

ORIGINAL RESEARCH

Surgical artefacts in oral biopsy specimens: Punch biopsy compared to conventional scalpel biopsy

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ABSTRACT

The presence of the handling artefacts in the oral biopsy specimens prevents accurate histopathological diagnosis of oral lesions. The aim of this study is to compare these artefacts ascribed to the technique of punch biopsy with those occurring in conventional scalpel / wedge biopsy and to suggest some techniques for minimizing the same. Fifty oral biopsies were performed, 25 each with a disposable biopsy punch and conventional incision with a scalpel. Significantly fewer artefacts were found in the punch biopsy group compared with the incisional biopsy group.

Key words: Biopsy, punch biopsy, scalpel / wedge biopsy, surgical artefacts

INTRODUCTION

There are many ways that the exact interpretation of tissue specimens by the pathologist can be compromised. The potential pitfall in the histological interpretation being the presence of artefacts in the microscopic sections. Artefact refers to "an artificial structure or tissue alteration on a prepared microscopic slide - the result of an extraneous factor".^[1,2] These artefacts result in alteration of normal morphologic and cytologic features or even lead to the complete uselessness of the tissue.^[3] Artefacts occur at several stages: during surgical removal, fixation, processing, embedding or staining.^[1]

The purpose of this study is to compare the number and type of handling artefacts, produced by the mucosal punch biopsy and the conventional incisional biopsy techniques at the time of surgical removal of the tissue. Some techniques for alleviating these problems have also been suggested.

MATERIALS AND METHODS

Fifty patients took part in the study and these patients were entered randomly into the punch biopsy or incisional biopsy. There were 25 patients each in the punch biopsy and the incisional biopsy groups. All biopsy procedures were carried out on the representative areas of the lesion, under infiltration of local anesthesia (1 ml of 1: 80,000 lignocaine and adrenaline). A disposable punch of 4 mm diameter was introduced into the oral cavity and rotated through the mucosa. The specimen was grasped with a blunt forceps and the base was released with a scissors. The incisional scalpel biopsies were carried out using a standard technique: a suture was placed within the mucosa that is to be removed. The ends of the suture were gently pulled

using an artery forceps and an elliptical incision enclosing the area of interest was carried out.

In both the cases, a single suture was placed after the biopsy. The biopsied tissues thus obtained in either technique were immediately introduced into a wide-mouthed container and fixed in a copious amount of 10% formalin solution for 24h.

All the specimens were processed and embedded in paraffin wax under the same protocol.^[4] They were later sectioned routinely at 5 µm and stained with hematoxylin and eosin. Six serial sections were prepared and all the sections were considered for the study. Further, an experienced pathologist observed all the six slides for artefacts under different magnifications (5x, 10x, 20x) and scored them according to the histological proforma provided. The mode value of the individual parameter after the scoring was considered for statistical analysis. After tabulation, the results were statistically analyzed using the Chi-square test.

RESULTS

The range of histological diagnoses for punch and incisional biopsies is listed in Table 1. The maximum mean depth and width of the biopsies is tabulated in Table 2. The incisional biopsy had, as expected, a greater depth and width range as compared to the punch biopsy group. The artefacts identified on histopathological examination are listed in Table 3. Statistical analysis was done using Chi-square test. The number of artefacts in the punch biopsy group was found to be significantly less than in incisional biopsy group (Chi-square test $P < 0.05$) with respect to the parameters: crush, splits and fragmentation.

Table 1: Histological diagnoses

	Punch (N=25)	Incisional (N=25)
Lichen planus	3	5
Leukoplakia	3	4
Oral submucous fibrosis	15	4
Squamous cell carcinoma	2	8
Lichenoid reaction	1	0
Non-diagnostic cases	1	4

Table 2: Dimensions of the biopsy specimens

	Punch (N=25)	Incisional (N=25)
Depth (mm)		
Range	3-6	3-13
Mean	5.4	7.0
Width (mm)		
Range	3-4	2-8
Mean	3.72	6.2

