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BIOPSY – A REVIEW OF LITERATURE

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INTRODUCTION

The clinical presentation of any pathology in the oral cavity can alter the mucosal surface or submucosal structures. The diagnosis of such pathology depends on the history, examination, laboratory studies, biopsy and other diagnostic procedures. In 16th century, Sir Marcello Malphigi formulated the basic microscopic technique of utilizing the living tissues. He termed it *Bios-living, Opsis-visualizing*. Later, Sir Georianni Morgahni in the early 17th century popularized this method through his book *'The site and causes of diseases'* which laid the foundation for physiologic anatomy.[1] The term "biopsy" was introduced into medical terminology in 1879 by Ernest Besnier.

Biopsy according to Tiecke RW in 1965 is the removal of tissue for examination, microscopic analysis, chemical analysis, and bacterial analysis or a combination of all four. The term is used most frequently to indicate removal of tissue from a living subject for analysis. Chiles DG in 1987 stated that biopsy is the removal and examination usually microscopic, of tissue from the living body, performed to establish precise diagnosis.[2] Pederson GW in 1988 defined biopsy as the removal of a tissue specimen either totally or partially for microscopic examination and diagnosis.[3] It was defined as the removal of a piece of tissue from the living body for diagnosis by microscopic examination by Tomlins Christopher DC in 1998.[4] In 2002 NA Malik, defined biopsy as the removal of tissue from a living subject for laboratory evaluation and analysis.

A biopsy is indicated to confirm a clinical impression of a lesion that is nonresponsive to conservative therapy after 10 to 14 days. To determine the nature of any intraosseous lesion and abnormal tissues removed from the oral cavity.[5,6] It is advised in the case of persistent hyperkeratotic changes in surface tissues, lesions that interfere with local function (e.g. Fibroma) and any lesion that has the characteristics of a malignancy (e.g. Erythroplakia).[7]

Surface lesions demonstrate a color/texture change e.g., white, red or pigmented, ulcerated, fissured or both and proliferative (fibroma, papilloma). Whereas subsurface lesions occurring in the soft tissue if present superficially, distort surface continuity, e.g., Mucocele. Those that are deep are detected by palpation. Hard tissue lesions if present superficially, distort surface continuity. Deeper lesions are detected by x-rays, e.g., radiopaque and radiolucent changes.

Relative contraindications for biopsy are inflammatory lesions of allergic, viral, fungal or bacterial etiology, normal anatomical and racial variations, e.g., linea alba, physiological racial pigmentation, leukoedema, exostoses etc and lesions caused by recent trauma. The absolute contraindications include, pulsatile lesions or those suggestive of a vascular nature, intrabony radiolucent lesions, pigmented lesions and biopsy from a difficult location or site and underlying vital structures.[8]

BIOPSY

- Technique used
- Incisional
- Excisional

Material employed

- Scalpel
- Punch
- B – forceps
- Electroscalpels
- Co2 laser scalpels

Clinical timing of sampling

- Intraoperative
- Extraoperative

Sampling location
- Salivary glands
- Bone
- Lymph nodes
- Other head and neck tissues

Processing of the sample
- Frozen
- Embedded in paraffin or methacrylate
- Fresh sample

Tissue obtained as
Scrapings
- Exfoliative cytology.
- Brush biopsy

Cell aspirate
- Fine needle aspiration cytology.

Tissue piece
- Incisional biopsy
- Excisional biopsy
- Curetting
- Frozen section.

Tissue core
- Fine needle cutting biopsy
- Tru-cut needle biopsy
- Vin Silverman needle biopsy.

Exfoliative cytology
Tissue scrapings are collected in exfoliative cytology. Rudolf Virchow (1955) described that "Every cell is derived from a cell and that human disease processes were essentially disease of the cells." If malignant or other disease processes affect the area, the deeper cells lose their cohesiveness and are exfoliated along with the superficial cells. The lesion is scraped with a moistened tongue depressor or spatula or cytobrush type instrument. The cells obtained are smeared on a glass slide and immediately fixed with a fixative spray or solution.[9]

It is advantageous as it is quickly obtained and sequential laboratory evaluation can be performed. It is indicated for the evaluation of recurrent oral cancers after treatment, mass screening of oral cancer, malignancy associated change in buccal squamous cells in patients with malnutrition and for vesicular lesions.

