ISSN: 0975-766X
CODEN: IJPTFI
Available Online through ***Review Article***
www.ijptonline.com

CHALLENGES IN GROSSING SPECIMENS: AN OVERVIEW

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Received on 12-02-2019 *Accepted on: 10-03-2019*

Abstract

Accurate and complete information in pathology reporting is essential since most cancer treatment decisions are based on pathologic findings. Grossing is a process by which pathological specimens are inspected with the naked eye to obtain diagnostic information, before being processed for further microscopic examination. The most important grossing skill is the ability to choose the most diagnostically valuable parts of the tissue. Knowledge of the precise site from which sections were taken for microscopic examination is of great importance, especially when determining the presence of tumor cells in the surgical margins.

This paper discusses the general requirements for biopsy grossing techniques that include optimal guidelines for handling of specimens and sampling the diagnostically valuable areas of the biopsy specimen.

Keywords: Biopsies; Grossing; Orientation, tumour margins

Introduction:

Biopsy, the most essential diagnostic tool, introduced by Carl Ruge and his associate Johann Veit in 1870's paved the way for surgical pathology. The need to establish a microscopic diagnosis prior to extensive mutilating procedures required for suspected malignant tumors is inevitable. The advent of cryostat and rising need for immediate diagnosis of rush biopsies (frozen sections) cannot underemphasize the importance of grossing resection specimens as it still plays a crucial role in deciding the clearance of tumour margins. Each specimen is unique and should be handled differently from the beginning of the surgery till it reaches the microscope for diagnosis.

The 21st century is heading towards digital pathology. A digitalized grossing system: EGROSS (Milestone Medical, Sorisole, Italy) incorporates the digital power of specimen identification and dissection documentation. This system also assures quality at the source of tissue selection and cassette generation from the gross room to its reporting. Another system: Anatomic Pathology Laboratory Information Systems (APLIS's) are also being adopted in many labs[1]. Though digitalization is being incorporated in many pathology labs, grossing and manual documentation of specimens still maintains its importance in pathology.

The orientation of specimens sent for histopathological examination is of upmost concern as the accuracy of the results relies on correct orientation by the pathologist. Specimens that contain both soft tissues and bone are handled in a different manner depending on the site and type of pathology present.

1. Clinical implications of biopsy

Surgeons' play a great role in specimen handling before it reaches the pathologist. Use of tinted disinfectants over the biopsy site can influence the tissue preparations in the laboratory. Local anaesthetics are advisable to be administered in areas adjacent to the biopsy site as intralesional injection generates epithelial vacuolation, connective tissue separation and haemorrhage[2]. The selection of accurate biopsy procedure is an important factor. The advancing use of laser/ electrocautery in surgeries is a boon to the patients but a disadvantage to the pathologist specifically when used for excision of premalignant and malignant lesions. These procedures, hampers diagnosis by not only producing tissue distortion via inducing carbonization, nuclear elongation and vacuolar degeneration[3], but also make the assessment of lesional margins difficult. Employment of electrocautery in parotid surgery causes acinar oncocytoid changes[4]. If laser surgeries are considered in treatment, it is better suggested to use a wider excision margin. Punch biopsy is considered better over scalpel biopsies though it produces fragmentation of tissue at the base[5]. Many other artefacts like compression/ crush created by excessive pressure[3] during surgery, or split artefacts by the use of blunt forceps, can be avoided with careful handling and with use of suitable instruments.

An accurate completely filled surgical pathology requisition form should accompany every specimen with all the necessary details viz; patient's name, age/sex, surgical details (type & site of biopsy, number of biopsied tissues), clinical and radiographic details and other additional information[6].

2. Handling of specimens

Handling of specimen is an important part and prior requite to grossing. It begins from the time of interruption of blood flow to the sample till it reaches the table for microscopy. There are many elements to be observed when handling the biopsied tissues (Table 1). They not only establish tissue diagnosis but are crucial in clinical management decisions and provide important prognostic data.

