Oral Pathology

Introduction

Biopsy (Principles and techniques)

Oral and maxillofacial pathology is the specialty of dentistry and the discipline of pathology that addresses the nature, identification and management of diseases affecting the oral and maxillofacial regions.

Surgical Pathology: is that specialty of pathology which deals with the diagnosis of diseases by microscopical examination of tissues taking by a surgeon ((Biopsy)).

Interpreting biopsies is one of the most important duties of the surgical pathologist, having taken a careful history and completed the clinical examination; the clinician is often in a position to formulate the diagnosis, or at least a list of differential diagnosis. In the latter case, the diagnosis is provisional and another opinion (consultation and referral) or investigation may be necessary to reach a firm diagnosis.

Biopsy is the removal of tissue from a living individual for a diagnosis by histopathological examination. The use of biopsy is not restricted to the diagnosis of the tumors, but is invaluable in determining the nature of any unusual lesion.' However not all lesions present a specific microscopic appearance and for this reason a definitive diagnosis cannot always be made. The need for special techniques in surgical pathology is sometimes needed to reach a final diagnosis.

Types of biopsy according to the size of tissue that to be biopsied:-

1- Incisional biopsies, only a portion of the lesion are sampled, and therefore the procedure is strictly of a diagnostic nature.
2- Excisional biopsy, the entire lesion is removed, usually with a rim of normal tissue, and therefore the procedure serves both a diagnostic and a therapeutic function.

Types of biopsy according to the instruments used to obtain them:-

- **Cautery biopsy.**
- **Cone biopsy.**
- **Core needle biopsy.**
- **Vacuum assisted biopsy.**
- **Endoscopic biopsy.**
- **Punch biopsy.**
- **Surface biopsy.**

1-Cautery Of these, the one usually least suitable for microscopic interpretation is that obtained with a cautery, because this instrument chars and distorts tissues.

2-Cone biopsy Cone Biopsy removes a piece of tissue which is cylindrical or cone shaped. Cone biopsy is performed to diagnose cervical cancer. Cone biopsy is often done following a pap smear, colposcopy (examination of the cervix under illuminated magnification), and a punch biopsy.

3-Core needle biopsy Core needle biopsy (or core biopsy) is performed by inserting a small hollow needle through the skin and into the organ or abnormality to be investigated. The needle is then advanced within the cell layers to remove a sample or core. Needle biopsy is also a type of percutaneous (through the skin) biopsy. The needle may be designed with a cutting tip to help remove the sample of tissue. Core biopsy is often performed with the use of spring loaded gun to help remove the tissue sample.

4-Vacuum Assisted Biopsy Core biopsy is sometimes suction assisted with a vacuum device. This method enables to removal of multiple samples with only one needle insertion. Vacuum assisted core biopsy is being used more and more in breast biopsy procedures

5-Endoscopic Biopsy Endoscopic biopsy is a very common type of biopsy that is done through an endoscope (a fiber optic cable for viewing inside the body) which is inserted into the body along with sampling instruments.

6-Punch Biopsy Punch biopsy is typically used by dermatologists to sample skin rashes, moles and other small masses. After a local anesthetic is injected,

7-Surface Biopsy Surface biopsy involves sampling or scraping the surface of a sore or tumor to remove cells for pathologic testing. Surface biopsy is often performed by dermatologists to remove a small piece of skin to test for carcinoma (cancerous tissue).

Some general rules for the biopsy procedure. The fact that they are so obvious makes it particularly bothersome that they are so often violated or ignored.

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

2. In ulcerated tumors, biopsy of the central ulcerated area may show only necrosis and inflammation. The most informative biopsy is likely to be one taken from the periphery that includes both normal and diseased tissue; however, the biopsy should not be so peripheral that only normal tissue is obtained.

3. The biopsy should be deep enough that the relationship between tumor and stroma can be properly assessed. Epithelia involved by carcinoma have a tendency to detach from the underlying stroma. This should be avoided whenever possible by careful handling of the tissue.

