Introductions and Slide Preparations

Pathology: is the study of disease, it describes the manifestations of the disease, its process and attempts to determine the cause (etiology) and underlying mechanism (pathogenesis).

It forms a bridge between basic science (including anatomy, histology, physiology and others) and clinical practice.

Disease: It is the "state in which an individual exhibits an anatomical, physiological, or biochemical deviation from the normal state".

Branches of Pathology are:

1. **General Pathology:**
   Deals with the general changes in all tissues in response to pathological stimuli like (inflammation, cancer, edema, aging ……. etc)
2. **Systemic pathology:**
   Study of diseases affecting specific organs and body systems like (CNS, CVS, GIT, respiratory tract, reproductive system & etc)

What are the important aspects to know about a disease?

- The general definition
- Epidemiology (where and when)
- Etiology (causes of the disease)
- Pathogenesis (evolution of the disease)
- Morphology (structural changes)
- Functional consequences
- Management
- Prognosis (outcome of the disease either good or bad)
- prevention

Pathophysiology or pathology focuses on only 4 aspects of a disease:

1. **the etiology:** causes of a disease, the etiological factors are:
   - Environmental:
     Physical agents, chemical agents, infections, immunological reactions and psychological agents.
   - Genetics (abnormal genes)
   - Indirect factors: like age, gender, race and habits
2. **the pathogenesis:** mechanisms of development of a disease
3. **the morphology:** structural alteration induced in cells and tissues. Those that can be seen with naked eye are called gross changes, and those that are seen under the microscope are called microscopic changes.
4. **functional consequences:** functional results of the morphologic changes as observed clinically, which they determine the clinical features (signs and symptoms)

Specimens in pathology are of two types:

1. **Autopsy:** it involves a gross examination and taking samples from various tissues of a dead corps (body), which is mostly used in forensic medicine
2. **Biopsy:** removal and examination of a tissue obtained from a living body. **There are 2 types of biopsy:**
   - **Incisional biopsy:** only a portion of the lesion is sampled, so the procedure is strictly diagnostic
   - **Excisional biopsy:** the entire lesion is removed usually with a portion of normal tissue, so the procedure serves both diagnostic and therapeutics

Diagnostic Histopathological Techniques:

1. Gross examination: by the naked eye to observe (color, size, surface and texture) of the tissue
2. Light microscopy: to observe the structural changes at the cellular level
3. Immunohistochemistry: to detect a specific antigen in the tissue
4. Electron microscopy:
5. Molecular biology: to detect abnormalities at the level of cellular DNA

Preparation of Histopathological slide:

The most widely used method of studying tissues is using histological slides. The tissue in the slide must reflect the actual nature of the tissue in the body, to insure that, tissues to be studied must pass through a series of steps before examination. There are 4 main sequential steps for preparation of slide:

1. **Fixation process:**

   Is a reaction between the fixative agent and proteins in the specimen which form a gel, so keeping every cell intact as their in vivo state.

   There several types of fixative agents:

   - Acetic acid
   - **10% formaldehyde (most widely used)**
   - Ethanol
   - Glutaryldehyde
   - Methanol
   - Picric acid
   - Osmic acid (osmium tetroxide)

   The purpose of the fixation process:

   a) Prevent autolysis and bacterial decomposition
   b) Preserve tissue details as nearly as possible to the living state
   c) Hardening the tissue by coagulating proteins
   d) Renders the tissue receptive to the subsequent staining
   e) Prevent cellular damage during subsequent procedure

2. **Tissue processing:**

   This process consists of 3 stages:

   - **Dehydration stage:**

     Removing the excess of fixative agent and water content from the tissue and replacing it with dehydrating fluid. Dehydration is done by using different concentrations of ethanol starting with 70% then 85% and finally 100% ethanol.

   - **Clearing stage:**

     Replacing the dehydrating fluid with another fluid that is miscible with both dehydrating and embedding medium. This stage is also important to make the tissue translucent by using a proper organic solvent like (xylene) or also called Histolene (the clearing solvent should be inert and non-harmful to the processed tissue).

   - **Embedding stage:**

     Is the process by which the tissue is submerged in a solidifying agent like liquefied paraffin wax, then left to solidify in order to provide support during sectioning.

3. **Sectioning process:**

   Is the process by which the paraffin wax embedded tissue is cut into a very thin layers (about 5 – 7 μm in thickness) using a mechanical instrument called a microtome

4. **Staining process:**

   The most common stain used in histopathological slide is H&E stain (Hematoxylin & Eosin), this type of stain is water and alcohol soluble.

   - Hematoxylin stains cellular nuclei blue to black
   - Eosin stains cytoplasm and intracellular components orange, red or pink

Procedure of staining:

- Deparaffinize with clearing agent and then hydrate to water. The tissue then is ready for staining
- Stain with hematoxylin first for 15 min end examine under the microscope. The excess of staining is washed with 0.1% HCL to the required shade.
- Then stain with eosin for 2 min.
- To make the process permanent, dehydrate with absolute ethanol for 2 min
- Clear with xylene or histolene for another 2 min
- Mount the slide with DPX (Distrene Dibutyl Phthalate Xylene) a synthetic mounting medium which acts as clear glue wax substance that adheres the cover slip to the stained section
- Finally place the mounted slide in an oven overnight
- Cool and then the slide is ready for examination