Table 1: Key Elements to Observe the Specimen (MICROPALMS).

Match specimen label with requisition form	**Parts or portion of the specimen**
Identify specimen by its label	**Appearance of the specimen and lesion**
Clinical information	**Lesion, location and extent**
Request of the clinician	**Margins of the specimen**
Orientation of the specimen	**Sampling and sections.**

2.1 Transportation and fixation of specimens

Biopsies are to be submitted to the laboratory in a fresh state immediately after resection. The amount of time that the tissue is left in contact with air should be reduced to a minimum[7]**.** They are to be transported in adequate free spaced containers, in10% buffered formalin with the volume of fixative at least twenty times that of the size of biopsy specimen, to ensure adequate fixation especially for large specimens. The search for a formalin substitute is on the way, including proprietary reagents. However, for the foreseeable future, replacement of formalin for the processing of biopsies is questionable[8]. An alternative method to the use of formalin called Tissue SAFE for short-term storage tissues has been developed by Milestone Medical (Sorisole, Italy) which uses a combination of vacuum storage (medical grade vacuum bag) and refrigeration. Tissues can be preserved up to 72 hours [9].

Fixation, besides its main purpose of preserving cellular structure, it also serves to ensure “hardening” of the specimen that is essential for manipulation during grossing and for preventing the specimen's mutilation during the cutting procedure[10].

The containers are to be stored in refrigerator at 4ºc to slow down autolysis if there is a delay in transportation of the specimens. Preservation of specimens in isotonic sodium chloride solution prevents the specimen from

drying out and allows many options for ancillary studies, but it can deteriorate tissues or alter the cellular morphology very rapidly leading to a misdiagnosis (Fig 1).

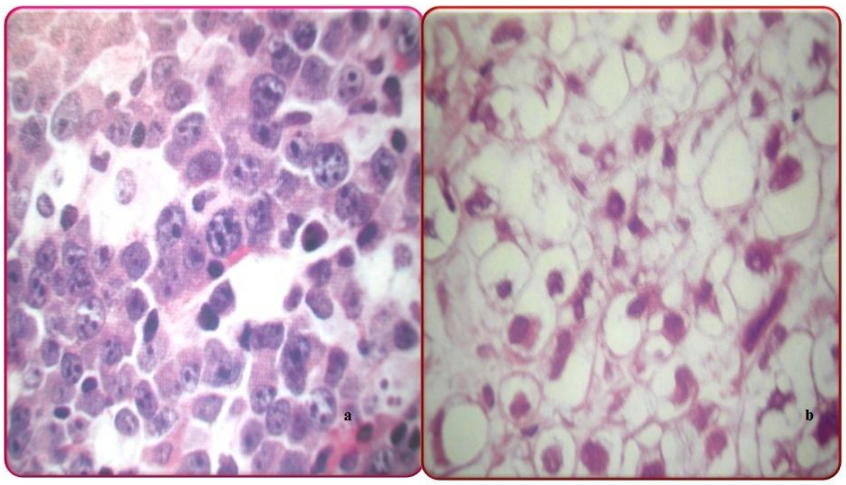

Fig 1: (a) Specimen transported in formlin - cell architecture maintained;

(b) Same specimen transported in saline: destruction of cell morphology leading to misdiagnosis.

2.2 Specimen accessioning

Accessioning is the main cornerstone in surgical pathology which connects the patient, specimen, report and the billing. Many crucial mistakes, besides specimen misidentification, occur at the initial pre-analytical and analytical phase during specimen accessioning and most of these errors occur within the grossing room[11]. Appropriate labeling is critical for successful and timely processing of biopsy material[12]. The specimens are accessioned by assigning a number that will identify each specimen for each patient (*E.g: No of the specimen – month – yr*). This main number should be placed on the requisition form as well as on the bottle. If multiple specimens are obtained from the same patient and the same surgery/procedure, all specimens from that patient are given the same number followed by a numerical or alphabetical designation [13] *e.g 1A, 1B; 2A, 2B/ 1.1, 1.2 etc.* With modern advancements in technology, bar coding and radio frequency chip technology is slowly taking over.

