BIOPSY AND CYTOLOGY

Biopsy

• Definition

  • Biopsy is the removal of a sample of living tissue for laboratory examination.

• Rationale

  • It is a dentist’s obligation to make a diagnosis, or see that a diagnosis is made, of any pathological lesion in the mouth.
  • Some lesions can be diagnosed clinically, and biopsy is not required e.g. recurrent aphthous ulceration, Herpes simplex lesions.
  • Many lesions cannot be positively diagnosed clinically and should be biopsied – or in some cases smeared for cytopathologic examination.
  • Pitfalls of relying on a strictly clinical diagnosis are numerous:
    • A loose tooth may be due to a malignancy, not to advance periodontal disease.
    • What appears to be a dentigerous cyst clinically e.g. may be an odontogenic tumor.
    • Harmless looking white patches may be malignant or premalignant. Red patches are even more important.
  • It has been said that “any tissue which has been removed surgically is worth examining microscopically”
    • Yet dentists in general do not follow this rule.
    • However, there are some tissues e.g. gingivectomies, where every specimen need not be submitted.
  • While cancer is one disease in which biopsy is important, there are many other lesions in which this procedure is useful.
  • It is a sensitive diagnostic tool.
• **Methods of Biopsy**
  • Excisional – entire lesion is removed, along with a margin of normal tissue.
  • Incisional – representative portion of the lesion is removed, along with a margin of normal tissue.
    • Electrocautery – tends to damage tissue and make interpretation difficult.
  • Punch
  • Needle and aspiration
  • Curettage – intraosseous
  • Exfoliative cytology.

• **The Oral Biopsy – Avoidable Pitfalls.**
  • **Tissue alteration before the biopsy is taken.**
    • Do not apply antiseptics e.g. iodine, or topical anesthetics to the surface of the lesion. This may leave an unnecessary deposit on the tissues.
    • Local anesthetic infiltrations should be injected around the periphery of the lesion – infiltration directly into the lesion will cause volumetric distortion.

• **Tissue alteration during the biopsy procedure.**
  • Impaction of bone dust, dentine dust and enamel fragments into some intrabony lesions is unavoidable.

  • **Electrosurgery.**
    • Produces significant coagulation damage, particularly at margins of biopsy specimen, and can completely ruin small biopsies.
    • Can be useful to control bleeding.

  • **Crush, puncture and tear artifacts.**
    • Avoid grasping tissue with forceps, hemostats – these produce crush and tear artefacts which make histologic interpretation difficult. Toothed forceps can leave puncture holes which resemble cysts.
• Insert a suture through the normal tissue which is to be included in the biopsy specimen, and apply gentle traction to the suture threads.

• **Unwanted additions.**
  - Occasionally unwanted tissues are inadvertently included rendering histologic interpretation difficult.
  - These include fragments of calculus or plaque, which may mimic Actinomycotic infection, starch granules from gloves, restorative and endodontic filling materials.

• **The Wrong Tissue.**
  - The biopsy must include all or part of the lesion, and a border of normal tissue.
  - Biopsies of epithelium only are non-diagnostic because the nature of associated connective tissue change cannot be assessed histologically.
  - A negative biopsy – one that does not comply with the clinical appearance or the history of the lesion – is not final; it simply means that the disorder in question was not found in the sections examined by the pathologist.

• **Insufficient Tissue.**
  - Biopsies should be no less than 2-3 mm in dimension.
  - Small biopsies are difficult to orient correctly and easy to lose in handling.
  - Shrinkage due to fixation and processing further reduces the size and usefulness of a tiny biopsy.
  - Balance: adequate tissue versus small as possible in the best interest of the patient.

• **Tissue alterations after the biopsy is taken.**

  • **Lack of fixative.**
    - Biopsy specimens should be immediately place in fixative solution, such as 10% buffered formalin in the biopsy kit.
• Volume of fixative should be at least 10 times the volume of tissue.
• If no formalin available, use 70% ethanol.
• If no ethanol available, use gin, vodka, rum or other spirit alcohol in a 20:1 volume proportion.
• Isopropyl alcohol (rubbing alcohol) or methyl alcohol (wood alcohol) should not be used.
• Water and saline are unsuitable transport media.
• Formalin and water in biopsy kit vial may evaporate, leaving a white powder which is the buffer. Adding water will therefore not help, and the tissue will not fix. Autolysis will occur, creating artifacts of non-fixation in the histological appearance.

• **Freezing.**
  - Tissue specimens mailed in winter are at risk of being frozen in transport producing significant freeze artifact which may make histologic diagnosis difficult.
  - 10% formalin freezes at -11°C, forming ice crystals which distorts the tissue architecture.
  - To reduce the risk, the following steps can be taken:
    - Do not put the biopsy in an outside mailbox overnight – drop it in an inside mailbox in the post office building.
    - Add ethanol to the formalin to lower the freezing point of the fixative solution – ethanol, and spirits such as vodka, gin etc. in equal volume to the formalin will lower the freezing point and reduce the risk of freeze artifact.

