

# Analysis of Histopathological Artifacts in Oral Biopsy Specimen- A Descriptive Cross Sectional Study

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## ABSTRACT

### Introduction

Arriving at the final diagnosis requires the histopathological examination of the biopsied lesion. Many a times, diagnosis of lesion may be hindered due to presence of artifacts in the slide. Having a thorough knowledge of these artifacts help to take the precautionary measures to avoid their occurrence. This study is an attempt to analyze histopathological slides from Department of Oral Pathology to identify the artifacts seen in oral biopsy specimens.

### Methods

This cross sectional study was conducted in Department of Oral Pathology, Dhulikhel Hospital from July 2021 to February 2022. Slides of all the oral biopsies during the study period were included in the study. The artifacts were divided into three groups: Artifacts related to surgeons performance, artifacts related to technicians performance and artifacts caused during transfer of sample to the laboratory. Then, the frequency distribution for each type of artifact was calculated.

### Results

A total of 280 slides were included in the present study. Artifacts related to technicians performance were seen in 89.3% slides whereas artifacts related to surgeons performance were seen in 76.4% slides. None of the slides showed artifacts related to transfer of sample to the laboratory. The most common artifact seen was eosin leaching (63.6%) followed by stain deposit (60%) and folds and wrinkles (40.7%).

### Conclusions

The findings of our study showed that various types of artifacts may be incorporated in biopsy specimen that create difficulty in diagnosing the lesion properly. Proper biopsy protocol and careful handling of sample to prevent technical errors may be helpful to reduce the frequency of artifacts.

**Keywords:** Artifacts; Biopsy; Diagnosis, Histopathology.

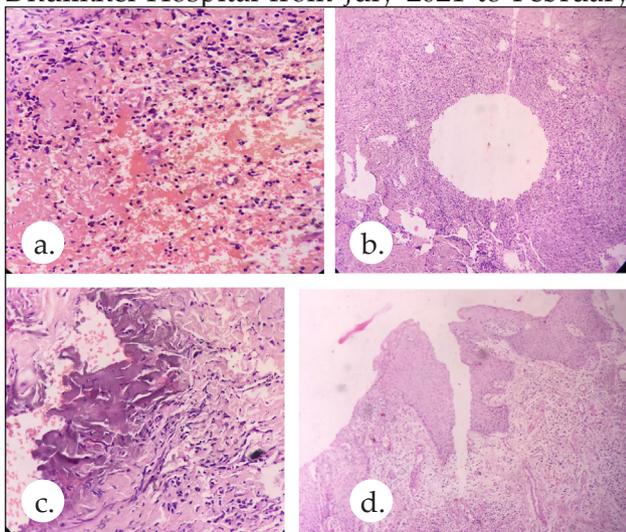
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## INTRODUCTION

Biopsy refers to the process of obtaining the tissue from living organism for the purpose of histopathological examination.<sup>1</sup> It is considered as the gold standard for diagnosis of the lesion.<sup>2</sup> However, various types of artifacts may be incorporated in the biopsy specimen that may sometimes create difficulty in diagnosing the lesion properly.<sup>3,4</sup> These artifacts are usually an artificial structure or tissue alteration seen on a prepared microscopic slide due to some extraneous factors.<sup>5</sup> Many a times, these artifacts may cause serious errors and misdiagnosis. Oral biopsies are of small size; as a result, artifacts are very likely to be incorporated during processing, microtomy and staining procedures. It is important to identify these artifacts during interpretation of prepared biopsy specimen.<sup>2,5</sup> Having adequate knowledge of artifacts helps to minimize or even avoid errors during diagnosis.<sup>2</sup> Thus this study is an attempt to identify the various types of artifacts in histopathological slides of oral biopsies.