3. General principles of gross examination:

The dissection, gross description and selection of sections for microscopic study is a crucial part of the pathologic examination and cannot be remedied if omitted or done poorly at the time of the initial work up. If

the microscopic description is inadequate, the slide can be reviewed and the microscopic diagnosis corrected; if the dimensions of the specimen are not recorded, the key sections not taken, and the proper special studies not performed at the time of the initial gross examination, the chances of acquiring this information may be lost forever. Large specimens must be cut open for examination as these surfaces provides diagnostic clues (Fig 2a, 2b). In cases like pleomorphic adenoma, the cut section has varied morphology. The cut surface is homogeneous and white or tan**.** It may have a glistening appearance/ soft/ gritty consistency suggestive of myxoid, cartilaginous or myxochondroid areas. Sectioned surfaces of ameloblastoma may show cystic areas sometime filled with keratinous material (fig 2 c). Cystic specimens are to be cut open through the lumen to know its contents which occasionally offers to provide an indication to diagnosis (Fig 2d).

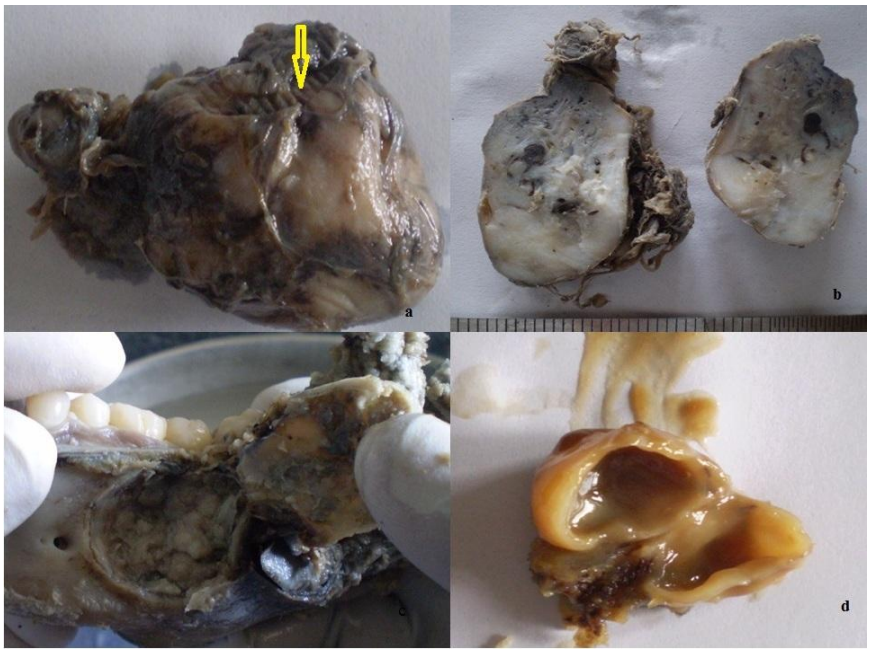

Fig 2: a,b: Cut surface of specimen showing whitish areas depicting fibrous tissue.
(2a: arrow: crush artefact cause by tissue holding forceps).
Fig 2 c: Cut surface of ameloblastoma - cystic areas filled with keratinous material.

3.1 Gross description

Proper identification and orientation of the specimen is always important and may be imperative for the adequate pathologic evaluation of a case. An accurate completed surgical pathology requisition form with the patient's identification, age, sex, essential clinical data, operation, surgical findings and tissue submitted should accompany every specimen. If such history is unavailable, the physician/surgeon should be contacted and the

pathologist has the prerogative and obligation, as a medical consultant, to review the chart, even examine the patient personally before rendering an opinion on a slide in cases where such information is essential.

