NAME: Nwoke Precious .O.

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COURSE TITLE: CELLULAR PATHOLOGY

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1.. Write explicitly on 5 diagnostic techniques used in pathology with illustrations and examples

Pathological diagnostic techniques include;

I.Immunohistochemistry

ii.Histopathology​

iii.Cytopathology

Iv.Cytogenetics

V. Autopsy

1. IMMUNOHISTOCHEMISTRY

Immunohistochemistry is the most common application of immunostaining. it involves the process of selectively identifying antigens (proteins) in cells of a tissue section by exploiting the principle f antibodies binding specifically to antigens in biological tissues. Immunohistochemistry takes its name from the roots “immune”, in reference to antibodies used in the procedure, and “histo” meaning tissue. Immunohistochemical staining is used widely in the diagnosis of abnormal cells such as those found in cancerous tumors. Specific peculiar markers are characteristics of particular cellular events such as proliferation or cell death (apoptosis). Immunohistochemistry is also used widely in the in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue. Immunohistochemistry is a technique that uses antibodies conjugated to enzymes to catalyze reactions to form detectable compounds to visualize and localize specific antigens in a tissue sample.

STEPS USED IN PERFORMING IMMUNOHISTOCHEMISTRY

The tissue and cells must be fixed using a chemical like formaldehyde. This stabilizes the structural properties of cells, preventing them from changing throughout the process

Cells need to be permeated using a detergent such as Triton X, which allows antibodies to enter tissue and bind to epitopes within the cell.

Primary antibodies against a protein of interest are added and secondary antibodies with enzymes like horseradish peroxides (HRP) conjugated to their Fc domain are added to target the primary antibody.

Enzymes like HRP can target certain substrates molecules like diaminobenzidine (DAB) and catalyze an oxidation which results in the creation of a colorful compound. This colorful compound will stay localized to the area near the protein of interest; a different color from the rest of the tissue.

Tissues are counter stained using a dye like hematoxylin to create contrast between the tissue stained using IHC and non colored regions for better visualization.

PURPOSE OF HISTOCHEMISTRY

The purpose of immunohistochemistry is to be able to see where specific antigens are expressed in a tissue sample. Localization of specific proteins are important for diagnostics in fields such as cancer or infectious diseases. IHC is often used in the diagnosis of certain forms of breast cancer.

SELECTION OF ANTIBODIES FOR IMMUNOHISTOCHEMISTRY

In IHC, there are primary and secondary antibodies used. Primary antibodies bind directly to the antigen, while secondary antibodies bind to the primary antibody. When selecting antibodies for IHC, there are three types of antibody preparation to choose from for IHC. They include

Monoclonal antibodies: they have a high specificity, reducing the number of false positive bindings, but often hive a much weaker stain.

Polyclonal antibodies: they result in greater staining and excellent signal, but can give false positives by binding unwanted sites.

Pooled monoclonal antibodies: they also give excellent staining, as well as high specificity however; there is limited availability for pooled monoclonal antibodies that do not bind noncompetitively.

2. HISTOPATHOLOGY

It is a compound of three Greek words “histo, pathos and logia” meaning “tissue, suffering and study of” respectively. It refers to a microscopic examination of tissues in order to study the manifestations of disease. Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by a pathologist, after the specimen have been processed and histological sections have been placed onto glass slides. In contrast, cytopathology examines free cells or tissue micro-fragments.

Histopathology involves the examination of sampled whole tissues under the microscope. Pieces of tissues rather than whole organs are removed as biopsies, which often require smaller surgical procedures that can be performed whilst the patient is still awake but sedated.

COLLECTION OF TISSUES​

Histopathological examination of tissues starts with the surgery, biopsy, or autopsy. The tissue is removed from the body or plant and often following expert’s dissection in the fresh state is placed in a fixative which stabilizes the tissues to prevent decay .The most common fixative is formalin (10% neutral buffered formaldehyde in water).