DISCUSSION

Previous studies have indicated that the oral mucosal punch biopsy is a safe and rapid method of obtaining tissue from the mouth, but have not sufficiently indicated the incidence of artefacts. The surgical removal in our study was found to be superior in punch biopsy group (96%) when compared to the incisional biopsy group (84%). This can be explained by the fact that the diameter of the punch is fixed at 4 mm whereas the width of the tissue removed by the scalpel biopsy is variable. Instead of a narrow and deep wedge of tissue, a broad and shallow wedge of tissue can be biopsied by the scalpel.^[5] Thus the interrelation between the epithelium and the connective tissue can be compromised. Further, our study showed that the oral punch biopsy provides specimens which were small but of an adequate size for diagnostic purposes. Due to improper surgical removal, one case and four cases each in punch and incisional biopsy groups respectively were non-diagnostic in this study [Figure 1]. Improper surgical removal can be avoided if: biopsy specimens have adequate depth and bulk; and if biopsy is performed on an appropriate representative area.^[1,6,7]

Four percent of the cases in the punch biopsy group and 12% of cases in incisional biopsy group showed curling artefact [Figure 2]. We found that the biopsy specimens which were thin or a narrow strip of mucosa exhibited curling on fixation. Also, in the study, difficulty in orientation of the tissue was noted as a problem whenever curling was seen. Due to this difficulty, tangential sectioning of the specimen was seen. Curling can be prevented if, after the biopsy, the tissue is placed with the mucosal surface up (epithelial surface down) on a piece of sterile paper (usually that which held the suture

Table 3: Number and location of different artefacts found on histological examination

Type of artefact	Punch (N=25)	Incisional (N=25)
Improper surgical removal		
None	24	21
Present	1	4
Curling		
Absent	24	22
Present	1	3
Orientation		
Good	23	23
Poor	2	2
Crush		
None	18	8
Base	1	4
Specimen	4	5
Combined	2	8
Hemorrhage		
None	14	14
Edge	1	2
Specimen	6	4
Combined	4	5
Splits		
None	15	5
Edge	8	9
Superficial	1	6
Combined	1	5
Fragmentation		
None	12	8
Deep	12	5
Superficial	0	2
Combined	1	10

material). This specimen can be allowed to remain unfixed for a short time while the incision is being sutured.^[2] Since curling is seen in thin biopsy specimens, adequate depth of the biopsy specimen can help in preventing this artifact.^[1] Though strictly speaking both improper surgical removal and curling do not directly depend on the type of biopsy technique used, we included these parameters as a part of our study because they occur during / immediately after the biopsy procedure and can be considered as surgical / handling artefacts.

Orientation [Figure 3] was not encountered as a problem during the diagnosis of the cases as we expected prior to the study. It was assumed that when the wedge-shaped specimen has similar dimensions for each major surface (top and the two sides), problem could arise in orienting the specimen. Since the tissue obtained by punch biopsy is approximately cylindrical in shape, it serves for effortless orientation, as the long axis of the tissue (which is easily recognized) need to be placed parallel to the blade. In our case, as the suture was attached to the epithelial surface of the incisional biopsy specimens, the problem in orienting the specimen was not encountered. Further, reliance was not placed on marking the mucosa with any colored solutions as such an application can interfere with tissue processing and staining procedures.^[1,2]

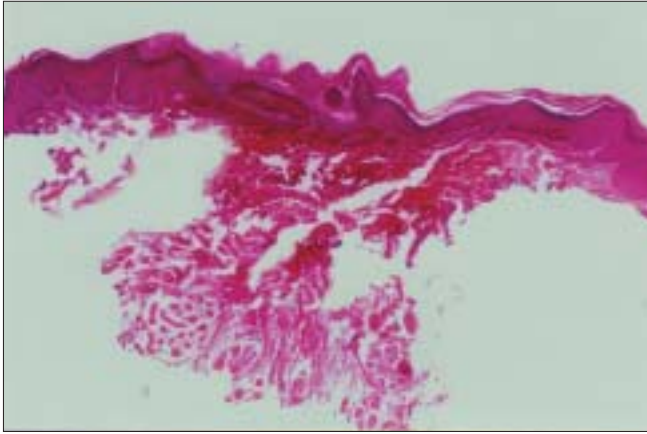


Figure 1: Photomicrograph showing improper surgical removal (H and E, 5x)

