

BIOPSY – A PRICELESS TOOL

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ABSTRACT

Biopsy is defined as the removal of tissue from the living organism for the purpose of diagnosis and treatment. Performing a good biopsy procedure needs appropriate training and good hand skill. There are various biopsy procedures and each procedure has its own advantages and disadvantages. Thus it is necessary for the dental practitioner to choose the appropriate biopsy procedure to arrive at a confirmatory diagnosis.

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INTRODUCTION

Biopsy is derived from a Greek word (By-Op-see), 'Bio'- meaning life and 'Opsy' – to look or vision. Thus biopsy is the removal of tissue from a living organism for the purpose of microscopic examination and diagnosis¹.

Tieckre RW (1965) defines biopsy as the removal of tissue for examination, microscopic analysis, chemical and bacterial analysis or a combination of all four. This term is used most frequently to indicate the removal of tissue from living subject for analysis. In 1870, **Ruge and Joham Vert** (Berlin) introduced surgical biopsy as an essential tool for diagnosis. And in 1889, **Emarch** put forward an argument that confirmation should be made before surgeries for malignancies. Later, **Williams Halsted** first introduced this principle in United States. Later in 1941, **George M Papanicolaou** studied exfoliated cells from female genital tract. The clinical presentation of any pathology can be mucosal surface change (change in texture, ulceration, proliferation) or it can be submucosal alterations (distortion or swelling produced by a mass). Hence the diagnosis of such pathology depends on the history, examination, laboratory studies and biopsy. Biopsy acts as a gold standard technique for a confirmatory diagnosis in the

field of pathology.² Thus the aim of this article is to review the various types of biopsy, indications, contraindications, advantages and disadvantages of various biopsy procedures and to impart awareness and knowledge among the general dental practitioners about the various techniques used.

Indications: Any lesion persisting for more than 2 weeks with no apparent etiological basis, Persistent hyperkeratotic changes on surface tissue (Eg: lips or oral mucosa), Lesion that interfere with local function (Eg: Fibroma), Any inflammatory lesion that does not respond to local treatment after 2 weeks (ie., after removing the local irritant). Any lesion that has characteristics of malignancy & bone lesions not specifically identified by clinical and radiographic findings.^{3,4}

Contraindications: There is no need to biopsy normal structures and inflammatory or infectious lesions that respond to local treatments (Examples: gingivitis & periodontal abscess).

Contraindicated in patients on anti-coagulant therapy and those with vascular lesions, over-whelming sepsis and local infection near the site, severe impaired lung function, multiple neurofibromas and patients on bisphosphonate therapy.^{4,5}

Objectives: Confirm a diagnosis made on plan as a self teaching aid and as a medical clinical findings, to determine the treatment record.

TYPES OF BIOPSY:⁶

S.NO	CHARACTERISTIC FEATURE	TYPES OF BIOPSY
1	Depending on the characteristics of the target lesion	<ul style="list-style-type: none"> • Direct (located superficially with an easy access) • Indirect (when the lesion lies deep and is covered by normal appearing mucosa)
2	Depending on the technique used	<ul style="list-style-type: none"> • Incisional biopsy • Excisional biopsy • Exfoliative cytology
3	Depending on the material employed	<ul style="list-style-type: none"> • Conventional scalpel • Punch • Electro scalpels • Carbondioxide lasers
4	Depending on the location of target lesion	<ul style="list-style-type: none"> • Salivary glands • Bone • Lymphnodes
5	Based on the purpose of biopsy	<ul style="list-style-type: none"> • Diagnostic • Experimental
6	Depending upon the clinical timing of the sample	<ul style="list-style-type: none"> • Pre-operative • Intra-operative • Post-operative
7	Hard tissue sampling techniques	<ul style="list-style-type: none"> • Drill biopsy • Trephine biopsy • Core needle biopsy
8	Soft tissue sampling techniques	<ul style="list-style-type: none"> • Scalpel biopsy • Punch biopsy • Shave biopsy • Imprint smear • Electrosurgery biopsy
9	Histologically	<ul style="list-style-type: none"> • Excisional biopsy • Incisional biopsy • Punch biopsy • Shave biopsy