4. Deeply seated lesions are sometimes accompanied by a prominent peripheral tissue reaction, which may be characterized by chronic inflammation, hyperemia, fibrosis, calcification, and metaplastic bone formation. If the biopsy is too peripheral, this may be the only tissue obtained. Similarly, in a mass of lymph nodes, a deep-seated node may show involvement by a malignant tumor, whereas a superficial node may show only nonspecific hyperplasia.

5. When several fragments of tissue are obtained, they should all be sent to the pathology department and all of them submitted for microscopic examination. Sometimes the smaller or grossly less impressive fragment is the only one that contains the diagnostic elements.

6. Crushing or squeezing of the tissue with forceps at the time of performance of the biopsy by the surgeon, at the time of the gross examination by the pathologist, or at the time of embedding by the histotechnologist should be carefully avoided. The artifacts resulting from it often render a biopsy impossible to interpret.

7. Once the biopsy is obtained, it should be placed immediately into a container with an adequate volume of fixative. The temptation on the part of the surgeon or the pathologist to turn it around, wash it, or scrape the surface should be resisted, since it will not provide any information of diagnostic significance but only create artifacts.

8. Depending on the presumed or known nature of the lesion, consideration should be given at the time of the biopsy to the possible need for special studies, such as touch preparations, electron microscopy, cytogenetics, molecular genetics, flow cytometry, or others.

For the above points to be fulfilled the following technical points to be considered by the surgeon in a biopsy procedure

1- Do not paint the surface of the area to be biopsied with iodine or a highly colored antiseptic
2- Local anesthesia should not be injected directly into the lesion but around the peripheries
3- Use a sharp scalpel to avoid tearing tissue.
4- Use care not to mutilationthe specimen whenholding it with forceps.
5- Remove a border of normal tissue if possible.
6- Fix immediately with 10% buffered formalin or 70% alcohol.
7- Put a land marks on tissue to indicate direction (e.g. sutures).
8- Labeling by name.

Indications for biopsy

- Any lesion that persist for more than 2 weeks with no apparent etiologic basis.
- Any inflammatory lesion that does not respond to local treatment after 10-14 days.
- Persistent hyperkeratotic changes in surface tissue.
- Any persistent tumescence, either visible or palpable beneath relatively normal tissue.
- Lesion that interfere with local function.
- Bone lesions not specifically identified by clinical and radiographic findings.
- Any lesion that has the characteristics of malignancy.
- Erythroplasia-lesion is totally red or has speckled red appearance.
- Ulceration-lesion is ulcerated or present as an ulcer persisted for more than 2 weeks.

- Growth rate-lesion exhibits rapid growth.
- Bleeding-lesion bleeds on gentle manipulation.
- Induration-lesion and surrounding tissue is firm to the touch.
- Fixation-lesion feels attached to adjacent structures.

Indications for incisional and excisional biopsy

Interpreting biopsies is one of the most important duties of the surgical pathologist. In ***incisional biopsies,*** only a portion of the lesion is sampled, and therefore the procedure is strictly of a diagnostic nature. In ***excisional biopsies***, the entire lesion is removed, usually with a rim of normal tissue, and therefore the procedure serves both a diagnostic and a therapeutic function. The decision whether to perform an incisional or an excisional biopsy depends primarily on the size of the lesion; the smaller it is, the more logical to take it out completely when first encountered. For large lesions, particularly those of deep soft tissues, an incisional biopsy is usually preferable because of the fact that the type and extent of excision vary considerably depending on the tumor type.

Diagnostic cytology

Diagnostic cytology, when performed by well-trained, experienced individuals, offers an extremely high degree of reliability. A positive cytological diagnosis of malignancy made under these circumstances should be given the same weight as one obtained from a surgical biopsy. The cytologist will make a certain number of false-negative diagnoses depending on the source of the material, but false-positive diagnoses should practically never occur, for they will in themselves invalidate the method.