Squamous cell Carcinoma remains the chief target for cytological investigation.[10] It sheds cells with less cohesion or poor adhesiveness. Oral red lesions usually considered as benign are also targets for cytological smearing. The technique followed is that a 90-degree angle of straight blade, cement spatula or the cotton swab is used for scraping of the lesion. It should be done while the tissue is stretched or taut. A single stroke is preferred over many small strokes. It is smeared on a standard glass slide (1x3 inches) with an etched label at one end. The cells should be evenly distributed over the central one third of the slide. The slide must be labeled.[11]

In the case of brush biopsy Brush Bx Kit is available which contains a Brush Bx instrument, pre-coded glass slide and test form, alcohol/carbowax fixative pouch and a pre-addressed submission container. Brush Bx Procedure involves a step by step procedure. Fixative is opened. Brush is wetted with water or saliva. Mild (flat ulcerated) to firm (thick keratinized) pressure is applied for 5 (flat)-10 (thick) rotations. The collected sample is spread immediately over entire glass slide. Fixative is squeezed onto the slide to fix the specimen.[12]

Fine needle aspiration cytology
Aspiration biopsy is the use of a needle and syringe to penetrate a lesion for aspiration of its contents for purpose of analysis. It is applicable to both intra osseous as well as soft tissue masses. It is used commonly in the case of, fluid filled cavities, vascular lesions, hematomas, empty cavities and cysts. Fine needle aspiration cytology was first introduced by Kun (1847). Greg and Gray in 1904 used this technique to obtain organisms in the lymph nodes. Franzem et al in 1956 gave the currently used technique of fine needle aspiration (needles of 21G or smaller). It is indicated in the case of localised disease, provides immediate results and is free of complications. It is a rapid and effective procedure to aid in diagnosis. A small sized needle avoids damage to vital structure. Cells obtained can be fixed, stained and examined within minutes. It requires little equipment, painless, anesthesia is not required, outpatient or bedside procedure, uncomplicated wound healing and readily repeatable. Success depends on obtaining a representative sample, experience is required for interpretation, definitive diagnosis is not always possible, false negative and false positive results are also obtained.[13]

Standard disposable needles (21 – 23G) are used and is attached to a standard 10-20 ml disposable syringe capable of producing good suction. Lesion is approached as vertically as possible and the needle is inserted into the lesion using no suction. Once entered into the lesion suction or negative pressure is applied and syringe is moved back and forth in the lesion for 10 to 15 times at different angulations making sure that the needle is within the lesion. Needle is withdrawn, air is drawn into the syringe and the aspirate is deposited on a clean-labeled microscopic slide. Usually 2-3 slides are prepared for each mass. One slide is immediately fixed in 95% ethanol solution and subsequently stained with Papanicolaou's or hematoxylin and eosin stain. Another slide is allowed to dry for staining with a May-Grunwald or Wright stain. After needle biopsy, direct pressure should be applied over the site to reduce the incidence of hematoma formation.[14]

Aspirates from enlarged lymph nodes can differentiate between, reactive hyperplasia or inflammation and malignant disease. Aspiration using 21 or 23G needles can be undertaken if the cortical bone is thin or absent. A larger bore 18G or small bur is used to gain access in case of deep seated lesions. Aspiration of salivary gland swelling is highly useful. In this case, incisional or cutting biopsies are contraindicated due to the risk of tumor seeding or fistula formation. The primary indication is to distinguish among benign, malignant and inflammatory lesions.[15]

Ultrasound guided aspiration procedure is non-invasive, quick, easy, and it can be performed with the patient under local anaesthesia. Advantage of this procedure is that the needle can be visualized in the organ and the organ can be scanned after biopsy for possible complications. Most importantly ionizing radiation is not used for imaging.[16]

Computed tomography guided procedure provides real-time CT images to guide a needle. It is indicated in the case of lymph nodes or masses that are not completely identifiable using ultrasound and for lesions near the skull base as CT is optimal for localizing these lesions. Radiation exposure and low soft tissue contrast are the limitations.[17]

Magnetic resonance imaging guided biopsy is advisable in cases of soft tissue lesion as MRI provides excellent soft tissue contrast and multiplanar imaging capability, and good vessel depiction. The mean procedure time is less than or equal to 10 minutes for aspiration as well as core biopsy.[17]