		<ul style="list-style-type: none"> • Trephine biopsy • Drill biopsy
10	Cytologically	<ul style="list-style-type: none"> • Fine needle aspiration cytology (FNAC) • Exfoliative cytology • Brush biopsy • Imprint smear

EXCISIONAL BIOPSY: Refers to complete excision or removal of the affected lesional area for diagnostic and therapeutic purposes. Here, the lesion is completely excised along with 2-3 mm margin of surrounding normal tissue.

Indications : For discrete lesions which are less than 1 cm in diameter.

Example : Fibromas, papillomas, mucoceles, pyogenic granuloma, verruca vulgaris.

Contraindications : In melanomas and hemangiomas.

Advantages : Offers dual advantage : both diagnostic and therapeutic.

Disadvantages: Difficult to perform in large lesions. Specimens likely to get crushed by using tissue forceps. Should be avoided in case when high grade malignancy is suspected.^{6,7}

INCISIONAL BIOPSY: Is a biopsy that samples only a representative or particular part of the lesion. If the lesion is large or has different locations, more than one area of the lesion needs to be sampled.

Indications: If the area under investigation is difficult to excise because of its extensive size (more than 1cm), suspected for malignancy and proximal to vital structures (Eg: nerve)

Principle: Representative areas of the lesion should be biopsied in wedge like fashion. The biopsy site should be selected in an area that shows complete tissue changes. The material should be taken from the edge of the lesion to include some normal tissue. Care must be taken to include an adequate amount of abnormal tissue

Deep narrow biopsy to be taken rather than a broad shallow one as superficial changes may be quite different from those deeper in the tissue. In incision biopsy, the tissue is removed in an elliptical wedge. The incision on the either side of the ellipse converge into a

‘V’ to join in deeper sublesional tissue. In either instance, a margin of normal tissue of at least 2-3mm is required except for the pigmented or vascular lesions, rapidly growing lesions with ill defined borders which require 5 mm of normal tissue.⁸

The length of the ellipse should be 3 times the width in all these instances, to assist in tissue closure thereby minimizing the possibility of wound dehiscence.^{8,9}

PUNCH BIOPSY: Is a variant of incisional biopsy where a special punch type of forceps is used for the removal of a portion of the lesion.

In this, a punch is held perpendicular to the lesion and gently rotated with a firm downward pressure. Thus the punch is pushed downward till the subcutaneous fat is reached. The incised column of tissue in the punch is lifted and the pedicle is cut. The tissue is then carefully removed from the punch.¹⁰

Advantages: Ease of technique, Useful in mass screening. Sutures may not be required if small diameter punch is made and produce a more satisfactory specimen in bound down tissue (Eg: Hard palate)

Drawbacks: May not be adequate for biopsy of deeper pathology, difficult to biopsy in the freely movable tissues (Eg: Soft palate, floor

of mouth) . The ulcerated areas may be difficult to punch.¹⁰

CORE NEEDLE BIOPSY: Is established as a safe procedure and is routinely performed under local anesthesia. Many pathologists believe that for histologic study, core tissue is more useful than cytologic material.^{11,12}

Here the specimens are obtained using larger 14-18 gauge needles and provide a tissue core for histological assessment.

Advantages: Cost effective and is a minimally invasive technique, used as a diagnostic procedure.

FINE NEEDLE ASPIRATION BIOPSY/ CYTOLOGY(FNAB/FNAC): In oral cavity, FNAB is indicated for biopsies of palpable nodules (deep mucosal abnormalities) not amenable to oral exfoliative cytology . FNAB is widely used in regions such as thyroid, lymphnodes and salivary glands.

The technique for aspiration of cells/ fluid/ tissue fragments using a fine needle for examination under a microscope. Mostly done on swellings or lumps located just below/under the skin.

Advantages: Is relatively painless & produces speedy results.