Fine needle aspiration (FNA)

The technique of fine-needle aspiration (FNA) was developed at Memorial Hospital in New York City in the 1920s. It is generally carried out with a ‘fine’ needle (OD 0.6–0.9 mm), sometimes under image guidance. There is no question that the procedure is, in most instances, inexpensive, safe, quick, and – when performed by experienced workers – quite accurate. It has contributed a great deal to transform cytology from a primarily screening tool to a powerful diagnostic technique**.**

Abrasive cytology (exfoliating cytology):

This method has provided very accurate results over the years for symptomatic patients, as good as or better than with the use of mucolytic agents or abrasive methods, this rather involved procedure precludes its use as a general screening method for unselected patients.

1. Cytology is not a substitute but an adjunct to the surgical biopsy.

2. It is quick ,simple, painless and bloodless procedure.

3. It helps as a check against false – negative biopsies.

4. It is especially helpful in follow-up detection of recurrent carcinoma in previously treated cases.

5. It is valuable for screening lesions whose gross appearance is such that biopsy is not warranted.

Laboratory techniques in histopathology

1-Fixation. Of the many fixatives that have been proposed, *10% buffered formalin* remains the best compromise under most circumstances. It is inexpensive, the tissue can remain in it for prolonged periods without deterioration, and it is compatible with most special stains, including immunohistochemical techniques, as long as the tissue is placed in fixative shortly (<30 min) after surgical removal, and overfixation (>24–48 hours) is avoided. Other fixative solutions are as follows:

Zenker fluid (which incorporates mercuric chloride) is an excellent fixative, one of the best that has ever been devised for light microscopic work, but it is expensive, requires careful disposal of the mercury

Bouin fixative (which contains picric acid) has been especially recommended for testicular biopsies, but Zenker fluid results in almost identical preparations. Bouin, Zenker, and B-5 are excellent fixatives for routine work and for most immunohistochemical stains, but the preservation of nucleic acids is very poor

2-Laboratory tissue processing

These refer to any treatment of tissues necessary to impregnate them with a solid medium to facilitate the production of sections for microscopy.

1- labeling of tissue
2- completion of fixation process
3- gentle and complete dehydration to remove aqueous fixative and any tissue watere.g. Ethanol and alcohol
4- Clearing with a substance which is totally miscible with both the dehydrating agent which precedes it and the embedding agent which follows it. e.g. Xylene
5- Embedding e.g. wax, resins and agarwe have two types of tissue processing. Manual and automated tissue processing
6- microtomy-is the sectioning of tissue blocks by microtome
7- staining either by ordinary stains (hematoxylin and eosin) or special stains

Special stains

Of the hundreds of 'special' stains listed in the classic texts dealing with histologic techniques, the surgical pathologist will find a relatively small minority to be of real diagnostic utility at present. This is especially true since the advent of immunohistochemistry, which has rendered many of them obsolete. Those most commonly used at present are the following:

1. ***Periodic acid–Schiff (PAS) stain.*** This is an extremely useful and esthetically pleasing technique, and makes evident most types of fungi and parasites.
2. ***Stains for microorganisms****. These include techniques for gram-positive and gram-negative bacteria, acid-fast mycobacteria, fungi, and parasites.*

3. ***Argentaffin and argyrophilic stains.*** Silver stains are mainly used for the identification of neuroendocrine cells and their tumors, but also for the demonstration of reticulin fibers, melanin, and calcium.
4. ***Amyloid stains.*** The mysteriously named Congo red followed by examination with both standard and polarized light (the notorious apple green birefringence) is regarded as the most reliable and practical technique to detect amyloid.
5. ***Reticulin stains.*** Reticulin stains demonstrate both 'reticular fibers' and basement membrane material.
6. ***Trichrome stain.***The main value of this group of stains is in the evaluation of the type and amount of extracellular material**.**
7. ***Phosphotungstic acid–hematoxylin (PTAH) stain.***
8. ***Stains for hemosiderin (Perls), melanin (Fontana–Masson), and calcium (von Kossa).***
9. ***Stains for neutral lipids.***
10. ***Mucin stains.*** since it demonstrates mucosubstances of neutral, slightly acidic, and highly acidic types