As with all procedures FNAC has a few complication and hazards such as hematoma formation, which is the commonest complications of the procedure. Firm finger pressure for 2-3 minutes will prevent this complication. Local dissemination by seeding of malignant cells along the needle tract is possible. The limitation of FNAC is that only a small population of cells is sampled, thus the reliability of test depends on adequacy of sample and its representative character.[18]

Incisional biopsy

Incisional biopsy / diagnostic biopsy samples only a particular or representative part of the lesion. It is useful in the cases requiring multiple biopsies. It is indicated in the case of extensive, potentially malignant lesions, for central bony lesions, chronic non-healing ulcer or squamous cell carcinoma, leukoplakia/ erythroplakia, lichen planus, bullous lesions (pemphigus, pemphigoid etc), granulomatous diseases (crohn‟s disease, orofacial granulomatosis, ulcerative colitis, tuberculosis) and minor salivary gland tumors (in palate). It is contraindicated in pulsatile/ vascular lesions and in pigmented lesions.[19]

Representative areas of the lesion (the area that shows complete tissue changes) should be biopsied in wedge fashion from the edge of the lesion including some of the normal tissue. Deep narrow biopsy should be considered rather than broad, shallow one, because superficial changes may be different from those deeper in the tissues. Necrotic areas should be avoided.[20] Electrosurgery can also be used for cutting and coagulation of tissue using very high- frequency, low-voltage electrical currents. A blended current is used. B-forceps (Reusable Olympus FD-6C-1B Hot Biopsy Forceps) offer a large 5 mm cup opening size for efficient collection of tissue.[21,22]

Excisional biopsy

Excisional biopsy is the removal of the lesion in total at the time of surgical diagnostic procedure. Entire lesion is made available for pathological examination and a complete excision is performed which also serves as a definitive treatment for few lesions. Entire lesion along with 2 to 3 mm of normal appearing surrounding tissue is excised. Surface excision is done with a simple elliptical approach. In case of deep soft tissue lesions a modified elliptical incision that is combined with deeper dissection is advocated. Anesthesia is administered around the periphery of the lesion followed by isolation and immobilization with a traction suture, hook or forceps. An elliptical area is marked and a no.15 sharp scalpel blade is used to make a firm single stroke incision down to the connective tissue layer to form a “V” at the base of the lesion. The length of the incision should be three times its width. Closure of the area of incision is achieved with a suture. Firm pressure for few minutes aids in hemostasis. Fixation is to be done immediately by placing the specimen in 10% formalin or 70% alcohol.[23]

Curettage biopsy

Curettage biopsy is indicated for intraosseous lesions that lie in cavities and for friable cellular lesions like sinuses and fistulae. Although the sample produced is usually soft tissue, it may also include bone fragments. If extremely small segments of tissue is obtained, the specimen should be centrifuged and the sediment used for investigations.[23]

Punch biopsy

Punch biopsy is an alternative technique of tissue removal applicable to both incisional and excisional biopsy. It is indicated for total removal of small lesions and fixed tissue such as firmly attached palatal tissue. The technique is used for oral mucosal malignancies where there is requirement of multiple biopsies. Contraindicated as a definitive surgical excision procedure for suspected malignant lesions and cases of vascular lesions. It is fast with low incidence of post-surgical morbidity. Suturing is usually not required. Any mucosal surface accessible to the biopsy punch can be biopsied. It is used primarily for epithelial or superficial mesenchymal lesions. Adequate representative tissue

deeper than the superficial lamina propria is difficult to obtain. Freely movable mucosa that cannot be well supported such as the floor of the mouth and soft palate may preclude the technique. Various types of biopsy punches are available such as Keye biopsy punch and belt-driven punch. The biopsy site is anesthetized and gently blotted with sterile gauze. The edge of the blade of the biopsy punch is placed on the site and rotated back and forth using moderate pressure to an appropriate depth until the external bevel is not visible and creates a clearly defined surgical margin. The tissue is then grasped with some atraumatic forceps and the base of the tissue core is released using a scalpel blade or fine curved scissors. Punch size varies from 2-6 mm in diameter.[24,25,26]

