Stereomicroscope as an aid in grossing and histopathological diagnosis: A prospective study

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INTRODUCTION
Histopathological examination of tissues is considered as a gold standard for the diagnosis of lesions.\textsuperscript{[1]} Macroscopic examination (grossing) of the tissue specimen plays an important role in arriving at an accurate histopathological diagnosis.\textsuperscript{[2]} Grossing is considered as an essential, but often mostly neglected step in histopathology. Pathologists rarely receive specimens with sufficient tissue and related clinical and radiological data.\textsuperscript{[3]} However, it is a challenging expertise for the pathologist to have an intense observation, experience and association between macroscopy and microscopy. It forms a bridge not only between the pathologist and the patient but also involves a close coordination and harmony between the surgeon, pathologist and the histotechnologist.\textsuperscript{[4,5]}

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For microscopic evaluation, correct identification and orientation of the specimen are most important. An accurate grossing procedure helps in reducing the errors and also provides valuable information regarding the specimen for achieving a definitive diagnosis. However, the significance of this step is often overlooked and neglected. Any mistake in this vital step may lead to an erroneous diagnosis as erroneous orientation of specimens may either cause delay in diagnosis or create a diagnostic dilemma.\(^6\)

For the difficulties come across at the time of specimen orientation, imaging might be useful which includes digital photography, radiographic examination and stereomicroscopy. Stereomicroscopic observation of specimen may help pathologists to give some critical clues about the nature of the specimen and the type of proliferation (mucosal or submucosal, papillary, presence of capsule or epithelium) seen in the specimen which might help in correct orientation of the specimen and also in diagnosis.\(^6\) Hence, we conducted this study to evaluate the role of stereomicroscope in routine grossing and its contribution to arrive at a definitive diagnosis.

**MATERIALS AND METHODS**

The present prospective study was conducted on 65 samples of the patients sent to the Department of Oral Pathology, Government Dental College and Hospital, Hyderabad. All the grossing specimens were observed between July 15, 2018, and September 15, 2018, after obtaining the institutional clearance (ethical clearance number: ECR/66/Inst/AP/2014/RR-17).

**Inclusion criteria**
1. Soft tissue specimens that include solid tumors, cystic lesions and surface lesions
2. Both incisional and excisional biopsies
3. Completely fixed tissues in 10% neutral buffered formalin (NBF)
4. Tissues with minimal dimensions
5. Fragmented tissues and tissues with forceps artifacts.

**Exclusion criteria**
1. Hard tissue specimens
2. Tissues with incomplete fixation.

**Methodology**
Specimens received were properly labeled and fixed in 10% NBF for 24 h. The tissue was cleared from blood clots. All the specimens were observed under naked eye and stereomicroscope (Lawrence and Mayo, India Pvt. Ltd.) [Figure 1].

The principle of stereomicroscope is that it can generate 3D images using two independent optical channels. This zoom lens which measures the modulated beams form an angle between 12°–15° and the magnification is imaged by eyepieces. Two beams of light in the stereomicroscope are angulated but not parallel. It utilizes light that is naturally reflected from the object, allowing it to deal with thick or opaque samples. A small number of models can use transmitted light, where the bulb or mirror will reside beneath the object itself [Figure 2].\(^7\) We conducted our study based on the criteria given in the Table 1.\(^3\)

The received tissues were grossed under naked eye [Figure 3] in daylight and were also observed under a stereomicroscope where we could appreciate the surface details of the tissue. Based on the stereomicroscopic evidence, they were embedded and sections were stained with hematoxylin and eosin stain and observed under a compound microscope. Among the total 50 samples, 17 were solid tumors, 12 were cystic lesions and 21 were surface lesions.

**RESULTS**

Total numbers of cases in our study were 65 and out of which, 50 cases met our criteria. They were categorized into solid tumors, cystic and surface lesions [Table 2].

Solid tumors included in our study were fibroma, pyogenic granuloma, peripheral ossifying fibroma and drug-induced

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**Table 1: Criteria for grossing\(^2\)**

| Color       | Interpretation                        |
|-------------|---------------------------------------|
| White       | Calcification/bone                    |
| Gray white  | Fibrosis                              |
| Yellow      | Fat, necrosis                         |
| Brown/black | Hemosiderin, melanin                  |
| Red         | Hemorrhage, blood vessel congestion   |

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**Figure 1: Stereomicroscope**
Shobhita, et al.: Stereomicroscope as an aid in grossing

Gingivitis. Cystic lesions were odontogenic keratocyst, dentigerous cyst, orthokeratinized odontogenic cyst, radicular cyst, mucocele and lymphangioma, while surface lesions were epithelial dysplasias and oral squamous cell carcinomas. Details of few cases that were considered in our study are as follows.