Immunohistochemistry

immunohistochemistry is the application of immunologic principles and techniques to demonstrate molecules in cells and tissues. The original method, brilliantly conceived by Coons, consisted of labeling with a fluorescent probe an antibody raised in rabbits and searching for it (and therefore for the antigen against which the antibody was directed) in tissue sections examined under a fluorescent microscope following incubation. The technical improvements that supervened in subsequent years have been responsible for these methods becoming a staple of the histopathology laboratory.
The most important diagnostic applications of immunohistochemical marker that have been applied widely to surgical pathology problems, whether as diagnostic aids, prognostic or predictive indicators, or as histogenetic probes are listed as follows:-

Actin. It is an extremely useful marker for the identification of smooth muscle cells and myofibroblasts

Albumin. Albumin comprises about one half of the blood serum proteins. It is potentially a good marker for hepatocellular and hepatoid carcinomas,

P53. Mutations of the *TP53* tumor-suppressor gene represent the most common genetic alteration in human tumors

S-100 protein. This is a family of acidic, dimeric, calcium-binding proteinsIts main use is in the evaluation of peripheral nerve sheath and melanocytic tumors

Desmin. This muscle-type intermediate filament (MW 55?000) is found in cells of smooth and striated muscle and in a lesser amount in myofibroblasts. Therefore it has been primarily used for the identification of smooth muscle and skeletal muscle tumors.

CD34. This marker stains normal and neoplastic endothelial cells, as well as a variety of soft tissue neoplasms, including dermatofibrosarcomaprotuberans, solitary fibrous tumor

Digital pathology and Telepathology

The era of digital pathology has arrived to surgical pathology. It has done so mainly through the many anatomic pathology information systems now on the market and the various devices that exist to capture digital images of gross and microscopic specimens, which can be integrated with the respective pathology reports. This has also allowed for these images to be transmitted electronically to any part of the globe. The latter, in short, is what is meant by *telepathology*. This can be done at various levels, from the e-mail attachment of a few static photographs to sophisticated systems that duplicate almost to perfection the examination of slides under the microscope and are, therefore, accurately referred to as *virtual microscopy*. These instruments allow the remote user to move the microscopic field in any direction, to change magnifications, and even to change the focus, the latter function being particularly useful for cytologic preparations. This can be achieved by moving the components of a microscope located elsewhere by remote control or by scanning the desired images and performing the above operations on those images

Surgical pathology report

The delivery of a specimen to the surgical pathology laboratory initiates a complex series of events that culminates in the issuance of the final pathology report. The surgical pathology report should describe, as thoroughly but also as concisely as possible, all the relevant gross and microscopic features of a case, and should also interpret their significance for the clinician. It should be accurate, prompt, and brief.

The usual surgical pathology report is composed of five major fields

* The first, which follows the demographics information, is designated as ‘History’, and contains the essential clinical data known to the pathologist at the time he dictates a description of the gross specimen(s), such as sex and age of the patient, symptoms, surgical findings, and type of surgery. It should also list previous biopsies on the same patient, if any had been taken

* The second field, designated as ‘Gross’, contains the gross description of the specimen(s). This should be precise and thorough, because once the gross specimen is discarded, and unless a picture has been taken, this description remains the only document by which the gross features of the case can be evaluated.

* The third field is termed ‘Microscopic’. We regard this as an optional feature of the report, which in many cases is unnecessary. When included, it should be short and to the point
* The fourth and most important field of the report is the ‘Diagnosis’. Each specimen received should have a separate diagnosis. It is preferable to divide each diagnosis into two parts, separated by a dash. The first lists the organ, specific site in that organ, and operation; the second gives the morphologic diagnosis (e.g., Bone, femur, biopsy – Osteosarcoma).

* The fifth field, which is optional, is a ‘Note’ or ‘Comment’. Here, the pathologist may mention the differential diagnosis, give the reasons for his diagnostic interpretation, make some prognostic and

therapeutic considerations about the entity, clarify some aspects of the case, and include selected references.

If a frozen section has been performed, the information regarding the organ biopsied, the diagnosis given, the names of the pathologist(s) who performed the procedure, and the final diagnosis corresponding to *the frozen sample* should be included in the report, either as a separate field (which we prefer) or incorporated into the History or Gross fields.