Frozen section

Frozen section is taken from the mucosal and deep surfaces of the defect intraoperatively. It is indicated to make an immediate surgical therapeutic decision, to determine whether a lesion is benign, malignant or non-neoplastic, for establishing the adequacy of clearance of margin after resection and for ascertaining metastatic involvement of regional lymph nodes. Freezing microtome using CO_2 gas or a refrigerated microtome (cryostat) is used to freeze the section. Biopsy tissue is frozen in a mixture of isopentene and solid carbon dioxide at -70^{O}C. Sections of 5-7µm are made on a refrigerated microtome adhered to a glass slide at room temperature, fixed with formal acetic alcohol (50ml formalin, 450ml 90% alcohol and 25ml of glacial acetic acid) and stained with haematoxylin and eosin. The procedure is completed within 5-10 mins from the time of receiving specimen till it is stained. The remainder of tissue is stored in 10% buffered formaldehyde.[27,28,29]

Errors in diagnosis can be due to sampling by the surgeon or pathologist, interpretation by the pathologist or a difference in communication between the two.[30,31]

Fine needle cutting biopsy and True cut needle biopsy

Fine needle cutting biopsy is a procedure in which a Monopty biopsy instrument (18G needle) is used in performing the biopsy. It has an accuracy of 88%. It can provide accurate information about cell type and tissue characteristics in head and neck lesions. Major complications associated with this is the possible spread of tumor cells along the large-bore needle track. Tru-cut needle biopsy consists of wide bore 14G and consists of a long 15.2cm cannula and trocar with a 2cm notch at the tip of the trocar. Local anesthetic is injected around the site followed by a stab incision with a scalpel. Cannula is inserted into the specimen and the trocar fully retracted until the specimen is within the notch. This is later used for further investigation.[32,33,34]

Scissors biopsy

Scissors biopsy is one of the ways to remove skin tissue for a biopsy specimen. It involves snipping off a growth that is attached to the skin with a stalk or which is pedunculated. Depending on lesion size and morphology, anaesthesia may or may not be necessary. The armamentarium needed are small forceps with teeth and a pair of sharp curved or straight iris scissors. The lesion is lightly grasped with forceps with a gentle pulling upward traction which provides a firm cutting surface and allows clear visualization of the lesion base. Bleeding after this procedure is usually minimal and can be easily controlled by application of 35% aluminium chloride solution.[36]

Shave biopsy

Shave biopsy is a procedure in which a scalpel or razor blade is used to scrape the lesion. It is performed superficially or deeply. Shave excision usually extends to the level of the middle dermis, with the subcutaneous tissue is left undisturbed. Seborrheic keratoses or fibrous papules are best biopsied using this technique.[37]

Bone biopsy

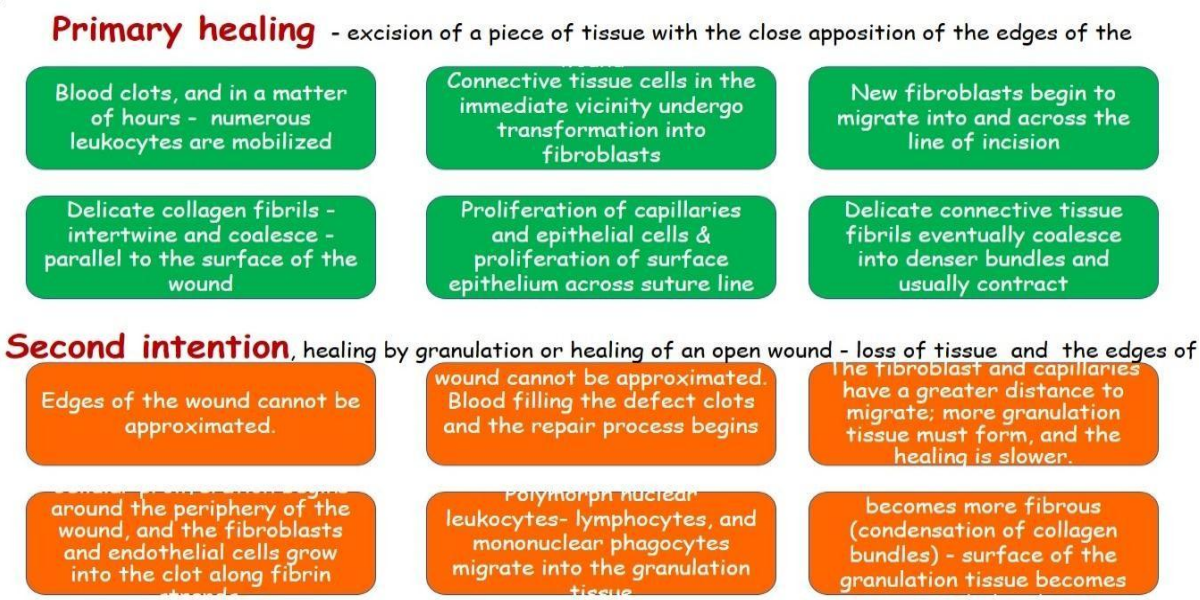
Intraosseous or hard tissue biopsy technique is indicated when a lesion is large, perforating into soft tissues, or suspected of malignancy. Area of the jaws where the lesion is present is located and palpated. If smooth and firm, it indicates no expansion or erosion of the cortical plate. Whereas a spongy feel to the jaw indicates an erosion or thinning of the cortical plates. In case of a radiolucent lesion, aspiration is done before surgical exploration. The choice of flap depends chiefly on the size and location of the lesion. Incisions are kept over sound bone for closure. For lesions within the jaw a cortical window is created to facilitate the procedure. If the cortical plate is intact, a rotating bur should be used to remove an osseous window. It is then enlarged with a rongeur. The osseous window specimens are submitted for histopathologic examination along with the primary specimen. A central lesion that may have eroded the cortical plate of the jaw may require a flap elevation in an area away from the lesion and over sound bone.[35]