For the general inspection of the specimen, the specimen should be placed on the cutting board, in an anatomic position, and the following information is recorded: type of specimen, structures included with the specimen, dimensions, weight, shape, and color. One must document the number of fragments; the type of biopsy, e.g., needle or core biopsies; and whether the specimens represent tissue or foreign material. The use nonspecific terms such as ‘‘multiple’’ or ‘‘numerous’’ for the number of fragments should be avoided. If only a few fragments are present, the dimensions of each can be specified; this is helpful at sign-out to ensure that all tissue has been examined microscopically[13]. In many surgical excisions, where the microscopic diagnosis of the lesion is already known; for a clearance report, the extent of the lesion, invasion of neighboring structures, presence of tumor at the surgical margins, vascular invasion, lymph node metastases if requested is recorded for the treatment and follow-up purpose.

a. *Specimen photography*

Photographs of the specimens should be taken prior to sampling. For most specimens the external appearance is merely that of a non-descript mass, whereas the cross section will demonstrate the important gross features of the lesion. Photographing of cut surface provides more information that the external surface of the intact tumor (providing some information on overall size and configuration) (Fig 2a, b, c, d). Rulers should be used only when reference to size is indicated. (Fig 3a) The specimen should be properly oriented, centered and framed. As indefinite storage of gross specimens is inconvenient, the gross photograph often remains, together with the gross description which is the best permanent documentation of the gross features of a lesion.

3.3 Specimen roentgenography

Roentgenographic examination of surgical specimens sometimes provides vital information. Specimens particularly suitable for this type of examination include bone lesions and calcified soft tissue masses. Radiopaque foreign bodies (metal clips) can be spotted and these have to be removed from the specimen as they can damage the microtome knives during microtomy. Radiological and pathologic correlations can be made by

injecting radiopaque material within the lumina of ducts or vessels while radiographing the specimen. To some extent the diagnostic clue is obtained that adds on to the final definitive diagnosis. (Fig 3a, b, c).

