DOI: 10.7860/JCDR/2012/4967.2703

Review Article

Dentistry Section

How to Help the Oral Pathologist in Making an Accurate Diagnosis

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ABSTRACT

A biopsy is a valuable diagnostic tool in clinics. In this procedure, the sample tissue is obtained in order to study its structure both macro and microscopically, thus making it possible to establish a final diagnosis. Some clinicians are surprised and disappointed when the report from the oral pathologist comes as "not from the representative area and "inadequate specimen size", instead of a final diagnosis. To avoid such disappointments and to make the best utilization of the skills of the oral pathologist, certain ideas have been suggested. Right from the history taking to the clinical examination to the operative findings, the clinician can pave the way to a conclusive histopathological diagnosis.

Key Words: Biopsy,Oral pathology,Diagnosis,Tissue

INTRODUCTION

A biopsy is the removal of tissue from the body for examination and examining it under a microscope can assist in the diagnosis. The word, 'biopsy' has originated from the greek words, 'bios' -life and 'opsis'- vision of life [1]. A biopsy is considered to be the gold standard of diagnostic procedures [2]. This procedure helps in confirming or denying a diagnosis. An oral biopsy is essential for a definitive diagnosis of the diseases which occur in the oral mucosa [1]. An oral biopsy is not limited to the diagnosis of tumours but it is also of great usefulness for determining the natures of all types of lesions [3]. Planning before performing a biopsy is essential in helping the pathologist in arriving at a proper diagnosis. Basic knowledge and technical skills are required to perform a good biopsy. The accuracy of a proper biopsy starts from the history taking and the clinical examination themselves. Thereafter, the administration of local anaesthesia, the method which is adopted to remove the tissue, the adequate size and the depth of the tissue from the representative site and the subsequent fixation method, influences a good biopsy. The various ways by which the quality of a biopsy can be improved, has been discussed along with other measures to minimise a useless biopsy [4].

The History Taking and the Clinical Examination

More often than not, little things make a big difference. So is the part of the history taking in a biopsy. Obtaining an accurate history is the first step towards doing a diagnostic biopsy. An evaluation of the previous surgical experience and hospitalizations, the current medication and habits like smoking and alcohol consumption are essential. Obtaining this information is pivotal in arriving at a correct diagnosis. The past surgical history will help the oral pathologist in understanding whether the lesion has a recurrent nature. Medications have profound effects on the oral health. So, a detailed drug history should be obtained. A history of epilepsy and a subsequent medication with phenytoin will provide a clue to the gingival enlargement which is sent for the biopsy. Any familial disease like tuberculosis or malignancy will help the pathologist in confirming the diagnosis. Other available diagnostic data such as the radiological findings, laboratory tests or any other special investigations like ultrasound or CT scan will give a good picture of the lesion which has to be biopsied.

The high technology investigations do not diminish the need for a clinical examination.Together with the history taking, a clinical examination tends to aid in the diagnosis. A thorough and a systematic examination of the oral mucosa forms an integral part in the detection of oral lesions. So, a detailed examination of the lesion should be carried out by inspection and palpation. The colour, consistency and the texture should also be observed. A yellow colour nodule in the buccal mucosa will lead the diagnosis towards a xanthoma. The description of a pedunculated, cauliflower-like growth will suggest that the lesion could be a papilloma. The positivity of blanching under pressure will help in distinguishing a vascular lesion from a pigmented lesion. These clinical details will help the oral pathologist in the orientation during a histopathological examination. The clinical presentation should be documented and it should be sent to the oral pathologist along with the specimen without the omission of any of the details. Additionally, it is also desirable to have the previous biopsy numbers, to enable a comparison to be made if necessary, especially in cases of recurrent lesions and to evaluate the progression or the regression of dysplasia and oral squamous cell carcinoma [4].

The Administration of Local Anaesthesia

A local anaesthetic solution should not be injected into the tissue which is to be removed, as it can cause artificial distortion of the specimen. A peripheral anaesthesia is indicated to avoid the application of excessive pressure to the tissue and the artificial distortion. A needle insertion at the biopsy site can also produce bleeding with extravasation which can mask the normal cell architecture. Moreover, it can cause connective tissue separation with vacuolization, causing the interpretation to be difficult for the oral pathologist [5].

