

# Evaluation of Artifacts in Oral and Maxillofacial Histopathological Slides

## Original Article

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Received: Sep 1, 2016  
Accepted: Nov 16, 2016

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## Abstract

### Introduction:

One of the reasons that cause a pathologist to make an incorrect diagnosis includes artifacts leading to tissue changes and artificial structures in microscopic slides. The aim of this study was to examine the artifacts in histopathological slides in Zahedan School of Dentistry.

### Materials and Methods:

In this cross-sectional study, all the available slides in Oral and Maxillofacial Pathology Archive at the School of Dentistry during 1999–2015 were evaluated for existing artifacts. The artifacts found in the slides were divided into the following three categories: 1) caused by a surgeon's performance, 2) resulting from sample transfer to laboratory, and 3) arising from a technician's performance. Then, the frequency of each type of artifact was calculated in numbers and percentages and analyzed with the proportions of two populations test.

### Results:

All the 658 histological slides contained artifacts. Moreover, 96.5% of the artifacts were associated with a technician's performance, and 90.4% were associated with a surgeon's performance. The proportion of difference between the two types of artifacts was statistically significant ( $P$  value = 0.001). The most common artifacts resulting from technicians' performances included formalin pigmentation, folding, and bubble. However, the most common artifacts resulting from surgeons' performances were split, crush, and hemorrhage.

### Conclusion:

The results of this study indicated that there was a high frequency of artifacts in oral histological slides. Retraining courses seemed to be helpful for practitioners involved in preparation of slides, in addition to increased cooperation of surgeon–pathologist, which can reduce the risk of artifacts.

### Key words:

•Artifacts •Biopsy •Oral Pathology •Mouth

## Introduction

The term “artifact” is derived from the Latin words art and factum. Artifact refers to the artificial structure or tissue change caused by external agents on a microscopic slide.<sup>(1)</sup> In fact, artifacts lead to a change in the normal morphological or cytological form, which might subsequently result in histological misinterpretation and incorrect diagnosis.<sup>(2)</sup> Artifacts occur for different reasons, and one among them is the sampling procedure, for example. It has been demonstrated that punch biopsy would leave significantly fewer artifacts compared with incisional scalpel biopsy.<sup>(3, 4)</sup> In a relevant study, the degree of artifacts in the margins of CO2 laser samples was reported to be higher than that with steel instruments.<sup>(5)</sup>

Artifacts might be minor involving a small part of samples without interfering the ability of pathologists to provide a precise diagnosis. Sometimes, however, the artifact is large enough to completely cover the sample, leading to inapplicability of a slide for diagnostic purposes.<sup>(6)</sup> Based on the recommendations by the American Academy of Oral and Maxillofacial Pathology, all abnormal tissues extracted from the oral and maxillofacial area must be sent to a pathologist for examination. The microscopic evaluation of slides is the gold standard for diagnosing most of the various pathologies.<sup>(7, 8)</sup> The appropriate preparation of tissues for microscopic evaluation is related to the measures taken by the surgeon, his/her associate, and the histotechnician to decrease the risk of artifacts.<sup>(9)</sup> In a study on the Archives of Pathology Department at Babol School of Dentistry, only one slide had no artifact among 154 slides. Moreover, 91.6% of the slides had artifacts resulting from a surgeon's performance, 99.4% from a technician's performance, and 0.06% from improper sample transfer.<sup>(10)</sup>

It has been reported that theoretical and practical training workshops could enhance the ability of general dental practitioners in oral biopsy.<sup>(11)</sup>

Therefore, it is crucial to gain a complete understanding of artifacts and take appropriate preventive measures.<sup>(1)</sup> Since artifacts frequently occur in oral biopsy,<sup>(12, 13)</sup> and there are very few studies comprehensively exploring the oral biopsy artifacts,<sup>(10)</sup> it was felt that a detailed in-

vestigation on the prevalence of artifacts in oral biopsies should be conducted.

Thus, the current study was designed to examine the histological artifacts in the Archives of Pathology at Zahedan School of Dentistry to facilitate plans for curtailing the problem.

