NAME: Bamisaye Mary Odunayo

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COURSE: CELLULAR PATHOLOGY

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.

ANSWER

1. Some diagnostic techniques include:
2. **Cytopathology**: Cytopathology is a diagnostic technique that examines cells from various body sites to determine the cause or the nature of disease. Cell samples cystoscopy.

An example of a cytopathology test is pap test. Specific tests, such as the Papanicolaou test, also known as the Pap test or fine-needle aspiration, can be used to gather cells from specific sites for diagnosis. Analysis of cell samples is often a rapid process, but requires specialist training to correctly identify cells that pose potential danger to a patient, such as pre-cancerous, cancerous or infected cells. The Pap test is a diagnostic technique that utilizes a sample of cells taken from the cervix. Two methods can be used in a Pap test; conventional and automated liquid tests. In a conventional Pap test, cells are placed on a glass slide, and then fixed and stained with a combination of dyes. The cells are inspected using light microscopy for abnormalities, such as morphological or nuclear feature changes. In the newer automated liquid Pap test, cell samples are placed in a vial of preservative for analysis. This screening method has proven to be more efficacious in diagnosis when compared to a traditional Pap test. The Pap test is a diagnostic technique that utilizes a sample of cells taken from the cervix. Two methods can be used in a Pap test; conventional and automated liquid tests. In a conventional Pap test, cells are placed on a glass slide, and then fixed and stained with a combination of dyes. The cells are inspected using light microscopy for abnormalities, such as morphological or nuclear feature changes. In the newer automated liquid Pap test, cell samples are placed in a vial of preservative for analysis. This screening method has proven to be more efficacious in diagnosis when compared to a traditional Pap test.

1. **Cytogenetics**: Cytogenetics is the study of chromosomes and their structure.

Cytogenetic testing involves the analysis of cells in a sample of blood, tissue, amniotic fluid, bone marrow, or cerebrovascular fluid to identify any changes in an individual’s chromosomes.

There are three major methods of cytogenetic testing:

* Routine karyotyping
* Fluorescent in situ hybridisation (FISH)
* Comparative genomic hybridisation (CGH) and array comparative genomic hybridisation (aCGH).

karyotyping

Karyotyping was one of the first methods of chromosome analysis. This method uses light microscopy and standardised staining procedures on cells in the metaphase portion of the cell cycle, when the chromosomes are lined along the equator of the cell prior to separation and are most condensed.

To make the chromosomal analysis more effective and efficient stain have been developed to bind with DNA and produce characteristic banding patterns to identify different chromosomes. The stain most commonly used is Giemsa dye. Through this process, the chromosomes can be organised into a karyogram of 23 pairs and any abnormality involving aneuploidy (an abnormal amount of chromosomes) and large translocations (where parts of chromosomes are transplanted between each other) can be identified.

Fluorescent in situ hybridisation (FISH)

FISH is a well-known diagnostic cytogenetic test in both congenital and acquired disease. FISH has a much higher resolution than routine karyotyping, especially when used on interphase cells (the phase cells remain in when not in mitosis).FISH uses fluorescent probes with complementary base sequences to locate the presence or absence of specific portions of DNA on chromosomes. The probe and target DNA must be denatured with heat or chemicals to break hydrogen bonds in the DNA and to allow hybridization to occur once the two samples are mixed. The fluorescent probes form new hydrogen bonds with their complementary base pairs on the DNA, and these can then be detected via microscope. FISH is commonly used to detect specific chromosomal deletions or translocations associated with pediatrics conditions or cancers. Examples include the deletion on chromosome 22 DiGeorge syndrome and the translocation of part of a gene on chromosomes 22 and 9 in chronic myeloid leukemia.

Comparative Genomic Hybridization

CGH is a method of molecular cytogenetic testing that detects chromosomal copy number variants (portions of the genome where sections of genes are doubled or tripled) without the need for cell culturing. It was first developed to identify such changes in tumors. CGH uses 2 genomes; the test sample and a control, both of which are fluorescently labeled to differentiate between the two. The two samples are denatured and mixed together, allowing hybridization of metaphase chromosomes. The intensity of the fluorescent signal of the labeled test DNA relative to the control DNA can then be plotted along each chromosome, which shows the loss or gain of genetic material and allows identification of any copy number variants. CGH differs from other methods of cytogenetic testing in that it does not rely on a specific target, nor does it require previous knowledge of the region under examination . Instead, CGH can quickly scan a whole genome for chromosomal imbalances and it is useful in cases where the diagnosis is not known. One limitation of CGH is the size of the genetic alteration that it can identify; the resolution of CGH is poor at approximately 5–10 megabases.