Tissue stabilization

Tissue stabilization is necessary while performing a biopsy. Soft tissue biopsies in the oral cavity are frequently performed on movable structures, such as the lips, buccal mucosa, soft palate, and tongue. An assistant's fingers pinching the lip on the both sides of the biopsy area can immobilize the lips. It also aids in hemostasis by compressing the labial arteries. Heavy retraction sutures or towel clips can also be used. The chalazion clamp is a tissue stabilization clamp which serves two important functions of providing a firm surface to work and yields nearly complete hemostasis. Sutures can be placed in the center of the ringed opening before the clamp is loosened.[38,39] Healing of biopsy wound can be by primary healing or secondary healing. Edges of the wound can be brought into apposition,

often by suturing. Lesion must fill in gradually with granulation tissue. (Table 1)

Table 1: Wound healing after biopsy.



Complications of biopsy

Complications of biopsy include immediate or subsequent hemorrhage. Highly vascular tissue bleed for a longer time. Bleeding can be from a large friable tumor mass, where an adjacent blood vessel of moderate size is accidentally severed, and allowed to retract. When the wound becomes infected it presents with late secondary hemorrhage. Infection occurs when tumors on the various skin or mucosal surfaces are biopsied. There is always a possibility that already present bacteria, may thus gain access to the depths of the tumor and to the adjacent normal tissue. Thus, an aseptic technique must always be followed. Poor wound healing may be due to ischemia of the skin overlying a tumor mass. Implantation of tumor cells or spread of tumor cells is also possible**.** Prominent reasons for local tumor cell contamination are, prolonged and unnecessary manipulation of tissue, careless protection of the tissue during the incision or excision of malignant neoplasm and failure to change contaminated drapes, instruments or material when indicated. Certain of the vital structures must be avoided in needle biopsy and adequate surgical exposure is essential when the biopsy is taken with a scalpel. Other complications include, post-operative pain, paresthesia in the lips or the tongue, swelling and bruising in the tongue, lips and buccal mucosa. Biopsy procedures in the floor of the mouth can lead to submandibular or sublingual duct damage. Removal of mucoceles from the lip carries the risk of further adjacent gland damage and recurrence.[40,41,42,43]

CONCLUSION

For entities of uncertain etiology, a biopsy provides the simplest and most reliable means of obtaining the perfect diagnosis. In the concern of patient's welfare, correct diagnosis is of extreme importance. A carefully selected, performed and interpreted biopsy is critical in rendering an accurate diagnosis. When considering biopsy, a little forward planning and thought can greatly improve the diagnostic value obtained. Careful handling of the tissue and prompt appropriate fixation will enable a confident histological diagnosis to be reached. Inadequate care at any stage could result in a non-diagnostic biopsy and may necessitate the patient having a repeat procedure with its ensuing physical and psychological morbidity.

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