Case 1
A 35-year-old female patient came with the chief complaint of swelling on the right cheek for 3 years. Clinically, it was a solitary swelling, whitish pink in color, roughly oval in shape with smooth surface. On palpation, it was soft in consistency and nontender. An excisional biopsy was carried out and we received a single grayish yellow soft tissue bit. Under naked eye, it was a solid tissue, which could not be described. On stereomicroscopic examination, outer layer and central fibrous component were easily distinguished. On histopathology, atrophic epithelium with underlying densely collagenized stroma was appreciated and diagnosed as fibroma [Figure 3].

Case 2
A 20-year-old male patient had a complaint of swelling on the right cheek for 3 years. Clinically, it was a solitary swelling, whitish pink in color, roughly oval in shape with smooth surface. On palpation, it was soft in consistency and nontender. An excisional biopsy was carried out and we received a single grayish yellow soft tissue bit. Under naked eye, it was a solid tissue, which could not be described. On stereomicroscopic examination, outer layer and central fibrous component were easily distinguished. On histopathology, atrophic epithelium with underlying densely collagenized stroma was appreciated and diagnosed as fibroma [Figure 3].

Table 2: All the cases observed in our study with their naked eye and stereomicroscope findings

| Type of lesion | Number of samples | Naked eye findings | Stereomicroscopic findings |
|---------------|------------------|--------------------|---------------------------|
| Solid tumors  | 17               | A well-circumscribed specimen | A thin lining surrounding the central fibrous component |
| Cystic lesions| 12               | A lumen with cystic wall | A lumen with surrounding cystic capsule |
| Surface lesions| 21              | Lack of differentiation between epithelium and connective tissue in most of the cases | Outer epithelium and underlying connective tissue could be differentiated |

it showed fibrocellular stroma with a well-defined 6–8 layered cystic epithelium, which was parakeratinized showing surface corrugations and palisaded basal cells with hyperchromatic nuclei. A diagnosis of odontogenic keratocyst was made [Figure 4].

Case 3
A 31-year-old male patient came with a chief complaint of swelling on the lower lip for 15 days. Intraoral examination revealed a well-defined swelling, which was translucent, nontender and compressible on palpation. On excisional biopsy, we received multiple grayish brown soft tissue bits. Under naked eye, capsule with lumen filled with mucous material was observed. Stereomicroscopic examination showed distinct brownish white outer surface and lumen filled with mucoid-type material. Microscopically, it showed fibrous capsule with mucinophages and few mucin pooled areas, along with salivary gland tissue at some areas and was diagnosed as extravasation mucocele [Figure 5].

Case 4
A 16-year-old male patient had a complaint of swelling on the lower lip for 1 month. Intraorally, it was a solitary swelling, whitish pink in color, roughly oval in shape with smooth surface. On palpation, it was soft in consistency and nontender. An excisional biopsy was carried out and we received a single grayish yellow soft tissue bit. Under naked eye, sac-like structure filled with mucoid-like material was noticed. On histopathological examination, a hyperkeratotic epithelium with flat to irregular rete ridges and large irregular and dilated lymphatic channels lined by endothelial cells were seen subepithelially, which was diagnosed as Cavernous Lymphangioma [Figure 6].

Case 5
A 60-year-old male patient reported with chief complaint of pain in the lower front tooth region for 1 month. Intraorally, an ulceroproliferative growth was noticed extending from the edentulous region of 41–46. On incisional biopsy, we received a single grayish brown soft tissue bit. Unlike naked eye, the stereomicroscopic examination showed a distinct white surface with whitish brown stroma. Microscopically, highly cellular connective tissue stroma with atypical epithelial cells arranged in the form of islands with dysplastic features and keratin pearl formation were seen. Overlying
epithelium was hyperkeratotic with dysplastic features. This was diagnosed as well-differentiated oral squamous cell carcinoma [Figure 7].

**Case 6**

A 33-year-old male patient reported with chief complaint of burning sensation on left cheek for 2 months. A white patch was noticed extending from corner of the mouth to area opposite to 36 intraorally. On incisional biopsy, we received a single creamish brown soft tissue bit, which when observed under naked eye showed creamish brown tissue. On stereomicroscopic examination, it showed outer whitish layer with central creamish brown stroma. Microscopic examination revealed an orthokeratotic
stratified squamous epithelium showing dysplastic features extending up to middle one-third with normal underlying fibrocellular connective tissue and was diagnosed as moderate dysplasia.

**Case 7**
A 35-year-old female patient reported with growth in the upper left back tooth region for 10 years. On intraoral examination, a solitary sessile growth and slightly reddish in color with smooth surface was evident. On palpation, it was nontender and firm in consistency. On excision, we received a single creamish white soft tissue bit which was indescribable under naked eye. On stereomicroscopic examination, an outer creamish brown layer with central creamish white stroma with white opaque specks was observed. Microscopic examination revealed a parakeratinized stratified squamous epithelium with underlying fibrocellular connective tissue containing ossifications and was diagnosed as peripheral ossifying fibroma.