**3. Immunohistochemistry:**

Immunohistochemistry is a technique that uses antibodies conjugated to enzymes that catalyze reactions to form detectable compounds to visualize and localize specific antigens in a tissue sample. The root “histo-” specifically applies to biological tissue, so the process is only immunohistochemistry if it is being done in an organic tissue. In contrast, immunocytochemistry applies the same process to individual cells. Immunohistochemistry can be performed in a few steps. First, 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. Next, cells need to be permeated using a detergent such as Triton X, which allows antibodies to enter the tissue and bind to epitopes within the cell. Primary antibodies against a protein of interest are added, and secondary antibodies with enzymes like horseradish peroxidase (HRP) conjugated to their Fc domain are added to target the primary antibody. Enzymes like HRP can target certain substrate 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 the antibody was targeted to, staining the area near the protein of interest a different color from the rest of the tissue. Lastly, tissues are counter stained using a dye like hematoxylin to create contrast between the tissue stained using IHC and the non-colored regions for better visualization.

**4. Hematology**:

Hematology is the study of blood and blood disorders. Hematologists and hematopathologists are highly trained healthcare providers who specialize in diseases of the blood and blood components. These include blood and bone marrow cells. Hematological tests can help diagnose anemia, infection, hemophilia, blood-clotting disorders, and leukemia.

Common hematological tests are Complete blood count (CBC), which includes:White blood cell count (WBC),Red blood cell count (RBC), Platelet count, Hematocrit red blood cell volume (HCT),Hemoglobin concentration ( This is the oxygen-carrying protein in red blood cells.),Differential white blood count, Red blood cell indices (measurements). This tests are used to test for anemia, certain cancers of the blood, inflammatory diseases, and to monitor blood loss and infection. Platelet count can be used to diagnose and/or to monitor certain types of bleeding and clotting disorders. Prothrombin time (PT) Partial Thromboplastin Time (PTT) and International Normalized Ratio (INR) are used to evaluate bleeding and clotting disorders and to monitor anticoagulation (anticlotting) therapies.

**5. 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. The principal aims of an autopsy is to determine the cause of death, mode of death, manner of death, the state of health of the person before he or she died, and whether any medical diagnosis and treatment before death was appropriate.

There are four main types of autopsy:

* Medico-legal or forensic or coroner's autopsies seek to find the cause and manner of death and to identify the decedent. 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 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 are performed by students of anatomy for study purpose only.
* Virtual or medical imaging autopsies are performed utilizing imaging technology only, primarily magnetic resonance imaging (MRI) and computed tomography (CT)

2.

• Adaptations are reversible changes in the size, number, phenotype, metabolic activity, or functions of cells in response to changes in the environment. Cells must constantly adapt, even under normal conditions, to changes in the environment. Pathologic adaptation may share the same underlining mechanisms, but they provide the cells with the ability to survive in their environment and perhaps escape injury.

Cellular adaptation is a state that lies between normal, unstressed cell and the injured, stressed cell. When cells are injured, one of two patterns will generally result: reversible cell injury leading to adaptation of the cells and tissue, or irreversible cell injury leading to cell death and tissue damage. When cells adapt to injury, their adaptive changes can be atrophy, hypertrophy, hyperplasia, or metaplasia. When a cell is unable to adapt to cell injury, cell death occurs.

One such example is radiation that causes lipid peroxidation, or the breakdown of lipid-based cellular structures due to oxidation. Regardless of cause, cells may undergo necrosis, or cell death, as a result of an irreversible injury. Also, inability of cells to adapt to oxygen deficiency due to anemia, blunt force trauma, toxins, and drugs can all cause either reversible or irreversible injury to a cell and even an entire organ or tissue.