Selection of the Tissue

Biopsies are performed to establish a diagnosis and to determine whether the lesion has been completely removed. A biopsy should be done so as to help in the removal of the representative

portion of the target area, along with a portion of the normal healthy tissue. The biopsy should also have sufficient depth and it should have a surrounding margin to ensure adequate clearance, especially if it is the case of a carcinoma, as this can yield useful prognostic information [4]. The centres of the larger tumours should be avoided in the biopsy, as they are often necrotic and will not help in the diagnosis [6]. In the biopsies which are performed to confirm the mucocutaneous lesions such as Lichen planus, an area of the non-erosive lesional tissue should be chosen [4]. A sample from an erosive area will often show non-specific inflammatory changes and it will not confirm the diagnosis. For the vesiculobullous lesions, the site of the biopsy should be adjacent to the bulla, where the epithelium is still intact. An early lesion should be selected for the biopsy, which is not older than 48 hours [6]. The samples which are taken in this manner will be beneficial for the oral pathologist in interpreting and rendering a correct diagnosis. An unrepresentative sample is of no use to the pathologist and it will usually lead to a repeat of the biopsy procedure, causing unnecessary discomfort to the patient. If the lesion is extensive or if it has varying appearances such as an erythroplakia or a speckled leukoplakia, different samples should be obtained and they should be placed in separate and adequately identified containers. Model diagrams of the lesions which specify the locations of the lesions should also be attached along with the data.

Size of the Tissue

In the case of an incisional biopsy, the quantity of the tissue is as important as the quality of the tissue. An insufficient tissue will not aid in the diagnosis. Further, the shrinkage which occurs during the fixation, causes the tissue to become even smaller. It also presents a difficulty in the orientation and a risk of being lost while handling. In case of excisional biopsies, the advancing front of the tumour should be evaluated.

Depth of the Tissue

An adequate depth of the sample is another important criteria which can yield a good tissue section. An adequate depth of the tissue should be obtained, to include the epithelium and a few millimetres of the underlying lamina propria. The biopsies which contain the epithelium become non-diagnostic, as the connective tissue changes cannot be assessed. In oral submucous fibrosis, the epithelial biopsy will not render any valid information. The hyalinization in the connective tissue will suggest the diagnosis of oral submucous fibrosis. The accurate evaluation of the local invasion in the biopsies which are taken for the epithelial neoplasia, becomes difficult in a shallow specimen. An inadequate depth also causes the narrow strip of the delicate mucosa to fold on or to curl on itself during the fixation. This is referred to as a curling artefact. Curling and bending of the tissue makes the correct orientation difficult during the embedding procedure [7]. Since curling of the tissue is seen in the thin biopsy specimens, an adequate depth can help preventing this artefact [8].

The Biopsy Technique

The correct handling of the tissue is necessary for a proper interpretation. A crush artefact occurs due to the incorrect use of forceps in handling the tissue, either at surgery or at the pathologist's table. A dull scissors or a knife contributes to this artefact. Some types of tumour cells such as small undifferentiated carcinomas are particularly prone to develop crush artefacts and such artefacts can render a tumour biopsy uninterpretable. The lymph nodes are also very susceptible to the damage which occurs due to crushing with forceps or other surgical instruments. A crush artefact produces a marked distortion of the cells, with streaking of the nuclei. The specimen should be obtained by means of a clean cut, taking care during the extraction to avoid tearing or compression, as this could also cause alterations. Firm grasping of the tissue with a toothed forceps leaves puncture holes that can mimic mucosal pits or epidermoid cysts [6]. The incorrect use of the forceps produces the formation of pseudomicrocysts which are apparently lined with the surface epithelium, which is forced inwards by the teeth of the instrument [7]. To avoid this, small atraumatic forceps should be used. The surrounding stroma is also compressed, making the interpretation difficult [5]. The forceps should be used to hold the tissue in the normal adjacent area rather than in the lesional tissue. Using a suture during an incisional biopsy can produce minimal artefacts. A suture usually prevents unwanted movements of tissue, especially while a biopsy is being taken from the mobile structures such as the tongue. Suture traction with the subsequent use of a scalpel can result in crushing and fragmentation of the tissue [8]. In an excisional biopsy, the cut should slightly exceed the depth of the lesion. If the tissue is removed with excessive force, the epithelium and the connective tissue components may suffer important damage. An oral punch biopsy is said to provide biopsy specimens which are of adequate size for diagnostic purposes and which have a fewer artefacts than a conventional incisional biopsy [9].

If electrocautery is used, the heat which is generated by it can give rise to alterations such as tissue protein coagulation, resulting in an amorphous epithelial and connective tissue appearance [7]. In such situations, the epithelial cells become fusiform and hyperchromatic. A fulgration artefact is an important problem which is induced during the electrosurgical or the laser cutting of the tissue. It is a heat produced, marked alteration in both the epithelium and the connective tissue. The epithelial cells appear detached and the nuclei assume a spindled, palisading configuration. The separation of the epithelium from the basement membrane was also observed. The fibrous connective tissue, fat and the muscle had an opaque, amorphous appearance [5]. The resulting effect of a layer of carbonized tissue, a zone of thermal necrosis and a zone of tissue which exhibits thermal damage makes a histopathological interpretation more difficult [3]. To avoid the biopsy from becoming non-diagnostic, a wide incisional margin which is far away from the clinical boundary of the lesion is preferred [7].