## Materials and Methods

This cross-sectional study was approved by the Moral Committee of Zahedan University of Medical Sciences (code IR.ZAUMS.REC.1395.91). All the slides available in the Archives of Oral and Maxillofacial Pathology at Zahedan School of Dentistry between May 1999 and September 2015 were examined. From 973 slides, 315 slides that were isolated from the lamella were not assessed. All slides were stained with hematoxylin and eosin (H&E). The eligible slides (658 slides) were selected and an oral and maxillofacial pathologist evaluated the slides for the presence or absence of artifacts as well as their types. The artifacts found in the slides were divided into the following three categories: 1) arising from a surgeon's performance, 2) arising from sample transfer to laboratory, and 3) arising from a technician's performance.<sup>(14)</sup>

The artifacts arising from the surgeon's performance included hemorrhage, inadequate depth of the specimen, crush, split (tissue rupture), and foreign bodies. The artifacts arising from the technician's performance included remaining wax, formalin pigmentation, folding, tangential cutting (entrapment of soft tissue within the epithelium), staining artifacts, bubble, presence of water, bone artifacts, and those caused by blunt blade.<sup>(1, 2, 15)</sup> Finally, each type of artifact was described in terms of percentage, and the relationship between artifacts and the technician's/surgeon's performance was determined through the proportional difference between the two populations test.

## Results

This study focused on a total of 658 histopathological slides available in the archive of Oral and Maxillofacial Pathology. The results revealed that all the slides contained artifacts. Eighty-six slides had only one type of artifact, 23 slides had only artifacts associated with a surgeon's performance, 63 slides had only artifacts associated with a technician's performance, and 572 slides

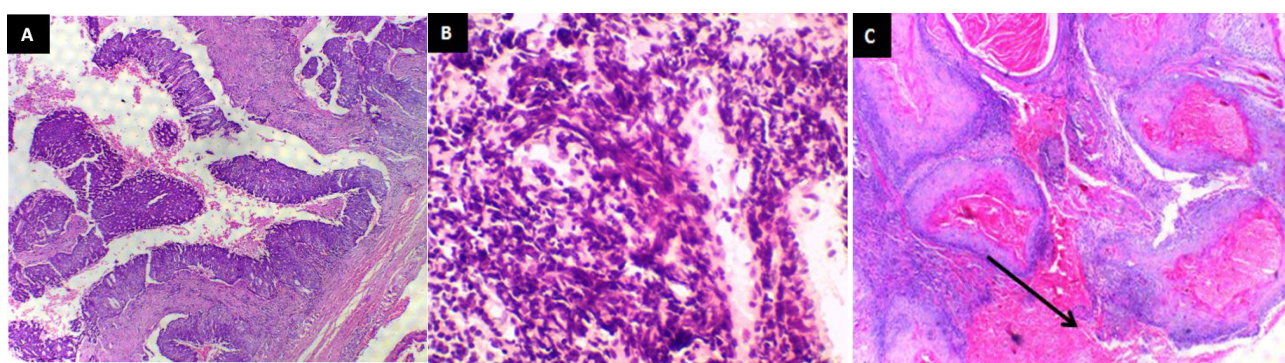


showed both types of artifacts (artifacts associated with a surgeon's and a technician's performance). Overall, a total of 595 slides (90.4%) contained artifacts associated with a surgeon's performance, whereas 635 slides (96.5%) contained artifacts associated with a technician's performance, and this difference was statistically significant ( $P < 0.001$ ). No case of artifact was found associated with the transfer of samples to the laboratory. The most common artifacts resulting from a surgeon's performance were related to split (86%), whereas it was formalin pigmentation (90.9%) in case of the technician's performance. Table 1 illustrates the types of artifacts related to the performance of surgeons and technicians separately. Artifacts left during staining included stain sediments (36.2%) and unstained areas (4.7%). Artifacts resulting from blunt blade contained chatters (thick and thin areas parallel to the blade edge) and scratch lines in 145 and 295 cases, respectively. Bone tissue artifacts included the dislocation of bone tissue from the connective tissue (14.4%), bone dust particles (11.4%), inadequate calcification (2.6%), and over-decalcification (1.1%).