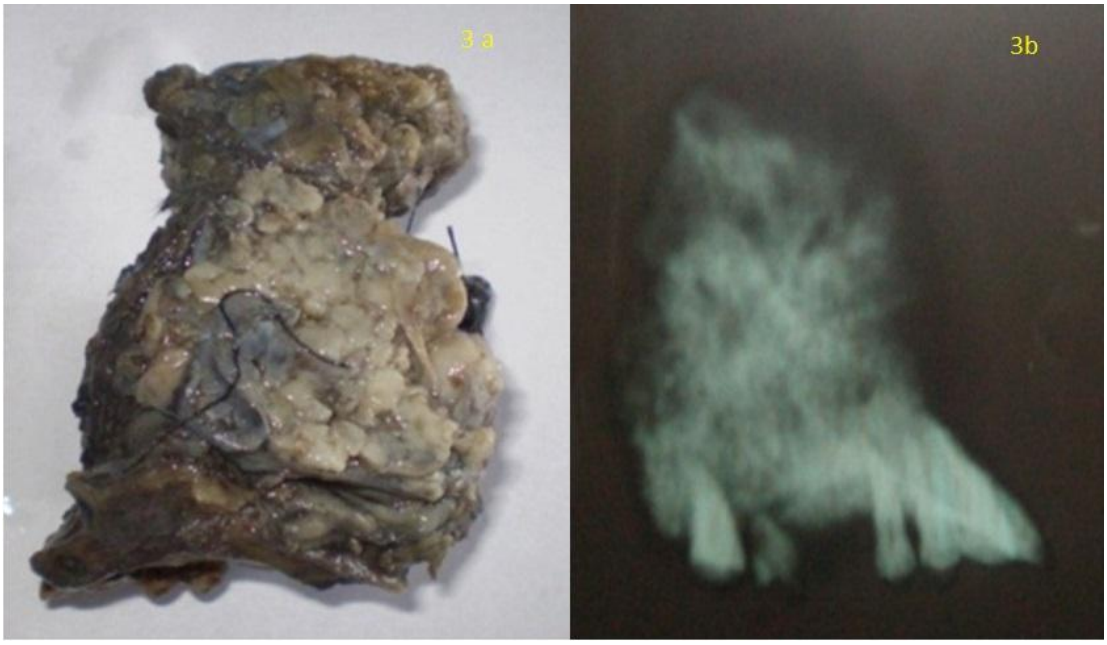

Fig 3a, b: Specimen and its radiograph. The radiograph gives a clue to diagnosis (sunburst appearance).

4. Sampling / grossing for histologic examination

Tissues submitted for histology must not be more than few mm thick and not larger than the dimensions of the cassette used, otherwise paraffin infiltration into the specimen will be inadequate. Small fragments of tissue must be wrapped in thin/tissue/filter paper or else placed between small porous cushions of the cassette. The nature of the case, appearance of the gross specimen and sagacity should dictate the amount of required tissue. Knowledge of the precise site from which sections were taken for microscopic examination is of great significance, especially when determining the presence of tumor cells in the surgical margins.

Every biopsy grossing description in the pathology report must include the statement "entirely submitted." This is the fundamental rule of grossing small biopsies. The completeness of submission depends in great extent on the grossing person, especially if the specimen contains numerous fragments or uncountable material[10].

The specimens are to be placed in its proper anatomic orientation prior to grossing. Larger specimens are best oriented by the surgeons by placement of a suture at the top[6]. Only one suture is necessary as all the other margins are easy to deduce once the superior/medial (north/east) margin is indicated. The margins can also be

labeled with indelible ink with respect to the orientation. This is useful if a tumor comes close to, but does not involve a margin.

Vertical/perpendicular, horizontal/parallel, and oblique (Mohs method) are three major grossing methods that employed used for evaluating tumor margins. Vertical sections can be made transversely (bread loaf method) or longitudinally (bread loaf cross method) through the tumor or peripheral/perimeter sections taken from the edges of the excised specimen[14]. Transverse bread-loaf sectioning will state clearly the relationship of tumor to the margins. For large resection specimens serial sectioning becomes impractical. Adoption of a combination of bread-loaf and peripheral sectioning not only shows the relationship of the tumor to the margin but also gives a 100% clearance of the margins.

Bread-loaf sectioning is commonly used in many laboratories for small specimens[6]. Excisional biopsies 10mm or less is sectioned transversely in a 'sliced bread/bread-loaf' pattern. The first section is obtained from the centre of the lesion followed by sectioning the whole specimen every 2 - 3 mm (*Fig. 4a, b*). The most important sections are the central ones as the margins are likely to be involved with the tumor centrally.

