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Review Article

Biopsy techniques: A review

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ABSTRACT

A biopsy is a medical procedure that involves extracting a small tissue sample from a living individual for diagnostic examination. Biopsies can be broadly categorized into two types: tissue and liquid biopsies. Tissue biopsy entails removing a sample for microscopic analysis, while liquid biopsy is a minimally invasive technique that analyzes biological fluids, primarily blood, for cancer-related materials, including circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs). Both methods aim to provide representative samples with minimal patient discomfort. Biopsies are crucial diagnostic tools in various medical fields, aiding in the diagnosis and management of conditions such as cancer, inflammatory diseases, and skin disorders. However, inadequate techniques and poor handling can lead to misdiagnosis. This review highlights recent biopsy techniques and outlines the general principles governing oral biopsies, emphasizing the importance of thorough patient assessment and proper procedural protocols.

1. Introduction

A biopsy is a medical procedure in which a small sample of tissue is taken from the body of a living person for examination and diagnosis. The term was coined by Dr. Ernst Henry, a French dermatologist.[1] Broadly, there are two types of biopsy- tissue biopsy and liquid biopsy. Tissue biopsy involves the removal of a sample of tissue from a specific area for microscopic examination. Whereas, liquid biopsy is a minimally invasive procedure that analyzes biological fluids, typically blood, to detect cancer-related materials. It includes methods such as circulating tumor DNA (ctDNA) analysis, circulating tumor cell (CTC) isolation, and exosome analysis.[2] Regardless of the method employed, the goal is to obtain a representative sample for the pathologist to analyze, while minimizing discomfort for the patient during the procedure.[3] Biopsies are versatile diagnostic tools used across various medical fields to obtain tissue samples for analysis, helping to diagnose and guide treatment for numerous conditions such as cancer diagnosis, inflammatory diseases, cysts, skin conditions, neurological tumors etc. Inadequate biopsy technique, improper selection of the biopsy site, and poor handling and documentation of clinical information can result in artifacts and misdiagnosis of patients' oral lesions.[4] This review article enumerates the recent biopsy techniques and the general principles of oral biopsy.

2. Principles of Oral Biopsy

Clinically, dentists and oral surgeons should assess the patient's medical and dental history. They must carefully review systemic diseases, including seizures, asthma, cardiovascular conditions (such as myocardial infarction

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and angina pectoris), stroke, rheumatoid fever, human immunodeficiency virus (HIV), tuberculosis, hepatitis, and others. History of the oral lesion should also be known to further evaluate the duration and growth rate of the lesion. Before biopsy, informed consent should be signed by the patients, explaining the procedure and the possible complications including pain, discomfort, bleeding, swelling, infection, and even scarring and esthetic concern, especially when biopsy has to be done on lip.[5] Local anesthetic agent should not be injected directly into the lesional tissues, particularly true for biopsy of immune-related mucocutaneous disorders.[6] Sharp scalpel blade is required for biopsy such as no. 15 blade.

The steps involved in biopsy surgery include selecting the biopsy sites, preparing the surgical field, administering anesthesia, stabilizing the tissue, making an incision or excision of the lesion, handling the biopsy specimens, achieving haemostasis, and closing the surgical wound.[7–9]

Various types of biopsy techniques are–

2.1. Tissue biopsy

1. Incisional Biopsy, 2. Excisional Biopsy. 3. Exploratory Biopsy, 4. Punch Biopsy, 5. Curettage Biopsy, 6. Unplanned Biopsy, 7. Needle Biopsy, 8. Imprint Cytology, 9. Shave Biopsy, 10. Fine Needle Cutting Biopsy, 11. Exfoliative cytology.

2.1.1. Incisional biopsy

Particular or representative part of the lesion is sampled. It is undertaken when the lesion is extensive in size and it cannot be excised (>1cm in dia) or whenever malignancy is suspected.[10]

A wedge shaped, deep, narrow tissue is taken 2-3mm in size. In cases of pigmented or vascular lesions, rapidly growing lesions with ill-defined borders require margins of 5mm normal tissue.

2.1.2. Excisional biopsy

This technique refers to the removal of the entire lesion alongwith normal tissue surrounding the lesion to ensure complete removal.

It is generally employed with smaller lesions (<1cm size).[11]

2.1.3. Exploratory biopsy

This procedure is performed to investigate an internal lesion by removing all exposed portions of the tissue. It is commonly used for intraosseous lesions of the mandible and maxilla.

2.1.4. Punch biopsy

This procedure can be classified as either incisional or excisional, although it is typically a variant of an incisional biopsy. It utilizes specialized punch forceps to remove a portion of the lesion. This method is effective for obtaining small tissue samples from difficult-to-reach lesions or from larger lesions where complete excision is not advisable. However, because this technique can cause distortion of the lesion, it is rarely used in the oral cavity.

In this technique, the punch is positioned perpendicular to the skin and gently rotated while applying firm downward pressure. It is pushed down until it reaches the subcutaneous fat. The column of tissue captured in the punch is then lifted, and the pedicle is severed. Finally, the tissue is carefully extracted from the punch.[12]

2.1.5. Curettage biopsy

This method is mainly used for intraosseous lesions and very friable cellular lesions, where only small amounts of surface material are required for evaluation. Small tissue samples are centrifuged, and the sediment is placed in agar media, which is then sectioned into tissue blocks. This technique is effective for lesions such as actinic keratosis, superficial squamous cell carcinoma, superficial basal cell carcinoma, and warts. However, it is not suitable for inflammatory dermatoses or pigmented lesions.

2.1.6. Unplanned biopsy

A suspicious tissue is obtained unexpectedly as a result of surgical procedure

2.1.7. Needle biopsy

This procedure is technically classified as an incisional or punch biopsy and is primarily used to obtain tissue samples from deep-seated lesions, such as those located within the bone or in hard-to-reach areas.