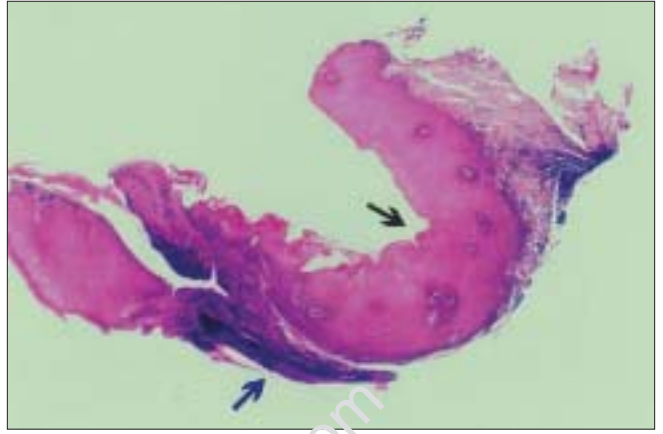


Figure 2: Photomicrograph showing curling (black arrow) and crush artefact (blue arrow) (H and E, 5x)

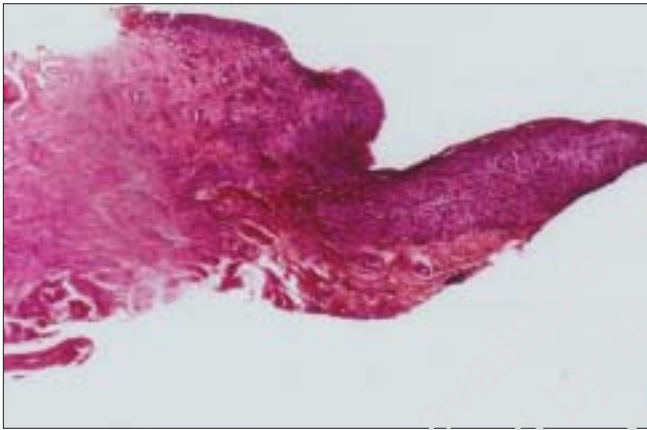


Figure 3: Photomicrograph showing crenation artefact (H and E, 5x)

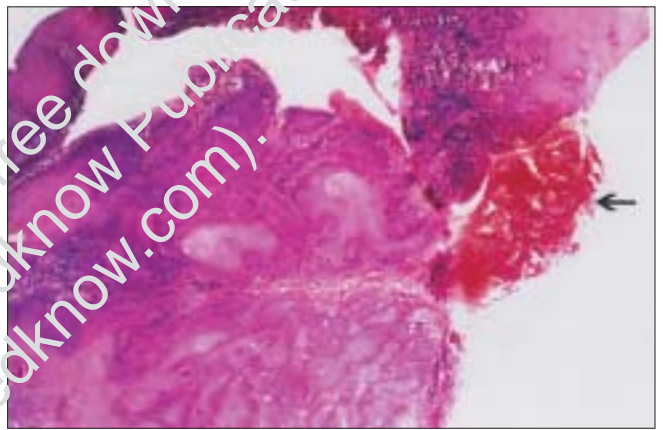


Figure 4: Photomicrograph showing hemorrhage artefact (arrow) (H and E, 5x)

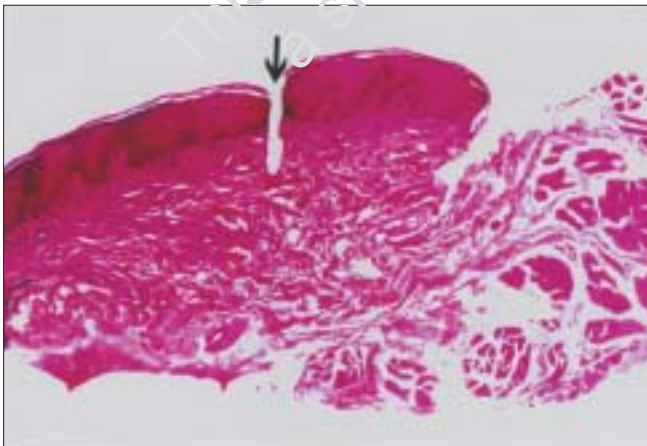


Figure 5: Photomicrograph showing splits artefact (arrow) (H and E, 5x)

