Histopathology

Compound of three Greek words: histos "tissue", pathos "suffering", and logia "study of".

It is the examination of tissues from the body under a microscope to study the signs and characteristics of disease (manifestations of disease).

Histology is the study of tissues, and **pathology** is the study of disease.

In clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides.

In contrast, **cytopathology** examines free cells or tissue micro-fragments (as "cell blocks").

Method of Biopsy Taking:

1. Incisional biopsy

* It is performed when removal of entire lesion is impossible.

* Often performed prior to major surgical procedure.

* Is strictly a diagnostic nature.

2. Excisional biopsy

* In this technique, the entire lesion is removed, usually with a rim of normal tissue.

* It is performed when the lesion smaller in size.

* The procedure serves the diagnostic and therapeutic function.

3. Punch biopsy

* It is done by biopsy forceps.

* It is performed in the lesion of uterine cervix, oral cavity, esophagus, stomach, intestine and bronchus.

4. Core needle biopsy

* It is done with special type of wide bore biopsy needle.

* It permits a percutaneous approach to internal structures.

5. Curettage biopsy

* Curetting is usually done for diagnosis of endometrial disease.

Some General Rules for the biopsy Procedure:

1. The larger the lesion, the numerous the biopsies that should be taken from it because of the fact that diagnostic areas may be present only focally.

2. In ulcerated tumour, Biopsies should be taken from the periphery that includes normal and diseased tissue.

3. Crushing or squeezing of the tissue with forceps should be carefully avoided.

4. Once the biopsy is obtained, it should be placed immediately into container with adequate volume of fixative.

Handling of Specimen

* Specimen should be transported in glass, plastic or metal container or in a plastic bag in 10% formalin. If formalin is not available at hand, place the specimen in refrigerator at 4ºC to slow down autolysis.

* The container should have an opening larger enough so that the tissue can be removed easily after it has hardened by fixation.

General Principle of Gross Examination:

1. Proper identification and orientation of the specimen.

2. Unlabelled specimen should never be processed.

3. A properly completed histopathology requisition form containing patient name, age, sex, relevant clinical data, surgical findings, nature of operation and name of tissue submitted.

4. Careful search and examination of all the tissue submitted in order.

5. Place the specimen on cutting board in an anatomic position and record the following information:

a. Types of specimen b. Structure included c. Dimensions
d. Weight e. Shape f. Colour
g. Consistency h. Surgical margin, whether included or not involved by tumour

6. Measurements are usually given in centimetre unless the specimen is very small in which mm can be used.

Sampling for Histopathological Examination:

* Tissue submitted for histopathology must not be more than 3 mm thick and not larger than the diameter of slides used. Most specimens from solid tissues are cut in the form of pieces measuring 10 to 15 mm on the slides and 2 to 3 mm in thickness.

* Discrete areas of calcification or ossification should be taken out and should be decalcified in nitric acid.

Fixation

This is the process by which the constituents of cells and tissue are fixed in a physical and chemical state so that they will withstand subsequent treatment with various reagents minimum loss of architecture.

* This is achieved by exposing the tissue to chemical compounds, call fixatives.

* The broad objective of tissue fixation is to preserve cells and tissue components in a "**life-like state**".

Mechanism of action of fixatives:

* Most fixatives act by precipitating proteins.

* No fixative will penetrate a piece of tissue thicker than 1 cm.

* For dealing with specimen thicker than this, following methods are recommended:

1. Solid organ: Cut slices as necessary as but not thicker than 5mm.

2. Hollow organ: Either open or fill with wool soaked in fixative.

3. Large specimen: Inject fixative along the vessels or bronchi as in case of lung so that it reaches all parts of the organ.

Properties of an Ideal Fixative:

1. Prevents autolysis and bacterial decomposition.

2. Preserves tissue in their natural state and fix all components.

3. Make the cellular components insoluble to reagent used in tissue processing.

4. Preserves tissue volume.

5. Avoid excessive hardness of tissue.

6. Allows enhanced staining of tissue.

7. Should be non-toxic and non-allergic for user.

8. Should not be very expensive.

Amount of fixative fluid:

* This should be approximately **10-20** times the volume of the specimen.

* Fixative should surround the specimen on all sides.

Classification of Fixatives:

A. Tissue fixatives

a. Buffered formalin b. Buffered glutaraldehyde c. Zenker's formal saline
d. Bowen's fluids

B. Cytological fixatives

a. Ethanol b. Methanol c. Ether

C. Histochemical fixatives

a. Formal saline b. Cold acetone c. Absolute alcohol

Factors affecting fixation:

There are a number of factors that will affect the fixation process:

- **Buffering**

- **Penetration**

- **Volume**

- **Temperature**

- **Concentration**

- **Time interval**

Staining:

* Staining is a process by which we give colour to a section.

* There are hundreds of stains available.

Classification of Stains: Generally the stains are classified as:

A. **Acid Dyes:**

* In an acid dye the basic component is coloured and the acid component is colourless.

* Acid dyes stain basic components: e.g. eosin stains cytoplasm red.

B. **Basic Dyes:**

* In a basic dye the acid component is coloured and the basic component is colourless.

* Basic dyes stain acidic components: e.g. basic fuchsin stains nucleus blue.

C. **Neutral Dyes:**

* When an acid dye is combined with a basic dye a neutral dye is formed.

* As it contains both coloured radicals, it gives different colours to cytoplasm and nucleus simultaneously. This is the basis of Leishman stain.

Haematoxylin and Eosin staining (H & E):

It is the most common used routine stain in histopathology laboratory.

Special Stains:

* Used in addition to H & E staining to selectively stain cells and cellular components

* Used when needed

1. **PAS** (Periodic Acid Schiff) stain: This stain demonstrates glycogen.

2. **Stains for micro-organism**:

a. **Gram-stain**: used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells violet or red.

b. **Ziehl_Neelsen stain**: this stain detects acid fast bacilli.

c. **PAS stain**: used for fungi, amoeba and Tricomonas.

d. **Modified Giemsa** (2% Giemsa in water): used for Helicobacter pylori.

3. **Congo-red**: used for identification of amyloid.

4. **Sudan-Black**: used for fat staining.

5. **Masson's Trichrome**: used for differentiation of connective tissue and used to differentiate between collagen and smooth muscle in tumor.

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