**Case 8**
A 50-year-old male patient reported with chief complaint of pain and burning sensation on lower right back cheek region for 2 months. A whitish patch was observed on buccal mucosa bilaterally which was nonscrapable and tender. On incisional biopsy, we received a single creamish brown soft tissue bit, which under naked eye showed outer whitish layer and brownish stroma. These two layers were differentiated more clearly on stereomicroscopic examination. On histopathological examination, an orthokeratinized stratified squamous epithelium with dysplastic features in lower one-third was observed with underlying normal fibrocellular connective tissue and was diagnosed as mild dysplasia [Figure 8].

**DISCUSSION**
There is a need to establish standard criteria to record the macroscopic features of specimens during grossing in order to properly cut the specimen and orient them appropriately. Stereomicroscope is an optical multipurpose instrument invented to observe tissues at low magnification. It is also called as dissecting microscope. Stereomicroscopes have two major types of magnification systems. The first one is fixed magnification system where the primary magnification is attained by a set of paired objective lenses with a set degree of magnification. The second one is panptic or zoom magnification system, which enabled a variable degree of magnification across a set range. Zoom magnification systems can accomplish additional magnification by usage of auxiliary objectives which augment total magnification by a set factor. The total magnification in both the systems can be increased or decreased by changing the eyepieces.

Apart from observing specimen in histopathological lab for clarity, stereomicroscope is also used to carry out procedures such as microsurgery, dissection and inspection of fracture surfaces. It is also used in the field of endodontics for measuring the microleakage and the depth of filling material after root canal treatment. Stereomicroscope has also been used in the fields of prosthotodontics and also forensic dentistry identification process by macroscopic examination of teeth. However, the primary use in our case is to...
observe tissue specimens in a three-dimensional view and can be used for laboratory purposes on daily basis.

The term “Grossing” means examining and dissecting surgical specimens received in the laboratory and is the first step in arriving at confirmative diagnosis. Grossing is the first and important step in the surgical pathology.[10] The ultimate purpose of grossing is obtaining accurate and diagnostically appropriate sections with correct orientation.[4]

Our findings are similar to the study by Shah et al. They carried a retrospective study on 5 cases to evaluate the efficacy of stereomicroscope in routine grossing and its role in arriving at a final diagnosis. They found that stereomicroscopic images aided in recording obscure details of specimen, correct orientation of the specimen and in the final diagnosis.[6] Our’s is the second study which evaluated gross specimens using stereomicroscope. In contrast to their study, ours is a prospective study on a larger sample of 50 cases. We found that stereomicroscope is especially useful in surface lesions as they need accurate orientation for final diagnosis. In our 21 surface cases, we could accurately diagnose the lesions as mild, moderate or severe dysplasia and frank squamous cell carcinoma of different grades. In addition, we also compared the features of each and every sample with naked eye and under stereomicroscope.

In our study, we followed the guidelines given by Rao and Premalatha in the review “Grossing in oral pathology.”[14] One of the most important steps in arriving at an accurate histopathological diagnosis is macroscopic examination, which is highly ignored in most of the routine laboratories due to inadequate knowledge. Improper grossing technique will result in wrong diagnosis, which may be fatal in many cases.[14] It is always advisable to use recent technologies such as stereomicroscope as an aid in grossing in order to arrive at an accurate diagnosis.

Stereomicroscope helps us to clearly visualize, orient and record the macroscopic features of the specimens which are considered as the fundamental requirements of gross examination.[4,6] We also observed similar findings in our studied cases. As in case one, stereomicroscopic examination of the specimen revealed an atrophic outer layer and a central fibrous component, which was not evident with the naked eye. The stereomicroscopic findings correlated with the histopathological findings of atrophic surface epithelium and underlying proliferating fibrous component, leading to a diagnosis of fibroma. In case 2, stereomicroscopic examination revealed a cystic lining and a distinct brownish white capsule, which correlated with histopathological findings of fibrocellular stroma with a well-defined 6–8-layered parakeratinized cystic epithelium with surface corrugations and palisaded basal cells with hyperchromatic nuclei, leading to a diagnosis of odontogenic keratocyst. In this case, we could also make a distinction between the normal and abnormal tissue by means of stereomicroscope mainly in thin linings of odontogenic cysts.

In case 5, stereomicroscopic examination showed a distinct white surface with whitish brown stroma. We could properly orient the tissue and microscopically, dysplastic features and keratin pearls could be appreciated prompting to a diagnosis of well-differentiated oral squamous cell carcinoma. All our cases showed that macroscopic examination of the specimen under stereomicroscope revealed a diagnostically valuable information, which helps in arriving at an accurate final diagnosis.

After thorough search of literature, to best of our knowledge, ours is first prospective study carried out on 50 samples. In our study, we could properly identify the nature of lesions and arrive at a proper diagnosis with the help of stereomicroscope. Hence, we recommend usage of stereomicroscope routinely in histopathological laboratories as an aid in grossing of surgical specimens.
CONCLUSION

Correct grossing and proper orientation plays a vital role in definitive diagnosis. Stereomicroscope can be used as an adjunct to the naked eye grossing for both incisional and excisional biopsies.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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