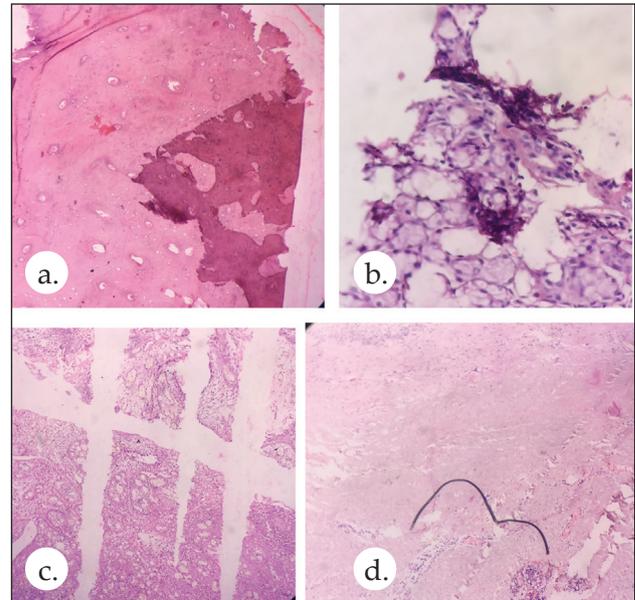
## METHODS

This descriptive cross sectional study was conducted in Department of Oral Pathology, Dhulikhel Hospital from July 2021 to February



**Figure 1.** Various types of artifacts related to surgeon's performance. A: Hemorrhage (H & E; 40x), B: Forcep injury (H & E; 10x), C: Coagulation of proteins (H & E; 40x), D: Split (H & E; 10x).

The artifacts related to technicians performance include: chattering, tangential section of epithelium, folds and wrinkles, knife scoring, stain deposit, eosin leaching, air bubble, contamination of mounted section, overstain and understain (Figure 2).



**Figure 2.** Various types of artifacts related to technician's performance. A: Folding (H & E; 10x), B: Stain deposit (H & E; 40x), C: Knife scoring (H & E; 10x), D: Foreign body contamination (H & E; 10x).

Artifact caused during transfer of sample to the laboratory includes autolysis.<sup>6</sup> All the slides were examined using Olympus Optical microscope (CX22LED) and the obtained data were analysed using SPSS version 23.

## RESULTS

A total of 280 slides were included in the present study and all the slides consisted of some sorts of artifacts. Artifacts related to technicians performance were seen in 250 (89.3%) slides whereas artifacts related to surgeons performance were seen in 214 (76.4%) slides. None of the slides showed artifacts related to transfer of sample to the laboratory (Table 1).

<b>Table 1.</b> Frequency distribution based on the cause of artifacts	
	n (%)
Artifacts related to technicians performance	250 (89.3)
Artifacts related to surgeons performance	214 (76.4)
Artifact related to transfer of sample to the lab	0 (0%)

The most common artifact seen in the present study was eosin leaching 178 (63.6%) followed by stain deposit 168 (60%) and folds and wrinkles 114 (40.7%). All the three artifacts were related to technicians performance. Other artifacts related to technicians performance consisted of knife scoring 64(22.9%), under stain 38(13.6%), air bubble 32(11.4%), foreign body contamination 12(4.3%), tangential section of epithelium 12(4.3%), overstain 6(2.1%) and chattering 4(1.4%).

The most frequent artifacts related to surgeons

performance comprised of split 94 (33.6%) followed by fragmentation of tissue 68 (24.3%). Coagulation of protein was seen in 54(19.3%), forcep injury/voids 54(19.3%), hemorrhage 44(15.7) and separation of connective tissue bands due to injection of anesthesia in 16 (5.7%) (Table 2).

## DISCUSSION

Artifacts in histopathological slides are often encountered by the pathologist. Many a times these changes may lead to alteration in tissue details leading to difficulty in diagnosis of the lesion.<sup>7</sup> It is therefore very important to identify these artifacts while interpreting the histopathological slides.