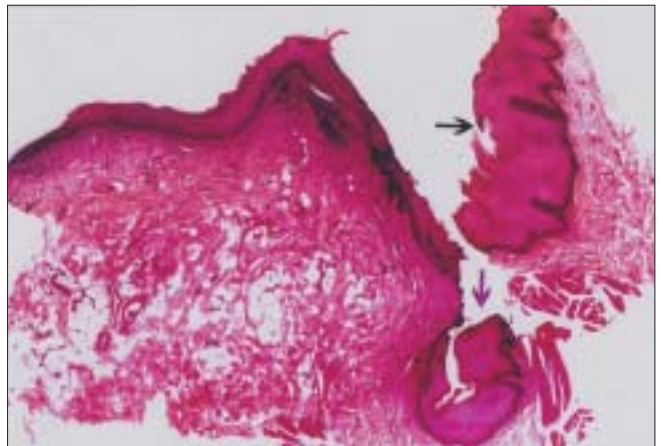


Figure 6: Photomicrograph showing fragmentation artefact (black arrow) and curling artefact (pink arrow) (H and E, 5x)

The crush artefact [Figure 2] was seen to be significantly less in punch biopsy group as compared to incisional biopsy group (Chi-square test $P < 0.05$). Previous studies have shown that punch biopsy specimens have less number of crush artefacts as compared with the incisional biopsy specimens; although they have indicated that the crushing of the base of the tissue is more in punch biopsy when compared to the scalpel / wedge biopsy.^[8] However, we found that punch biopsy specimens showed fewer number of crush artefacts in the base as well as in the specimen proper as compared to the incisional biopsy specimens. This could be attributed to the fact that we used blunt forceps instead of toothed forceps during punch biopsy and the tissue was handled only at the base of the specimen, where usually fat or muscle is present. Previous studies also indicate that the suture traction with the subsequent use of scalpel produced crushing of the biopsy specimen.^[9]

Since intralesional injection was avoided, care was taken to deposit the solution away from the biopsy site and excessive pressure on the tissues was not applied during the biopsy procedure, there was no difference between the two groups in terms of hemorrhage artefact [Figure 4]. On the other hand, significant difference between the two groups in terms of split artefact (Chi-square test $P < 0.05$) was noted. The use of scalpel, which can result in multiple cuts into the tissue could be a factor which leads to the splitting of the tissue both in the superficial and in the edge of the tissue [Figure 5]. Also, previous studies have shown that the use of excessive force during traction by the suture can result in splitting of the tissue.^[9] On the other hand, since scalpel and suture traction was not used in the punch biopsy group, splitting was seen significantly less in these specimens.

Fragmentation artefact [Figure 6] was found to be significantly less in punch biopsy specimens as compared to incisional biopsy specimens (Chi-square test $P < 0.01$) under the study. Fragmentation in the deep portions of the tissue was observed to be greater than those in the incisional biopsy group. This can be attributed to the use of scissors at the tissue base for releasing the core of the tissue in the punch biopsy technique. Whereas, in case of scalpel / wedge biopsy, the incisions on either side of the ellipse converge in a “V” to join in deeper sublesional tissues. Thereby, the chances of fragmenting the tissue at the base are reduced in the incisional biopsy group. Whereas, superficial fragmentation of the tissue was found to be greater in this group due to the use of suture traction and subsequent use of scalpel.

The histological diagnoses for group I and group II included: lichen planus, leukoplakia, oral submucous fibrosis, squamous cell carcinoma and lichenoid reaction.

CONCLUSION

Several papers have reported that artefacts are common in oral mucosal biopsy specimens.^[2,3,8] Therefore, it was felt that a reduction in artefacts caused by tissue handling could be achieved with the disposable biopsy punch and yet still provide sufficient material for diagnostic purposes. Though the punch biopsy had its own disadvantages, we found that it produced biopsy specimens with fewer artefacts as compared to the conventional scalpel / wedge biopsy. Through this clinico-pathologic study, we wish to alert the clinician about the possible diagnostic pitfalls arising due to faulty handling of the tissue. These artefacts may go unnoticed clinically but can create potential diagnostic problems to the pathologist during histopathological examination. This study was done to suggest an alternative biopsy technique (to the conventional scalpel / wedge biopsy), which is simple, fast and safe with the resulting specimen having fewer artefacts.

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