2.1.8. Imprint cytology

In this technique, the biopsied tissue is sliced in half, and the cut surface is pressed onto a slide. The slide is then stained to examine the exfoliated cells.

2.1.9. Shave biopsy

This technique is used for raised lesions and involves obtaining a sample with either a scalp blade or a double-edge razor blade, cutting through the lesion along with the surrounding skin. Care must be taken to avoid excessive traction on an exophytic lesion, as this could lead to permanent depression at the biopsy site. This method yields shallower specimens and often heals with minimal cosmetic defects. Shave biopsy is indicated for:

1. Benign exophytic lesions
2. Superficial inflammatory lesions

However, it is contraindicated in suspected melanomas.

2.1.10. Fine needle cutting biopsy (FNCB)

Utilizes a 12 or 16-gauge needle with a trocar to obtain tissue cores, which are examined through routine histological methods. This outpatient procedure is straightforward, associated with minimal infection risk and rapid healing, and is generally well-tolerated by patients, particularly those who have undergone major head and neck surgeries. FNCB allows surgeons to obtain tissue for histological diagnosis without requiring a surgical biopsy, and its results are often easier for practicing pathologists to interpret compared to fine needle aspiration cytology (FNAC). However, there are some drawbacks, including the risk of false-negative results and potential tumor dissemination. The primary clinical indication for FNCB in head and neck surgery is to differentiate between reactive changes and recurrent malignancy, as confirming cervical metastasis from previously treated oral carcinoma does not compromise the success of future surgeries. Additionally, it may be employed for the initial evaluation of isolated, asymptomatic neck swellings that could be inflammatory or neoplastic. Although its use for salivary gland swellings is somewhat controversial, evidence suggests that the risk of tumor dissemination is minimal. Furthermore, determining the nature of salivary gland tumors—such as distinguishing between pleomorphic adenoma and malignant tumors—can be advantageous for surgical planning[13].

2.1.11. Exfoliative cytology

This is the microscopic examination of cells collected from the surface of an organ or lesion following appropriate staining techniques.

Neoplastic cells are less cohesive than normal cells and typically shed from the surface of a lesion or into secretions. These cells can be collected by scraping the lesion's surface and evaluated for changes such as dysplasia. The technique involves first cleaning the surface of the lesion to remove debris and mucins. Then, using a metal cement spatula or a moistened tongue blade, the surface is gently scraped multiple times, collecting material at the edge of the instrument. This material is then spread evenly over a microscopic slide and immediately fixed with 95% alcohol. After air drying, the slide is stained with Papanicolaou (PAP) stain. Exfoliative cytology is particularly useful for diagnosing oral lesions such as herpes simplex, herpes zoster, and pemphigus vulgaris.

Exfoliative cytology has several benefits: anesthesia is not required, making it particularly useful for detecting virally infected cells, acantholytic cells, and candidal hyphae. The procedure is quick, simple, painless, and bloodless. Additionally, specialized techniques such as immunohistochemistry can be performed on the samples. Interpretation of exfoliative cytology is as follows:

1. Class I (normal): only normal cells are observed.
2. Class II (atypical): presence of minor atypia due to inflammation. No signs of malignancy.
3. Class III (intermediate): wider atypia suggestive of severe dysplasia, carcinoma-in-situ or cancer.
4. Class IV (suggestive of cancer): shows few epithelial cells with malignant changes. Biopsy is mandatory.
5. Class V (positive for cancer): cells show characteristic malignant. Biopsy is mandatory.

However, it has its limitations, as it is often unreliable for confirmatory cancer diagnoses due to the high incidence of false-negative results.

The minimal requirements for the procedure are as follows:

1. Blade handle and No. 15 blade
2. Fine tissue forceps (preferably Adson forceps)
3. Retractor suitable for the site
4. Syringe and local anaesthetic
5. Sutures, if needed
6. Hemostatic agents (such as silver nitrate or absorbable gelatin sponge)
7. Needle holder
8. Curved scissors
9. Gauze sponges
10. Specimen bottle containing 10% neutral buffered formalin
11. Biopsy data sheet
12. Punch for punch biopsy

2.2. Liquid biopsy

Liquid biopsy technology refers to the molecular analysis of liquid samples. These minimally invasive procedures provide a way to obtain tumor-derived information from body fluids. While various body fluids can be analyzed, blood is the most commonly used for liquid biopsies. Liquid biopsies are primarily utilized in various diseases, most notably in cancer. They are commonly used for monitoring breast, lung, colorectal, prostate, and melanoma cancers, helping to assess treatment response and detect recurrence through circulating tumor DNA (ctDNA) analysis.[14]

Procedure- A blood sample is typically drawn using standard venipuncture techniques. Other fluids like urine, saliva, or pleural fluid can also be used, depending on the clinical context. The collected sample is processed promptly to isolate components of interest. For example, centrifugation for blood. The analytes that are isolated such as Circulating Tumor DNA (ctDNA), Circulating Tumor Cells (CTCs), exosomes and analysis is done by various molecular techniques.[15]

3. Conclusion

When planning a biopsy, careful preparation and consideration can significantly enhance the diagnostic

value obtained. Different tissue types and sites require specific techniques tailored to the disease or lesion in question. The selection of the biopsy site should be based on the nature of the condition. Proper handling of the tissue and prompt fixation are crucial for achieving an accurate histological diagnosis. Any inadequacies at any stage may lead to a non-diagnostic biopsy, necessitating repeat procedures that can impose both physical and psychological burdens on the patient. The challenges for clinician are to provide a representative tissue specimen that is adequate in quantity, properly fixed and without artifacts or distortion.

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5. Conflict of Interest

None.

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