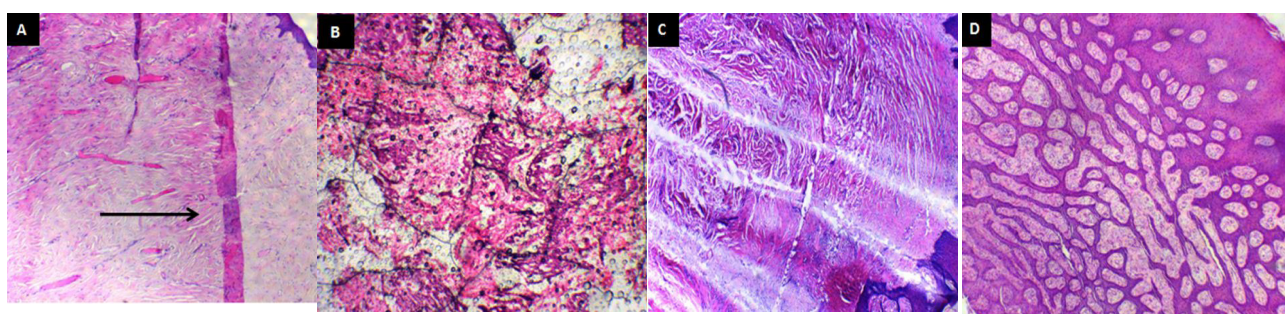
Figures 1 and 2 show the various artifacts in the histological slides pertaining to the technician's and surgeon's performances.

**Table 1.** Different types of artifacts related to the surgeon's and technician's performances

Artifact classification	Artifact name	Number (%)
Related to the surgeon's performance	Split	566 (86)
	Crush	197 (29.9)
	Hemorrhage	181 (27.5)
	Inadequate depth of the specimen	21 (3.2)
	Foreign body	12 (1.8)
Related to the technician's performance	Formalin pigmentation	598 (90.9)
	Curling/folding	572 (86.9)
	Bubble	490 (74.5)
	Blunt blade	440 (66.9)
	Staining	269 (40.9)
	Tangential sections	160 (24.3)
	Bony tissue artifact	95 (14.4)
	Presence of water in the section	88 (13.4)
	Residual wax	51 (7.8)



**Figure 1.** Various artifacts in the histological slides pertaining to the surgeon's performance. (A) Split artifact (H&E staining; 40×), (B) Crush artifact (H&E staining; 400×), and (C) Hemorrhage artifact (H&E staining; 40×).



**Figure 2.** Various artifacts in the histological slides pertaining to the technician's performance. (A) Folding artifact (H&E staining; 40×), (B) Bubble artifact (H&E staining; 100×), (C) Artifacts associated to blunt blade (H&E staining; 40×), (D) Tangential sections (H&E staining; 40×).

## Discussion

The results of this study showed that all the slides contained artifacts, which were almost consistent with the results reported by Seify et al.<sup>(10)</sup> It seems that artificial changes in the sample of oral lesions might be due to the small size and rapid tissue dehydration as oral pathologists are faced with more difficulty in making a correct diagnosis.<sup>(10)</sup> Most of the artifacts observed in this study resulted from a technician's performance, which was consistent with the finding reported by Seify et al.<sup>(10)</sup>

Formalin pigmentation was the most common artifact arising from a technician's performance, the frequency of which was greater than that observed in Seify's study (59.4%).<sup>(10)</sup> All the collected biopsies were supposed to be rinsed with physiological saline and immediately fixed. A good fixative should rapidly penetrate into the tissue to prevent tissue autolysis and maintain the cellular details. The best fixative for optical microscopic examination is 10% formaldehyde in water.<sup>(15)</sup> Formalin can build intermolecular bridges and cross-link between the terminal groups in proteins to prevent autolysis.<sup>(7,9)</sup> Formalin pigment, also known as acid hematin pigment,<sup>(16)</sup> is commonly found in biopsies where formalin would be fixed through acid pH or in environments at a high temperature and humidity. Furthermore, it was found that formalin pigment in tissues with heavy bleeding was more common. Neutral buffered formalin is suggested to prevent the formation of formalin pigment, especially for long-term fixation in environments at a high temperature and humidity.<sup>(17)</sup>

Curling/folding is the second most common artifact found in the oral tissues. Most of the samples delivered to the archive at the Zahedan School of Dentistry are incisional biopsies. It was found that the risk of artifacts in incisional biopsies would tend to be higher because when the tissue was small, the shrinkage process due to formalin fixation led to reduction in size, thus bending the tissue and making its proper orientation difficult during embedding.<sup>(1)</sup>

These artifacts were similarly common in Seify's study.<sup>(10)</sup> It would be beneficial to transfer the sample slices to a new water bath, or a small amount of detergent could be added to the water bath for reducing such artifacts.<sup>(6)</sup>

The third most common artifact resulting from a technician's performance was the bubble, which was similarly one of the most common artifacts in the earlier study.<sup>(10)</sup> These artifacts occurred due to trapped air bubbles beneath the lamellae, thus leading to cracked areas. Therefore, the lamella failed to stick properly and made staining alteration.<sup>(2)</sup> Such a condition can be prevented through adequately thickening the adhesive material or removal of air bubbles from under the lamella during mounting.<sup>(1)</sup>