In our present study, none of the slides were free of artifacts. All the slides consisted of some sorts of artifacts related to surgical or laboratory

<b>Table 2.</b> Frequency distribution of various types of artifacts seen in present study.		
	Type of Artifact	n (%)
Artifacts related to technicians performance	Eosin leaching	178(63.6)
	Stain deposit	168(60)
	Folds and wrinkles	114(40.7)
	Knife scoring	64(22.9)
	Understain	38(13.6)
	Air bubble	32(11.4)
	Foreign body contamination	12(4.3)
	Tangential section of epithelium	12(4.3)
	Overstain	6(2.1)
	Chattering	4(1.4)
Artifacts related to surgeons performance	Split	94(33.6)
	Fragmentation of tissue	68(24.3)
	Coagulation of protein	54(19.3)
	Forcep injury/voids	54(19.3)
	Hemorrhage	44(15.7)
	Separation of connective tissue bands due to injection of anesthesia	16(5.7)

procedure. However, none of the slides showed artifacts related to transfer of sample to the laboratory. Most of the artifacts observed in this study were related to technicians performance which is in accordance to the findings of Seify et al.<sup>6</sup> Artifacts due to technician's performance can be created at any stage of tissue handling in laboratory such as: processing, embedding and staining.<sup>4</sup> Eosin leaching, stain deposit and folds/wrinkles were the commonest artifacts observed in our study due to technicians performance. Stain deposits usually occur due to use of old or unfiltered dye solution. Eosin leaching may be due to presence of water in the sections.<sup>8</sup> If eosin stained sections are washed in tap water with an acidic pH, there might be leaching of stain into the mounting media. This artifact is more common in humid areas which may be due to atmospheric moisture being absorbed by alcohols and xylene substitutes.<sup>8</sup>

In a similar study done by Kargahi et al. folding was the commonest artifact and this may occur due to use of dull blade.<sup>9</sup> Regular replacement of blade may be useful to avoid such artifacts. Other artifacts seen in our study related to technicians performance includes: knife scoring, under stain, air bubble, foreign body contamination, tangential section of epithelium, overstain and chattering. Knife scoring occurs due to nick in knife and is avoided by sharpening the knife. Air bubble entrapment may occur when mounting medium is too thin and can be avoided by using mounting media of adequate thickness.<sup>10</sup> In our study, 4.3% cases showed foreign body contamination, which is similar to the findings of Rafieyan et al. This may occur when the water bath is contaminated with dust, hair or residual cells of previous sections and is best avoided by frequent cleaning of the water bath.<sup>7</sup> Tangential section of epithelium may give impression of pseudoinvasion and may lead to false diagnosis. Hence careful evaluation of

section and identification of the artifact is must. In our study, only 4.3% cases showed this artifact which is in contrast to the result of Rafieyan et al. wherein this artifact was observed in 43.7% cases.

About 2.1% cases in our study showed overstaining and 1.4% cases showed chattering. Overstaining may be due to prolonged staining duration where as chattering may be due to several reasons such as loosely attached microtome knife or tissue block, steep cutting angle and hard tissue/wax or presence of calcification in the tissue.<sup>8,10</sup> It is the visible record of knife vibration which appears as narrow parallel spaced evenly across the tissue specimen. This can be prevented by altering the tissue thickness, changing orientation and soaking the block face in water or detergent.<sup>10</sup>

In our study, we observed that split and fragmentation of tissue were the most common artifacts related to surgeon. This is in accordance to the results obtained by Seify et al and Saravani et al.<sup>6, 11</sup> Moule et al<sup>12</sup> and Seoane et al<sup>13</sup> also reported high incidence of split and fragmentation in their study. This type of artifact may occur due to use of toothed forcep and can be best avoided by using blunt forcep.<sup>8</sup> This also holds true for void artifact seen in the slide.<sup>10</sup> We also observed coagulation of protein in 19.3% cases. This occurs due to use of electrocautery during biopsy which led to dehydration of tissue. This can be prevented by using cutting instead of coagulation electrodes while obtaining the biopsy specimen. This will produce low milliamperere current and there will be less tissue damage.<sup>8,10</sup>

As mentioned by Rastogi et al, intralesional injection of anaesthetic solution may produce hemorrhage and separation of connective tissue bands.<sup>10</sup> In our study 15.7% cases showed hemorrhage and only 5.7% cases showed

separation of connective tissue bands. These artifacts can be prevented by injecting the anesthesia 3-4mm away from the biopsy site.<sup>10</sup>

Thus in present study we observed that majority of artifacts are related to technician and few are related to surgeon. However, with adequate skill, careful attention and proper handling of specimen in laboratory, these artifacts can be prevented. Nevertheless, our study represents slides from only one institution and needs to be validated by further studies with larger number of samples.

