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COURSE TITLE: CELLULAR PATHOLOGY

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QUESTION

1. Write explicitly on 5 diagnostic techniques use in pathology, relevant illustrations and examples required.
2. Cellular Adaptation precedes cell death, discuss. Diagrams essential.

5 DIAGNOSTIC TECHNIQUES USED IN PATHOLOGY

The pathologist uses the following techniques to the diagnose diseases:

* Cytopathology
* Hematopathology
* Immunohistochemistry
* Cytogenetics
* Autopsy
1. **CYTOPATHOLOGY**



**The images above show two examples of results from a standard Pap test.**

**Cells that are cancerous or pre-cancerous are clearly distinct from normal samples.**

Cytopathology is a branch of pathology that studies and diagnoses diseases on the cellular level. Cytopathology is generally used on samples of free cells or tissue fragments, in contrast to histopathology, which studies whole tissues.

APPLICATIONS OF CYTOPATHOLOGY:

 The main applications of cytology include the following:

1. Screening for the early detection of asymptomatic cancer

For example, the examination of scrapings from cervix for early detection and prevention of cervical cancer.

1. Diagnosis of symptomatic cancer

Cytopathology may be used alone or in conjunction with other modalities to diagnose tumors revealed by physical or radiological examinations.

It can be used in the diagnosis of cysts, inflammatory conditions and infections of various organs.

1. Surveillance of patients treated for cancer

For some types of cancers, cytology is the most feasible method of surveillance to detect recurrence. The best example is periodic urine cytology to monitor the recurrence of cancer of the urinary tract.

**CYTOPATHOLOGIC METHODS**

There are different cytopathologic methods including:

1. FINE-NEEDLE ASPIRATION CYTOLOGY (FNAC)

In FNAC, cells are obtained by aspirating the diseased organ using a very thin needle under negative pressure. Virtually any organ or tissue can be sampled by fine-needle aspiration. The aspirated cells are then stained & are studied under the microscope. Superficial organs (e.g. thyroid, breast, lymph nodes, skin and soft tissues) can be easily aspirated. Deep organs, such as the lung, mediastinum, liver, pancreas, kidney, adrenal gland, and retroperitoneum are aspirated with guidance by fluoroscopy, ultrasound or CT scan. FNAC is cheap, fast, & accurate in diagnosing many diseases.

1. EXFOLIATIVE CYTOLOGY

Refers to the examination of cells that are shed spontaneously into body fluids or secretions. Examples include sputum, cerebrospinal fluid, urine, effusions in body cavities (pleura, pericardium, peritoneum), nipple discharge and vaginal discharge.

3. ABRASIVE CYTOLOGY

Refers to methods by which cells are dislodged by various tools from body surfaces (skin, mucous membranes, and serous membranes). E.g. preparation of cervical smears with a spatula or a small brush to detect cancer of the uterine cervix at early stages. Such cervical smears, also called Pap smears, can significantly reduce the mortality from cervical cancer. Cervical cancer is the most common cancer in Ethiopian women.

1. **HEMATOPATHOLOGY**

Hematopathology or hemopathology is the study of diseases and disorders affecting and found in blood cells, their production, and any organs and tissues involved in hematopoiesis, such as bone marrow, the spleen, and the thymus. Diagnoses and treatment of diseases such as leukemia and lymphoma often deal with hematopathology; techniques and technologies include flow cytometry studies and immunohistochemistry.

**HEMATOPATHOLOGY TECHNIQUE**

* FLOW CYTOMETRY

Flow cytometry (FCM) is a technique used to detect and measure physical and chemical characteristics of a population of cells or particles.

Flow cytometry is routinely used in basic research, clinical practice, and clinical trials. Uses for flow cytometry include:

* Cell counting
* Cell sorting
* Determining cell characteristics and function
* Detecting microorganisms
* Biomarker detection
* Protein engineering detection
* Diagnosis of health disorders such as blood cancers

A flow cytometry analyzer is an instrument that provides quantifiable data from a sample. Other instruments using flow cytometry include cell sorters which physically separate and thereby purify cells of interest based on their optical properties.

* IMMUNOHISTOCHEMISTRY

Immunochemistry is the identification of a certain antigen in a histological tissue section or cytological preparation by an antibody specific to that antigen. Immunohistochemistry refers specifically to histological tissue sections.

1. **IMMUNOHISTOCHEMISTRY**



**Chromogenic immunohistochemistry of a normal kidney targeting the protein CD10.**

Immunochemistry is the identification of a certain antigen in a histological tissue section or cytological preparation by an antibody specific to that antigen. Immunohistochemistry refers specifically to histological tissue sections.

**IMMUNOHISTOCHEMISTRY TECHNIQUES**

* Immunohistochemistry Techniques uses antibodies, reagents and stains for the diagnosis and research of cancer.
* Immunohistochemistry Techniques uses different methods and approaches. The specimen needs to be well fixed. One of the most popular fixatives is 10% Neutral Formalin and Zinc Formalin.
* Also in immunohistochemistry, a transport solution is needed to transport the specimen. The most popular is Michel's Immunofluorescence Working.
* Immunohistochemical techniques detect antigens in tissue sections by means of immunological and chemical reactions. This technique is highly sensitive and specific and can detect a wide variety of antigens in multiple animal species.

1. **CLINICAL GENETICS (CYTOGENETICS)**

**A metaphase cell positive for the BCR/ABL rearrangement using FISH**

Cytogenetics is essentially a branch of genetics, but is also a part of cell biology/cytology (a subdivision of human anatomy), that is concerned with how the chromosomes relate to cell behavior, particularly to their behavior during mitosis and meiosis.