Artifacts caused by blunt blade in the present study were present in 66.9% of the slides. Other studies assessed only some types of blunt blade artifacts and consequently their frequency was less than that observed in our study.<sup>(10, 18)</sup> Blunt blade artifacts (chatters and scratches) can arise from loose connection of blade or tissue blocks to the machine, improper angle of the cutting blade, unhealthy blade, hard wax, and calcified materials in the tissue.<sup>(2)</sup>

In this study, stain sediments and unstained areas were observed in 36.2% and 4.7% of the slides, respectively, while these two artifacts were present in 8% and 10% of slides, respectively, in the study by Shah et al.<sup>(18)</sup> It should be noted that Shah et al. examined the slides obtained from three different surgical techniques, and they had fewer sample size than the present study. Stain sediments come from altered nature and intensity of old dyes or dye pollution, which can be prevented by dye filtering and staining in appropriate time and temperature.<sup>(1,19)</sup> In addition, the creation of unstained areas can be avoided by filling the staining container sufficiently.<sup>(6)</sup>

Split contributed to most of the artifacts in the histological slides caused by the surgeon's performance, which was consistent with the results obtained by Seify et al.<sup>(10)</sup> Nevertheless, Camacho Alonso et al. reported that there was no split in the punch biopsy of normal oral mucosa. Apparently, this type of artifact is influenced by the type of tools used for the biopsy procedure and the type of biopsied tissue.<sup>(20)</sup> This artifact was caused by the application of toothed forceps that penetrate the tissue.<sup>(2)</sup> Nowadays, it has been observed that forceps B can lead to a far lower number of artifacts in biopsy of oral lesions.<sup>(21)</sup> The second artifact resulting from a surgeon's performance was crush. In a study, Seoane et al. showed that samples delivered by general



dental practitioners (27.1%) contained significantly more cases of crush compared to those delivered by oral and maxillofacial surgeons (10.2%).<sup>(22)</sup> This type of artifact occurs prior to fixation when the sample is placed using forceps with extreme force.<sup>(2)</sup> The crushed cells are seen as dark chromatin stripes, which may be confused with dysplastic lesions.<sup>(1)</sup> This artifact is more common in inflammatory lesions.<sup>(22)</sup> The occurrence of such artifacts can be prevented through the application of appropriate forceps and careful handling of samples, particularly those with delicate bases.<sup>(1)</sup> Segal et al. reported that biopsy through scissors led to significantly lower cases of crush compared with forceps.<sup>(23)</sup> Another artifact potentially occurring due to a surgeon's performance is hemorrhage, which was highly prevalent in Seify's study.<sup>(10)</sup> Injecting a large amount of anesthetic solution into the biopsy area can cause hemorrhage, which covers the cell structures.<sup>(6)</sup> Hemorrhage was observed in 8.5% and 19.8% of the samples delivered by oral and maxillofacial surgeons and general dental practitioners, respectively. Moreover, oral and maxillofacial surgeons found significantly lower cases of hemorrhage in their samples.<sup>(22)</sup> These artifacts can be prevented by injecting the anesthesia 3–4 mm away from the main area of the lesion and the surroundings in four different directions.<sup>(1)</sup>

The strength of this study was the high volume of examined slides in comparison with similar studies. Since most slides of pathology archive at the Zahedan School of Dentistry are prepared

from incisional biopsy, we cannot compare the artifacts arising from different types of surgical procedures. It is recommended that in the future studies artifacts of punch biopsy and biopsy with different types of laser be evaluated.

## Conclusion

This study demonstrated that there was a high frequency of artifacts in the histopathological oral and maxillofacial slides. Moreover, 96.5% of the artifacts were associated with a technician's performance, and 90.4% were associated with a surgeon's performance, and this difference between the two types of artifacts was statistically significant. The most common artifacts resulting from technicians' performances included formalin pigmentation, folding, and bubble. However, the most common artifacts resulting from surgeons' performances were split, crush, and hemorrhage. Therefore, increased cooperation of surgeons, technicians, and pathologists and retraining courses for laboratory technicians and clinicians with varying degrees of education (i.e., general dental practitioners, residents, and specialists) may be helpful to reduce the artifacts.

## Acknowledgement

The authors gratefully acknowledge the laboratory technician of Zahedan School of Dentistry. This study was a student research project (Grant No: 95-7802) supported and funded by Zahedan University of Medical Sciences.

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