• **The Dentists Responsibility.**
  - To ensure that a biopsy is taken when necessary, to submit tissue removed surgically for examination. This involves judgment, but it is a legal responsibility.

  • The obtaining of the tissue:
    - To obtain a representative piece of tissue.
    - Not too small.
• In as good condition as possible.
• Use a sharp blade.
• Do not rip or crush tissue with forceps.
• Do not inject directly into the lesion – may distort it.
• Do not paint the lesion with iodine.
• Avoid electrocautery.
• Avoid freezing.
• Fix immediately in 10% formalin
• Place the tissue on a small piece of paper to avoid its curling.

• Provide pathologist with history:
  • Supply pertinent information.
  • Too much history is far better than too little.
  • Name, age, sex, occupation.
  • Clinical appearance and location of lesion.
  • History of lesion.
  • Pertinent medical history.
  • Clinical impression or comments.
  • Radiographs – send them (or copy) to pathologist (preferable) or provide good description.
  • Photographs if possible.

• Most dentists are capable of taking biopsies but are reluctant to do so. If a dentist feels that he/she is not qualified to perform this procedure, he/she should refer the patient promptly to an oral surgeon or someone else who is qualified.

• The Pathologists Responsibility:
  • Examine the tissue grossly.
  • Decide the location from which sections should be cut.
  • Examine and report on these sections.
  • The report:
    • Gross description.
• Microscopic description.
• Diagnosis.

• **Interpretation of the report.**
  • Read it all – it can be a learning process.
  • Pathologists are not infallible – it is an interpretive science.
  • A negative biopsy – one that does not comply with the clinical appearance or the history of the lesion – is not final; it simply means that the disorder in question was not found in the sections examined by the pathologist.
  • It is in order to ask the pathologist to review the sections if the clinical impression does not agree with the pathologists report.
  • Sometimes it may be necessary to do a repeat biopsy.

• **Dangers of biopsy.**
  • Causing metastasis.
    • While this is possible, the value of reaching a definitive diagnosis completely outweighs the objection.
    • Excision is better than incision.
    • Do not treat tissues roughly.
    • Do not biopsy a lesion which you are sure is cancer – send it to the cancer surgeon or cancer clinic.
  • Hemangioma can be dangerous – send to oral surgeon.
Cytology

• Both normal and malignant epithelial surfaces continually desquamate superficial cells.
  • These can be demonstrated on special stains.
  • This is exfoliative cytology.

• Technique:
  • Scrape area with spatula or tongue depressor, or brush.
  • Smear cells on glass slide, and fix immediately with 95% ethyl alcohol or spray fixative.
  • Two slides are preferred for each lesion.
  • Provide clinical history.

• Interpretation
  • Slides are examined by pathologist.
    • Normal:
      • Smears from oral mucous membranes consist almost exclusively of
        • Superficial cornified squames – pink or yellow.
        • non-cornified squames – green.
    • Infection and inflammation:
      • Leukocytes.
      • Distorted or degenerating cells.
      • Increased nuclear size.
    • Malignant cells:
      • Mainly nuclear changes.
      • Enlargement of nucleus.
      • Hyperchromatism.
      • Thick nuclear borders.
      • Clumped chromatin.
      • Variation in size and shape.
      • Epithelial cells tend to clump together.
These changes correspond to the cytological changes seen in a tissue section. However, the emphasis in cytological examination is on cellular changes. In biopsy, we can study tissue patterns and inter-relationships as well as cellular changes.

• **Indications:**
  1. **General.**
     - Tumors and thick white lesions should be biopsied and not smeared.
     - Ulcers and erythroplakias can be smeared more readily. It is easier to get to the deep cells which are the ones required.
     - In dental practice, the prime application should be for innocuous appearing lesions for which biopsy is not planned or indicated.

  2. **Specific indications.**
     - Innocuous appearing lesions for which biopsy does not seem to be indicated.
     - Patient refuses biopsy.
     - After radiation/chemotherapy as a follow-up procedure.
     - Lesions suspected of being viral conditions:
       - Herpes simplex.
       - Herpes zoster.
       - Pemphigus.
       - Sex chromatin studies.
       - Candidiasis.

• **Contraindications:**
  1. Do not smear a lesion suspected of being cancer – biopsy it.
  2. Do not smear any tumor.

  Do not attempt to smear any but the thinnest of white lesions.

• **Disadvantages:**
  - Not as reliable as biopsy.
• A negative cytology report does not preclude the presence of a neoplasm. If the lesion persists, a biopsy should be performed.

• False positives and false negatives are a problem.

• Although it is quick to perform, it requires a painstaking thorough examination by the pathologist.

• **N.B. Cytology is not, and was never meant to be, a substitute for biopsy.**