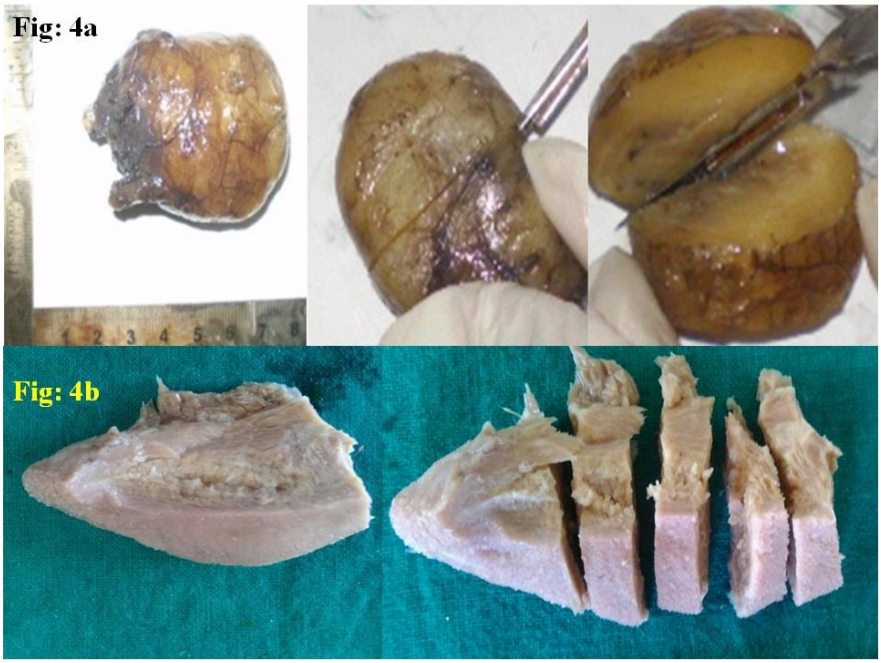

Fig 4a, b: Sliced bread pattern of sampling with the center of the lesion followed by sectioning the whole specimen every 2 - 3 mm.

Small core biopsies (2mm) are not to be bisected. A uniform thickness of the section is an obvious requirement for processing, but it is difficult to achieve under conditions of insufficient fixation or inadequate hardening of the specimen[15].

4.1 Complex oral resection specimens/ radical neck dissection specimens

A wide spread sampling is required for such specimen to grade the oral carcinoma, to detect perineural, metastatic, transcapsular & vascular spread and to define areas where the tumour extends to a few mm of margin. Sampling begins with the core of the lesion. Two/ three samples are taken. Assessment of all surgical margins, both the mucosal and deep, is required. Slices of about 4 mm covering ideally all margins of the specimen are made, and the most 'patient-adjacent' section is first examined (*Fig. 5a, b*)[16]. Radially oriented blocks at right angles to the resection margin is more appropriate to show the anatomical relationships of tumour, margin & underlying tissue more accurately. If tumour extends to within a few mm of the margin a series of parallel contiguous blocks should be taken at its closest point. The deep margin is investigated in one or more central sections of the tumour, depending on the macroscopic closest distance between the tumour and the margin, and is reported separately[16]. At least one representative section should be taken from normal structural component of the specimen[12].