**CYTOGENETICS TECHNIQUE**

Some techniques used include:

* Karyotyping
* Quinacrine banding
* Reverse banding
* C-banding and NOR Stains
* High-Resolution banding
* Fluorescent In Situ Hybridization (FISH).
* KARYOTYPING

The routine chromosome analysis (Karyotyping) refers to analysis of metaphase chromosomes which have been banded using trypsin followed by Giemsa, Leishman, or a mixture of the two. This creates unique banding patterns on the chromosomes.

**CHROMOSOME-BANDING TECHNIQUES**

* QUINACRINE BANDING (Q-BANDING)

Quinacrine banding (Q-banding) was the first staining method used to produce specific banding patterns. This method requires a fluorescence microscope and is no longer as widely used as Giemsa banding (G-banding).

* REVERSE BANDING(R-BANDING)

Reverse banding, or R-banding, requires heat treatment and reverses the usual black-and-white pattern that is seen in G-bands and Q-bands. This method is particularly helpful for staining the distal ends of chromosomes.

* C-BANDING AND NUCLEOLAR ORGANIZING REGION STAINS (NOR STAINS).

C-banding and nucleolar organizing region stains (NOR stains). These latter methods specifically stain certain portions of the chromosome. C-banding stains the constitutive heterochromatin, which usually lies near the centromere, and NOR staining highlights the satellites and stalks of acrocentric chromosomes.

* HIGH-RESOLUTION BANDING

High-resolution banding involves the staining of chromosomes during prophase or early metaphase (prometaphase), before they reach maximal condensation. Because prophase and prometaphase chromosomes are more extended than metaphase chromosomes, the number of bands observable for all chromosomes increases from about 300 to 450 to as many as 800. This allows the detection of less obvious abnormalities usually not seen with conventional banding.

* FLUORESCENT IN SITU HYBRIDIZATION

Fluorescent in situ hybridization (FISH) refers to using fluorescently labeled probe to hybridize to cytogenetic cell preparations.

In addition to standard preparations FISH can also be performed on:

1. Bone marrow smears
2. Blood smears
3. Paraffin embedded tissue preparations
4. Enzymatically dissociated tissue samples
5. Uncultured bone marrow
6. **AUTOPSY**

An autopsy (post-mortem examination, obduction, necropsy, or autopsia cadaverum) is a surgical procedure that consists of a thorough examination of a corpse by dissection to determine the cause, mode, and manner of death or to evaluate any disease or injury that may be present for research or educational purposes.

**AUTOPSY TECHNIQUES**

There are four major autopsy techniques which differ chiefly in the methods used in removal of the organs and the order in which they are opened.

The four major techniques are:

* Virchow Technique
* Rokitansky Technique
* En Masse Technique
* En Bloc Technique
* VIRCHOW TECHNIQUE

In the Virchow technique, the organs are removed one by one and dissected as removed. This approach is good for demonstrating pathological change in individual organs, especially in high- risk autopsies or where permission is limited to one organ. This organ can be immediately removed and examined. The disadvantage of this technique is that relationships between various organs may be hard to interpret.

* ROKITANSKY TECHNIQUE

This procedure is characterized by in situ dissection, in part combined with en bloc removal. The term “Rokitansky technique” is used erroneously by many pathologists to designate the en masse technique.

* EN MASSE TECHNIQUE

Thoracic, cervical, abdominal, and pelvic organs are removed en masse and subsequently dissected into organ blocks. This is the best technique for preserving the vascular supply and relationships between organs. Another advantage is that the body can be made available to the undertaker quickly, without having to rush the dissection and risk obscuring findings or destroying important specimens. The major disadvantage is that the organ mass is often awkward to handle, and the autopsy is difficult to perform without an assistant.

* EN BLOC TECHNIQUE

Various modifications of the en bloc technique are widely used. Thoracic and cervical organs, abdominal organs, and the urogenital system are removed in functionally related blocks. This procedure is a compromise between the Virchow and en masse techniques, preserving anatomical relationships sufficiently for most cases while being simpler for one person to execute.

CELLULAR ADAPTATION PRECEDES CELL DEATH, DISCUSS.

In cell biology and pathophysiology, cellular adaptation refers to changes made by a cell in response to adverse or varying environmental changes. The adaptation may be physiologic (normal) or pathologic (abnormal). Four types of morphological adaptations include atrophy, hypertrophy, hyperplasia, and metaplasia.

While,

Cell death is the event of a biological cell ceasing to carry out its functions. This may be the result of the natural process of old cells dying and being replaced by new ones, or may result from such factors as disease, localized injury, or the death of the organism of which the cells are part. Apoptosis or Type I cell-death, and autophagy or Type II cell-death are both forms of programmed cell death, while necrosis is a non-physiological process that occurs as a result of infection or injury.

DISCUSSION

Cellular adaptation is the ability of cells to respond to various types of stimuli and adverse environmental changes. These adaptations include hypertrophy (enlargement of individual cells), hyperplasia (increase in cell number), atrophy (reduction in size and cell number), metaplasia (transformation from one type of epithelium to another), and dysplasia (disordered growth of cells). Tissues adapt differently depending on the replicative characteristics of the cells that make up the tissue. For example, labile tissue such as the skin can rapidly replicate, and therefore can also regenerate after injury, whereas permanent tissue such as neural and cardiac tissue cannot regenerate after injury. If cells are not able to adapt to the adverse environmental changes, cell death occurs physiologically in the form of apoptosis, or pathologically, in the form of necrosis.