Inexpensive technique with little equipment. Can be done as an outpatient or a bed side procedure.

Disadvantages: Oral cavity provides limited space to perform cutting movements required during FNAB procedure, also leading to traumatic artefacts in the excised specimen.^{13,14}

Indications: In situations of doubt rather than a precise diagnosis. Is most useful when the investigating clinician asks a series of questions rather than expecting an absolutely precise diagnosis. Such questions might include

Whether the condition is reactive, cystic or neoplastic, if neoplastic, is it likely to be benign or malignant . Is it primary or metastatic. If cystic , could it be of salivary gland origin

In non-palpable lesions, area difficult to biopsy but can be localized by CT, MRI & ultrasound ,to rule out vascular lesions prior to open surgery.In cases where biopsy is contraindicated on medical background, used as a diagnostic screening test at community level for head and neck masses, in cases of known tumors to assess the effect of treatment.

Principle: FNAB is usually performed using a 10 ml/20 ml syringe with 23 or 25 gauge needle.

After that the needle is inserted into the lesion and a vacuum is created and maintained while the operator proceeds with cutting movements at different angles throughout the lesion. Once the sufficient material is seen in the hub of the needle, the pressure is released and needle is withdrawn from the tissue. The needle is removed from the syringe which is filled with air. The needle is repositioned back onto syringe placed near the surface of 2 glass slides on which the collected material is deposited.

When aspirating cysts, cystic content must be fully drawn and then aspirated again to obtain material from capsule. Aspirates with large amount of blood contents must be discarded.¹⁴

Note: The use of narrower gauge needles reduces the need of local anesthesia and quantity of blood in the specimen.

BRUSH BIOPSY:

Introduced in 1991, as an alternative to conventional exfoliative cytology for investigating persistent oral epithelial lesions not considered suspicious for carcinoma. Today, the preferred screening tool is the

brush biopsy technique which enables a trans – epithelial capture of cells.

With this method, a brush is rotated against the tissue until there is a slight bleeding, which indicates that the brush has reached the basement membrane. The cellular aggregate on the brush is transferred to the glass slide, fixed and analysed.

Oral CDX kit consist of an oral brush biopsy instrument , a pre-coded glass slide and a matching coded test, requisition form, an alcohol fixative pouch and a pre-addressed container.

Clinical indications: Use of brush biopsy with computer aided analysis has wide clinical application in cases of leukoplakia, erythroleukoplakia and erythroplakia. Pinkness of tissue/redness of biopsy site is the evidence of proper technique. The cellular material is collected on brush which is then transferred to bar coded slide and rapidly flooded with fixative to avoid drying, stained by PAP/H&E. The slides are then scanned by an automated computer driven microscope system.¹⁵

Neural network based image processing software is designed for the detection of oral mucosal pre-malignant and malignant cells. Abnormality in cell morphology, altered cell

size, degree of keratinization, nuclear size and intensity are analysed.

The specimens were classified into 4 categories:

Negative: No epithelial abnormality

Atypical: Abnormal epithelial changes of uncertain diagnostic significance

Positive: Definitive cellular evidence of epithelial dysplasia/carcinoma

Inadequate: Incomplete trans-epithelial biopsies were excluded.

Is a non-invasive procedure for the diagnosis of oral mucosal lesions & also a method for determining the presence of cellular atypia (Eg: In oral epithelial dysplasia) ,a Oral computed assisted brush biopsy.^{15,16}

Advantages: Simplified technique that provides excellent diagnostic material & well suited to diagnose oral manifestation of mucocutaneous & vesiculoulcer disease.

Simple screening tool, fast, inexpensive and safe chairside technique.

EXPLORATORY BIOPSY:

Routinely done only after tests & blood work reveal no abnormalities. Used to make

diagnosis when typical imaging techniques fail to assure at an accurate diagnosis.

Eg: Endoscopy which uses a camera & minimal incisions instead of more invasive techniques.