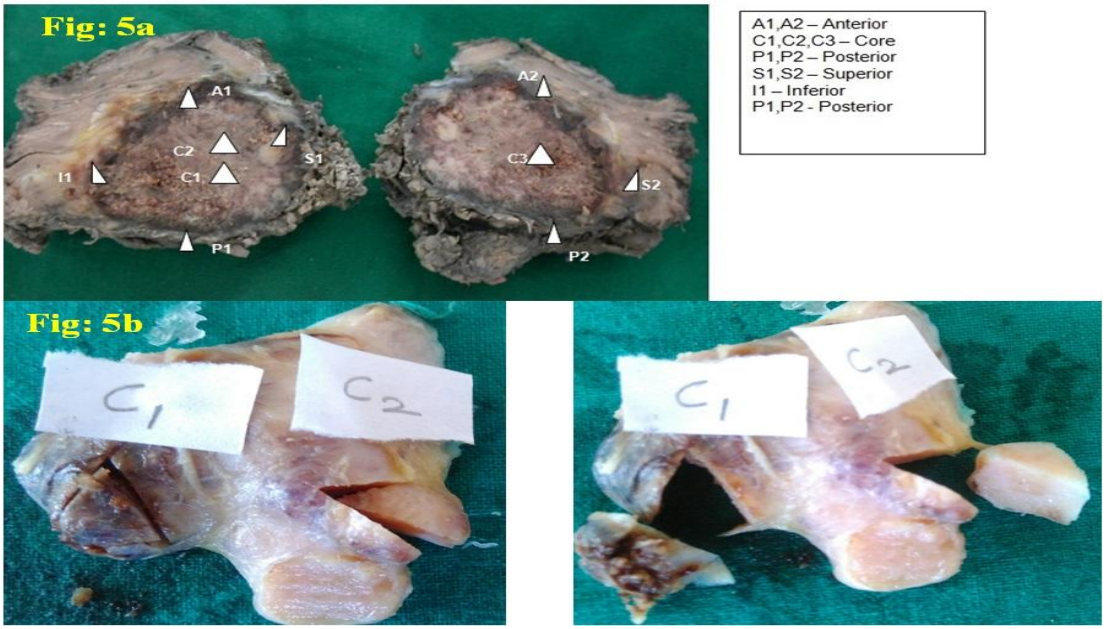

Fig 5: Sampling from the core of the lesion, followed by margins. A wedge shaped section is obtained from specimen.

When the margins appear free of tumor, one must not develop a false sense of security as no method is perfect and persistence of tumor or recurrence of tumor still can occur. Clinicians must be able to differentiate the terminologies when the tumors are to be reported as “near”, “close to”, “at”, “approximately” or “in” the margin [17]. If the tumor does not appear to be in margin, one must rule out the possibility of misorientation, fragmentation, incomplete sectioning of the tissue, excessive facing of block containing peripheral sections or sectioning of wrong side of peripheral section[18].

4.11 Lymph node sampling in resection specimens

The sentinel node is the first lymph node draining lymphatic fluid from the tumor; therefore, if tumor cells are metastasizing through the lymphatics, the sentinel node is usually the first lymph node involved **[13].** Lymph nodes are not advised to be removed from the primary site of tumour and if the extent of tumour at primary site is not clear initially itself as this prevents removal of further blocks from well defined sites. They are to be sectioned & identified by using the Memorial – Sloane-Kettering classification. Positive nodes are be sampled close to excision margins & in continuity with the margin. Lymph nodes are bisected perpendicular to long axis (*Fig. 6*) and for each anatomic group the number of nodes, the size of the largest node, and any gross features are to be described. Lymph nodes larger than 5mm should be serial sectioned at 2-3mm intervals[12]. 25 -60 nodes are usually identifiable in full radical neck dissection. Lymph nodes with gritty white (presence of keratin) areas suggest infiltrate or metastasis.

Fig. 6: Perpendicularly bisected lymph nodes, showing tumour infiltration.

4.12 Bone sampling in resection specimens

Bony excision margins are left till soft tissue blocks have been reported. Bone provides support in correct anatomic position for the partly dissected tissues. The most important determinant of bony invasion is the tumour size.

Longitudinal sections are to be avoided and transverse sections are more optimal[19]. If invasion is suspected, then entire bone resection margin is sampled with the blocks sawed parallel to the margin and decalcified in 30-40% formic acid. In case where bony structures are not involved, the remaining soft tissue is carefully dissected out from the bone, as a single specimen.

5. Orientation and embedding of sections

The ultimate goal of grossing is a correct diagnostically sound section with proper orientation. Accurate orientated embedding is the last step where grossing can directly influence specimen processing. All the sampled specimens should be embedded with cut surfaces facing downwards. Very small specimens should not be bisected; instead, the whole specimen should be embedded on edge[13]. The orientation of cyst linings (*Fig. 7a,b*) stands out as an exception when compared to that of the tumor or any other samplings. They are to be oriented in radial manner.

Fig 7a, b: **Radial orientation for cyst lining (Radicular cyst).**

A variety of techniques has been used for embedding orientation of biopsies. One technique involves sticking a specimen to slabs of Gelfoam with cyanoacrylate[20]. Agar-agar has been used for orientation of biopsies and small specimens[21, 22]. Sectional cassettes with fluoropolymer platforms[23] or silicone pads for automatic embedding orientation are still at the proposal and testing stage.

6. Conclusion

The Pathologic diagnosis should relate with the history and the post-operative diagnosis provided by the clinician, and the gross description by the pathologist. Selection of site for sampling is of uttermost importance for a proper diagnosis.

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