Histopathologists provide a diagnostic service for cancer; they handle the cells and tissues removed from suspicious lumps and bumps, identify the nature of the abnormality and, if malignant, provide information to the clinician about the type of cancer, its grade and for some cancers, its responsiveness to certain treatments. With the help of sophisticated imaging techniques, biopsy tissue can now be obtained from previously inaccessible sites such as the pancreas or retro peritoneum, the membrane lining the abdominal cavity.

Specimens received by the pathology laboratory require tissue preparation then are treated and analyzed using techniques appropriate to the type of tissue and the investigation required. For immediate diagnosis during a surgical procedure, a frozen section is performed.

• Larger specimens include whole organs or parts thereof, which are removed during surgical operations. Examples include a uterus after a hysterectomy, a large bowel after a colectomy or tonsils after a tonsillectomy.

• Pieces of tissue rather than whole organs are removed as biopsies, which often require smaller surgical procedures that can be performed whilst the patient is still awake but sedated. Biopsies include excision biopsies, in which tissue is removed with a scalpel or a core biopsy, in which a needle is inserted into a suspicious mass to remove a slither or core of tissue that can be examined under the microscope.

• Fluid and very small pieces of tissue can be obtained via a fine needle aspiration (FNA). This is performed using a thinner needle than that used in a core biopsy, but with a similar technique. This type of material is usually liquid rather than solid.

3. CYTOPATHOLOGY

It is a diagnostic technique that examines cells from various body sites to determine the cause or the nature of disease. The first cytopathology test developed was the Pap test, which has been widely utilized in the last 50 years for screening and diagnosing of cervical cancer and its precursors. Cytopathology is generally used on samples of free cells or tissue fragments in contrast to histopathology, which studies whole tissues. Cytopathology is frequently, less precisely called cytology which means the study of cells. Cytopathology is used commonly to investigate diseases involving a wide range of body sites, often to aid in the diagnosis of some infectious diseases and other inflammatory conditions. For example, a common application of cytopathology is the Pap smear, a screening tool used to detect precancerous cervical lesions that may lead to cervical cancer. Cytopathlogic tests are sometimes called smear tests because the samples may be smeared across a glass microscopic slide for subsequent staining and microscopic examination. However, cytology samples may be prepared in other ways, including cytocentrifugation. Different types of smear tests may also be used for cancer diagnosis. In this sense, it is termed a cytological smear.

CELL COLLECTION

There are two methods of collecting cells for Cytopathlogic analysis; exfoliative cytology and intervention cytology.

EXFOLIATIVE CYTOLOGY: in this method, cells are collected after they have been either spontaneously shed by the body or manually scraped off of a surface in the body. An example of spontaneous exfoliation is when cells of the pleural cavity are shed into the pleural or peritoneal fluid. This fluid can be collected via various methods for examination. Examples of mechanical exfoliation include pap smears where cells are scraped from the cervix with a cervical spatula, or bronchial brushings, where a bronchoscope is inserted into the trachea and used to evaluate a visible lesion by brushing cells from its cells from its surface and subjecting them to Cytopathlogic analysis. After sampling, two main techniques can be used; conventional cytology and liquid based cytology. With the latter, the sample is placed in a liquid that is then processed for further investigations.

INTERVENTION CYTOLOGY

In intervention cytology, the pathologist intervenes into the body for sample collection.

Fine needle aspiration; or fine needle aspiration cytology involves the use of a needle attached to a syringe to collect cells from lesion or masses in various body organs by micro coring, often with the application of negative pressure to increase yield. FNAC can be performed under palpation guidance on a mass in a superficial region like the neck, thyroid or breast; FNAC may be assisted by ultrasound or CAT scan for sampling of deep seated lesions within the body that cannot be localized via palpation.

4. AUTOPSY

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. Autopsies are performed for either legal or medical purposes. Autopsies can be performed when any of the following information is desired.

PURPOSE OF AUTOPSY

• To determine if death was natural or unnatural​

• Injury source and extent on the corpse

• Manner of death must be determined

• Time since death

• Establish identity of the deceased

• Retain relevant organs

• If it is an infant, determine live birth and viability

For example, a forensic autopsy is carried out when the cause of death may be a criminal matter, while a clinical or academic autopsy is performed to find the medical cause of death and it is used in cases of unknown or uncertain death or for research purposes. Autopsies can be further classified into cases where external examination suffices, and those where the body is dissected and internal examination is conducted. Permission from next of kin may be required for internal autopsy in some cases. Once an internal autopsy is complete, the body is reconstituted by sewing it back together.

