: fine needle aspiration cytology or biopsy. A 10 ml syringe with a 22-23 gauge needle used to aspirate material.
Aspiration

All superficial “lumps”
Breast growths
Thyroid nodules
Enlarged lymph nodes
Aspiration

Deep lesions may be aspirated under imaging guidance (US - CAT)

Fine Needle aspiration Cytology

In general, the definitive diagnosis of any mass can be established by:

Open biopsy,
Tissue core needle (Tru-cut) biopsy,
Fine needle aspiration biopsy.

Compared to FNA, Tru-cut biopsy is a more traumatic procedure which should be performed under local anaesthesia. It requires more time and special equipment that are more expensive. Pain, discomfort and bleeding are common complications.
Fine Needle aspiration Cytology

FNAC, on the other hand, provides many advantages to the surgeons:

It is an easy, reliable, cost effective diagnostic technique which can give rapid results.

The procedure could be performed in an office setting without anaesthesia. It is usually not more painful than a venipuncture and can be repeated immediately if the acquired material is inadequate.
Equipments and Procedure of FNAC:

When reduced to its simplest terms, FNA consists of:

- Using a needle and syringe to remove material from a mass.
- Smearing it on a glass slide.
- Applying a routine stain.
- Examining it under the microscope.
CYTOLOGY SAMPLE PREPARATION

A) PROCESSING
TECHNICAL ASPECTS

B) FIXATIVES

- 95% ethanol is a routine fixative
- Cytofix spray
- Special fixatives according to sample type
TECHNICAL ASPECTS- stains

C) STAINS

**Routine**

**Fixed slides:**

- **Papanicolaou (PAP):** All smears
- **Hematoxylin and eosin:** FNAC & rest of sediment
TECHNICAL ASPECTS- stains

C) STAINS

**PAP Stain**

- 3 colours *(pink-blue-orange)* + different shades
- colour & shade determines degree of keratinization
- nuclear details clearer than HE
PAP smear showing normal Intermediate & superficial Sq.cells

Pleomorphism;
Irregular N & C outline

Cancerous Cell
TECHNICAL ASPECTS-stains

Unfixed slides

Giemsa

Toluidene blue or Diff Quick
TECHNICAL ASPECTS-stains

Special stains

1. **PAS**: for glycogen – mucous – fungus

2. **Silver stains**: Fungus – inclusions

3. **Immunohistochemical stains & Tumour markers**
EXAMINATION OF SAMPLE & DIAGNOSIS

CYTOLOGIST

1) The cytologist examines the gross appearance of the sample and describes it: color, volume and whether sample is clear or turbid.

2) The cytologist then performs the microscopic examination & diagnosis:
EXAMINATION OF SAMPLE & DIAGNOSIS

**Low power** is important for:

- Determination of adequacy (are there enough cells & are the cells representative of the tissue being examined?)
- Pattern & background
- Cell types
EXAMINATION OF SAMPLE & DIAGNOSIS

High power + Oil immersion are important for:
The determination of the benign or malignant nature of cells examined depending on cytoplasmic features & nuclear details:

1- N/C ratio
2- Cell & nuclear shape and size
3- Cell & nuclear membranes
4- Nucleoli-mitotic figures-chromatin pattern
5- Others: Inclusion bodies
   Bacteria/fungi/parasites
   Artifacts
Criteria of Malignancy

How can we detect the presence of malignant cells cytologically?

Nuclear Changes

Nuclear Hypertrophy
Nuclear Size & Shape Variation
Hyperchromatism and Chromatin Irregularity
Multinucleation
Irregularity of Nuclear Membrane
Irregular and Prominent Nucleoli
Abnormal Mitosis
Cytoplasmic Changes in Malignant Cells

Scantiness of Cytoplasm

Cytoplasmic Boundries (sharp & distinct in Squamous cell ca & indistinct in undifferentiated ca)

Variation in Size & Shape

Cytoplasmic Staining (deep orange in keratinizing squamous ca or basophilic in immature poorly differentiated ca)

Cytoplasmic Inclusions (melanin pigments in melanoma)
Changes in Cells as a Group in Malignancy

Cellular Phagocytosis or Cannibalism (indicating rapid growth of cells within a narrow cavity)

Lack of Cellular Adhesion (due to abnormalities in desmosomes)

 Bloody Background (fresh blood is meaningless, but when RBCs are ingested by histiocytes or blood obtained without trauma)

Foreign Cellular Structures (ex. psammoma Bodies)

Degeneration and Inflammation (Tumour Diathesis)
Normal Cell

Cancerous Cell
Fig. 614 Differentiated nonkeratinizing epidermoid carcinoma cell in sputum. This well preserved cell of epidermoid type is characterized by a fibrillar structure of the cytoplasm, condensed chromatin, rigid nuclear contour and irregular nucleolus.

Fig. 36 Malignant cells shed in a loose cluster from giant cell carcinoma of the lung. Note following findings in full of “malignant criteria” (1) marked nuclear enlargement, (2) marked increase in N/C ratio, (3) pronounced hyperchromatism with coarse chromatins, (4) prominence of nucleoli, and (5) variation in size and shape of nuclei.
Ca bladder

High nuclear-cytoplasmic ratio and pleomorphism
CYTOLOGY

LOVE IT
OR
LEAVE IT

Thank you