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COURSE CODE:NSC 308

COURSE NAME: CELLULAR PATHOLOGY

MATRIC NO: 17/MHS02/097

DEPT: NURSING SCIENCE

LEVEL: 300L

A. Histopathological techniques

Histopathological examination studies tissues under the microscope. During this study, the pathologist looks for abnormal structures in the tissue. Tissues for histopathological examination are obtained by biopsy. Biopsy is a tissue sample from a living person to identify the disease. Biopsy can be either incisional or excisional. Once the tissue is removed from the patient, it has to be immediately fixed by putting it into adequate amount of 10% Formaldehyde (10% formalin) before sending it to the pathologist.The purpose of fixation is:

1. To prevent autolysis and bacterial decomposition and putrefaction

2. To coagulate the tissue to prevent loss of easily diffusible substances

3. To fortify the tissue against the deleterious effects of the various stages in the preparation of sections and tissue processing.

4. To leave the tissues in a condition which facilitates differential staining with dyes and other reagents.

Once the tissue arrives at the pathology department, the pathologist will exam it macroscopically (i.e. naked-eye examination of tissues).

Then the tissue is processed to make it ready for microscopic examination. The whole purpose of the tissue processing is to prepare a very thin tissue (i.e. five to seven μm or one cell thick tissue) which can be clearly seen under the microscope. The tissue is processed by putting it into different chemicals. It is then impregnated (embedded) in paraffin, sectioned (cut) into thin slices, & is finally stained. The stains can be Hematoxylin/Eosin stain or special stains such as PAS, Immunohistochemistry, etc...

The Hematoxylin/Eosin stain is usually abbreviated as H&E stain. The H&E stain is routinely used. It gives the nucleus a blue color & the cytoplasm & the extracellular matrix a pinkish color. Then the pathologist will look for abnormal structures in the tissue.And based on this abnormal morphology he/she will make the diagnosis. Histopathology is usually the gold standard for pathologic diagnosis.

B. Cytopathologic techniques

Cytopathology is the study of cells from various body sites to determine the cause or nature of disease.

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.

2. 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.

3. 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.

Advantages of cytologic examination

Compared to histopathologic technique it is cheap, takes less time and needs no anesthesia to take specimens. Therefore, it is appropriate for developing countries with limited resources like Ethiopia. In addition, it is complementary to histopathological examination.

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.

2. 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.

C. Hematological examination

This is a method by which abnormalities of the cells of the blood and their precursors in the bone marrow are investigated to diagnose the different kinds of anemia & leukemia

C.Autopsy

Autopsy is examination of the dead body to identify the cause of death. This can be for forensic or clinical purposes.

The relative importance of each of the above disciplines to our understanding of disease varies for different types of diseases. For example, in diabetes mellitus, biochemical investigation provides the best means of diagnosis and is of greatest value in the control of the disease. Whereas in the diagnosis of tumors, FNAC & histopathology contribute much. However, for most diseases, diagnosis is based on a combination of pathological investigations.

D.Immunohistochemistry: this is a method used to detect a specific antigen in the tissue in order to identify the type of disease. IHC offers several distinct advantages when compared to traditional methods. This technique is rapidly expanding the diagnostic capability of pathologist. IHC permits rapid identification. The technique employs specific antibodies, which localize to the antigens of the etiological agent of interest. Since this technique uses formalin-fixed tissues, specimen transport is simplified, allowing retrospective studies and minimizing laboratory worker exposure to infectious agents. IHC is a sensitive and specific test methodology for many microorganisms, and unlike some traditional staining methods, they result in direct, highly interpretable visual evidence of the presence of an infectious agent within tissues. In addition, IHC detects organisms that are difficult to culture and those that cannot be cultured. IHC provides invaluable information for clinical diagnosis as well as for the study of pathogenesis. IDPB has developed many specific IHC assays for emerging or re-emerging infectious diseases. Currently, IDPB has diagnostic IHC assays for more than 100 etiologic agents, including viral, bacteria, parasitic and fungal organisms. For a number of agents, IHC tests may provide the only reliable methods of detection.

