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 DIAGNOSTIC TECHNIQUES USED IN PATHOLOGY

 There are several techniques used to diagnose diseases in pathology and they include:

1. Histopathology

2. Cytopathology

3. Hematopathology

4. Microbiological examination

5. Biochemical examination

**1. Histopathological techniques:** Histopathological examination has to do with the studying of tissues under the microscope. During this study, the pathologist looks for abnormal structures that could be present in the tissue. The tissues for used for histopathological examinations are obtained through biopsy.

 Biopsy is a tissue sample which is obtained from a living person in order 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 an adequate amount of 10% Formaldehyde (10% formalin) before sending it to the pathologist.

The purpose of this exact fixation is:

. To prevent autolysis and bacterial decomposition and putrefaction
. To coagulate the tissue to prevent loss of easily diffusible substances
. To fortify the tissue against the deleterious effects of the various stages in the preparation of sections and tissue processing.
. 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 embedded in paraffin, sectioned or cut into thin slices, and 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 colour & the cytoplasm & the extracellular matrix a pinkish colour. 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.
 **2. Cytopathologic techniques**: This 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:
. Screening for the early detection of asymptomatic cancer for example, the examination of scrapings from cervix for early detection and prevention of cervical cancer.
. 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 also be used in the diagnosis of cysts, inflammatory conditions and infections of various organs.

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

 CYTOPATHOLOGICAL METHODS

  There are different cytopathological methods which include:

 . 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.

. Exfoliative cytology: This 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.

. Abrasive cytology: This 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.

**3. 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 anaemia & leukaemia. Examples of tests carried out during a haematological examination includes; full blood count test, white blood cell (WBC) testing, red blood cell (RBC) testing, haemoglobin testing, renal profiling, vitamin B12 deficiency testing, blood film, etc. Blood film for instance, the blood is smeared over a glass slide with specific dyes and viewed under a microscope. The number, shape, and size of the blood cells and the presence of any abnormal cells or immature red blood cells are noted. The stain used for reticulocytes or immature red blood cells is Heilmeyer’s reticulocyte stain. Staining may flag up abnormally shaped red blood cells such as sickle cells or spherocytes.

 **4. Microbiological examination:** This is a method by which body fluids, excised tissue, etc. are examined by microscopical, cultural and serological techniques to identify micro-organisms responsible for many diseases. Examples of tests carried out during a microbiological examination include; aspirate culture and sensitivity, aspirate for AFB, cholera ag., chlamydia, CSF culture and sensitivity, ear culture and sensitivity, etc. when collecting specimens, the site of sampling, visual features like colour, viscosity, if the material is a liquid, etc. along with the odour, status of the collection site in some cases, method of collection, patient condition, must all be recorded. Specimens should be transported to the laboratory as soon as possible because excessive delay or exposure to extreme temperatures can compromise the results and must be avoided.

**5. Biochemical examination:** This is a method by which the metabolic disturbances of disease are investigated by assay of various normal and abnormal compounds in the blood, urine, etc. The biochemical features of this test includes data on growth at different temperatures, PH values, salt concentrations, and data on growth in the presence of various substances such as antimicrobial agents, presence or activity of various enzymes and with respect to metabolization. Examples of tests carried out during biochemical examination includes; Bile Esculin Agar, catalase test, Mannitol salt Agar (MSA), Taxos P (optochin sensitivity testing). Etc.

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.

 CELLULAR ADAPTATION PRECEEDS CELL DEATH

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. The diagram below will show this sequence;

**NORMALCELL**

 **(homeostasis)**

**REVERSIBLE**

**INJURY**

stress injurious stimulus

 mild transient

**ADAPTATION**

**CELL INJURY**

 Inability to

 Adapt severe progressive

**IRREVERSIBLE INJURY**

CELL DEATH

**APOPTOSIS**

**NECROSIS**