EXFOLIATIVE CYTOLOGY:

Is a microscopic examination of desquamated cells from the epithelial surfaces & used as an adjunct to biopsy. In **exfoliative cytology**, cells shed from body surfaces (such as inside of the mouth) are collected and examined.¹⁷

INDICATIONS: This technique is particularly used for diagnosing lesions of epithelial surfaces. It should be used as an adjunct, not as a substitute for incisional or excisional biopsy. Main role is screening of apparently innocuous lesions.

When surgical removal of tissue is either not feasible or refused by the patient, when large areas of mucosal change must be monitored for the dysplasia or dysplastic changes such as post-radiation changes, herpes and pemphigus and for repeated examination or follow-up cases of oral cancer.

TECHNIQUE:

Firstly cleansing the surface of oral lesion off the debris and mucin followed by vigorously

scraping the entire lesion with a metal spatula or moistened tongue blade.

The collected material is then quickly spread evenly on a microscopic slide, fixed immediately before smear dries. Fixative used is 95% alcohol, later it is stained and viewed under microscope.

Systemic study of smear¹⁶

Class I (normal) – only normal cells are observed

Class II (atypical) – presence of minor atypia due to inflammation . No signs of malignancy

Class III (intermediate) – wider atypia suggestive of severe dysplasia, carcinoma-in-situ or cancer.

Class IV (Suggestive of cancer) – shows few epithelial cells with malignant changes. Biopsy is mandatory

Class V (Positive for cancer) – cells show characteristic malignant changes. Biopsy is mandatory

Uses and limitations: This method is simple, quick, causes little or no discomfort and can be repeated as frequently as required.

LA not necessary. Most useful in detecting virally damaged cells, acantholytic cells and candidal hyphae, samples only surface cells

and provides no information about deeper tissues. Negative cytology report does not rule out cancer and that a repeat smear or biopsy is indicated in suspicious lesions.^{16,17}

SHAVE BIOPSY: When a lesion is raised, a shave biopsy can be obtained for selected lesions using either scalpel blade or double edge razor blade. The lesion is cut fresh with the surrounding lesion.

The specimens are less deep and wound often heals with less obvious cosmetic details.

Indications: Benign exophytic lesions, Superficial inflammatory lesions

Contraindications: Suspected melanomas.⁶

ELECTROSURGERY BIOPSY: Electrosurgery refers to cutting and coagulation of tissue using very high frequency and low voltage electric current.

-A blend current combines cutting and coagulation and is useful in producing a bloodless operative field.

- Excisions of lesions on the face are usually performed with only a cutting current to limit scarring at wound base, which can be produced due to thermal coagulation

The lesion is grasped with forceps through a loop electrode. The electrode is activated by going under lesion, removing the growth.⁶

TREPHINE BIOPSY: Also called as closed needle biopsy. A bone marrow trephine means removal of 1-2 cms core of bone marrow in one piece.

Indicated in suspected cases of cancers, lymphomas, myeloma and leukemia to study the bone marrow architecture.¹⁸

DRILL BIOPSY: A modified Ellis biopsy drill that fits into a straight hand piece is used to perform this procedure. The major disadvantage is that generation of heat during the procedure. Indicated for intra-osseous lesions.

IMPRINT SMEAR /TOUCH PREPARATION:

Dudgeon and Patrick reported a technique for examination of imprints of fresh tissue specimens.¹⁹

On a glass slide, freshly cut surface of specimen is gently pressed, without trusting motion. Slide is fixed and stained with hematoxylin and eosin or papanicolaou stain.¹⁹

Disadvantage is that depth of infiltration cannot be analyzed.

Conclusion

Various techniques of biopsies have been tried over the past and each method has its own pros and cons. Therefore, the clinician must choose the appropriate biopsy technique to arrive at a confirmatory diagnosis. Also treatment of any condition solely depends on the accurate diagnosis and thus histopathological diagnosis remains the gold standard. Hence, proper biopsy will aid in arriving at a confirmatory diagnosis and for evaluating the prognosis of patient.

Conflict of Interest Statement-

There is no conflict of interest.

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