e) Heamatological techniques: this is amethod by which abnormalities of the cells of the blood and their precursors in the bone marrow are investigated to diagnose the different kinds of anemia and leukemia. The examination is the first step to a haematological diagnosis and treatment of blood disorders such as anemia, abnormalities of the red blood cells, disease related to defective blood clotting, thromboembolic diseases. Such as thrombus formation, and immunoheamatological diseases. Further more it is used to diagnose and identify the best treatment for blood cancer, hodgkin’s disease, acute and chronic leukemias, myeloma and myeoproliferative disorders such as essential thrombocytothemia, polycythemia vera, and myelofibrosis. Others include heamatological disease of the ederly such as myelodysplasia and low malignant lymphoproliferative disorders, arterial thromboembolic disease, thrombophilia, thrombosis and clotting abnormalities. During the visit, a heamatologist collects information about the history and lifestyle of the patient such as nutrition, smoking habits, physical inactivity, pathologies, previous interventions, a family history of similar diseases, and a medication intake. A heamatologist then conducts a thorough clinical examination that can last between 20 and 40 minutes, during which the doctor feels the abdominal area, listens to the heart and lungs, and looks for enlarged lymph nodes. A heamatologist will view prior exams or prescribe them when necessary, to determine an appropriate course of action.

Cell injury underlies all diseases. So to understand diseases, one has to start by knowing what cell injury is. When a cell is exposed to an injurious agent (i.e. the causes of diseases discussed in chapter one), the possible outcomes are:

1. The cell may adapt to the situation or

2. They cell may acquire a reversible injury or

3. The cell may obtain an irreversible injury & may die. The cell may die via one of two ways: either by necrosis or by apoptosis. Which of these outcomes occur depends on both the injurious agent & on cellular factors. In other words, the result depends on the type, severity, & duration of the injury & on the type of the cell. This chapter covers the types of cellular adaptation, reversible cell injury, & cell death in that order.

Types of cellular adaptation

The types of cellular adaptation include hypertrophy, atrophy, hyperplasia, & metaplasia A.Hypertrophy

Hypertrophy is increase in the size of cells. Increased workload leads to increased protein synthesis & increased size & number of intracellular organelles which, in turn, leads to increased cell size. The increased cell size leads to increased size of the organ.

Examples: the enlargement of the left ventricle in hypertensive heart disease & the increase in skeletal muscle during sternous exercise.

B. Hyperplasia

Hyperplasia is an increase in the number of cells. It can lead to an increase in the size of the organ. It is usually caused by hormonal stimulation. It can be physiological as in enlargement of the breast during pregnancy or it can pathological as in endometrial hyperplasia.

C. Atrophy

Atrophy is a decrease in the size of a cell. This can lead to decreased size of the organ. The atrophic cell shows autophagic vacuoles which contain cellular debris from degraded organelles.

Atrophy can be caused by:

1. Disuse

2. Undernutrition

3. Decreased endocrine stimulation

4. Denervation

5. Old age

D. Metaplasia

Metaplasia is the replacement of one differentiated tissue by another differentiated tissue. There are different types of metaplasia.

Cell death

Cells can die via one of the following two ways:

1. Necrosis

2. Apoptosis

1. Necrosis

In necrosis, excess fluid enters the cell, swells it, & ruptures its membrane which kills it. After the cell has died, intracellular degradative reactions occur within a living organism. Necrosis does not occur in dead organisms. In dead organisms, autolysis & heterolysis take place. Necrosis occurs by the following mechanisms:

A. Hypoxia

B. Free radical-induced cell injury

C. Cell membrane damage

D. Increased intracellular calcium level

A. Hypoxia

Hypoxia is decreased oxygen supply to tissues. It can be caused by:

1. Ischemia

Ischemia is decreased blood flow to or from an organ. Ischemia can be caused by obstruction of arterial blood flow – the most common cause, or by decreased perfusion of tissues by oxygen-carrying blood as occurs in cardiac failure, hypotension, & shock.

2. Anemia

Anemia is a reduction in the number of oxygen-carrying red blood cells.

3. Carbon monoxide poisoning

CO decreases the oxygen-capacity of red blood cells by chemical alteration of hemoglobin.

4. Poor oxygenation of blood due to pulmonary disease.

The cell injury that results following hypoxia can be divided into early & late stages:

1. Early (reversible) stages of hypoxic cell injury

At this stage, hypoxia results in decreased oxidative phosphorylation & ATP

synthesis. Decreased ATP leads to:

a. Failure of the cell membrane Na – K pump, which leads to increased intracellular Na & water, which cause cellular & organelle swelling. Cellular swelling (hydropic change) is characterized by the presence of large vacuoles in the cytoplasm. The endoplasmic reticulum also swells. The mitochondria show a low amplitude swelling. All of the above changes are reversible if the hypoxia is corrected.

b. Disaggregation of ribosomes & failure of protein synthesis.