Using a carbon dioxide laser beam to procure the diagnostic biopsy specimens is compromised by the thermal cytological artefacts that include vacuolation of the superficial layer, detachment and shredding of the keratin, basal cell degeneration and separation from the lamina propia . These artefacts could be critical when the dysplastic changes are being assessed as the thermal damage which is induced along the laser treated margins would simulate a cytological atypia [10].

The artifacts can arise from the vacuum effect of a surgical suction tip on the tissue specimen that is being excised. This artifact is characterized by the formation of large, often pleomorphic and connective tissue vacuoles which resemble the traumatized adipose tissue [11]. So, appropriate care should be taken regarding the use of the suction tips.

Tissue Submission Free of Contamination

The foreign bodies which are present in the biopsy tissue makes the interpretation difficult [7]. The tissues which are contaminated with cotton can resemble fungal infections, where the cotton fibres seem like fungal hyphae. The cotton can also resemble an eosinophilic, amyloid-like substance [7]. A gingival abscess which contains a fragment of a calculus may mimic an actinomycotic infection in the histologic sections. Care should also be taken to prevent the contamination of the tissue with the starch from the surgeon's gloves. The starch in the specimens gives an appearance of small spherical calcifications on histopathology [12].

Fixation of the Obtained tissue

After obtaining the sample, washing with physiological saline is indicated followed by immediate fixation. A good fixing agent penetrates rapidly, it prevents autolysis and putrefaction and it preserves the cell details due to the stabilization of the proteins in the cell. The light microscopic studies use a 10% formaldehyde solution in water. For an optimum fixation, the amount of the fixing agent should exceed the tissue volume by a factor of 20 [5]. The volume and the concentration of the fixing agent is essential for a good tissue section. Insufficient formalin leaves the specimen dry and unfixed. An immediate and correct fixation of the specimen is needed to interrupt the autolysis and the putrefaction and to stabilize the cell proteins. When placed in alternative solutions such as saline or water results in autolysis of the tissue and artefactual change resembling that of pemphigus [7]. There is a lack of detail and a loss of cellularity due to the autolysis and the putrefaction. The cell cytoplasm and the nuclear structures appear completely indistinct [7].The fixation bottles should be securely closed, to prevent evaporation of the fixative [6]. They must be also labelled with tape by using a pencil for writing to prevent interchange of the specimens.

Other considerations

When a biopsy is given for a histopathological examination, the question of what the biopsy was being taken for, must be answered. The characteristics of the lesion (size, shape, colour, texture, consistency, the time of evolution, the associated signs and symptoms and the regional nodes) should be described in the patient's clinical records together with a presumed diagnosis and a possible differential diagnosis. The provisional clinical diagnosis is especially important in guiding the technique and the tissue handling which has to be used. Radiographs must be provided to the oral pathologist wherever necessary, especially in cases of bony lesions, to make interpretation easy and precise. An explanatory diagram of the biopsy area may be useful for this purpose [12]. A cordial relationship between the surgeon and the pathologist is important, so as to clear any doubts that may arise in the case. Freezing of the tissue prior to its fixation should be avoided. The freezing during transport is also not recommended, as a cytoplasmic condensation has been described to occur secondary to the cell dehydration following freezing. Marking the surgical margins with silk sutures will help the oral pathologist in orienting the specimens and also in identifying the margins, if any had residual lesions. Lastly, a biopsy of a poorly differentiated malignancy can cause spreading or "seeding" of the cancer cells along the path or the track which is made by the biopsy needle or the surgical instruments during the diagnostic procedures. Making a direct incision into the cancer tissue could destroy the physical barriers and facilitate the cancer cells in forming a metastatic colony at a distant site [13]. This could cause the malignancy which had been confined solely to the oral cavity to spread into the surrounding tissues, making a serious health concern even more problematic. Seeding of the tumour along a needle biopsy track is a recognized potential complication which has been reported in the literature.

CONCLUSION

An oral biopsy, though it is a simple procedure, may prove to be futile, if it is not performed properly. The general dental practitioners should be competent in performing biopsies, regardless of whether they prefer to do a biopsy or to refer [14]. A biopsy which is done with prior planning, along with a little technical skill, can greatly improve the diagnostic value. One of the important causes which underlie a delayed definitive diagnosis, involves a professional who can perform a biopsy but who performs the technique incorrectly due to a lack of practical experience [3]. The steps that have been discussed above will alert the dental practitioners and they will help them in obtaining good biopsy specimens and in enabling the oral pathologists in interpreting the cases accurately. A good history taking and a clinical examination, a peripheral anaesthesia, a correct handling of the tissue and the fixation and submission of the tissue without contamination will enable a conclusive histological diagnosis to be reached. Inadequate care at any stage could create potential diagnostic problems for the oral pathologists.

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FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Submission: **Aug 22, 2012**
Date of Peer Review: **Sep 04, 2012**
Date of Acceptance: **Sep 26, 2012**
Date of Publishing: **Jan 01, 2013**