## CONCLUSIONS

The findings of present study highlights the fact that histopathological slides are subjected to various surgical and technical errors. These errors appear in the form of various artifacts that might create difficulty in diagnosing the lesion. Careful attention and proper handling of specimen in laboratory along with consultation among surgeons, technicians and pathologists may be helpful to achieve a better diagnosis.

**Conflict of interest:** None

## REFERENCES

1. Mota-Ramírez A, Silvestre FJ, Simó JM. Oral biopsy in dental practice. *Med Oral Patol Oral Cir Bucal*. 2007;12(7):504–10. PMID: 17978774
2. Yanduri S, Pandey G, Kumar V, Suma S, Madhura M. Artifacts in oral biopsy specimens: A comparison of scalpel, punch, and laser biopsies. *Indian J Oral Heal Res*. 2016;2(2):100.
3. Alonso FC, Jornet PL, Torres MJJ, Domingo AO. Analysis of the histopathological artifacts in punch biopsies of the normal oral mucosa. *Med Oral Patol Oral Cir Bucal*. 2008;13(10):636–9. PMID: 18830171
4. Rafieyan S, Farsadeghi M, Firoozi P, Sokhansanj M. Frequency of Different Types of Artifacts among Oral and Maxillofacial Histopathological Slides in Zanzan Dental School from 2015 to 2017. *Avicenna J Dent Res*. 2019;11(4):116–9. doi: 10.34172/ajdr.2019.23
5. Shah A, Kulkarni M, Gabhane M. Artifacts in Oral Biopsies : A Study. 2012;3(4):217–20.
6. Seifi S, Mehdizadeh M, Bijani A, Alipour A, Nafarzadeh SH. An Investigation into the Frequency and Type of Artifacts in Oral Histopathologic Slides. *J Res Dent Sci*. 2014;10(4).
7. Taqi SA, Sami SA, Sami LB, Zaki SA. A review of artifacts in histopathology. *J oral Maxillofac Pathol JOMFP*. 2018;22(2):279. doi: 10.4103/jomfp.JOMFP\_125\_15. PMID: 30158787
8. Bindhu PR, Krishnapillai R, Thomas P, Jayanthi P. Facts in artifacts. *J Oral Maxillofac Pathol*. 2013;17(3):397–401. doi: 10.4103/0973-029X.125206. PMID: 24574659
9. Khan S, Tijare M, Jain M, Desai A. Artifacts in Histopathology: A Potential Cause of Misinterpretation. *Res Rev J Dent Sci*. 2014;2(2):23–31.
10. Rastogi V, Puri N, Arora S, Kaur G, Yadav L, Sharma R. Artefacts: A diagnostic dilemma - A review. *J Clin Diagnostic Res*. 2013;7(10):2408–13. doi: 10.7860/JCDR/2013/6170.3541. PMID: 24298546
11. Saravani S, Kadeh H, Shahsavari M, Shahrakipour M, Mosafer B.

Evaluation of Artifacts in Oral and Maxillofacial Histopathological Slides. *J Dentomaxillofac Radiol Pathol Surg.* 2016;5(3):11–6.

12. Moule I, Parsons PA, Irvine GH. Avoiding artefacts in oral biopsies: the punch biopsy versus the incisional biopsy. *Br J Oral Maxillofac Surg.* 1995;33(4):244–7.

doi: 10.1016/0266-4356(95)90010-1. PMID: 8736752

13. Seoane J, Varela-Centelles P, Ramirez JR, Romero MA, De La Cruz A. Artefacts produced by suture traction during incisional biopsy of oral lesions. *Clin Otolaryngol Allied Sci.* 2002;27(6):549–53. doi: 10.1046/j.1365-2273.2002.00619.x. PMID: 12472529

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