2. Late (irreversible) stages of hypoxic cell injury.

This is caused by severe or prolonged injury. It is caused by massive calcium influx & very low pH, which lead to activation of enzymes, which damage the cell membrane& organelle membranes. Irreversible damage to the mitochondria, cell membranes, & the nucleus mark the point of no return for the cell, that is after this stage, the cell is destined to die. Release of aspartate aminotransferase (AST), creatine phosphokinase(CPK), & lactate dehydrogenase (LDH) into the blood is an important indicator of irreversible injury to heart muscle following myocardial infarction.

B. Free radical-induced injury

Free radical is any molecule with a single unpaired electron in the outer orbital. Examples include superoxide & the hydroxyl radicals. Free radicals are formed by normal metabolism, oxygen toxicity, ionizing radiation, & drugs & chemicals, & reperfusion injury. They are degraded by spontaneous decay, intracellular enzymes such as glutathione peroxidase, catalase, or superoxide dismutase, & endogenous substances such as ceruloplasmin or transferrin. When the production of free radicals exceeds their degradation, the excess free radicals cause membrane pump damage, ATP depletion, & DNA damage. These can cause cell injury & cell death.

a. Cell membrane damage

Direct cell membrane damage as in extremes of temprature, toxins, or viruses, or indirect cell membrane damage as in the case of hypoxia can lead to cell death by disrupting the homeostasis of the cell.

b. Increased intracellular calcium level

Increased intracellular calcium level is a common pathway via which different causes of cell injury operate. For example, the cell membrane damage leads to increased intracellular calcium level. The increased cytosolic calcium, in turn, activates enzymes in the presence of low pH. The activated enzymes will degrade the cellular organelles.

Types of necrosis

The types of necrosis include:

1. Coagulative necrosis

2. Liquefactive necrosis

3. Fat necrosis

4. Caseous necrosis

5. Gangrenous necrosis

1. Coagulative necrosis

Cogulative necrosis most often results from sudden interruption of blood supply to an organ, especially to the heart. It is, in early stages, characterized by general preservation of tissue architecture. It is marked by the following nuclear changes: Pyknosis (which is chromatin clumping & shrinking with increased basophilia), karyorrhexis (fragmentation of chromatin), & karyolysis (fading of the chromatin material).

2. Liquefactive necrosis

Liquefactive necrosis is characterized by digestion of tissue. It shows softening & liquefaction of tissue. It characteristically results from ischemic injury to the CNS. It also occurs in suppurative infections characterized by formation of pus.

3. Fat necrosis

Fat necrosis can be caused by trauma to tissue with high fat content, such as the breast or it can also be caused by acute hemorrhagic pancreatitis in which pancreatic enzymes diffuse into the inflamed pancreatic tissue & digest it. The fatty acids released from the digestion form calcium salts (soap formation or dystrophic calcification). In addition, the elastase enzyme digests the blood vessels & cause the hemorrhage inside the pancreas, hence the name hemorrhagic pancreatitis.

4. Caseous necrosis

Caseous necrosis has a cheese-like (caseous, white) appearance to the naked eye. And it appears as an amorphous eosinophilic material on microscopic examination. Caseous necrosis is typical of tuberculosis.

5. Gangrenous necrosis

This is due to vascular occlusion & most often affects the lower extremities & the bowel. It is called wet gangrene if it is complicated by bacterial infection which leads to superimposed liquefactive necrosis. Whereas it is called dry gangrene if there is only coagulative necrosis without liquefactive necrosis.

Necrosis can be followed by release of intracellular enzymes into the blood, inflammation or dystrophic calcification. Inflammation will be discussed in the next chapter.

2. Apoptosis

Apoptosis is the death of single cells within clusters of other cells. (Note that necrosis causes the death of clusters of cells.) In apoptosis, the cell shows shrinkage & increased acidophilic staining of the cell. This is followed by fragmentation of the cells. These fragments are called apoptotic bodies. Apoptosis usually occurs as a physiologic process for removal of cells during embryogenesis, menstruation, etc… It can also be seen in pathological conditions caused by mild injurious agents.

Apoptosis is not followed by inflammation or calcification. The above mentioned features distinguish apoptosis from necrosis.

Pathologic calcification

Pathologic calcification is divided into 2 types:

1. Metastatic calcification

This is caused by hypercalcemia, resulting from hyperparathyroidism, milk-alkali syndrome, sarcoidosis etc…

2. Dystrophic calcification

This occurs in previously damaged tissue, such as areas of old trauma, tuberculous lesions, scarred heart valves, & atherosclerotic lesions. Unlike metastatic calcification, it is not caused by hypercalcemia. Typically, the serum calcium level is normal.