There are four types of autopsy

Medico-legal or forensic autopsies: it seeks to find the cause and manner of death and to identify the decadent. They are generally performed as prescribed by applicable law, in cases of violent, suspicious or sudden deaths, deaths without medical assistance or during surgical procedures.

Clinical or pathological autopsies: they are performed to diagnose a particular disease or for research purposes. They aim to determine, clarify or confirm medical diagnoses that remained unknown or unclear prior to the patient’s death.

Anatomical or academic autopsies: they are performed by students of anatomy for the purpose of study only.

Virtual or medical imaging autopsies: they are performed utilizing imaging technology only, primarily magnetic resonance imaging (MRI) and computed tomography (CT).

5. CYTOGENETICS

This is the study of genetic phenomena through the cytological analysis of chromosomes under the light or electron microscope. It has developed over the years from crude analysis of mitotic cells using simple strains, to an analysis of extended DNA fibers using a digital fluorescence microscopy and image analysis where the resolution maybe on the order of 1 kilo base. Cytogenetic techniques are central to the assignment and localization of genes to chromosomes and thus to the construction of genetic maps.

Cytogenetics is essentially a branch of genetics, but it is also a part of cell biology/cytology, that is concerned with how the chromosomes relate to cell behavior during mitosis and meiosis. Techniques used include; Karyotyping, analysis of G-banded chromosomes, other cytogenetic banding techniques include in situ hybridization and comparative genomic hybridization.

KARYOTYPING: the routine chromosome analysis (karyotyping) refers to analysis of metaphase chromosomes which have been banded using trypsin followed by Giemsa, Leishmanns, or a mixture of the two. This creates unique banding patterns on the chromosomes. The molecular mechanism and reason for these patterns is unknown, although it is likely related to replication timing and chromatin packing. Several chromosome-banding techniques are used in cytogenetics laboratories. 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 widely used as Giemsa banding (G-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 in staining the distal ends of chromosomes. Other staining techniques include the C-banding and the necleolar organizing regions 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 the NOR staining highlights the satellites and stalks of acrocentric chromosomes. High- resolution banding involves the staining of chromosomes during the prophase or early metaphase, before they reach a maximal condensation. This is 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.

FLOURESCENT IN SITU HYBRIDIZATION: it refers to using fluorescently labeled probe to hybridize to cytogenetic cell preparations. In addition to standard preparations, FISH can also be performed on;

• Bone marrow smears

• Blood smears

• Paraffin embedded tissue preparations

• Enzymatically dissociated tissue samples

• Uncultured bone marrow

• Cytopsin preparations

Advances now focus on molecular cytogenetics including automated systems for counting the results of standard FISH preparations and techniques for virtual karyotyping, such as comparative genomic hybridization arrays, CHG and Single Nucleotide polymorphism arrays.

2. Cellular adaptation precedes cell death discuss!!!

The diagram below explains this sequence. Cellular adaptation is the ability of cells respond to various types of stimuli and adverse environmental changes. These adaptations include Hypertrophy (enlargement of individual cells), Hyperplasia (increase in the number of cells), Atrophy (reduction in the number and size of cells), Metaplasia (transformation of one 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. Its cells are not able to adapt to the adverse environmental changes. Cellular adaptation could be normal (physiological) or abnormal (pathological).

When cells are injured, one or two patterns will gradually occur; reversible cell injury leading to adaptation of the cells and tissues, or irreversible cell injury leading to cell death and tissue damage. Injured cells may accumulate materials including fat, cholesterol, protein, glycogen or pigment. When cells are irreversibly injured and dying, specific nuclear changes may be visible including pyknosis, karyrrhexis and karyolysis. If large number of cells dies, tissue necrosis may occur. Observable patterns of necrosis include; coagulative, liquefactive, fibrinous, gummatous, fat, gangrene and caseous necrosis.

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